PHARMACOGNOSY & PHYTOCHEMISTRY - I

Chapter No.: III

Glycosides

By: Vipul Dhasade

INTRODUCTION

- Primary metabolites : Carbohydrates, Proteins, Amino acids, Lipids
- Secondary metabolites : Glycosides, Flavonoids, Alkaloids, Terpenoids, Volatile oils, Essential oils, Tannins, Resins.
- Phytopharmaceuticals: Significance/ Applications

Glycosides

They are secondary products formed from one or more sugar molecules added to a non-sugar biologically active molecule

- Sugar most commonly found is glucose
- Non-sugar molecule known as the aglycone and may be
  - amino acid derivatives
  - steroids
  - triterpenes

Glycosides

- Glycosides consist of

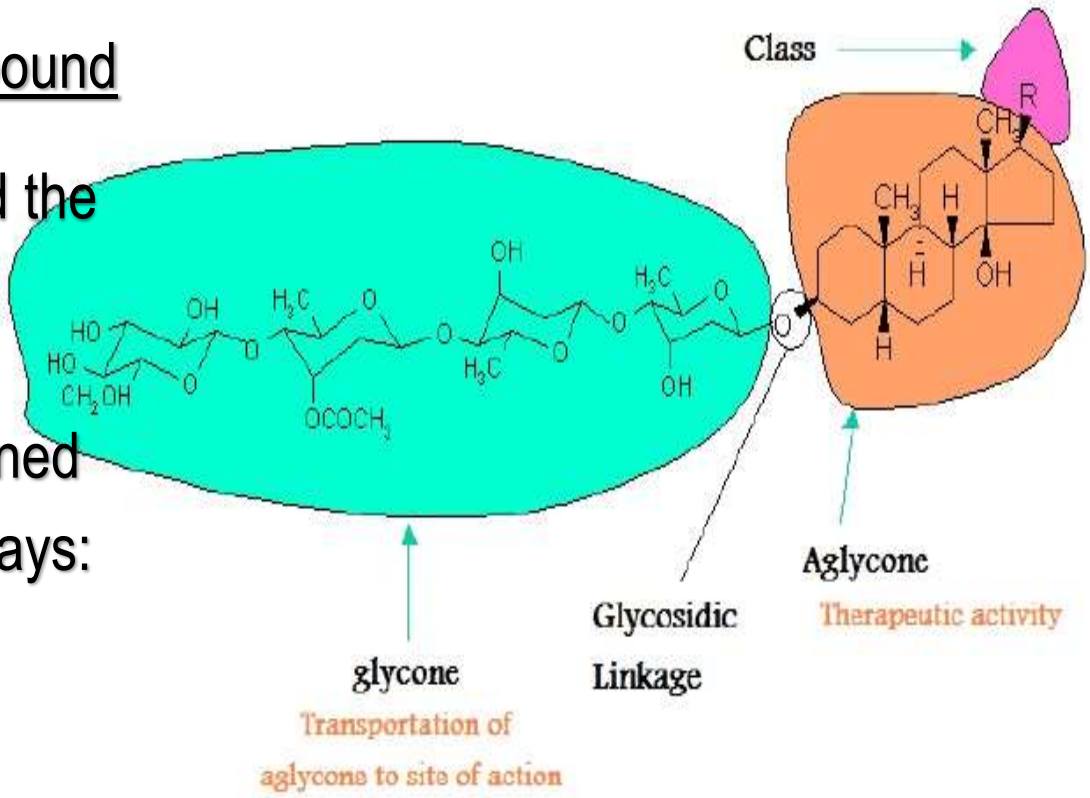
a sugar residue <u>covalently bound</u>

to a different structure called the

aglycone.

The sugar moiety can be joined to the aglycone in various ways:

1. Oxygen *(O-glycoside)*
2. Sulphur *(S-glycoside)*
3. Nitrogen *(N-glycoside)*
4. Carbon (*Cglycoside)*



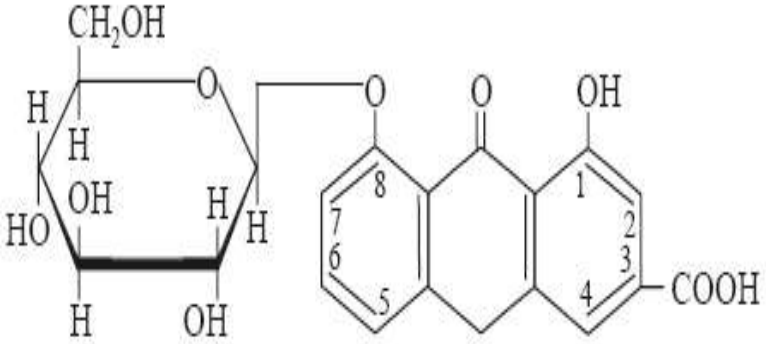
- Definition:: The organic compounds from plant or animal source which on enzymatic or acid hydrolysis give one or more sugar moieties along with non-sugar moieties.

Glycone -------- (Glycosidic linkage)-------- Aglycone (genin)

- Almost all plants that contain glycosides also contain enzymes that bring about their hydrolysis (**glycosidases**).
- α-Glycosides and β-glycosides are distinguished by the configuration of the hemiacetal hydroxyl group.
- The majority of naturally-occurring glycosides are β-glycosides.
- O-Glycosides can easily be cleaved into sugar and aglycone by hydrolysis with acids or enzymes.

1.1 O-Glycosides

- The **O-glycosides** are usually represented as follows:
- **Glycone---- (OH + H)---C—Aglycone…… *O-Glycoside***
- **Glycone---- C—Aglycone + H_2O**



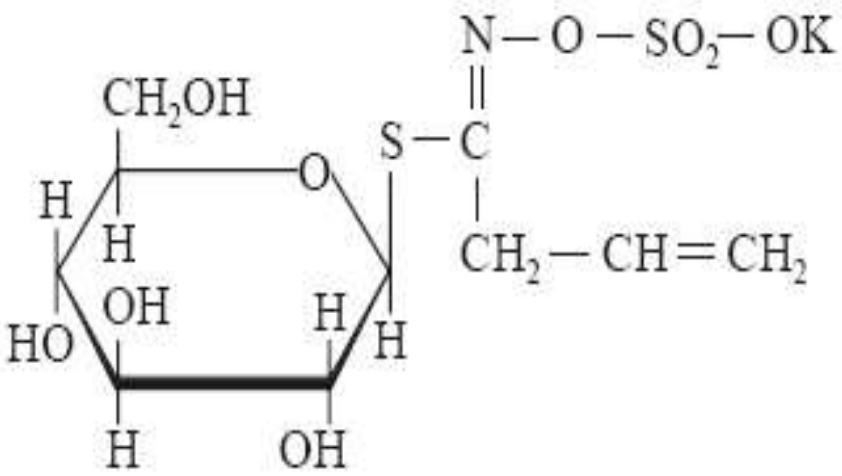
Rhein-8-Glycoside

- such as: senna, rhubarb and frangula.
- **Examples: Rhein-8-glucoside** obtained from rhubarb.
-

1.2 S-Glycosides

- The **S-glycosides** are normally designated as below:
- **Glycone---- (OH + H)---S—Aglycone…… *S-Glycoside***
- **Glycone---- S—Aglycone + H_2O**
- —SH $HO—C_6H_{11}O_5$ ——> $S—C_6H_{11}O_5$….. S-*Glycoside*

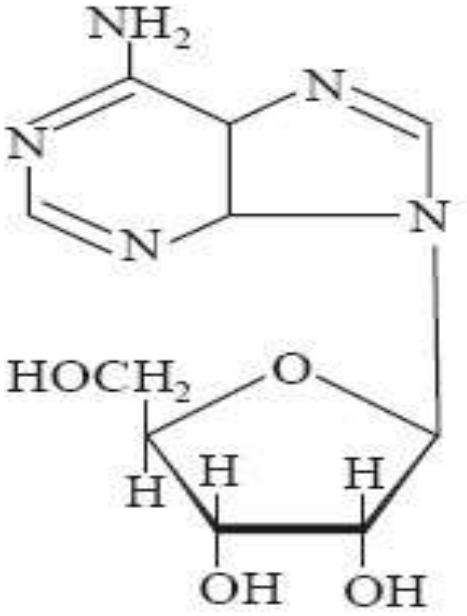
- such as: **Sinigrin,** obtained from
- black mustard seeds.
- (*i.e.*, **Brassica campestris** Family: *Cruciferae*)



Sinigrin

1.3 N-Glycosides

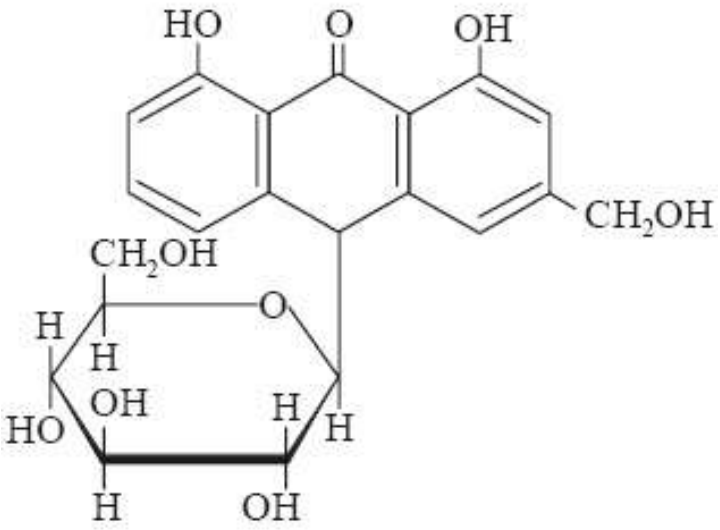
- The **N-glycosides** may be represented as shown below:
- **Glycone---- (OH + H)---N—Aglycone…… *N-Glycoside***
- **Glycone---- N—Aglycone + H_2O**
- =N—H HO—$C_6H_{11}O_5$ ——> =N—$C_6H_{11}O_5$ *N-Glycoside*
- The most typical example of N-glycosides is the nucleosides, wherein the respective amino group of the base ultimately reacts with —OH group ribose/deoxyribose.
- **Examples: Adenosine:** It is widely distributed in nature *e.g.*; from yeast nucleic acid.



Adenosine

1.4 C-Glycosides

- The **C-glycosides** may be designated as shown under:
- **Glycone---- (OH + H)---C—Aglycone…… *C-Glycoside***
- **Glycone---- C—Aglycone + H_2O**
- CH HO—$C_6H_{11}O_5$ ——> C—$C_6H_{11}O_5$ *C-Glycoside*
- C-Glycosides are present in a variety of plant substances, such as: **aloin** in Aloe; **cascaroside** in Cascara.
- E.g. **Aloin or Barbaloin:**



Aloin (or Barbaloin)

Physical Characters:

- Solids either amorphous or crystalline.
- Non volatile.
- Usually bitter in taste.
- Soluble in water and polar organic solvents.
- Reduce Fehling's solutions only after hydrolysis.

Properties:

- Glycosides are usually soluble in water and in polar organic solvents, where as aglycone are normally insoluble or only slightly soluble in water.
- It is often very difficult to isolate intact glycosides because of their polar character.
- Many important drugs are glycosides and their pharmacological effects are largely determined by the structure of the aglycone.

Extraction and Isolation

- Because of the wide range of physical and chemical properties of glycosides and other constituents associated with them, no common general method for their isolation is recommended.
- Water, methanol, water-ethanol and ethanol are the most common solvents for extraction of glycosides.

- **Precautions before extraction**
  - **Deactivation of enzymes:**
- **Drying** for 15-30 min at **100 ^{o}C** followed by slow drying at a low temperature.
- Dipping the fresh material into **boiling water** or **boiling alcohol** for 10-20 min.
- **Boiling** the fresh plant material with **acetone**.
- Carrying out the extraction at very low temp.
- **Freeze-drying** of the plant material **before extraction** (lyophilization).
- Carrying the extraction in the presence of $(NH_4)_2SO_4$.

Classification:

- According to chemical nature of aglycone part/ therapeutic activity exhibited by the same

1. Anthraquinone / anthracene Glycosides- Senna, Aloe, Rhubarb, Cascara
2. Cardiac / sterols Glycosides- Digitalis, Squill, Strophanthus, Quabain
3. Saponin Glycosides- Dioscorea, Shatavari, Brahmi, Ginseng,
4. Cynogenetic / cynophoretic Glycosides- Bitter almond, wild cherry bark
5. Isothiocynate Glycosides- Mustard (black)
6. Flavonoids / flavonol Glycosides- Silymarin, Gingko, Citrus fruits
7. Coumarins and Furanocoumarin Glycosides- Visnaga, Psoralea
8. Aldehyde Glycosides-Vanilla, Anantmul
9. Phenol Glycosides- Bearberry
10. Steroidal Glycosides-Solanum
11. Bitter Glycosidal / miscellaneous Glycosides- Gentain, Chirata, Quassia, Kalmegh, Guduchi

Classification:

- **Atom from the aglycone involved in the glycosidic linkage:**
  - **Aglycone- O- Sugar O-glycosides**
  - **Aglycone- C- Sugar C-glycosides**
  - **Aglycone- S- Sugar S-glycosides**
  - **Aglycone- N- Sugar N-glycosides**
- **Number of sugars:**
  - **One sugar monosides e.g. Salicin.**
  - **Two sugar Biosides e.g. Diosmin.**
  - **Three sugars Triosides e.g. Digoxin.**
- **Nature of the glycoside:**
  - **Primary glycosides: Originally present in the plant e.g. Purpurea A**
  - **Secondary glycosides: Resulted from removal of one sugar from the primary glycosides e.g. Digitoxin**

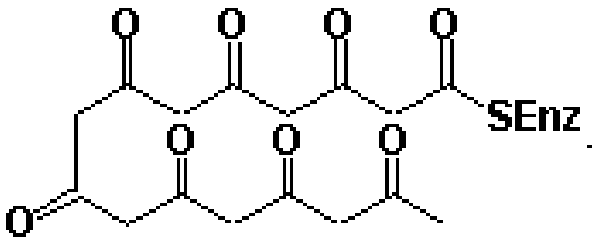
- **Type of the glycosidic linkage:**
  - **α- glycosides**
  - **β- glycosides**
- **Botanical source:**
  - **Digitalis glycosides**
  - **Senna glycosides.**
- **Therapeutic use:**
  - **Analgesic glycosides.**
  - **Purgative glycosides.**
  - **Cardiac glycosides.**
- **Chemical nature of the aglycone:**
  - **Flavone glycosides.**
  - **Steroidal glycosides.**
  - **Aldehydic glycosides.**
  - **Etc…….**

According to chemical nature of aglycone part/ therapeutic activity exhibited by the same

1. Anthraquinone / anthracene Glycosides-
   Example: Senna, Aloe, Rhubarb, Cascara

Major class of glycosides. Present mainly in Dicot plants. These group having aglycone moieties like anthraquinone, anthrone, dianthrone, anthronol, dianthronol. Oxanthronol.

Anthraquinone Glycosides



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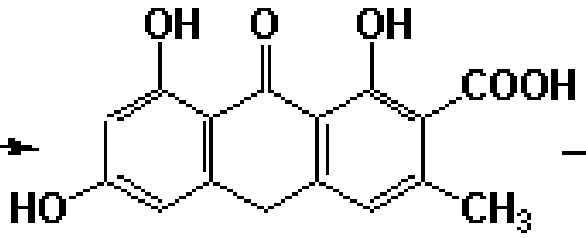
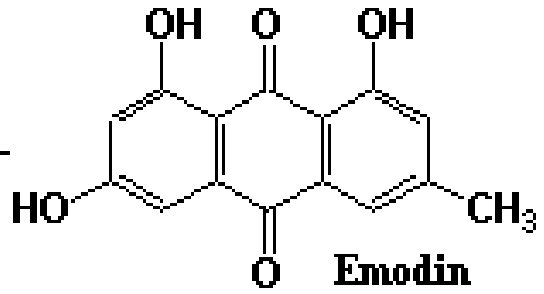
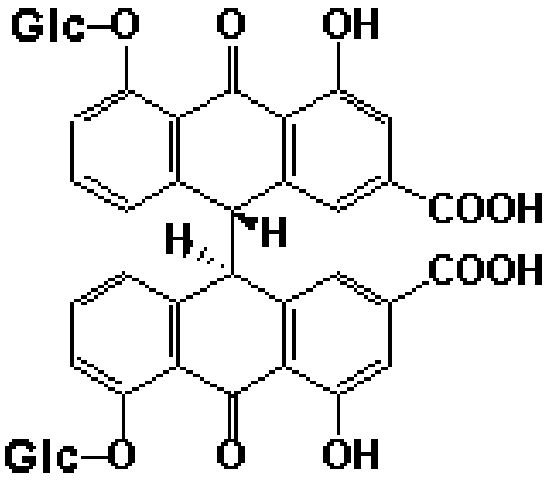


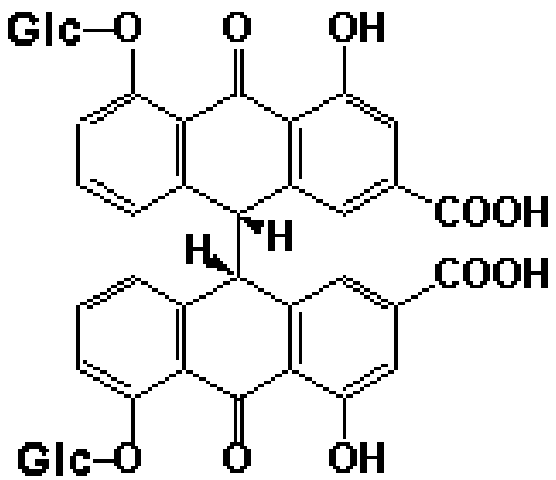
(anthrone)



(anthraquinone)

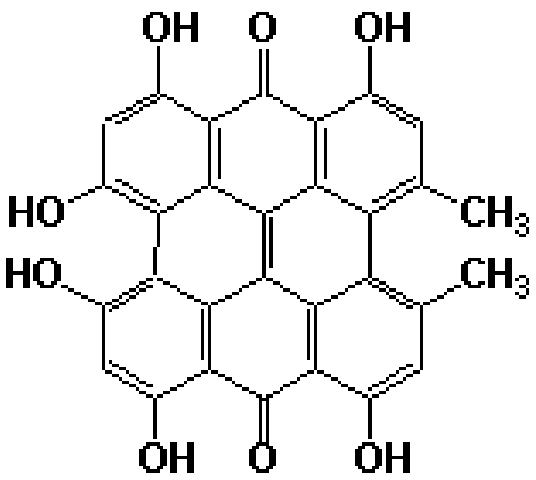


Sennoside A



Sennoside B

(bisanthrone)



Hypericin

(extended bisanthrone)

- Reduced derivatives of anthraquinones
  - Oxanthrones, anthranols and anthrones

- Compounds formed by the union of 2 anthrone molecules
  - Dianthrones

- <u>Aglycones:</u>
  - Chrysophanol/Chrysophanic acid → Rhubarb and Senna.
  - Rhein → Rhubarb and Senna
  - Aloe-emodin → Rhubarb and Senna
  - Emodin → Rhubarb and Cascara

Anthraquinones – Chemical Properties

- Anthraquinone derivatives: orange-red compounds
- Soluble in hot water/dilute alcohol.

- Identified via **Borntrager's test**
  1. Powdered drug – extrcted with ether
  2. Filter
  3. Made alkaline by adding ammonia/caustic soda
  4. Shake → Aqueous layer shows pink, red or violet colour – positive for anthraquinone derivatives

  – If the Anthraquinones are reduced (within the herb) or stable (glycosides) test will be negative

Senna

- Indian Senna
- Alexandrian Senna

Senna

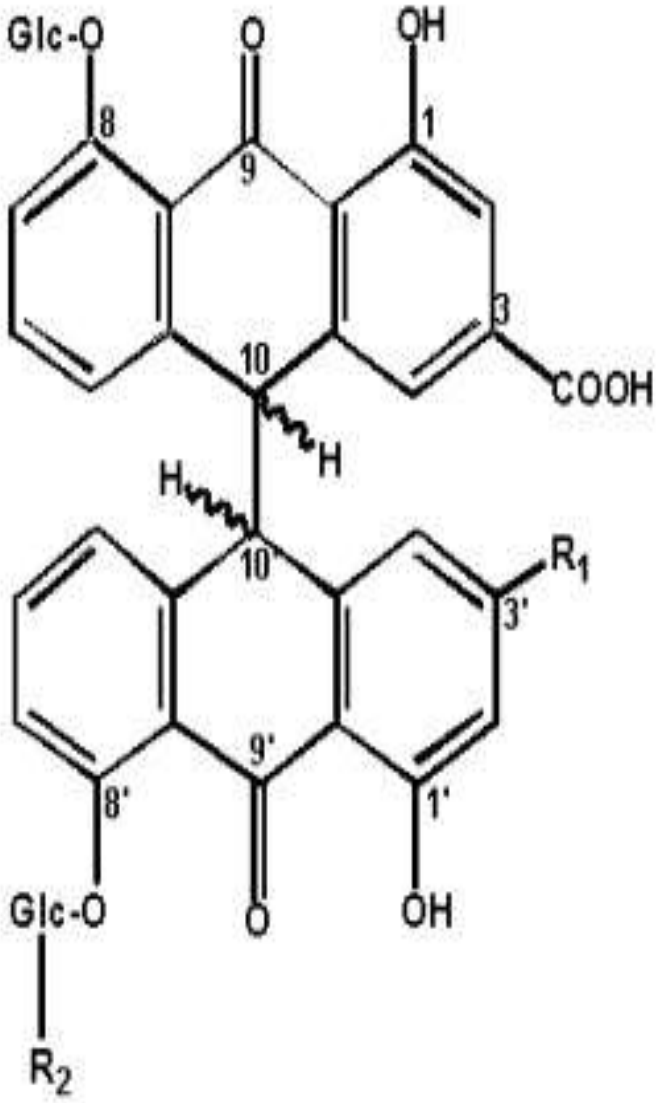
- Synonym: Senna Leaf; Sennae folium; Senna-ki-patti
- Biological Source: It Consists of the dried leaflets of *Cassia angustifolia* (Tinnevelly senna, / Indian Senna) or *Cassia acutifolia* (Alexandrian senna / Egyption)
- Family: Leguminosae

Senna - Constituents

- 2 Active glycosides: Sennoside A
  Sennoside B
  - Both hydrolyse: 2 molecules glucose + aglycones: Sennidin A and Sennidin B.

- Sennoside C & Sennoside D
- Rhein
- Aloe-emodin
- Palmidin A (Rhubarb)

Chemical Test: Borntrager's Test positive due to anthaquinone in leaves.



Compound	R_1	R_2	10-10'
Sennoside A	COOH	H	*threo*
Sennoside B	COOH	H	*erythro*
Sennoside C	CH_2OH	H	*threo*
Sennoside D	CH_2OH	H	*erythro*
Sennoside E	COOH	HOOC-CO	*threo*
Sennoside F	COOH	HOOC-CO	*erythro*

Senna - Uses

- Laxatives (habitual constipation or occasional use).
- Lacks astringent after-effect (Rhubarb)

Senna - Additional

- **Medicinal Actions**

✓ Vermifuge, diuretic, febrifuge

✓ Other uses: laxative candy (bitter taste).
  - ✓ Also used to treat flatulence, gout, fever.

✓ Topically: poultice prepared with vinegar to treat pimples.

✓ NOTE: Senna may cause urine to become reddish – no clinical significance.

- **Contra-indications**
- **Gout, colitis, GI inflammation.**

❑ **Should not be used with cardiac glycosides.**

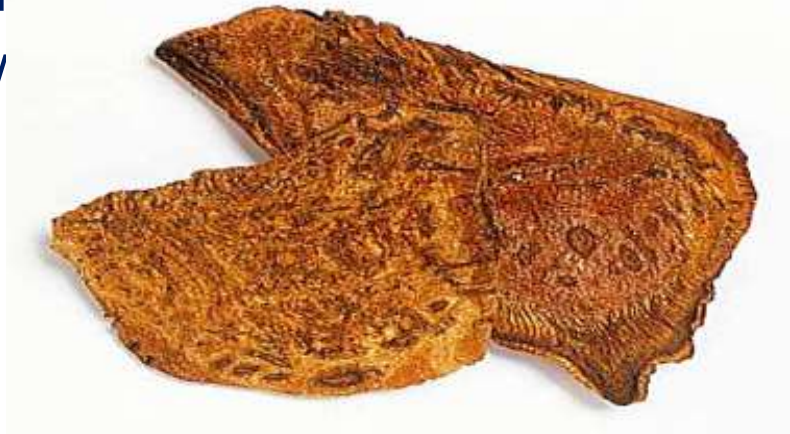
❑ **Overdose: nausea, bloody diarrhoea, vomiting and nephritis.**

❑ **Long-term use: dehydration & electrolyte depletion, worsening constipation and weakening intestinal muscles.**

Rhubarb

- Synonym: Radix rhei; Rheum; Revandchini
- **Bio. Source:** It consist of the dried rhizome of *Rheum emodi* Wall (Indian rhubarb) , *Rheum palmatum* Linn(Chinese rhubarb).
- **Family: - Polygonaceae**
- **Geographical Source:** Kashmir, Sikkim, China, Tibet, Korea etc.

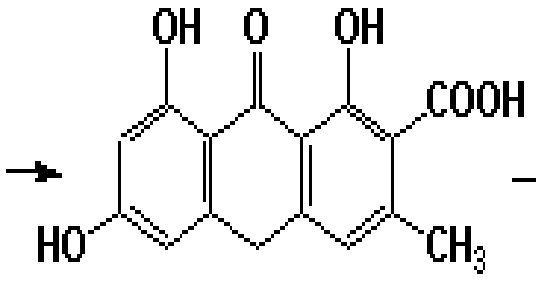
- **Collection and Preparation:** It collected from rhizomes 6-10 year old plants. Allow to dry and decorticated , cut longitudinally – transversely
- **Macroscopy:** Small pieces from rhizome 8-10cm length, 4cm thickness, yellow, brown colour observed.



Rhubarb – Chemical Constituents

1. **Anthraquinones without a carboxyl group** – chrysophanol, emodin, aloe-emodin & physcion.
2. **Anthraquinones with a carboxyl group**

   (rhein and its glycoside: glucorhein).
3. **Anthrones and dianthrones** of chrysophanol, emodin, aloe-emodin or physcoin.
4. **Dianthrone glucosides** of rhein (Sennosides A and B).
5. **Hetrodianthrones** derived from 2 different anthrone molecules: **Palmidin A** and **Palmidin B**.



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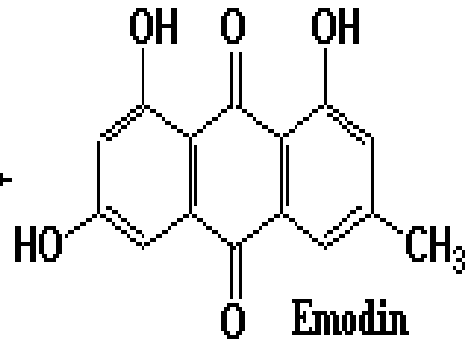
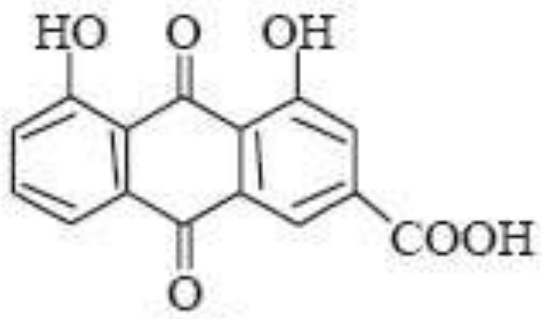


(anthraquinone)



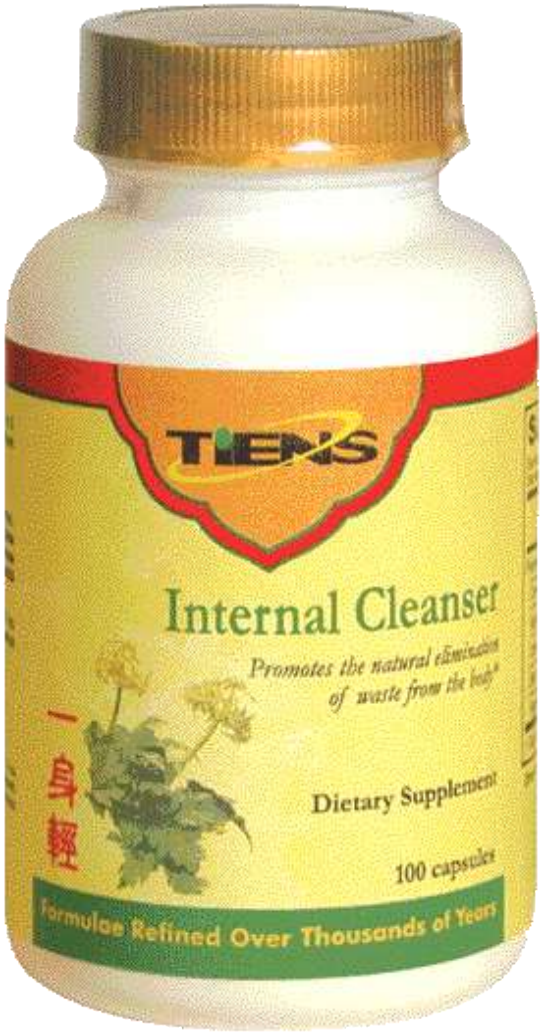
Rhein

Chemical Tests

1. The Rhubarb powder on being treated with ammonia gives rise to a **pink colouration**.
2. Rhubarb gives **a blood-red colouration with 5% potassium hydroxide**.
3. It gives a positive indication with **modified Borntrager's test** (see under Aloes).

Rhubarb - Uses

- ✓ Bitter stomachic
- ✓ Diarrhoea (low doses) – contains tannins
- ✓ Purgative (high doses) – followed by an astringent effect.
- ✓ Suitable only for occasional use, not for chronic constipation.



Rhubarb - Additional

- Etymology
  - Rheo – Greek, 'to flow', in reference to the purgative properties.
- Medicinal Actions
  - Anti-helminthic, anti-bacterial, anti-inflammatory, antiseptic.
- Topical Uses:
  - Poultice to treat boils, burns, wounds. Used to stop bleeding (tannins – stypic and astringent). Used as a mouthwash for oral ulcers.
- Caution
  - Leaves should be avoided – high calcium oxalate - toxic

Aloes

Aloes

- **Synonym:** Aloe, Musabbar, Kumari
- **Biological Source:** Aloe is the dried latex of leaves of various species of Aloes, namely:

✓ *Aloe barbadensis* Miller (or **Curacao Aloe**);

✓ *Aloe ferox* Miller (or **Cape Aloe**);

✓ *Aloe perryi* Baker (or **Socotrine Aloe**);

✓ *Aloe africana* Miller and A*loe spicata* Baker (or **Cape Aloe**).

✓ **All these species belong to the family Liliaceae.**

- **Geographical Source**

✓ Curacao, Barbados, Aruba : Curacao Aloes or Barbados Aloes and Bonaire (West Indian Islands)

✓ Cape Town (South Africa) : Cape Aloes

✓ Socotra and Zanzibar Islands : Socotrine or Zanzibar Aloes

✓ It is also cultivated in Europe and the North West Himmalayan region in India.

Preparation of Cape Aloes

- Cape Aloes are prepared from the wild plants *of Aloe ferox*.
- Leaves are cut transversely near the base.
- 200 leaves arranged around a shallow hole in the ground (lined with canvas or goatskin).
- Cut ends overlap & drain into the canvas.
- After 6hrs all the juice is collected.
- Transferred and Boiled for 4hrs on an open fire.
- Poured into tins while hot → solidifies.

Collection & Preparation of Cape Aloes

Aloes - Constituents

- C-glycosides
- Resins
- Glycosides
  - Aloin
    - Barbaloin
    - Isobarbaloin
- Aloe-emodin
- Cape Aloes:

Aloinoside A &

Aloinoside B (O-glycosides of barbaloin)

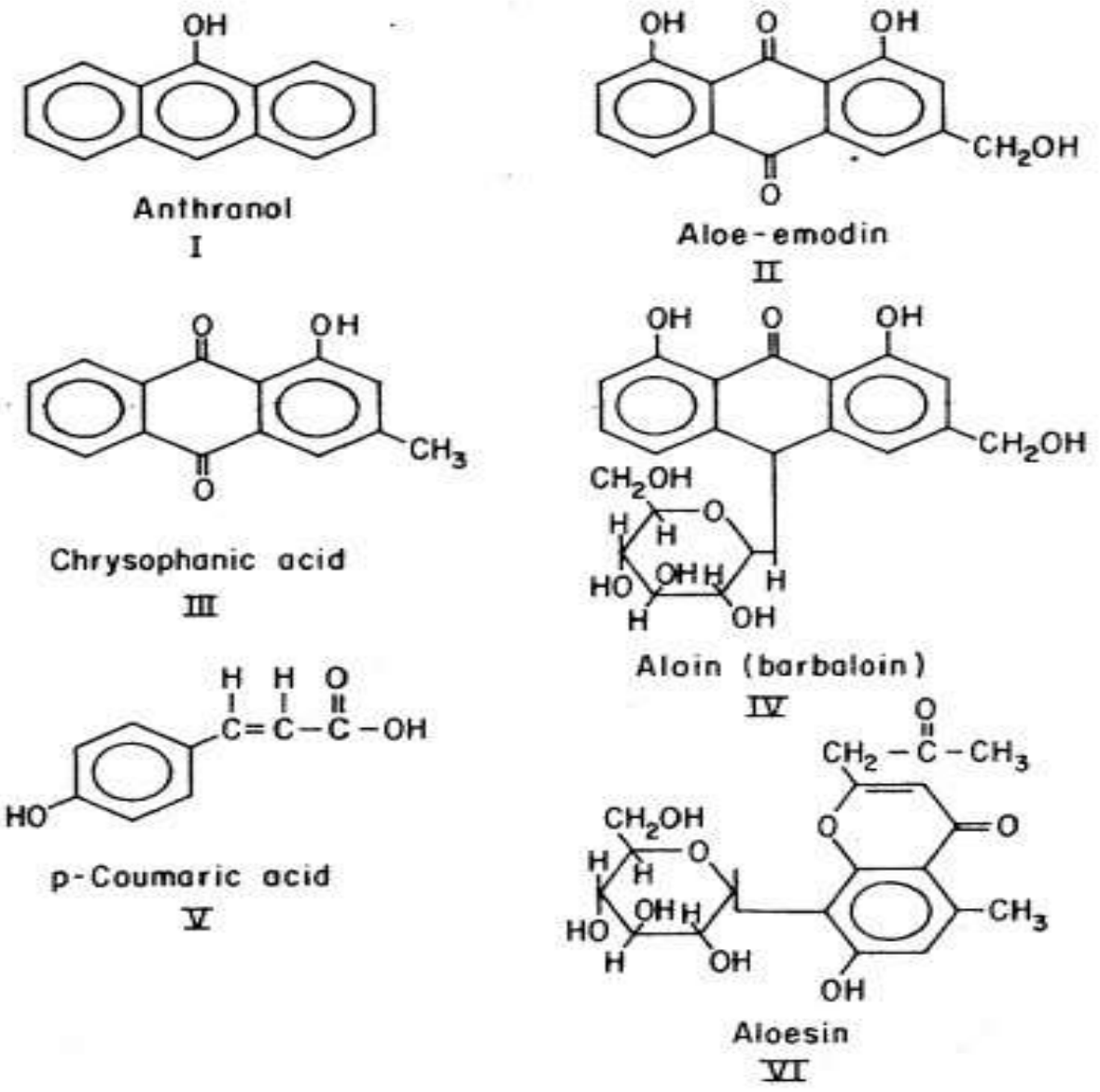


FIGURE 3. Structural formulas of compounds isolated from *Aloe barbadensis*.

Aloe Constituents & Chemical Tests:

- Unlike C-glycosides, O-glycosides of *Aloe* are not hydrolysed by heating with dilute acids or alkali.
- Can be decomposed with ferric chloride & dilute HLC - **Modified Borntrager's Test** – oxidative hydrolysis. .....Anthraquinones give a red colour when shaken with dilute ammonia.

- All Aloes give a strong **green fluorescence/ rose-pink** with borax (characteristic of anthranols) - General test for aloes.

Aloe Chemical Tests:

General test	Type	Observation
Bromine test 1gm sub powder + Δ boil in 10 ml water+Bromine sol	--	Pale Yellow ppt.
Schoenteten's Reaction(Borax test) 1gm sub powder+ Δ in 10 ml water+borax---Dilute with water	--	Green fluroscence
Special Test	**Type**	**Observation/ Colour**
1. Nitrous acid Test: Sodium nitrite + Acetic acid in aq. Solution of aloes	**Curacao**	**Dark Pink**
	Cape	**Faint Pink**
	Socotrine , Zinzibar	**No Change**
2. Nitric acid Test: Drop of Nitric acid + Aq. Sol. Of aloes	**Curacao**	**Brown**
	Cape	**Brown---=----Green**
	Socotrine	**Brownish yellow**
	Zinzibar	**Yellowish brown**
3. Cupraloin Test {Klunege's isobarbaloin test} Aq. Sol. Of aloe +$CuSO_4$+NaCl+90%Alcohol	**Curacao --- Cape ----**	**Wine Red Yellow Colour**

Aloe - Uses

- Purgative

- Seldom prescribed alone – activity is increased when administered with small quantities of soap or alkaline salts; Carminatives moderate griping tendency.

- Ingredient in Friar's Balsam.

Aloe – Additional Notes

- Medicinal Uses:
  - Anti-bacterial, anti-fungal, chologoge, emmenogogue, anti-inflammatory (juice), anti-inflammatory , demulcent, vulnerary, immune-stimulating (gel).

  Radiation burns (internal and external use)

- Contra-indications

  Pregnancy & lactation (internal uses)

- **Etymology**
  - **Name derives from Arabic *alu,* meaning shiny or bitter in reference to the gel.**
- **Other uses**
  - **reduce sweating and mask scent.**
  - **Used to break nail-biting habit.**

Aloe vera Products

- These are derived from the mucilage gel – parenchyma cells
- Should not be confused with aloes (juice of pericycle – juice used for laxative effect).
- Cosmetic industry (usefulness often exaggerated) - Used as suntan lotions, tonics and food additives.
- Mucilage = polysaccharide of glucomannans and pectin

Cascara

Other points

HERBAL DRUG TECHNOLOGY

Prof. Dr R.Y Patil

Department of Pharmacognosy

PDEAs Shankarrao Ursal college of Pharmaceutical Sciences and Research center Kharadi.

- Man has been using herbs and plants products for combating diseases since times immemorial.
- The Indian subcontinent is enriched by a variety of flora—both aromatic and medicinal plants. This is due to the wide diversity of climatic conditions in India ranging from deserts to swamplands.
- Numerous types of herbs have been well recognised and catalogued from the high ranges of the Himalayan tract up to the sea-shore of Kanyakumari.
- This extensive flora has been greatly utilized as a source of many drugs in the Indian traditional system of medicine.

Scope

- After study of Herbal drug technology subject
- Basic knowledge and understanding of herbal drug industry, the quality of raw material, guidelines for quality of herbal drugs, herbal cosmetics, natural sweeteners, nutraceuticals etc.
- The subject also emphasizes on Good Manufacturing Practices (GMP), patenting and regulatory issues of herbal drugs

Herbal drug technology has become a very important instrument for converting botanical materials into therapeutically useful products and medicines

- Thus, the area will gain increasing importance, which of course is obvious.
- However the countries which desire to bring herbal products into the global market and become successful, need to make continuous use of modern scientific techniques and integrate it with traditional knowledge and biodiversity.
- As India is trying to work towards achieving this goal, integration of herbal drug technology and undertaking research specifically in this area, assumes special significance.

UNIT- I

- **Herbs as Raw material**
- **Biodynamic Agriculture**
- **Indian Systems of Medicine**
- **Pharmacognosy in various systems of medicine**

Learning outcome

After completion of this chapter student should be able to

1. Understand herbs as raw material. Steps involved in Selection, identification and Processing of herbal raw material
2. Appreciate good agricultural practices in cultivation of medicinal plants
3. Explain the basic principles involved in traditional systems of medicine and role of Pharmacognosy.

Herbs as Raw material

- Over the past few decades interest in herbal medicines, has increased substantially in both developed and developing countries.
- Global and national markets for medicinal herbs have been growing rapidly, and significant economic gains are being realized.
- According to the Secretariat of the Convention on Biological Diversity, global sales of herbal products is about US$ 60000 million in 2000

- ***Herbs***

  Herbs include entire plant or any part of plant such as leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes, which may be entire, fragmented or powdered.

- Herbal drugs

  Plant or any part of plant, usually in unprocessed or crude form which have medicinal value.

  Their constituents and therapeutic activity may be known or unknown.

- **Herbal drug preparation-**
- These are processed form of herbs.
- Derived from herbal drugs by various techniques like extraction, purification, fractionation, fermentation.
- May be in the form of powder, extract, tincture, fixed oil, volatile oil, resins, gums.
- They may contain a mixture of various constituents
- However pure isolated compounds do not come under this category

- **Herbal medicinal products (Herbal finished products)**
- These are the medicinal products which exclusively contain herbal drugs or herbal drug preparation which are made from one or more herbs.
- They may contain excipients in addition to active ingredients.
- If more than one herb is used, the term ***mixed herbal product can also be*** used.
- In some countries, herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g. animal materials and mineral materials)

Sources of herbs

- **Wild source and Cultivated source**
- WILD SOURCES-

  Plants may be obtained from forests, plains, river banks etc.

  suitable for plants which are abundant in nature

  Advantages-

  Easy, economical, less time consuming, decreased cost labour.

  Disadvantages-

  Quality of plants cannot be predicted

  Plants are not uniform with respect to growth and yield, Collection for prolonged period may leads to depletion of source

CULTIVATION

- Important technical process, wherein human skill and scientific knowledge of plants needs to be used under various climatic conditions.
- Cultivation is the act of raising the plants
- It is an art, science and business of crop production
- ART- Ability and capacity to carry out the operation, management of labour, water.
- SCIENCE- utilizes all modern technologies developed on scientific principles e.g. breeding
- BUSINESS-aims at net maximum return through crop or crude drug production

Advantages

- Cultivation of medicinal plant plays important role in conservation of biodiversity and protect endangered species.
- In the pharmaceutical industry, where the active medicinal principle cannot be synthesized economically, the product must be obtained from cultivation of medicinal plant.
- Cultivation of medicinal plant ensures regular supply of raw material
- Systemic cultivation by breeding or clonal propogation help to develop high yielding varieties.

Cultivation Methods

Sexual method

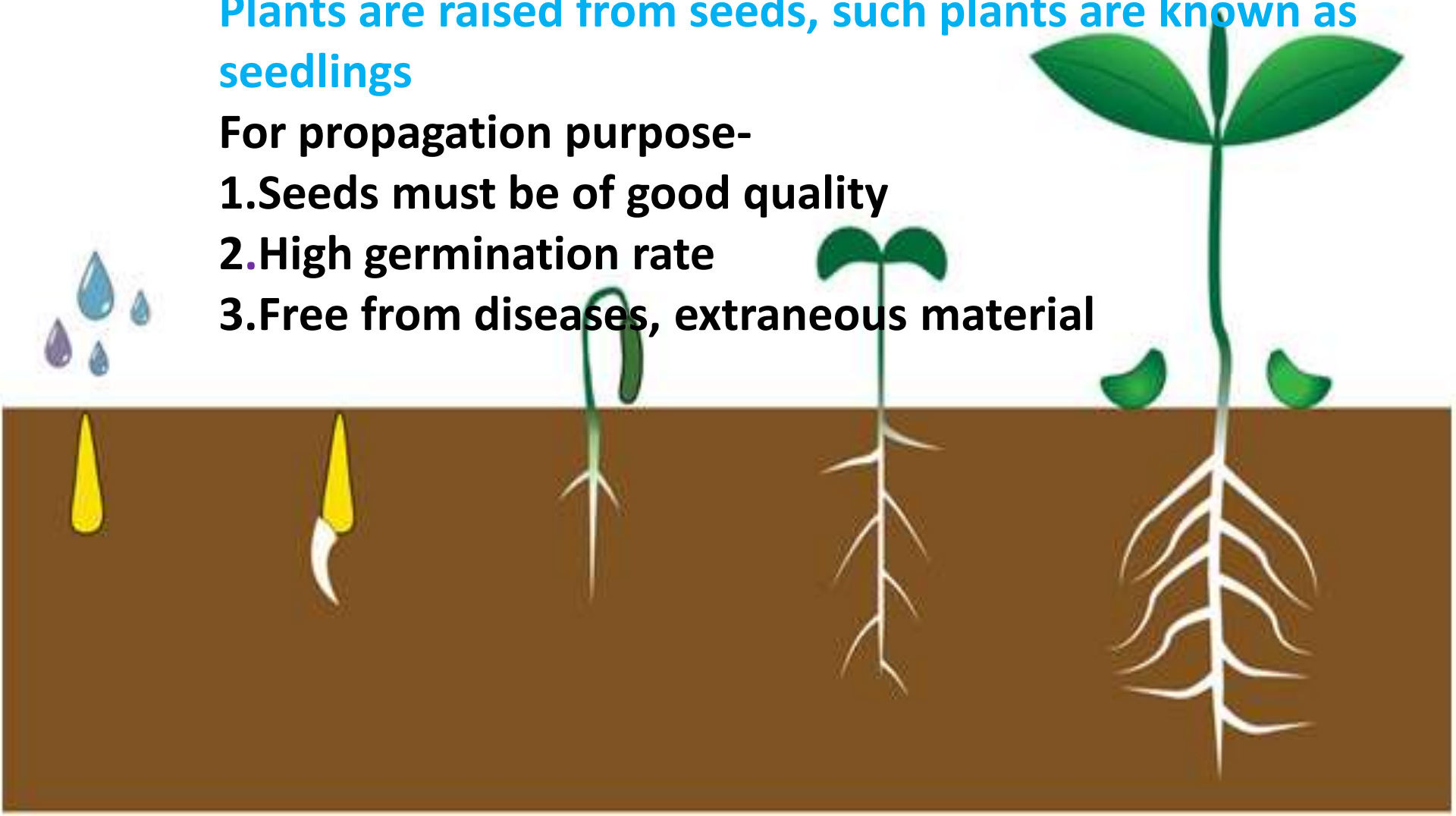
Plants are raised from seeds, such plants are known as seedlings

For propagation purpose-

1.Seeds must be of good quality

2.High germination rate

3.Free from diseases, extraneous material



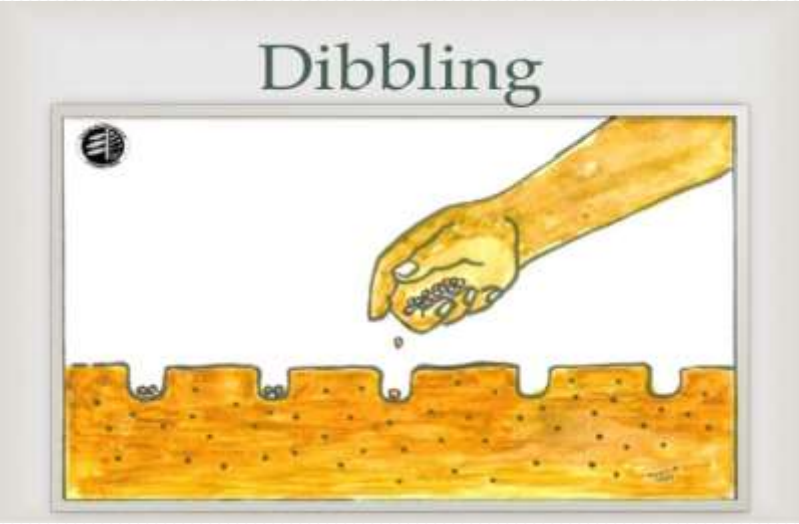
Method of Sowing the seeds

1. Broadcasting-

- Small seeds
- Scattered freely in well prepared soil
- If deeply sown may not get germinated
- E.g. Isapgol, linseed

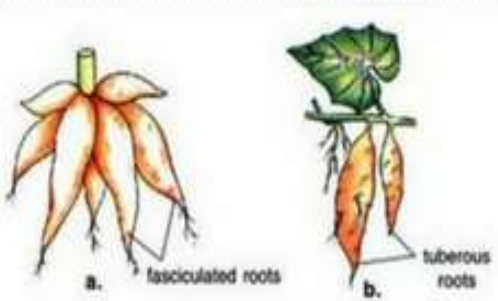
Broadcasting of rice seeds

- **2. Dibbling-**
- **Seeds of average size and weight**
- **Sown by placing in holes**
- **Number of seeds to be put in the holes vary from 3 to 5**
- **e.g. Castor, fennel.**

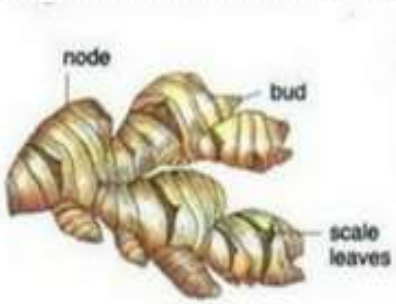


- **Asexual method**
- **Vegetative propagation**
- **Vegetative part like stem, root etc. are used**
- **Natural Methods of vegetative propagation-**
- **Sowing various part of plant in well prepared soil**
- **Bulbs-Garlic, Squill**
- **Corms- Colchicum**
- **Runners- Peppermint**
- **Stolon- Liquorice**
- **Rhizome- Ginger**
- **Tubers- Jalap, Potato**
- **Suckers- Mint**

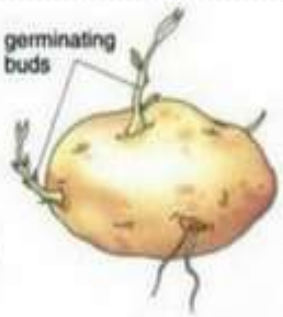
Vegetative Propagation Methods



Vegetative propagation by roots of *Dahlia* and sweet potato

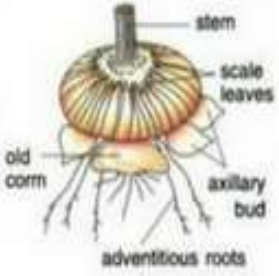


Vegetative propagation by rhizome in ginger

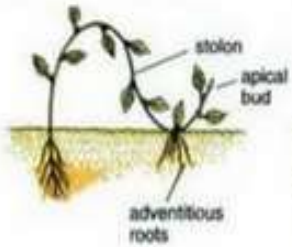


Vegetative propagation by tuber in potato

Vegetative propagation by bulb in onion

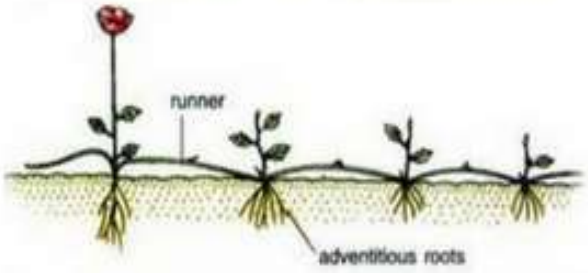


Vegetative propagation by corm in *Gladiolus*



Vegetative propagation by stolon

Vegetative propagation by sucker in *Chrysanthemum*



Vegetative propagation by runner in strawberry

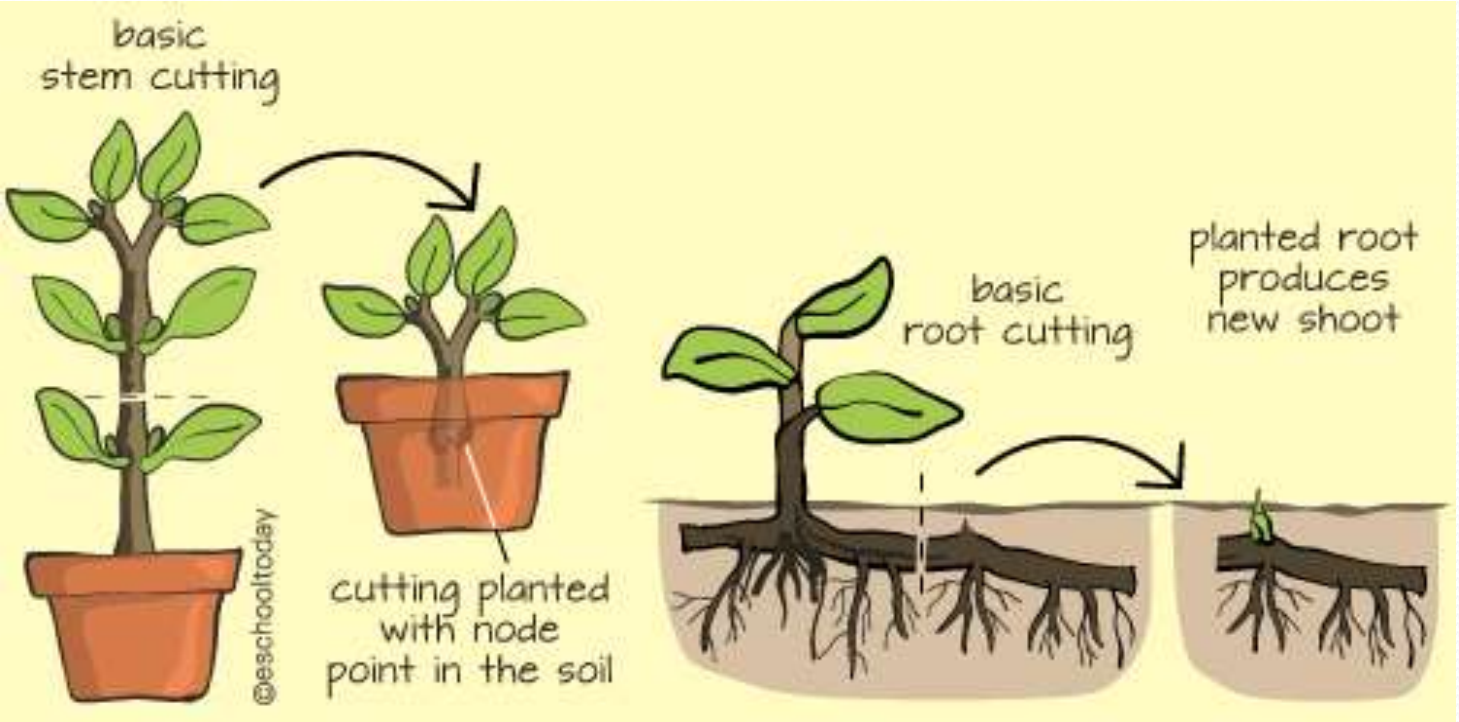
www.plantscience4u.com

Vegetative propagation by offset in water lettuce

www.plantscience4u.com

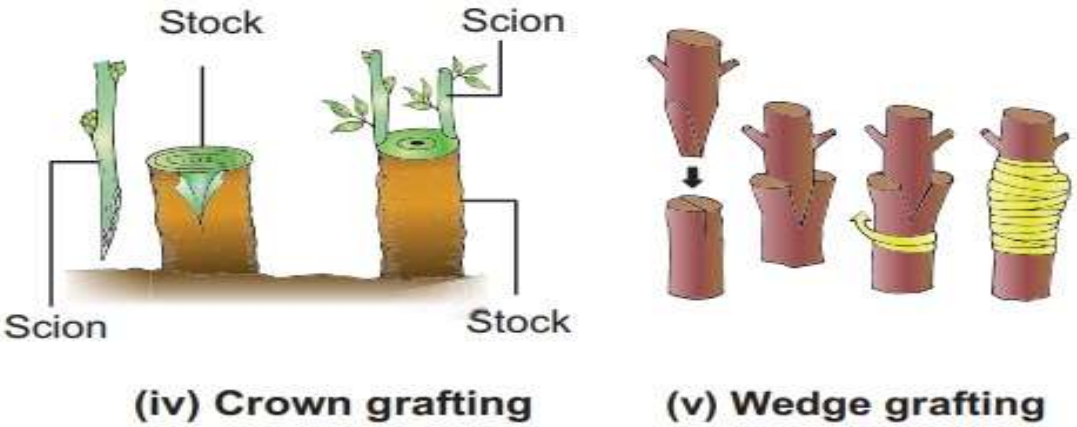
Artificial method of vegetative propagation

- Plantlets are produced from vegetative part of the plant
- 1. Cutting- Stem, root, Leaf etc.



a) Types of Grafting

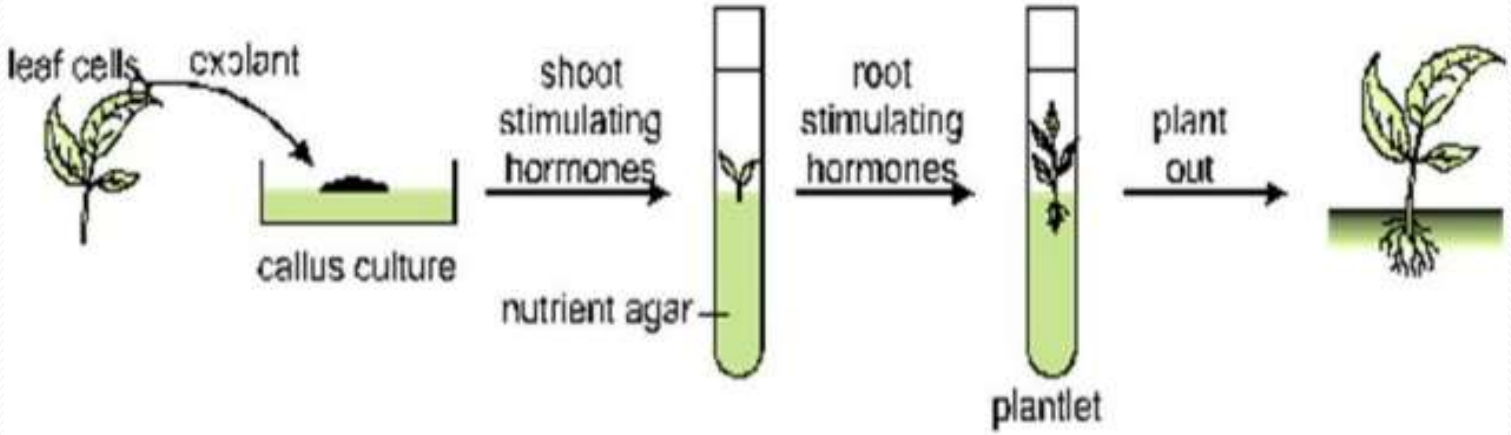
Figure 1.2(a): Artificial methods of vegetative reproduction in plants

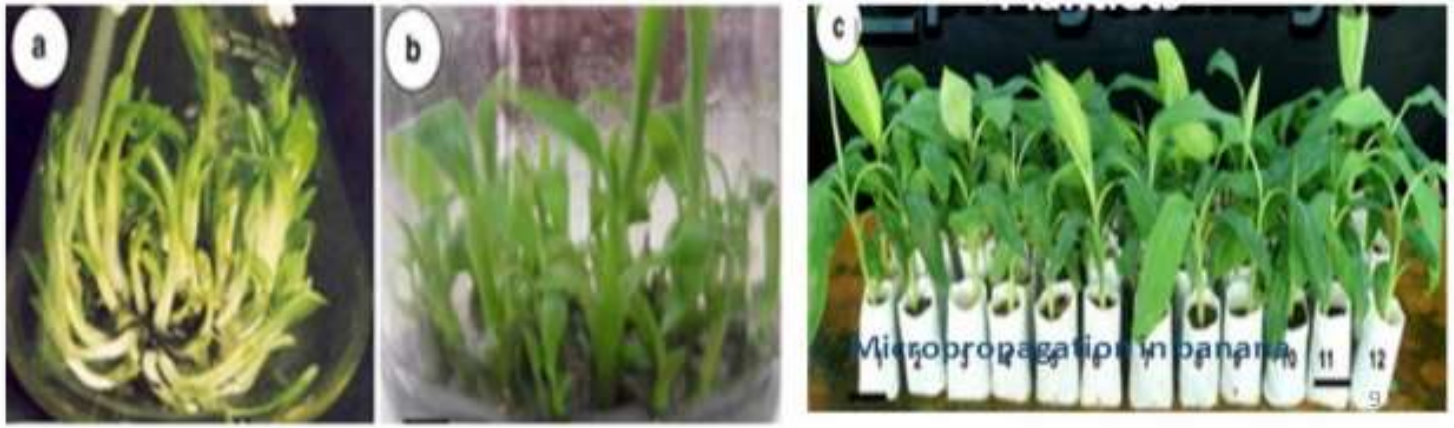


Aseptic method of Micropropogation

- Novel method for the propagation of medicinal plant
- Plants are developed in an artificial medium under aseptic condition
- Single cell, callus, embryo, root tips, shoot tip etc.
- They are also provided with nutritional and hormonal requirement

Micropropagation





Advantages of Asexual Propagation

- 1. No variation between the plant grown and plant from which it is grown, plants are uniform in growth and yielding capacity.
- 2. seedless varieties of fruits can only be propagated vegetatively e.g. Grapes, lemon, pomegranate
- 3.Plants start bearing earlier as compared to seedling tree
- 4. Budding/grafting encourages disease resistant variety

- Disadvantages
- 1. Short living
- 2. New varieties can not be evolved

Drugs isolated from Plant Parts

- Roots- Shatavari, Rauowlfia, Ipecac, Ashwagandha
- Woods- Quassia, Sandalwood.
- Barks- Cinchona, Kurchi, Cinnamon
- Rhizomes- Turmeric, Ginger
- Flowers- Clove, Saffron
- Fruits- Fennel, Amla, Hirda
- Leaves- Senna, datura, Vinca
- Seeds- Nux-vomica, Isabgol, castor

Selection of Herbal material

- According to WHO more than 60% population is directly or indirectly using plant based medicine.
- Hence it is very important to select right plant material for development of herbal drugs.
- Selection of plant material can be done by various approaches like

1. Randomized approach
2. Ecological approach
3. Chemo systemic approach
4. Ethno botanical approach

STEPS INVOLVED IN THE SELECTION, IDENTIFICATION AND PROCESSING OF HERBAL RAW MATERIAL

Selection of herbs-

The species or botanical variety selected for cultivation should be the same as that specified in the national pharmacopoeia or recommended by other authoritative national documents of the end-user's country.

In the absence of such national documents, the selection of species or botanical varieties specified in the pharmacopoeia or other authoritative documents of other countries should be considered.

In the case of newly introduced medicinal plants, the species or botanical variety selected for cultivation should be identified and documented as the source material used or described in traditional medicine of the original country.

Identification and Authentication of Herbal material

- The botanical identity – scientific name (genus, species, subspecies/variety, author, and family) – of each medicinal plant under cultivation should be verified and recorded.
- If available, the local and English common names should also be recorded. Other relevant information, such as the cultivar name, ecotype, chemotype or phenotype, may also be provided, as appropriate.
- In the case of the first registration in a producer's country of a medicinal plant or where reasonable doubt exists as to the identity of a botanical species, a voucher botanical specimen should be submitted to a regional or national herbarium for identification.
- Where possible, a genetic pattern should be compared to that of an authentic specimen. Documentation of the botanical identity should be included in the registration file.

Seeds and other propagation materials

- Seeds and other propagation materials should be specified, and suppliers of seeds and other propagation materials should provide all necessary information relating to the identity, quality and performance of their products, as well as their breeding history, where possible.
- The propagation or planting materials should be of the appropriate quality and be as free as possible from contamination and diseases in order to promote healthy plant growth. Planting material should preferably be resistant or tolerant to biotic or abiotic factors.
- Care should be taken to exclude extraneous species, botanical varieties and strains of medicinal plants during the entire production process. Counterfeit, substandard and adulterated propagation materials must be avoided.

Cultivation

- Cultivation of medicinal plants requires intensive care and management.
- The conditions and duration of cultivation required vary depending on the quality of medicinal plant materials required.
- If no scientific published or documented cultivation data are available, traditional methods of cultivation should be followed, where feasible.
- Otherwise a method should be developed through research.

Collection

- Collection practices should ensure the long-term survival of wild populations and their associated habitats.
- Management plans for collection should provide a framework for setting sustainable harvest levels and describe appropriate collection practices that are suitable for each medicinal plant species and plant part used (roots, leaves, fruits, etc.)
- Proper time should be selected, stage of growth must be suitable for collection so that maximum yield is obtained.
- Skilled labour should be employed

- Diseased plant should not be selected
- Due consideration should be given to season of collection

Processing of herbal raw material

- Involves various stages

A. Primary processing

1. Garbling (Sorting)-

- Preparation of drug for market by removing sand, dirt, extraneous matter, insect, stalk etc.
- Ensures the purity and cleanliness
- Done manually by hands.

2. Washing-

After garbling the raw material should be cleaned to remove traces of dirt, soil and other material from surface roots, rhizomes and tubers.

- After washing certain herbal raw material needs to undergo parboiling process, in which they are put into boiling water for short period.
- This may help in improving the storage life of the raw material and prevent insect/ moulds contamination
- Leaching-
- Some impurities can be removed by subjecting the plant material under running water.
- However it should be carried out under controlled condition to prevent loss of chemical constituents

- Drying-
- Plant material should be thoroughly dried after washing in order to prevent deterioration and degradation of the active constituents
- Must be dried as soon as possible to remove the moisture and reduce the damage due to microbial or mould infestation.
- Drying prevents activation of certain enzymes
- Drying facilitates grinding and milling of raw material
- Types of Drying 1. Natural Drying
  2. Artificial drying

- Natural drying

  a. Sun drying- most herbal raw material can be dried in open air under direct sunshine.

  Plant material is spread in thin layer

  b. Shade drying- some plant material cannot be directly exposed to sunlight, hence need to be dried under shade.

  this drying process is slow but helps in minimizing the loss of colour, volatile oil and aromatic components being from evaporated.

- Artificial drying-
- More rapid
- Necessary in rainy season and region where high humidity is present.
- Tray dryer, spray dryer, vacuum dryer are used

Secondary processing

- Steps applied to herb in addition to primary processing
- Differs from one herb to another depending on nature of active ingredient as well as therapeutic properties.
- 1. Cutting/ sectioning/ communition
- After drying herbal materials are processed by cutting and sectioning into smaller sizes which are convinient for storage as well as extraction.
- 2. Aging or sweating
- Storing the raw material for specified period after harvesting

- Aging is done under shade or sun for upto 1 year
- eg. Cascara bark should be aged for at least one year prior to use in medicinal preparation to reduce its irritant effect.
- Sweating is done by subjecting the herbal material at temp. between 45 to 65^{O}C with high humidity for a period ranging from one week to few month
- Herbal material is stacked between woolen blanket or other kind of cloth
- Sweating process is considered a hydrolytic and oxidative process

- Eg. Vanilla beans are subjected to sweating between woolen blanket for two month during which they lose upto 80% weight and develop characteristic desirable colour and odour.
- 3. Baking/ roasting-
- It is a process of drug heating in oven
- Temperature and duration vary from one material to another
- Nutmeg is roasted till they turn yellowish brown color

- 4. Frying-
- Herbal material put in a pan of frying and continuously stirred for a specific period under heat until external color changes.
- eg- liquorice root stir fried with honey
- 5.Fumigation-
- Sometimes harvested raw materials are subjected to fumes.
- Fumigation with sulphur dioxide is done for preserving color, improve appearance and prevent growth of insect and moulds

- 6. Extraction of herbal raw material
- Extraction is a process of separation in which chemical constituents present in the tissue are removed by using selective solvent (menstrum)
- Maceration
- Decoction
- Infusion
- Percolation
- Soxhlet extraction
- Counter current extraction

Pest and Pest control

(Assignment)

- Pest- Undesired animal or plant species
- Pesticides- Chemicals derived from synthetic and natural sources effective in small concentration against pests.

- Types of Pest-

1. Fungi and viruses
2. Insects
3. Weeds
4. Non- Insect pests

Methods of Pest control

1. Mechanical method
2. Agricultural Method
3. Biological Method
4. Chemical Method

WHO guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants

Objectives

1. Contribute to the quality assurance of medicinal plant materials used as the source for herbal medicines, which aims to improve the quality, safety and efficacy of finished herbal products
2. Guide the formulation of national and/or regional GACP guidelines and GACP monographs for medicinal plants and related standard operating procedures.
3. Encourage and support the sustainable cultivation and collection of medicinal plants of good quality in ways that respect and support the conservation of medicinal plants and the environment in general.

Structure

- The guidelines are divided into five sections:
- section 1 - a general introduction
- section 2 - good agricultural practices for medicinal plants
- section 3 - good collection practices for medicinal plants
- section 4 - outlines common technical aspects of good agricultural practices for medicinal plants and good collection practices for medicinal plants
- section 5- considers other relevant issues.

2. Good Agricultural Practices for Medicinal Plants

2.1.Identification/authentication of cultivated medicinal plants

2.1.1 Selection of medicinal plants

Where applicable, the species or botanical variety selected for cultivation should be the same as that specified in the national pharmacopoeia or recommended by other authoritative national documents of the end-user's country.

In the absence of such national documents, the selection of species or botanical varieties specified in the pharmacopoeia or other authoritative documents of other countries should be considered.

In the case of newly introduced medicinal plants, the species or botanical variety selected for cultivation should be identified and documented as the source material used or described in traditional medicine of the original country.

- **2.1.2 Botanical identity**

  The botanical identity – scientific name (genus, species, subspecies/variety, author, and family) – of each medicinal plant under cultivation should be verified and recorded. If available, the local and English common names should also be recorded.

- **2.1.3 Specimens**

  In the case of the first registration in a producer's country of a medicinal plant or where reasonable doubt exists as to the identity of a botanical species, a voucher botanical specimen should be submitted to a regional or national herbarium for identification.

  Where possible, a genetic pattern should be compared to that of an authentic specimen. Documentation of the botanical identity should be included in the registration file.

2.2 Seeds and other propagation materials

- Seeds and other propagation materials should be specified.
- Suppliers of seeds and other propagation materials should provide all necessary information relating to the identity, quality and performance of their products, as well as their breeding history, where possible.
- The propagation or planting materials should be of the appropriate quality and be as free as possible from contamination and diseases in order to promote healthy plant growth. Planting material should preferably be resistant or tolerant to biotic or abiotic factors.

2.3 Cultivation

- Cultivation of medicinal plants requires intensive care and management. The conditions and duration of cultivation required vary depending on the quality of medicinal plant materials required. If no scientific published or documented cultivation data are available, traditional methods of cultivation should be followed, where feasible. Otherwise a method should be developed through research.
- The principles of good plant husbandry, including appropriate rotation of plants selected according to environmental suitability, should be followed, and tillage should be adapted to plant growth and other requirements.

2.3.1 Site selection

- Medicinal plant materials derived from the same species can show significant differences in quality when cultivated at different sites, owing to the influence of soil, climate and other factors. These differences may relate to physical appearance or to variations in their constituents, the biosynthesis of which may be affected by extrinsic environmental conditions, including ecological and geographical variables, and should be taken into consideration.

2.3.2 Ecological environment and social impact

- The cultivation of medicinal plants may affect the ecological balance and, in particular, the genetic diversity of the flora and fauna in surrounding habitats. The quality and growth of medicinal plants can also be affected by other plants, other living organisms and by human activities.
- The introduction of non-indigenous medicinal plant species into cultivation may have a detrimental impact on the biological and ecological balance of the region. The ecological impact of cultivation activities should be monitored over time.

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- The social impact of cultivation on local communities should be examined to ensure that negative impacts on local livelihood are avoided.
- In terms of local income- earning opportunities, small-scale cultivation is often preferable to large-scale production, in particular if small-scale farmers are organized to market their products jointly. If large-scale medicinal plant cultivation is or has been established, care should be taken that local communities benefit directly from, for example, fair wages, equal employment opportunities and capital reinvestment.

2.3.3 Climate

- Climatic conditions, for example, length of day, rainfall (water supply) and field temperature, significantly influence the physical, chemical and biological qualities of medicinal plants.
- The duration of sunlight, average rainfall, average temperature, including daytime and night-time temperature differences, also influence the physiological and biochemical activities of plants, and prior knowledge should be considered.

2.3.4 Soil

- The soil should contain appropriate amounts of nutrients, organic matter and other elements to ensure optimal medicinal plant growth and quality. Optimal soil conditions, including soil type, drainage, moisture retention, fertility and pH, will be dictated by the selected medicinal plant species and/or target medicinal plant part.
- The use of fertilizers is often indispensable in order to obtain large yields of medicinal plants. It is, however, necessary to ensure that correct types and quantities of fertilizers are used through agricultural research.
- In practice, organic and chemical fertilizers should be used.

.....contd.

- Human excreta must not be used as a fertilizer owing to the potential presence of infectious microorganisms or parasites.
- Animal manure should be thoroughly composted to meet safe sanitary standards of acceptable microbial limits and destroyed by the germination capacity of weeds. Any applications of animal manure should be documented.
- Chemical fertilizers that have been approved by the countries of cultivation and consumption should be used.
- All fertilizing agents should be applied sparingly and in accordance with the needs of the particular medicinal plant species and supporting capacity of the soil. Fertilizers should be applied in such a manner as to minimize leaching.

2.3.5 Irrigation and Drainage

- Irrigation and drainage should be controlled and carried out in accordance with the needs of the individual medicinal plant species during its various stages of growth.
- Water used for irrigation purposes should comply with local, regional and/or national quality standards.
- Care should be exercised to ensure that the plants under cultivation are neither over- nor under-watered.

2.3.6 Plant Maintenance and Protection

- The growth and development characteristics of individual medicinal plants, as well as the plant part destined for medicinal use, should guide field management practices. The timely application of measures such as topping, bud nipping, pruning and shading may be used to control the growth and development of the plant, thereby improving the quality and quantity of the medicinal plant material being produced.
- Any agrochemicals used to promote the growth or to protect medicinal plants should be kept to a minimum, and applied only when no alternative measures are available. Integrated pest management should be followed where appropriate.
- When necessary, only approved pesticides and herbicides should be applied at the minimum effective level .

2.6.Personnel

- Growers and producers should have adequate knowledge of the medicinal plant concerned. This should include botanical identification, cultivation characteristics and environmental requirements.
- All personnel (including field workers) involved in the propagation, cultivation, harvest and post-harvest processing stages of medicinal plant production should maintain appropriate personal hygiene and should have received training regarding their hygiene responsibilities.
- Only properly trained personnel, wearing appropriate protective clothing (such as gloves, helmet, goggles, face mask), should apply agrochemicals.
- Growers and producers should receive instruction on all issues relevant to the protection of the environment, conservation of medicinal plant species, and proper agricultural stewardship.

Section 3.
Good Collection Practices For Medicinal Plants

- This section describes the general strategies and basic methods for small- and large-scale collection of fresh medicinal plant materials.
- Collection practices should ensure the long-term survival of wild populations and their associated habitats.
- Management plans for collection should provide a framework for setting sustainable harvest levels and describe appropriate collection practices that are suitable for each medicinal plant species and plant part used (roots, leaves, fruits, stems etc.)

3.1.Permission to collect

- In some countries, collection permits and other documents from government authorities and landowners must be obtained prior to collecting any plants from the wild. Sufficient time for the processing and issuance of these permits must be allocated at the planning stage. National legislation, such as national “red” lists, should be consulted and respected.

- For medicinal plant materials intended for export from the country of collection, export permits, phytosanitary certificates, Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permit(s) (for export and import), CITES certificates (for re-export), and other permits must be obtained, when required.

3.2 Technical planning

- Prior to initiating a collection expedition, the geographical distribution and population density of the target medicinal plant species should be determined.
- Distance from home base and quality of the target plant(s) available are factors to be considered.
- When the collection sites have been identified, local and/or national collection permits should be obtained, as indicated in section 3.1.
- Essential information on the target species (taxonomy, distribution, phenology, genetic diversity, reproductive biology and ethnobotany) should be obtained

……contd.

- Research on the morphology of the target medicinal plant species and variability of its populations should be carried out in order to develop a “search image” for the species.
- Copies of photographs and other illustrations of the target medicinal plant(s) from books and herbarium specimens, and ethnographical information (common or local names) of the target species and plant parts are useful field instruments, especially for untrained workers.
- Botanical keys and other taxonomic identification aids are useful at collection sites where either related species, or unrelated species of similar morphological characteristics, may be found.

……..contd.

- Rapid, safe and dependable transportation to carry personnel, equipment, supplies and collected medicinal plant materials should be arranged in advance.
- A collection team familiar withgood collecting techniques, transport, and handling of equipment and medicinal plant materials, including cleaning, drying and storage, should be assembled.
- Training of personnel should be conducted regularly. The responsibilities of all those involved in collection should be clearly set out in a written document. All stakeholders, in particular, manufacturers, traders and government, are accountable for the conservation and management of the targeted medicinal plant species.

3.3. Selection of Medicinal Plants for Collection

- Collectors of medicinal plants and producers of medicinal plant materials and herbal medicines should prepare botanical specimens for submission to regional or national herbaria for authentication.
- The voucher specimens should be retained for a sufficient period of time, and should be preserved under proper conditions.
- The name of the botanist or other experts who provided the botanical identification or authentication should be recorded. If the medicinal plant is not well known to the community, then documentation of the botanical identity should be recorded and maintained.

3.4. Collection

- Collection practices should ensure the long-term survival of wild populations and their associated habitats.
- The population density of the target species at the collection site(s) should be determined and species that are rare or scarce should not be collected.
- To encourage the regeneration of source medicinal plant materials, a sound demographic structure of the population has to be ensured.
- Management plans should refer to the species and the plant parts (roots, leaves, fruits, etc.) to be collected and should specify collection levels and collection practices. It is incumbent on the government or environmental authority to ensure that buyers of collected plant material do not place the collected species at risk.

.....contd.

- Medicinal plant materials should be collected during the appropriate season or time period to ensure the best possible quality of both source materials and finished products.
- It is well known that the quantitative concentration of biologically active constituents varies with the stage of plant growth and development.
- The best time for collection (quality peak season or time of day) should be determined according to the quality and quantity of biologically active constituents rather than the total vegetative yield of the targeted medicinal plant parts.

…….contd.

- Only ecologically non-destructive systems of collection should be employed. These will vary widely from species to species. For example, when collecting roots of trees and bushes, the main roots should not be cut or dug up, and severing the taproot of trees and bushes should be avoided. Only some of the lateral roots should be located and collected. When collecting species whose bark is the primary material to be used, the tree should not be girdled or completely stripped of its bark; longitudinal strips of bark along one side of the tree should be cut and collected.

- Medicinal plants should not be collected in or near areas where high levels of pesticides or other possible contaminants are used or found, such as roadsides, drainage ditches, mine tailings, garbage dumps and industrial facilities which may produce toxic emissions. In addition, the collection of medicinal plants in and around active pastures, including riverbanks downstream from pastures, should be avoided in order to avoid microbial contamination from animal waste.

……contd

- After collection, the raw medicinal plant materials may be subjected to appropriate preliminary processing, including elimination of undesirable materials and contaminants, washing (to remove excess soil), sorting and cutting. The collected medicinal plant materials should be protected from insects, rodents, birds and other pests, and from livestock and domestic animals.
- If the collection site is located some distance from processing facilities, it may be necessary to air or sun-dry the raw medicinal plant materials prior to transport.
- If more than one medicinal plant part is to be collected, the different plant species or plant materials should be gathered separately and transported in separate containers. Cross-contamination should be avoided at all times.

3.5. Personnel

- Local experts responsible for the field collection should have formal or informal practical education and training in plant sciences and have practical experience in fieldwork.
- They should be responsible for training any collectors who lack sufficient technical knowledge to perform the various tasks involved in the plant collection process.
- They are also responsible for the supervision of workers and the full documentation of the work performed. Field personnel should have adequate botanical training, and be able to recognize medicinal plants by their common names and, ideally, by their scientific (Latin) names.

……..contd.

- The collection team should take measures to ensure the welfare and safety of staff and local communities during all stages of medicinal plant sourcing and trade. All personnel must be protected from toxic and dermatitis-causing plants, poisonous animals and disease-carrying insects. Appropriate protective clothing, including gloves, should be worn when necessary.

Secondary Processing

- 1. Cutting/ sectioning/ communition
- 2. Aging or sweating
- 3. Baking/ roasting
- 4. Frying-
- 5.Fumigation
- 6. Extraction
- Removal of soluble materials from an insoluble residue, either liquid or solid, by treatment with a liquid solvent

MACERATION

- Solid material is mixed with menstrum in closed vessel
- Allowed to stand for 7 days shaking occasionally

DECOCTION

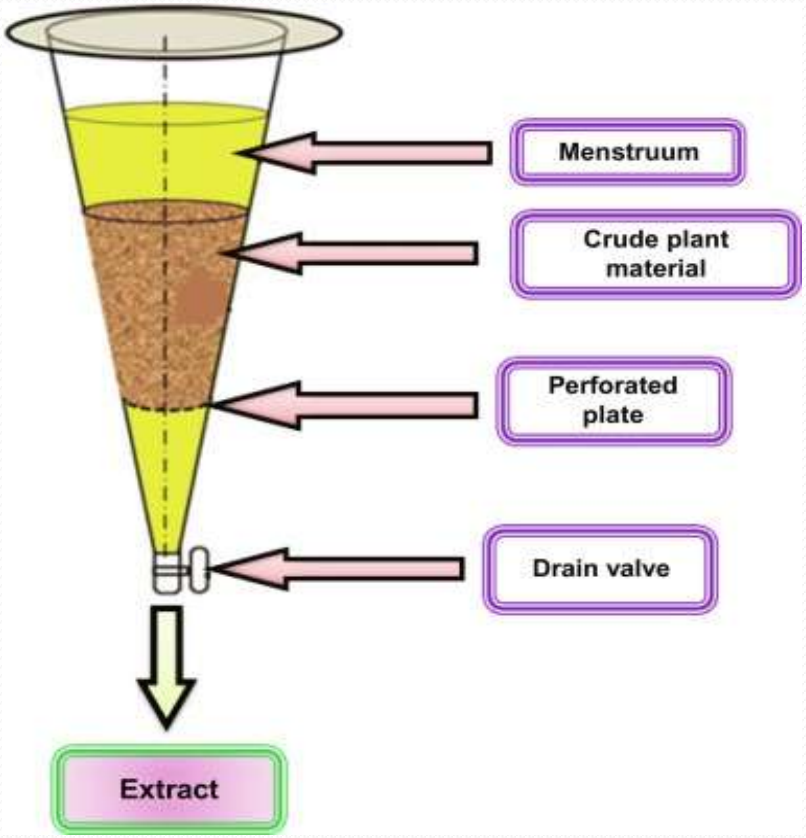
- Known quantity of drug is boiled with the menstrum for a specified period of time at the end supernatant liquid is collected
- Eg- Coffee or Tea decoction

INFUSION

- Weighed quantity of drug is kept in contact with menstrum for a specified period, the supernatant liquid is collected.
- Menstrum may either cold or hot

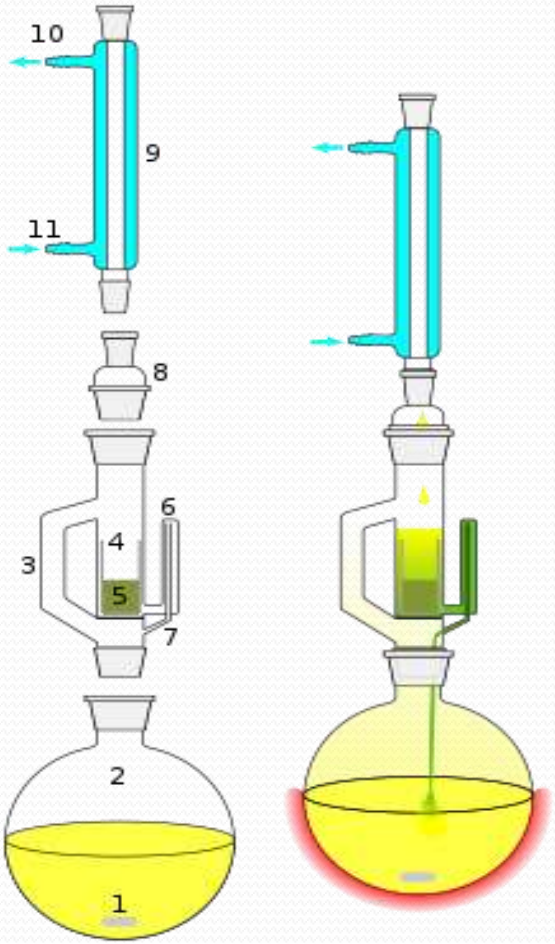
PERCOLATION

- Used most frequently to extract active ingredients in the preparation of tinctures



SOXHLET EXTRACTION

- Apparatus invented by Franz von Soxhlet a German chemist in 1879



COUNTER CURRENT EXTRACTION

- Method of multiple liquid-liquid extraction
- Permits the separation of substances with different distribution coefficients
- Lyman C. Craig designed apparatus

- https://youtu.be/pJ_U359lMD4

BIODYNAMIC AGRICULTURE

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BIODYNAMIC AGRICULTURE

- Type of agricultural technique where farm is considered as, living entity where sustainable, ecological methods are used for soil fertility, plant growth and livestock care.
- Includes various concepts drawn from ideas of Rudolf Steiner

Biodynamic agriculture is a form of alternative agriculture very similar to organic farming, but it includes various esoteric* concepts

*Loosely related ideas and movements developed in western

- Use of manure and compost which are prepared ecologically
- It is environment friendly, resource conserving, economical, socially supportive.
- This method do not use synthetic chemicals, fertilizers and pesticides
- It involves greater earthworm population and less energy is used for production as compared to other farming

Parameter	Organic Farming	Chemical, Inorganic / synthetic farming	Biodynamic Farming
Cost	Low	High	Low
Method	Need special Training	Easy to use	Training required
Fertilizers	Naturally occurring manures, green manures	Synthetic	Special preparations like BD500, BD501, BD502
Soil life	High	Low	High
Energy consumed	Medium	High	Low
Sustainable method	Yes	No	Yes
Crop rotation	May be	No	Yes

- In common with other forms of organic agriculture, biodynamic agriculture uses management practices that are intended to "restore, maintain and enhance ecological harmony".
- Central features include crop diversification, the avoidance of chemical soil treatments and decentralized production and distribution, and the consideration of celestial and terrestrial influences on biological organism

- In his "agricultural course" Steiner prescribed nine different preparations to aid fertilization, and described how these were to be prepared.
- Steiner believed that these preparations mediated terrestrial and cosmic forces into the soil.
- The prepared substances are numbered 500 through 508, where the first two are used for preparing fields, and the other seven are used for making compost.
- A long term trial evaluating the biodynamic farming system in comparison with organic and conventional farming systems, found that both organic farming and biodynamic farming resulted in enhanced soil properties, but had lower yields than conventional farming.
- Regarding compost development beyond accelerating the initial phase of composting, some positive effects have been noted.

Field Preparations

- **500**: A humus* mixture prepared by filling a cow's horn with cow manure and burying it in the ground (40–60 cm below the surface) in the autumn.
- It is left to decompose during the winter and recovered for use as fertilizer the following spring.

- **501**: Crushed powdered quartz stuffed into a cow's horn and buried in the ground in springtime and taken out in autumn. It can be mixed with 500 but is usually prepared on its own.
- The mixture is sprayed under very low pressure over the crop during the wet season, as a supposed antifungal

*(in soil science humus is organic matter that is amorphous and contributes to retention of moisture and nutrients)

Compost preparations

- **502**: Yarrow blossoms (*Achillea millefolium*) stuffed into the urinary bladders from red deer (*Cervus elaphus*), placed in the sun during summer, buried in the ground during winter, and retrieved in the spring.

- **Yarrow** or **common yarrow**, is a flowering plant family Asteraceae. It is native to temperate regions of the Northern Hemisphere in Asia and Europe and North America. It has been introduced as a feed for livestock in New Zealand and Australia, where it is a common weed of both wet and dry areas, such as roadsides, meadows, fields and coastal places.

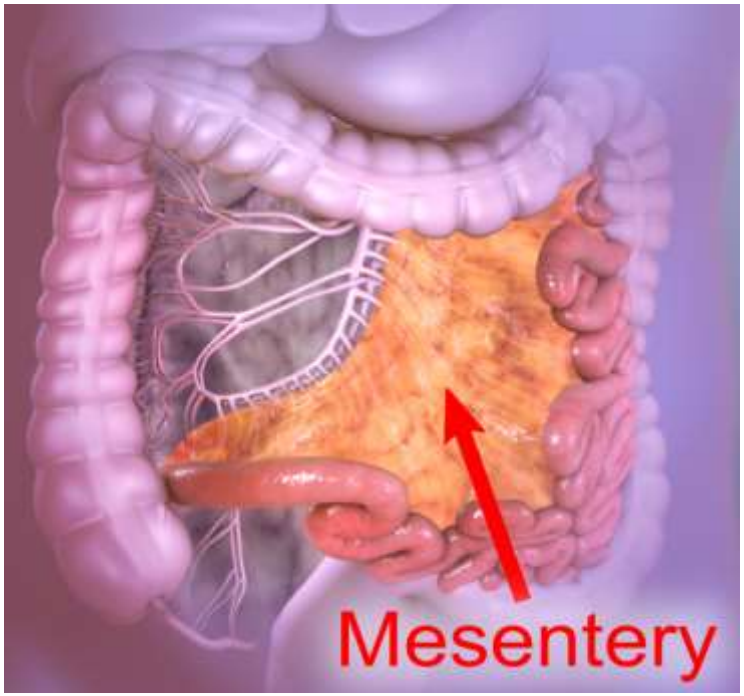
Yarrow and Red deer

- **503**: Chamomile blossoms (*Matricaria recutita*) stuffed into the small intestines of cattle, buried in humus-rich earth in the autumn, and retrieved in the spring.

- **504**: Stinging nettle (*Urtica dioica*) plants in full bloom stuffed together underground surrounded on all sides by peat for a year.

- **505**: Oak bark (*Quercus robur*) chopped in small pieces, placed inside the skull of a domesticated animal, surrounded by peat, and buried in the ground in a place near rain runoff.

- **506**: Dandelion flowers (*Taraxacum officinale*) stuffed into the mesentery of a cow, buried in the ground during winter, and retrieved in the spring.



- **507**: Valerian flowers (*Valeriana officinalis*) extracted into water.

508. Horsetail (Equisetum)

Good Agricultural Practices (GAP) in Cultivation of Medicinal Plants

- In India about 22% of the production of medicinal plants sourced through cultivation
- Tremendous scope for improvement in cultivation
- Guidelines described for GAP are intended to streamline the cultivation of medicinal plants as per the well regulated method and follow a systematic way in cultivation process as it is important for the production of good quality plant material

- **Selection of medicinal plants**
- The species or botanical variety selected for cultivation should be the same as that specified in the national pharmacopoeia.
- In the case of newly introduced medicinal plants, the species or botanical variety selected for cultivation should be identified and documented as the source material used or described in traditional medicine of the original country
- **Botanical Identity-**
- The botanical identity – scientific name (genus, species, subspecies/variety, author, and family) – of each medicinal plant under cultivation should be verified and recorded.
- **Seeds and other propagation materials**
- Should be free from any contamination & disease
- Should be resistant to environmental stress
- Sub standard, Inferior quality, adulterated, mixed varieties should be avoided

- **CULTIVATION**
- **Site selection**
- Medicinal plant materials derived from the same species can show significant differences in quality when cultivated at different sites, owing to the influence of soil, climate and other factors.
- **Ecological environment and social impact**
- The introduction of non-indigenous medicinal plant species into cultivation may have a detrimental impact on the biological and ecological balance of the region. The ecological impact of cultivation activities should be monitored over time, where practical.

- The social impact of cultivation on local communities should be examined to ensure that negative impacts on local livelihood are avoided.
- In terms of local income- earning opportunities, small-scale cultivation is often preferable to large-scale production, in particular if small-scale farmers are organized to market their products jointly.
- If large-scale medicinal plant cultivation is or has been established, care should be taken that local communities benefit directly from, for example, fair wages, equal employment opportunities and capital reinvestment.

- **Climate**
- Climatic conditions, like length of day, rainfall (water supply) and field temperature, significantly influence the physical, chemical and biological qualities of medicinal plants.
- The duration of sunlight, average rainfall, average temperature, including daytime and night-time temperature differences, also influence the physiological and biochemical activities of plants, and prior knowledge should be considered

- **Soil**
- The soil should contain appropriate amounts of nutrients, organic matter and other elements to ensure optimal plant growth and quality.
- Optimal soil conditions, including soil type, drainage, moisture retention, fertility and pH, will be dictated by the selected medicinal plant species and/or target medicinal plant part.
- It is, however, necessary to ensure that correct types and quantities of fertilizers are used through agricultural research. In practice, organic and chemical fertilizers are used.

- Animal manure should be thoroughly composted to meet safe sanitary standards of acceptable microbial limits and destroyed by the germination capacity of weeds.
- Chemical fertilizers that have been approved by the countries of cultivation and consumption should be used.

Good collection practices

- **Permission to collect**
- Collection permits and other documents from government authorities and landowners must be obtained prior to collecting any plants from the wild.
- For medicinal plant materials intended for export from the country of collection, export permits, phytosanitary certificates, Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permit(s) (for export and import), CITES certificates (for re-export), and other permits must be obtained, when required.

- **Technical planning**
- Prior to initiating a collection expedition, the geographical distribution and population density of the target medicinal plant species should be determined.
- Distance from home base and quality of the target plant(s) available are factors to be considered.
- A collection team familiar with good collecting techniques, transport, and handling of equipment and medicinal plant materials, including cleaning, drying and storage, should be assembled

- **Selection of medicinal plants for collection**
- The species or botanical variety selected for collection should be the same as that specified in the national pharmacopoeia or recommended by other authoritative national documents of the end-user's country, as the source for the herbal medicines concerned
- The stability of the natural habitat(s) and the maintenance of sustainable populations of the target species in the collection area(s) must be ensured.

- **Collection**
- Collection practices should ensure the long-term survival of wild populations and their associated habitats.
- The population density of the target species at the collection site(s) should be determined and species that are rare should not be collected.
- To encourage the regeneration of source medicinal plant materials, a sound demographic structure of the population has to be ensured.
- Management plans should refer to the species and the plant parts (roots, leaves, fruits, etc.) to be collected and should specify collection levels and collection practices.

- Only ecologically non-destructive systems of collection should be employed.
- For example, when collecting roots of trees and bushes, the main roots should not be cut or dug up, and severing the taproot of trees and bushes should be avoided.
- Only some of the lateral roots should be located and collected.
- When collecting species whose bark is the primary material to be used, the tree should not be girdled or completely stripped of its bark; longitudinal strips of bark along one side of the tree should be cut and collected.

Personnel

- Local experts responsible for the field collection should have formal or informal practical education and training in plant sciences and have practical experience in fieldwork.
- All collectors and local workers involved in the collection operation should have sufficient knowledge of the species targeted for collection and be able to distinguish target species from botanically related and/or morphologically similar species.
- The collection team should take measures to ensure the welfare and safety of staff and local communities during all stages of medicinal plant sourcing and trade.
- All personnel must be protected from toxic and dermatitis-causing plants, poisonous animals and disease-carrying insects. Appropriate protective clothing, including gloves, should be worn when necessary.

Pest and Pest control

- Pest- Undesired animal or plant species
- Pesticides- Chemicals derived from synthetic and natural sources effective in small concentration against pests
- Types of Pest-

1. Fungi and viruses
2. Insects
3. Weeds
4. Non- Insect pests

- 1.Fungi and Viruses
- Different types of fungi are known to occur on medicinal plants
- *Ascochyta atropae* causes formation of greyish white spot which further cause necrosis of leaves

- *Cercospora atropae causes* round brown spots with chestnut colored margin on both sides of leaf

- *Phytophthora nicotianae* is a dreadful disease occurring on belladonna in which dropping of young leaves and drying of whole apical portion occurs
-

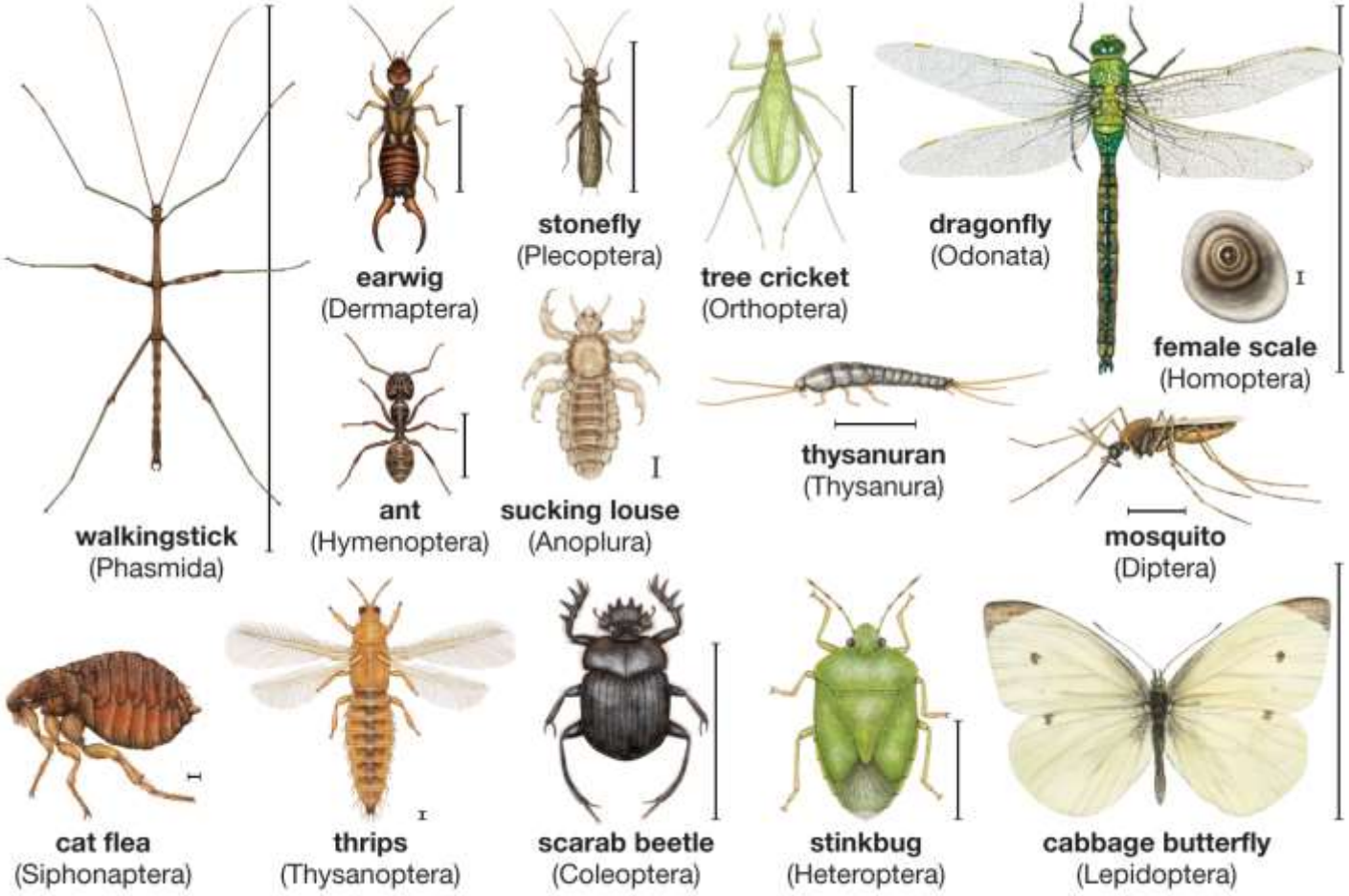
- Several other pathogenic fungi infest the medicinal and aromatic plants causes disease like
- *Pythium spinosum*
- *Currularia prasadii*
- *Septoria digitalis*
- *Ascochyta kashmiriana*
- *Peronospora hyoscyami*
- *Cercospora jasminicola*

- Different viruses are responsible for plant diseases
- Mosaic causing necrosis of leaves, petioles and stem of Solanaceous plant
- Tobacco mosaic virus

- Tobacco ring spot virus

Insects

- Total number of insect in world is larger than the total number of species of all other forms of life put together.
- Throughout the world about one million species of insects have been reported
- Adult form and in certain instances earlier stages like larvae is also destructive



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- Weeds-
- A weed is a undesired plant.
- Weeds are considered as dreadful pests because losses due to them are estimated to be more than those causing due to other pests and diseases combined together.
- Loss of nutrients, water, light & space increase in cost of labour
- Some plants grown as weed may causes allergy, dermatitis or may be poisonous in nature.

- Non insect pest-
- Grouped in two categories
- Vertebrates like rats, monkeys, bird, rabbits, deer, pigs etc.
- Invertebrates like crab, snail, nematodes etc.
- The rodents have sharp and growing incisor teeth thereby causing considerable spoilage of stored crude drugs

Methods of Pest Control

- Mechanical method
- Agricultural method
- Biological method
- Chemical method

Biopesticides /Bioinsecticides

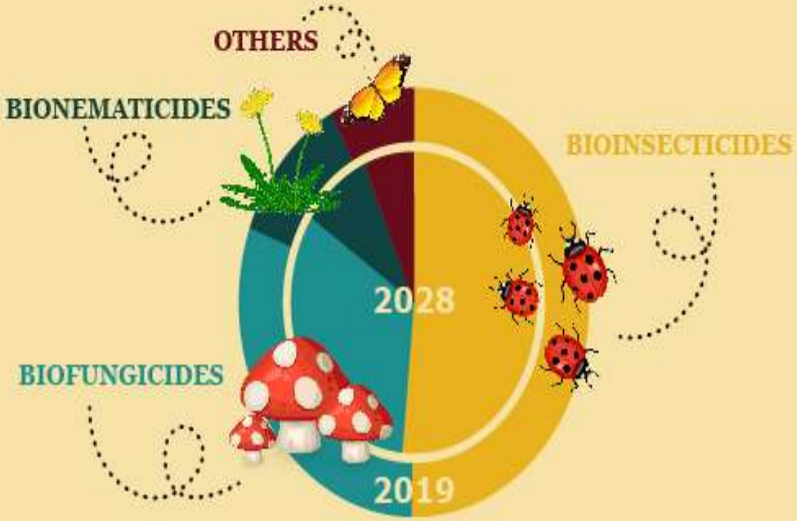
- These are the pesticides obtained from natural sources like microorganisms, plants, animals, insects and certain minerals.
- As a harmful effect of chemical pesticides evident, there is growing need of research and development for biological alternative.
- Indian Government, Ministry of Agriculture has taken initiative under Dr. M.S. Swaminathan in 2004.
- In 2006, use of biopesticides was 12-15%

GLOBAL BIOPESTICIDES MARKET FORECAST 2020-2028

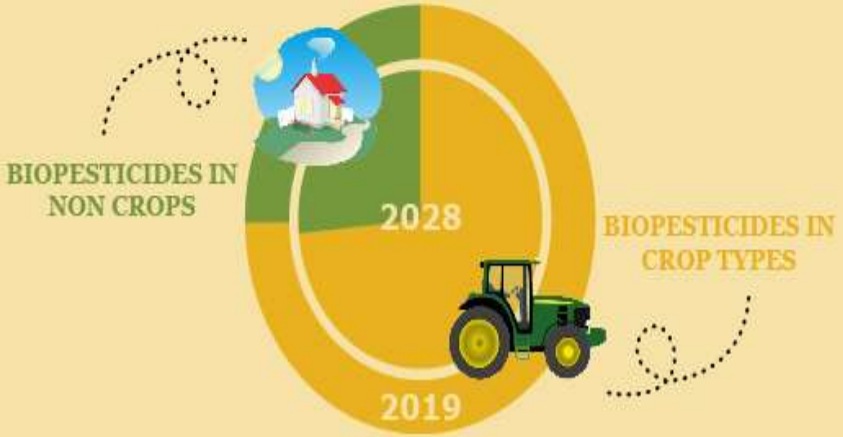
TOP COMPANIES

- ANDERMATT BIOCONTROL AG
- BASF SE
- BAYER CROPSCIENCE AG
- BIOWORKS INC.

MARKET BY PRODUCT TYPE

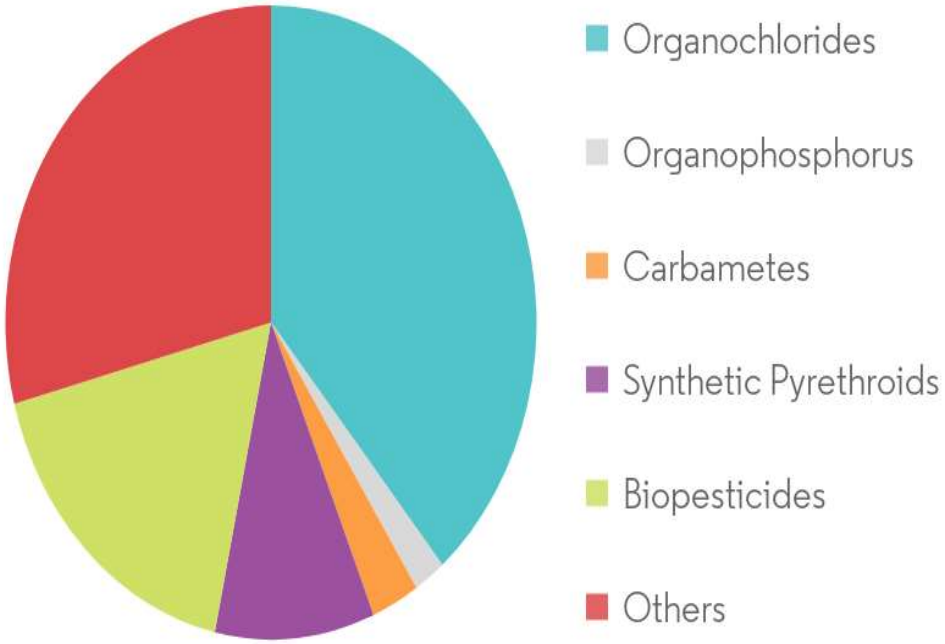


MARKET BY APPLICATION



www.inkwoodresearch.com

India Biopesticide Market: Pesticide Consumption in %, By Type, 2019



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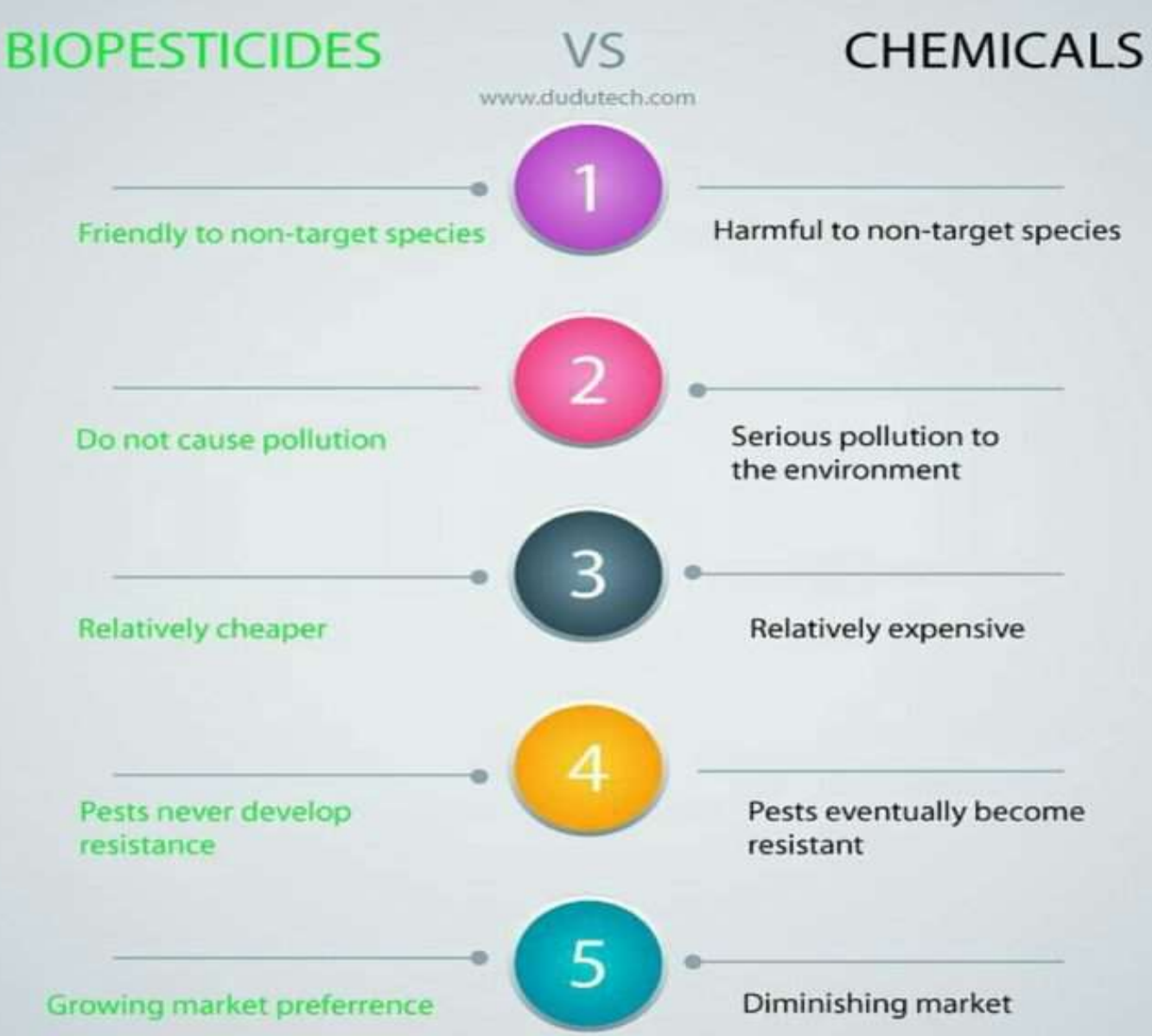
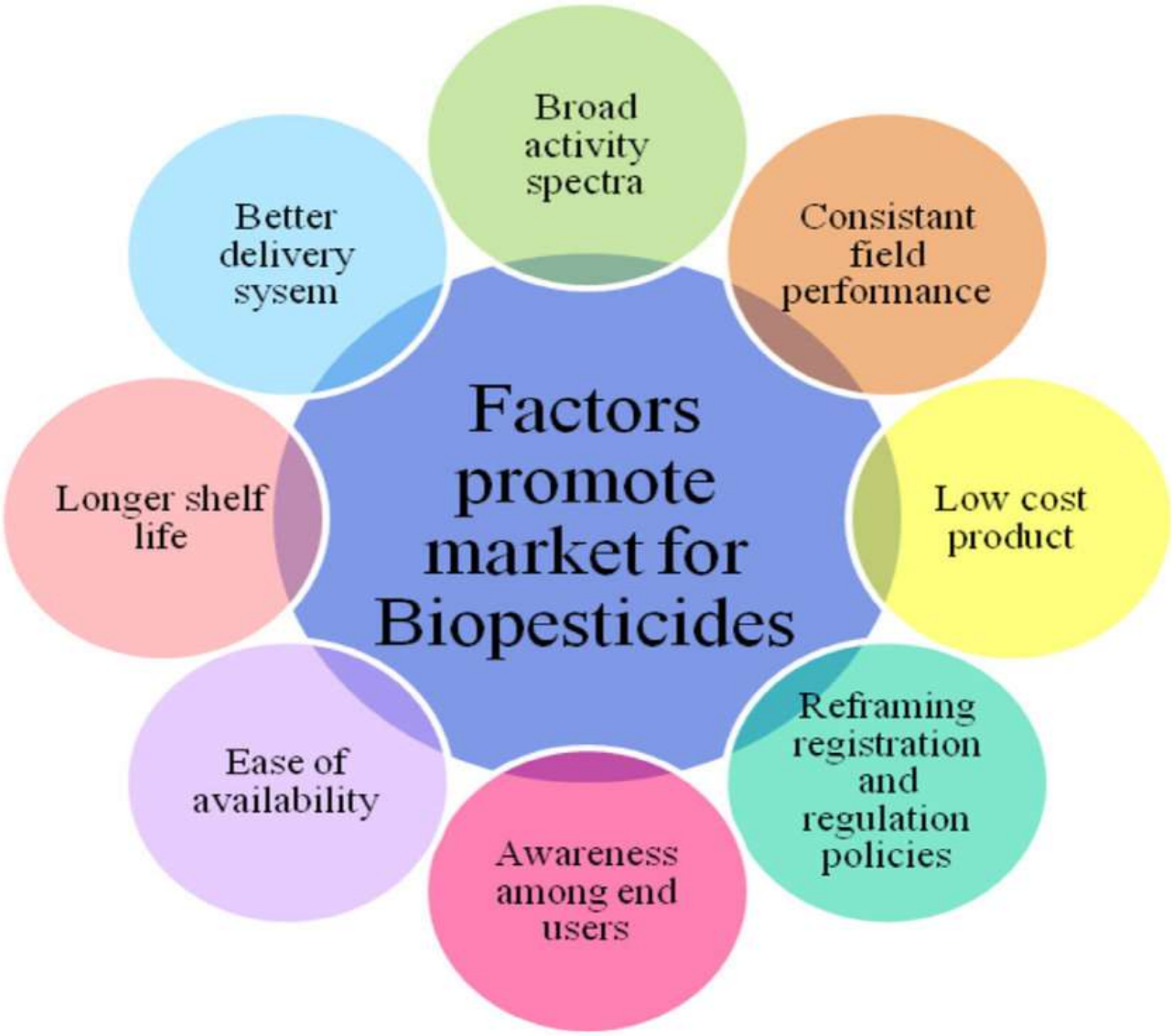

BIOPESTICIDES
VS
CHEMICALS
www.dudutech.com
1
Friendly to non-target species
Harmful to non-target species
2
Do not cause pollution
Serious pollution to the environment
3
Relatively cheaper
Relatively expensive
4
Pests never develop resistance
Pests eventually become resistant
5
Growing market preferrence
Diminishing market


Factors promote market for Biopesticides
Broad activity spectra
Consistant field performance
Low cost product
Reframing registration and regulation policies
Awareness among end users
Ease of availability
Longer shelf life
Better delivery sysem

- Advantages of biopesticides-
- 1. Non toxic to plants as well as human
- 2. Highly specific in action
- 3. Biodegradable and do not leave any toxic residue
- 4. Development of resistance is less common
- 5. Less expensive and can be grown alongwith cultivated medicinal plant.
- 6. Ecofriendly and do not affect soil fertility

Types of biopesticides

- Microbial pests-
- They consist of microorganisms, it can control different kind of pests and are relatively specific for its target pest.
- It is reported that some fungi are used to control weed and pests.
- Biochemical pests-
- These are naturally occurring chemical substances obtained from insect and animal which have ability to control the pest by non toxic mechanism e.g.- sex pheromones

- Plant pesticides-
- Various plants are reported to posses pesticidal and insecticidal properties. They can be grown along with cultivated plants. Used in the powdered form or chemical constituents are extracted to be used as pesticides.
- E.g. Neem, Tobacco, Pyrethrum, Derris

Common biopesticides available in India

Sr. No.	Name of biopesticide	Average production (approx.)
1.	Neem 300 PPM	Over 1,000,000 L
2.	Neem 1500 PPM	Over 250,000 L
3.	*Bacillus thuringiensis*	Over 50,000 kg
4.	Nuclear Polyhedrosis Virus	Over 500,000 L.E
5.	*Beauveria*	--
6.	Pheromone traps	Over 500,000
7.	Lures	Over 2 million
8.	*Trichogramma*	1 million
9.	*Chrysoperla* and other biocontrol insects	--

Biopesticides	Pest
Bacillus thuringiensis	Diamondback moth
Beauveria	Mango hoppers, coffee borers
Trichogramma	Sugarcane borers
Trichoderma	Rots in various crops
Neem	White fly on cotton

Indian Systems of Medicine

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AYURVEDA

- Prevalent for last 5000 years in India
- Most noted system in the world
- Based on the hypothesis that everything in the universe is made up of five basic elements

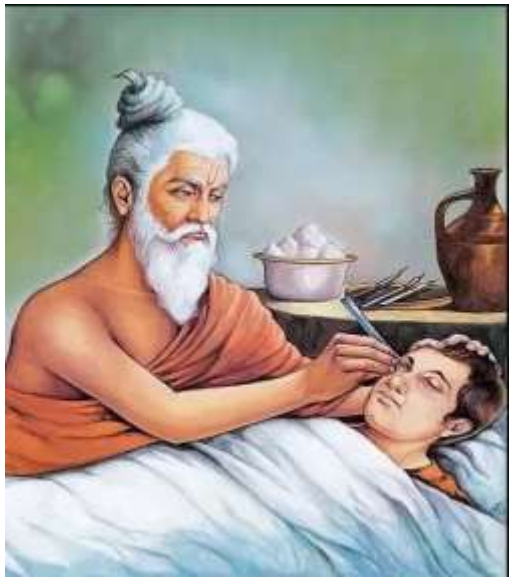
  1. Space 2. Air 3. Energy 4. Liquid 5. Solid
- They exist in human body in combined form
- Vata- Space & air
- Pitta- Energy & liquid
- Kapha- Liquid & solid
- Vata, Pitta & Kapha together are called as TRIDOSHA (three pillars of life)

- It is believed that they are in harmony with each other
- But in every human being one of them is dominating which in turn is called as PRAKRUTI of that person
- TRIDOSHA exist in human body in seven forms called SAPTDHATU
- 1. Rasa (lymph) 2.Rakta (Blood)
- 3. Meda (Adipose tissue) 4.Mamsa (Flesh)
- 5. Majja (Nervine Tissue) 6.Asthi (Bone)
- 7. Shukra (Reproductive Tissue)

- These tissues are subject to wear and tear so that MALA (Excretory material) is formed.
- When Tridosha, Saptdhatu and Mala are in balance with each other is called healthy condition.
- While imbalance causes pathological condition
- It is hypothesized that the five characters of medicinal herbs Rasa, Guna, Virya, Vipaka and Prabhava can be applied to treat various pathological conditions.

- Authentic information on ayurveda has been compiled by ancient Indian medicine practitioners

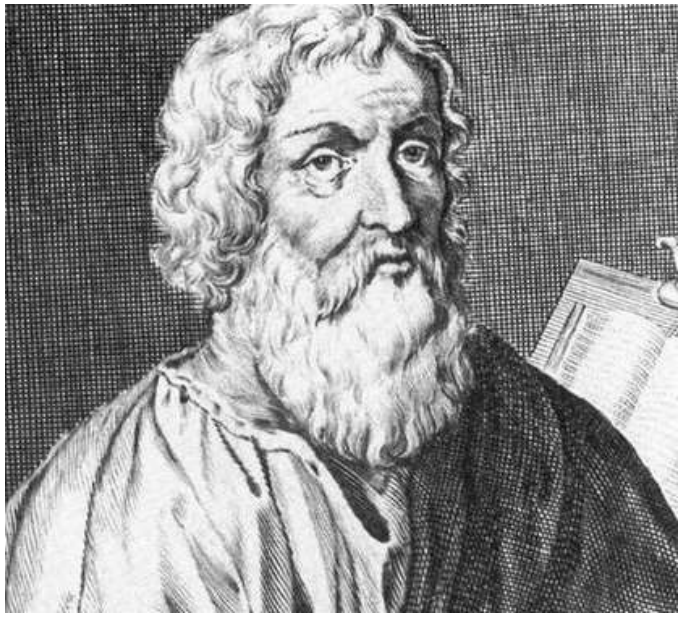
Maharshi Charak



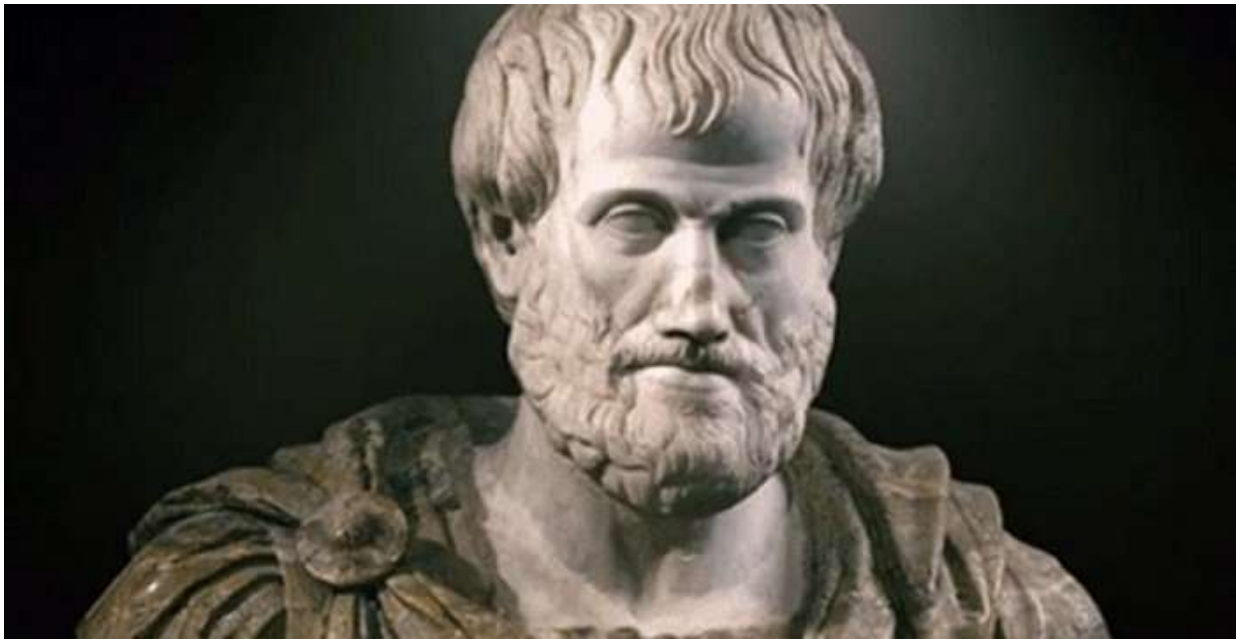
Maharshi Sushruta

UNANI SYSTEM OF MEDICINE

- Roots of this system go deep to the times of the well known Greek Philosopher Hippocrates



Aristotle, Greek philosopher ‘Father of Natural History’ made valuable contributions to Unani system of medicine.



- Unani system of Greek origin was further carried to Persia (Iran) and then developed by Arabian Physicians
- Based on two theories

1. Hippocratic theory of four Humours
2. Pythagorian theory of four proximate qualities

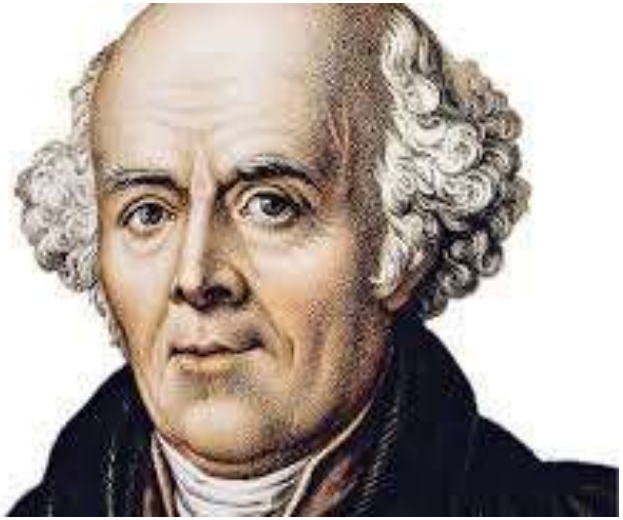
Four humours- Blood, Phelgm, yellow bile, black bile

Four qualities- hot, cold, moist and dry they are represented as earth, water, fire and air

- The Greek ideas were put by Arabian Physicians as seven working principle (Umur-e-Tabia) and included elements, temperaments, humours, organs, life, spirit, energy and actions.
- It is believed that these principles are responsible for the body constitution and its health as well as the diseased conditions.
- Unani system of medicine aims at treating the cause of disease and not its symptoms.
- Through history of patient is recorded in addition to his pulse, urine and stool examination.
- The drugs are polyherbal formulations and their collective effect is considered

HOMOEOPATHIC SYSTEM OF MEDICINE

- In comparison to other traditional system of medicine, Homoeopathy is newer one and has been developed in the eighteenth century.



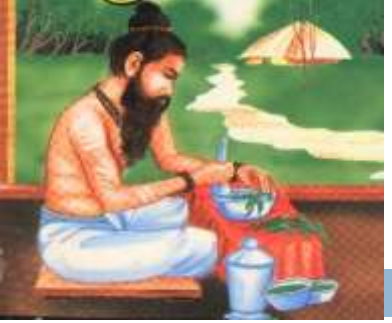
Samuel Hahnemann

(German Physician)

- Samuel Hahnemann proposed that the cause of disease itself can be used for its treatment.
- He put forth the law of similars which says that like cures like (Similae similibus curentur)
- He showed that cinchona can produce symptoms of malaria
- He succeeded in getting relevant results with a large number of extracts prepared from plant, animals and minerals.
- He compiled all these observation in 'The Organon of Medicine'

- In Homoeopathic system, the drug treatment is not specified, but the choice of drug depends on the symptoms and the clinical conditions of the patient. This is based on the concept of proving and prover.
- In healthy person called prover, the symptoms created by different doses of drug extract are noted which is called proving, and it specifically considers physical, mental and emotional changes of the prover

- Consequently these symptoms are compared with the patient with similar symptoms and accordingly same type of extract is given for the treatment.
- During the treatment the drug extracts are extremely diluted which is believed to cause potentiation and enhancement of curative effect.
- The drugs are extracted in the form of mother tincture which is further diluted in terms of decimal or centesimal potencies.



SIDDHA SYSTEM OF MEDICINE

- Siddha-Achievment
- Siddhars- saintly personalities who attained proficiency in medicine through practice of Bhakti and Yoga
- This is the system of pre Vedic period identified with Dravidian culture
- Like ayurveda this system also believes that everything in the universe is made up of five basic elements namely earth, water, sky, fire and air

- The identification of causative factors of disease is done through pulse reading, colour of body, study of voice, urine examination, status of digestive system and examination of tongue.
- The literature of Siddha system is mostly in Tamil

AYURVEDIC DOSAGE FORMS

- Classification

Ayurvedic Dosage Forms		
Solids	Semisolids	Liquids
Churna Vatika (Pills) Modaka (Boluses) Phalavarti	Kalka (Ointment) Avleha (Soft extract)	A. Aqueous Swarasa Kasaya (extracts) B. Oily Taila Ghrita Mantha C. Spiritous Sura, Asava, Arishhta

CHURNA

- Churna is the fine powder of drug or drugs.

- Drugs mentioned in the formula are cleaned & dried

- They are finely powdered and sieved
- If more than one drug is present then each one is separately powdered, sieved, accurately weighed and all mixed together
- The powder is fine to the extent of at least 80 mesh sieve
- It should not adhere together or become moist
- The fine powder has better therapeutic value

- Churna retains potency for one year and should be stored in air tight containers
- Commonly used churna include Narayana churna, Triphala churna, Sitopaladi churna etc.
- Evaluation-

1.Organoleptic properties-

a. colour

b. odour

2. Taste

- 3. Loss on drying at 105 degree Celsius
- 4. Total Ash
- 5.Acid insoluble ash
- 6.Particle size
- 7.Test for heavy metals- lead, cadmium, mercury, Arsenic.
- 8.Microbial contamination- Total bacterial count, Total fungal count.
- 9.Test for specific pathogen
- 10.Pesticide residue

Vati and Gutika

- Medicines prepared in the form of tablets or pills are known as vati and gutika

- These are made up from one or more drugs of plant, animal or mineral origin

Method of preparation

- The drugs of plant origin are dried and made into fine powders, separately.
- Minerals are made into ‘Bhasmas’ unless otherwise mentioned.
- These are put into ‘Khalva’ (Mortar) and ground to soft paste with prescribed fluids.
- When the mass is properly ground and it is in condition to be made into pills ‘sugandhi dravya’ like kasturi, karpura are added and ground again

- The final pill mass should not stick to finger when rolled.
- It can be dried in shade or sun
- Vati or gutika made from plant drugs when kept in air tight container can be for two years.
- Vati or gutika made from minerals can be used for indefinite period
- It should not lose thheir original colour, smell, taste and form.
- Eg- Gandhka vati, Mritsanjivani gutika, chandraprabha vati

Evaluation

- Description- colour, odor.
- Weight variation
- Disintegration time- NMT 15 Min.
- Assay
- Test for heavy metals- lead, cadmium, mercury, Arsenic
- Microbial contamination- Total bacterial count, Total fungal count
- Test for specific pathogen
- Pesticide residue

BHASMA

- Bhasma is the powder of substance obtained by calcination
- It is applied to the metals and mineral products which are prepared by special processes in closed crucibles in pits and with cow dung cake (puta)

❖ Method of preparation-

- ✓ First stage- Sodhana
- ✓ Second stage- Marana

□ 1.Sodhana-

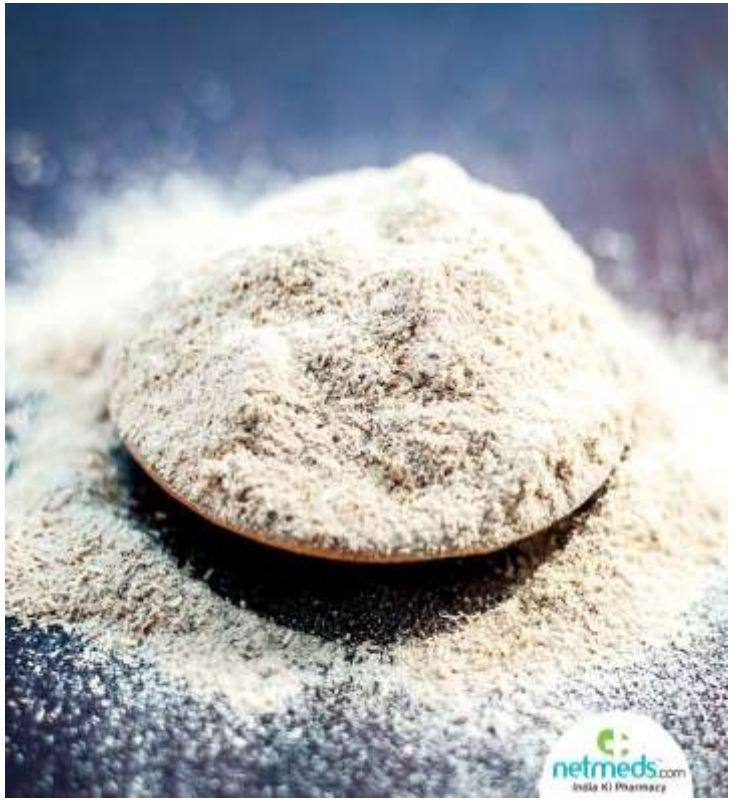
- ➢ Bhasmas are prepared from minerals, metals and animal products.
- ➢ In ayurveda the process of purification is called ‘Sodhana’
- ➢ During Sodhana thin sheets of the metal is heated and immersed in taila or gomutra.

2. Marana-

- The purified drug is put into Khalva (Stone mortar and pestle) and ground with juices of the plant or kasaya of the drugs.
- It is mixed for particular time and then small cakes are made
- Cakes are dried well under sunlight and placed in one single layer shallow earthen plate and closed with another plate.
- The edge is sealed with clay smeared cloth in seven consecutive layer and dried.

- A pit is dug into open space
- Half of the pit is filled with cow dung cake
- The sealed earthen plate is placed in it and remaining space is filled with more cow dung cakes.
- Fire is put on all sides of pit.
- When the burning is over it is allowed to cool completely, the earthen plates are removed and the seals are opened and contents are taken out.
- The medicine is ground into fine powder in khalva.

- Generally Bhasmas are yellowish, black, dark white, grey, reddish black and red
- It is preserved in air tight container.
- They maintain their potency indefinitely, they have no characteristic taste
- Eg- Abhraka Bhasma, tamra bhasma, suvarna bhasma etc.


netmeds.com
India Ki Pharmacy

- Evaluation
  - Description- colour, odour, taste
  - Particle size - # 200-300
  - Loss on Drying- at 105 degree
  - Total Ash
  - Acid insoluble ash
  - Water soluble ash
  - Assay
  - Floating on water

ASAVA AND ARISTA

- Medicinal preparation made by soaking the drug either in powder form or in the form of decoction, in a solution of sugar or jaggery for specified period of time
- During this process fermentation takes place and alcohol is generated which facilitates the extraction of active principle and also acts as preservative.
- Method of preparation
  - Drugs mentioned in the formula are coarsely powdered and kasaya is prepared

Dabur
द्राक्षासव
आसव - अरिष्ट
DRAKSHASAVA
ASAVA - ARISHTA

साधु
अर्जुनारिष्ट
SAADU
Arjunarishta

- Kasaya is added in fermentation vessel
- Sugar, jaggery or honey is dissolved, boiled and added
- The mouth of vessel is covered with earthen lid
- A constant temperature is maintained for fermentation by keeping the container either in special room or in an underground cellar or heap of paddy.

- The main difference between Asava and Arishta is involvement of the heat.
- Generally, Asava formulations are prepared without making decoctions and Arishta formulations are prepared by making decoctions. However, it is not a universal principle and there are a few exceptions.
- They have longer shelf life and have no expiry. In Ayurveda it is believed old Asava and Arishta are more beneficial

- The filtered Asava and Arista should be clear without froth at the top.
- Asava and Arista should not be sour, they have characteristic aromatic alcoholic odour.
- Asava and Arista can be kept indefinately, if stored in well stoppered bottle
- E.g.- Arvindasav Abhyarista

  Vasakasava Vidangarista

- Evaluation-
  - Description- colour, odour
  - pH
  - Specific gravity
  - Total Solids
  - Alcohol content
  - Reducing sugar
  - Non reducing sugar
  - Test for heavy metals-lead, cadmium, mercury, arsenic

- Microbial contamination- total bacterial count, total fungal count
- Test for specific pathogens-*E.coli, Salmonella spp. S. aureus, Pseudomonas aeruginosa*
- Pesticide residue

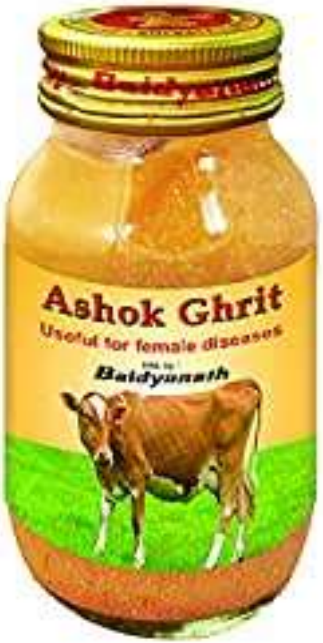
GHRITA

- Ghritas are preparation in which ghee is boiled with prescribed kasayas (decoction) and kalkas(paste) of the drugs according to formula.
- This process ensures absorption of the active therapeutic principles of the ingredients used.
- Method of Preparation
- Three essential components

  1. Drava dravaiga (a liquid which may be one or more as Kasayas
  2. Kalka-(A fine paste of the drugs)
  3. Sneha dravya (taila)

- Kalka (01) : Sneha (04) : Drava dravaiga (16)
- The kalkas and drava are mixed together
- Sneha is added, boiled and stirred well continuously so that kalka does not adhere to the vessel.
- When all Drava dravaiga have been evaporated the moisture in the kalka will also begin to evaporate.
- At this stage it should be stirred more often and carefully to ensure that kalka does not stick to the bottom of vessel

- Ghrita generally solidify when cooled
- It will have the colour, odour and taste of the drugs used
- It is preserved in glass, polythene or aluminium container
- Ghrita preparations for internal use keep their potency for about 16 months.
- Ghrita is to be taken after warming.
- E.g.Amrita ghrita, Eladi ghrita, Triphala ghrita etc.


Ashok Ghrit
Useful for female diseases
Baidyanath

GOSEVA
Serve cow to save yourself
PANCHAGAVYA GHRITA

Evaluation

- Description- colour, odour
- Refractive index at 25^0C
- Viscosity
- Iodine value
- Saponification value
- Peroxide value
- Test for heavy metals
- Microbial contamination- total bacterial count, total fungal count
- Test for specific pathogens-E.coli, Salmonella spp. S. aureus, Pseudomonas aeruginosa
- Pesticide residue

TAILA:

- Tailas are preparations in which tail (oil) is boiled with prescribed Kasaya and Kalka of drugs according to the formula
- This process ensures absorption of the active therapeutic principles of the ingredients used.
- Taila have the colour odour and taste of the drugs used and have the consistency of the oil.
- E.g. Narayana taila, Bhringraj taila, Pinda taila

Evaluation

- Description- colour, odour
- Weight/ml
- Refractive index at 25^0C
- Viscosity
- Iodine value
- Saponification value
- Peroxide value
- Test for heavy metals
- Microbial contamination- total bacterial count, total fungal count
- Test for specific pathogens-E.coli, Salmonella spp. S. aureus, Pseudomonas aeruginosa
- Pesticide residue

AVLEHA

- Avleha is a semisolid preparation of drugs prepared with the addition of jaggery or sugar and boiled with prescribed drug juice or decoction.
- Method of preparation
- Avleha mainly contains 1. Kasaya 2. Jaggery/sugar 3. powder or pulps of certain drugs 4. ghee or oil 5. honey
- Jaggery or sugar is dissolved in the liquid and strained to remove foreign particles
- This solution is boiled over moderate fire

- When the paka is thready when pressed between two fingers or when it sinks in water without getting easily dissolved, it should be removed from the fire
- Fine powders of drugs are then added in small quantities and stirred continuously and vigorously to form homogeneous mixture.
- Ghee or oil if required is added while the preparation is still hot and mixed well.
- Honey is added when preparation is cool and mixed well.

- The avaleha should neither be hard nor be a thick fluid
- There should not be growth of fungus
- The colour and smell of the preparation depends on the drug used.
- Avaleha should be kept in glass or porcelain jars.
- Avaleha should be used within one year
- E.g. Draksavleha, Vasavleha

Evaluation

- Description- colour, odour, taste
- Loss on drying at 105^0C
- pH
- Total ash
- Total solid
- Fat content
- Reducing sugar
- Total sugar
- Test for heavy metals- lead, cadmium, mercury, Arsenic
- Microbial contamination- total bacterial count , total fungal count
- Test for specific pathogens
- Pesticide residue

Role of Pharmacognosy in Ayurveda

- Pharmacognosy- systematic and scientific study of crude drugs
- In Indian System of Medicine, herbal drugs play an important role in preparing or in manufacturing the Ayurvedic formulations
- The drugs may comprise of whole plant or different parts of the plant, which are used **singly or in combination to** make compound formulations.
- Majority of the crude drugs are obtained from the **plant** sources where as very less percentage are derived from animal and mineral sources.

- Pharmacognostic study of crude drug helps in
- Development of Pharmacopoeial standard eg. Ashwagandha- Foreign organic matter- NMT 2% Total ash- 07%, Acid Insoluble ash- NMT 1.2%

- One of the important aspect of the pharmacognosy is to identify correctly the crude drugs used as such in medicine or as sources for commercial exploitation by drug industries, pharmacies or for exporter.
- The current Pharmacognostical methods to identify a herbal plant are based on their chemical analysis or by morphological examination of the plants and by microscopical/anatomical studies.

- Anatomical characters/structure are most likely to provide evidence for correct identification concerned with the inter relationships of larger groups such as families or in helping to establish the real affinities of genera of uncertain taxonomic studies. Anatomy some times proves very much helpful for individual identification.
- diagnostic characters like presence or absence of cluster crystals of Calcium oxalate, Prism cry stals, Raph i de bundles (Acicular crystals), Rosette crystals of calcium oxalate, trichomes of simple glan- dular and non-glandular type.

- Some *times* in establishing the botanical identity of some market samples of medicinal plants/drugs anatomy or microscopical studies plays an important role and also in checking adulteration, substitution for genuine drugs.
- The different macro and microscopical characters that are present in the different parts of the plant or drug plays a major role in the authentication of the drug. More over each drug will be having its own identifying characteristic.

Pune District Education Association's Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre.

AFFINITY CHROMATOGRAPHY

Presented by : Dr. Vijaya U. Barge
(Vice Principal & Professor)

Learning Objectives :

- After completing this sub-unit the students should be able:
- To study the principle involved in Affinity chromatography.
- To study the components of affinity medium.
- To study the steps involved in Affinity chromatography.
- To study the applications of Affinity chromatography.

Contents :-

- Introduction
- Principle
- Types of affinity chromatography
- Theory
- Advantages
- Disadvantages

Introduction :-

- **Affinity chromatography :-**
- It is a unique technique in the sense that it is based on the specific biological function of the biomolecules of interest.
- This feature also makes affinity chromatography suitable for the selective separation of active biomolecules and their isolation from the inactive or denatured forms .

Principle :-

- Affinity chromatography involves the covalent attachment of an immobilized biochemical called affinity ligand to a solid support. When sample passed through the column , only a solute that selectively binds to the complementary ligand is retained ; other sample components elute without retention.
- The retained solutes can be eluted from the column by changing the mobile phase condition
- By changing mobile phase composition means change in pH or ionic strength of the molecules of interest can be isolated in pure form.

Diagram :-

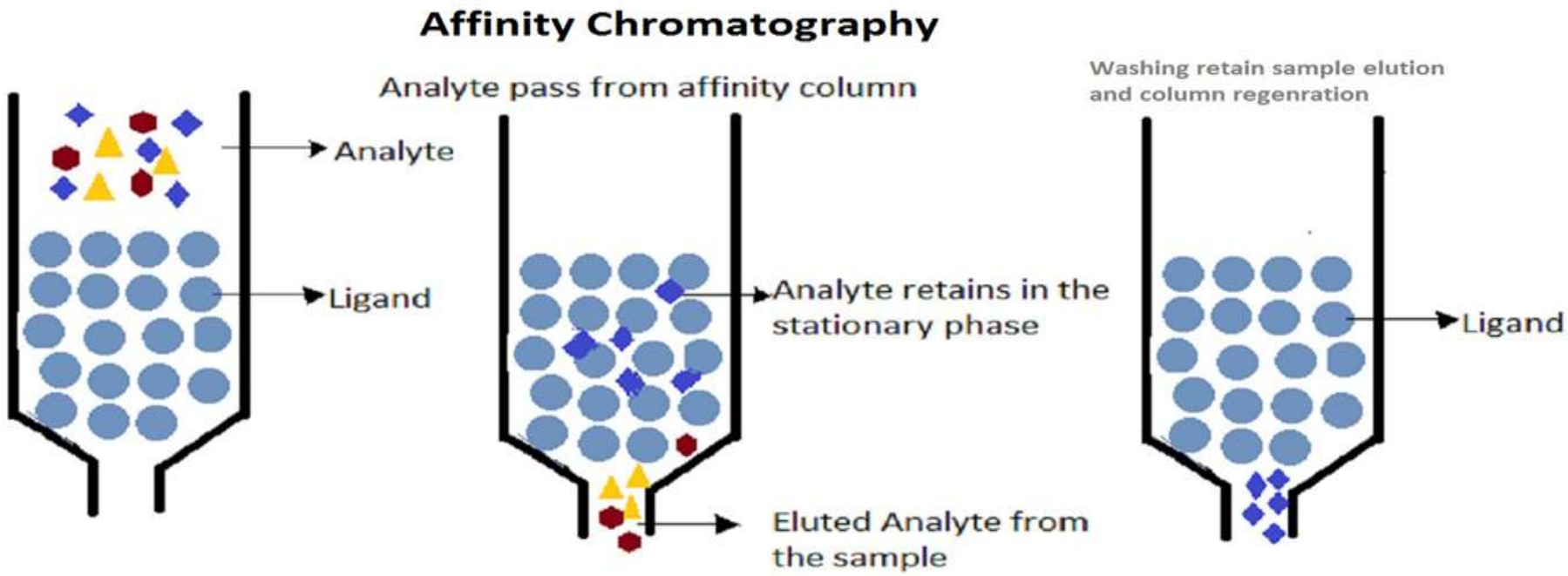


Fig. principal of affinity chromatography

Types of affinity chromatography :-

- Based on the nature of receptor present on matrix are as follows :-
- 1. Bio-affinity chromatography.
- 2. pseudo affinity chromatography.
    - a. Dye-affinity chromatography
    - b. Metal-affinity chromatography

- **1.bio-affinity chromatography :-** In this type of affinity chromatography , biomolecules used as receptor present on matrix and it has the biological phenomeonon such as antibody – antizen enzyme substrate or enzyme inhibitor.
- **2.pseudo –affinity chromatography :-**
- a.Dye-affinity chromatography :- In this chromatography dyes are utilizes as ligands in order to target protein.
- B.metal-affinity chromatography :- this is the most widely used method to purify the proteins according

Theory :-

- By affinity chromatography highly selectivety separation of bio-molecules can be achived throught their specific interaction.
- The separation exploit the “lock and key” binding that prevalent in bio logical system
- The stationary phase for affinity chromatography is solid such as agarose to which the affinity ligand is immobilised.
- Agarose is very popular because of its porous meshwork and it permits the high degree of ligand substitution and is more accessible to larger molecules.
- The ligands fixed to the stationary phase reversibly binds the desired biomolecule present in the mobile phase. This material can be eluted from the column by changing the composition of the mobile phase.

List of some of the commonly used interactions in affinity chromatography :-

Enzyme	Substrate analogue or inhibitor
Antibody	Antigen (virus , cells)
Nucleic acid	Complementary nucleic acid
Nucleic acid	Histone or other nucleic acid binding protein
Hormone	Hormone receptor
Glutathione	Glutathione S-transferase (GST) fusion protein
Metal chelate	His-tag fusion protein

Instrumentation :-

1.STATIONARY PHASE :-

- Instrumentation of affinity chromatography is same as per the high performance liquid chromatography.
- Stationary Phase has variety of materials like as organic gels such as beaded agarose , cellulose, dextran, combination of these polymers. The basic requirements of stationary phase.

1. the stationary phase should be sufficiently hydrophilic to avoid nonspecific binding of solutes and stable to most water compatible organic solvents.

2. a porous stationary phase permits a high degree of ligand substitution and is more accessible to larger molecules.

2. Affinity ligand :-

- The affinity ligands can be antibodies , enzyme inhibitors or other molecules that reversibly and bioselective bind to complementary analyte molecules in the sample.
- **Affinity ligands are of two types :-**

- *1.specific ligand :-* specific ligands bind only to one particular solute. The advantage of specific ligand is high selectivity by using short columns to perform seperations by step elution in less than 1 min.

- *2.Group specific :-* Group specific ligand bind to certain group of solutes. These can be separated in one step as a group or separated from each other using isocratic or gradient elution techniques. For better separation longer or more efficient columns must be used.

3. Sample preparation :-

- The sample must be clear solution free from solid particles. This can be achieved by centrifugation or filtration. Protein solutions be centrifuged at least 10000 g. cell lysates should be centrifuged at 40-50000 g.
- The factors affecting the interactions between the desired target protein and the matrix- bound ligand ,should also be determined.
- Sample components interfering with the target protein and/or ligand should be removed before loading onto the column.

Equilibration with a buffer facilitating the specific interaction :-

- The chromatography column is washed with 3-4 column volumes of the starting (binding) buffer. The sample must also be equilibrated with this starting binding buffer (if necessary , via solvent exchange or dialysis).

binding of the molecule of interest and Wash Out of the unbound material :-

- During sample loading, considered the strength of the interaction. In case of high- affinity samples, a high flow rate may be applied. In case of a weak interaction and/or slow equilibration process, reduce the rate of sample loading. After sample application, the column should be further washed with binding buffer until all unbound components are removed.
- **Regeneration :-** After successful completion of the elution, the column can be washed with several column volumes of binding buffer, and it can be then be reused. For long term storage, one must ensure that the column is not exposed to bacterial or fungal infection. The toxic compound sodium azide can be used to prevent such infections.

Advantages :-

- High specificity , reproducible , simple
- Target molecules can be obtained in a highly pure state
- Single step purification
- The matrix can be reused rapidly.
- The matrix is a solid, can be easily washed and dried.
- Give purified product with high yield.
- Affinity chromatography can also be used to remove specific contaminants,such as proteases.

Disadvantages :-

- Time consuming method
- More amounts of solvents are required hence becomes expensive.
- Intense labour requirement
- Non-specific adsorption cannot be totally eliminated, it can only be minimized.
- Limited availability and high cost of immobilized ligands.
- Proteins get denatured if required pH is not adjusted.

Applications:-

- Separation of mixture of compounds.
- Removal of impurities or in purification process.
- In enzyme assays
- Detection of substrates
- Investigation of binding sites of enzymes
- In in vitro antigen-antibody reactions
- Detection of single nucleotide polymorphisms and mutations in nucleic acids.

Referance :-

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3. Modern pharmaceutical Analytical Techniques by DR. Shashikant D. Barthate , DR. MD. Rageeb MD. Usman , Poonam A. Salunke , Shital S. patil.

THANK
YOU!

Alkaloids

S.Y.B.Pharm- Sem- IV

CONTENT

- Definition
- History
- Introduction
- Chemistry
- Classification
- Occurrence
- Drugs
- Biosynthesis

Origin, History, Introduction

- the term "alkaloid" (alkali-like) is commonly used to designate basic heterocyclic nitrogenous compounds of plant origin that are physiologically active.
- The term alkaloid ot Pflanzenlkalien was coined by Meissner, a German pharmacist, in 1819.
- The mankind has been using alkaloid for various purposes like poisons, medicines, poultices, teas etc.
- The French chemist, Derosne in 1803, isolated Narcotine.

Alkaloids

Definition: the term “alkaloid” (alkali-like) is commonly used to designate basic heterocyclic nitrogenous compounds of plant origin that are physiologically active.

Deviation from Definition:

- **Basicity: Some alkaloids are not basic e.g. Colchicine, Piperine, Quaternary alkaloids.**
- **Nitrogen: The nitrogen in some alkaloids is not in a heterocyclic ring e.g. Ephedrine, Colchicine, Mescaline.**
- **Plant Origine: Some alkaloids are derived from Bacteria, Fungi, Insects, Frogs, Animals.**

Distribution and occurrence:

- Rare in lower plants.
- Dicots are more rich in alkaloids than Monocots.
- Families rich in Alkaloids: Apocynaceae, Rubiaceae, Solanaceae and Papaveracea.
- Families free from Alkaloids: Rosaceae, Labiatae

Distribution in Plant:

- All Parts e.g. Datura.
- Barks e.g. Cinchona
- Seeds e.g. Nux vomica
- Roots e.g. Aconite
- Fruits e.g. Black pepper
- Leaves e.g. Tobacco
- Latex e.g. Opium

Effects of alkaloids on humans

- High biological activity
- Produce varying degrees of physiological and psychological responses - largely by interfering with neurotransmitters
  - others interfere with membrane transport, protein synthesis or other processes
- In large doses - highly toxic - fatal
- In small doses, many have therapeutic value
  - muscle relaxants, tranquilizers, pain killers, mind altering drugs, chemotherapy

Psychoactive alkaloids

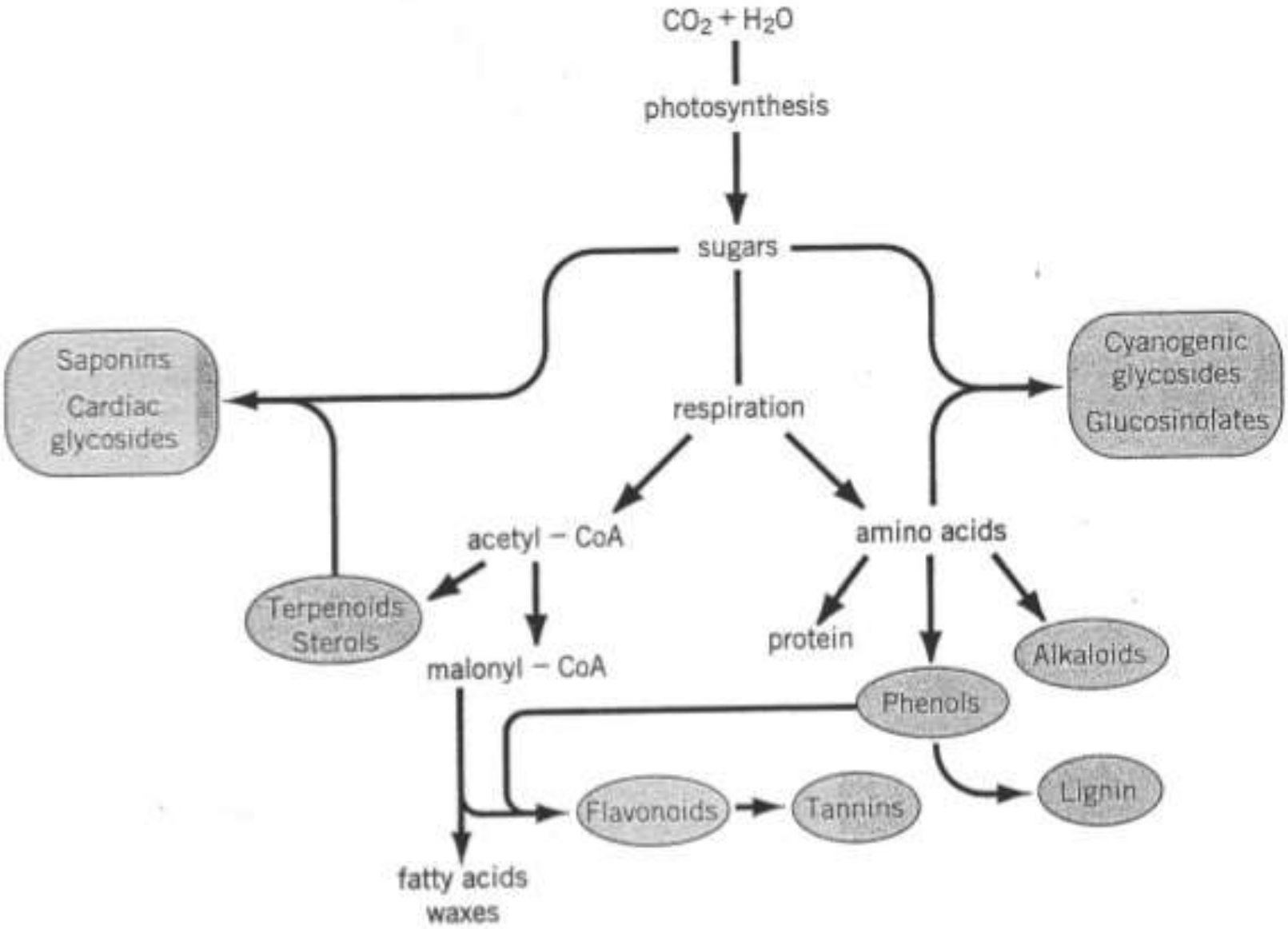
- Although only a small percent are psychoactive, these get much focus
- Affect the central nervous system - often by influencing neurotransmitters
- Categories of psychoactive cmpds
  - Stimulants
  - Hallucinogens
  - Depressants
- May also be narcotic (*addictive*)

Psychoactive alkaloids

Stimulants	Hallucinogens	Depressants
Cocaine	Tropane alkaloids	Morphine
Ephedrine	Mescaline	Codeine
Caffeine	Psilocybin	Heroin
	Ergot alkaloids (LSD)	

Alkaloid synthesis

- Most alkaloids are synthesized from a few common amino acids (tyrosine, tryptophan, ornithine or argenine, aspartic acid, & lysine)
- Nicotinic acid precursor for part of nicotine
- Purine precursor for caffeine
- Some alkaloids synthesized from terpenes - along mevalonic acid pathway
- Generally classified by the predominant ring structure and/or carbon skeleton


$CO_2 + H_2O$
photosynthesis
sugars
Saponins
Cardiac glycosides
respiration
Cyanogenic glycosides
Glucosinolates
acetyl – CoA
amino acids
Terpenoids
Sterols
malonyl – CoA
protein
Alkaloids
Phenols
Lignin
Flavonoids
Tannins
fatty acids
waxes

Function in Plants

- They may act as **protective** against insects and herbivores due to their bitterness and toxicity.
- They are, in certain cases, the final **products of detoxification** (**waste products**).
- **Source of nitrogen** in case of nitrogen deficiency.
- They, sometimes, act as **growth regulators** in certain metabolic systems.
- They may be utilized as a **source of energy** in case of deficiency in carbon dioxide assimilation.

Chemical tests

- Wagner's test: (I_2/kI):Reddish brown precipitate .
- Mayer's: ($HgCl_2$ Creamy precipitate with True alkaloid.
- Hagger's test: (Picric acid) Yellow precipitate with True alkaloid.
- Dragendorff: (Potassium Bismuth Iodide) Reddish Brown precipitate .
- Tannic acid solution: different alkaloid colored

Physical Properties:

I- State:

- **Most** alkaloids are crystalline **solids**.
- Few alkaloids are amorphous solids e.g. emetine.
- **Some** are **liquids** that are either:

  Volatile e.g. nicotine and coniine, or

  Non-volatile e.g. pilocarpine and hyoscine.

II- Color:

The **majority** of alkaloids are **colorless** but **some** are **colored** e.g.:

- Colchicine and berberine are yellow.
- betanidine is orange.
- The salts of sanguinarine are copper-red.

III- Solubility:

- Both **alkaloidal bases** and their **salts** are **soluble in alcohol**.
- Generally, the **bases** are **soluble in organic solvents** and **insoluble in water**

  Exceptions:

  - Bases **soluble in water**: caffeine, ephedrine, codeine, colchicine, pilocarpine and quaternary ammonium bases.
  - Bases **insoluble or sparingly soluble in certain organic solvents**: morphine in ether, theobromine and theophylline in benzene.
- **Salts** are usually **soluble in water** and, **insoluble or sparingly soluble in organic solvents.**

  Exceptions:

  - Salts **insoluble in water**: quinine monosulphate.
  - Salts **soluble in organic solvents**: lobeline and apoatropine hydrochlorides are soluble in chloroform

IV- Isomerization:

- Optically active isomers may show different physiological activities.
- *l*-ephedrine is 3.5 times more active than *d*-ephedrine.
- *l*-ergotamine is 3-4 times more active than *d*-ergotamine.
- *d*- Tubocurarine is more active than the corresponding *l*- form.
- Quinine (*l*-form) is antimalarial and its *d*- isomer quinidine is antiarrythmic.
- The **racemic (**optically inactive) *dl*-atropine is physiologically active.

Chemical Properties:

I- Nitrogen:

- Primary amines $R\text{-}NH_2$ e.g. Norephedrine
- Secondary amines $R_2\text{-}NH$ e.g. Ephedrine
- Tertiary amines $R_3\text{-}N$ e.g. Atropine
- Quaternary ammonium salts $R_4\text{-}N$ e.g *d*-Tubocurarine

II- Basicity:

- $R_2\text{-}NH > R\text{-}NH_2 > R_3\text{-}N$
- Saturated hexacyclic amines is more basic than aromatic amines.

Nomenclature:

Trivial names should end by **"ine"**. These names may refer to:

- The **genus** of the plant, such as Atropine from *Atropa belladona*.
- The plant **species**, such as Cocaine from *Erythroxylon coca*.
- The **common name** of the drug, such as Ergotamine from ergot.
- The name of the **discoverer**, such as Pelletierine that was discovered by Pelletier.
- The **physiological action**, such as Emetine that acts as emetic, Morphine acts as narcotic.
- A prominent **physical character**, such as Hygrine that is hygroscopic.

Prefixes and suffixes:

Prefixes:

- **"Nor-"** designates N-demethylation or N-demethoxylation, e.g. norpseudoephedrine and nornicotine.
- **"Apo-"** designates dehydration e.g. apomorphine.
- **"Iso-, pseudo-, neo-,** and **epi-"** indicate different types of isomers.

Suffixes:

- **"-dine"** designates isomerism as quinidine and cinchonidine.
- **"-ine"** indicates, in case of ergot alkaloids, a lower pharmacological activity e.g. ergotaminine is less potent than ergotamine.

IV- Stability:

- **Effect of heat**:

  Alkaloids are decomposed by heat, except **Strychnine** and **caffeine** (**sublimable**).

- **Reaction with acids:**

  1- Salt formation.

  2- Dil acids hydrolyze Ester Alkaloids e.g. Atropine

3- Conc. acids may cause:

- Dehydration:

  Atropine → Apoatropine

  Morphine → Apomorphine

- Demethoxylation:

  e.g. Codeine

- **Effect of Alkalies:**

1- Dil alkalis liberate most alkaloids from their salts e.g. NH3.

2- They may cause isomerization (racemization) of alkaloid as the conversion of hyoscyamine to atropine.

3- They also can **form salts with alkaloids containing a carboxylic group** e.g. narceine.

4- Strong alkalis: such as aqueous NaOH and KOH form salts with phenolic alkaloids.

5- Strong alkalis cause hydrolysis of Ester alkaloids (e.g. atropine, cocaine and physostigmine) and **Amide alkaloids** (colchicines).

6**- Strong alkalis cause opening of lactone ring.**

Qualitative test for Alkaloids:

Precipitation Reagents:

They are used to:

1- Indicate the absence or presence of Alkaloids

2- Test for complete of extraction

Disadvantages: Some non alkaloids interfere such as Proteins, lactones, coumarins

- **Colour Reagents:**

1- Froehd's Reagent: Phosphomolybdic acid

2- Marqui's Reagent: Formaldehyde/ Conc. H_2SO_4

3- Mandalin's Reagent: Sulphovanidic acid

4- Erdmann's Reagent: Conc. HNO_3/Conc. H_2SO_4

5- **Mecke's Reagent:** Selenious acid / conc. H_2SO_4

6- **Shaer's Reagent:** Hydrogen peroxide / conc. H_2SO_4

7- **Rosenthaler's Reagent:** Potassium arsenate / conc. H_2SO_4

8- **Conc. HNO_3**

Identification of Alkaloids:

- Melting point
- Colour test
- Optical Rotation
- Microcrystal test
- HPLC, GC, GC-MS
- UV, IR, NMR, MS.

Quantitative Determination of Alkaloids:

- **Volumetric methods:**
- These are based on reaction of alkaloidal bases with acids (Acid-Base titration).

  They include:
- **Aqueous titration:** This is carried by either:

  1- **Direct titration** of the alcoholic solution of the alkaloidal residue with standard acid, or

  2- **Back titration** by dissolving the residue in a known amount of standard acid and back titration of residual acid against standard alkali.
- **Non-aqueous titration:** This method is suitable for determination of weak bases e.g. Caffeine.

- **Gravimetric methods:**

These methods are recommended for determination of:

1- Very weak bases which can not be determined by volumetric methods e.g. caffeine and colchicine.

2- Mixtures of alkaloids that are obtained from the same plant but differ greatly in their molecular weight e.g. Cinchona and Rawolfia alkaloids.

They can be performed by either:

1- Direct Weighing of the alkaloidal mixtures

2- Precipitation of the total alkaloids and determination of the weight of the precipitate obtained.

The major **drawbacks** of the gravimetric methods are:

1- They are insensitive to microamounts of alkaloids.

2- They could not be applied in case of thermolabile and volatile alkaloids.

3- Lipophilic impurities in the residue are calculated as alkaloids.

- **Colourimetric Method:**

  e.g. Morphine + $NaNO_2$/HCl

  Ergot + p-dimethylaminobenzaldehyde

- **Spectrophotometric Methods.**

- **Polarimetric Method.**

- **Fluorimetric Method.**

- **Chromatographic Methods**

QUALITATIVE CHEMICAL TESTS FOR ALKALOIDS

General tests answered by all alkaloids are as follows:

1. ***Dragendorff's test:*** *To 2–3 mL of the alkaloid solution add few drops of Dragendorff's reagent* (potassium bismuth iodide solution). An orange brown precipitate is formed.

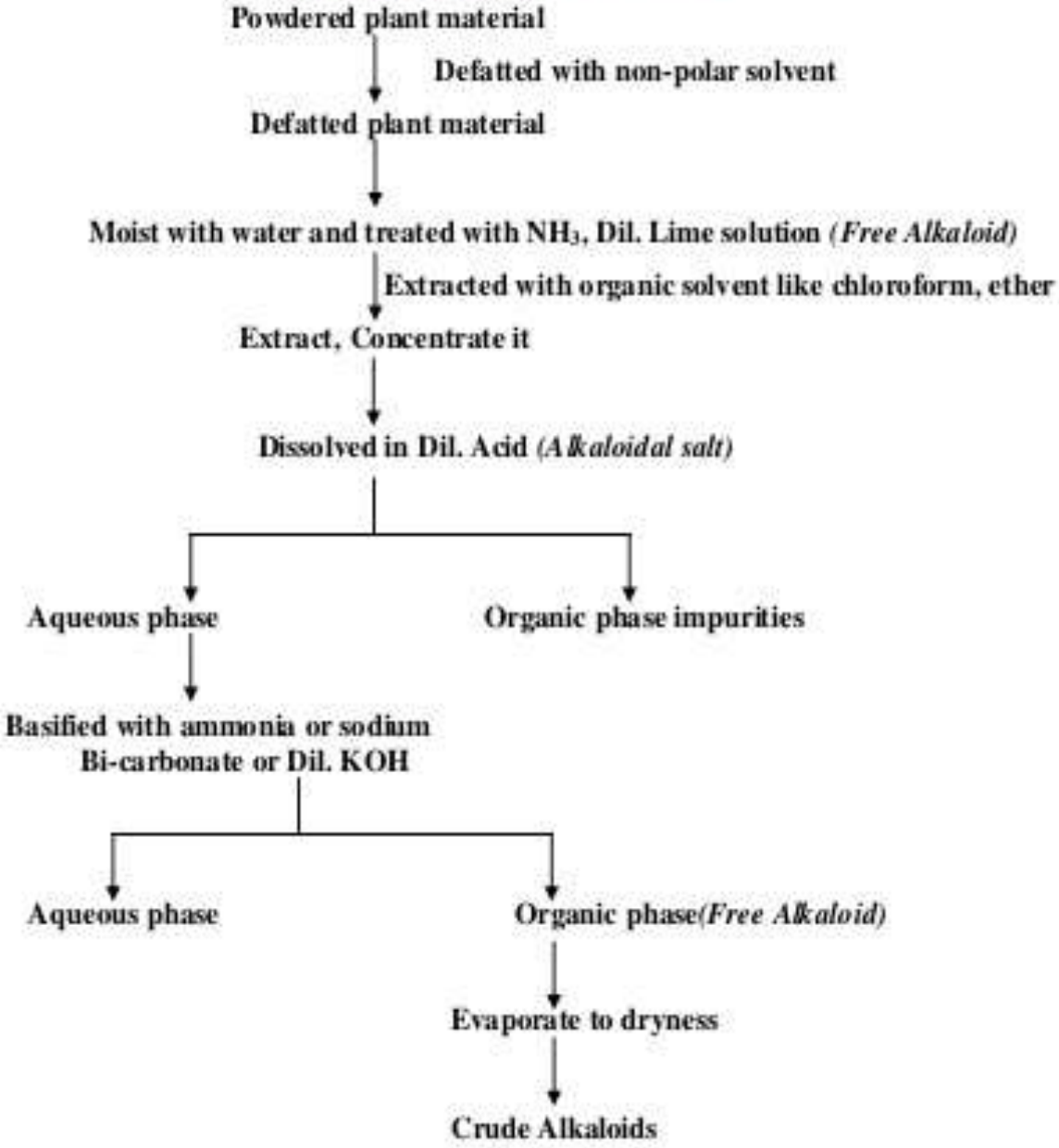
2. ***Mayer's test:*** *To 2–3 mL of the alkaloid solution add few drops of Mayer's reagent (potassium* mercuric iodide solution). White brown precipitate is formed.

3. ***Hager's test:*** *To 2–3 mL of the alkaloid solution add few drops of Hager's reagent (saturated* solution of picric acid). Yellow precipitate is formed.

4. ***Wagner's test:*** *To 2–3 mL of the alkaloid solution add few drops of Wagner's reagent (iodine–* potassium iodide solution). Reddish brown precipitate is formed.

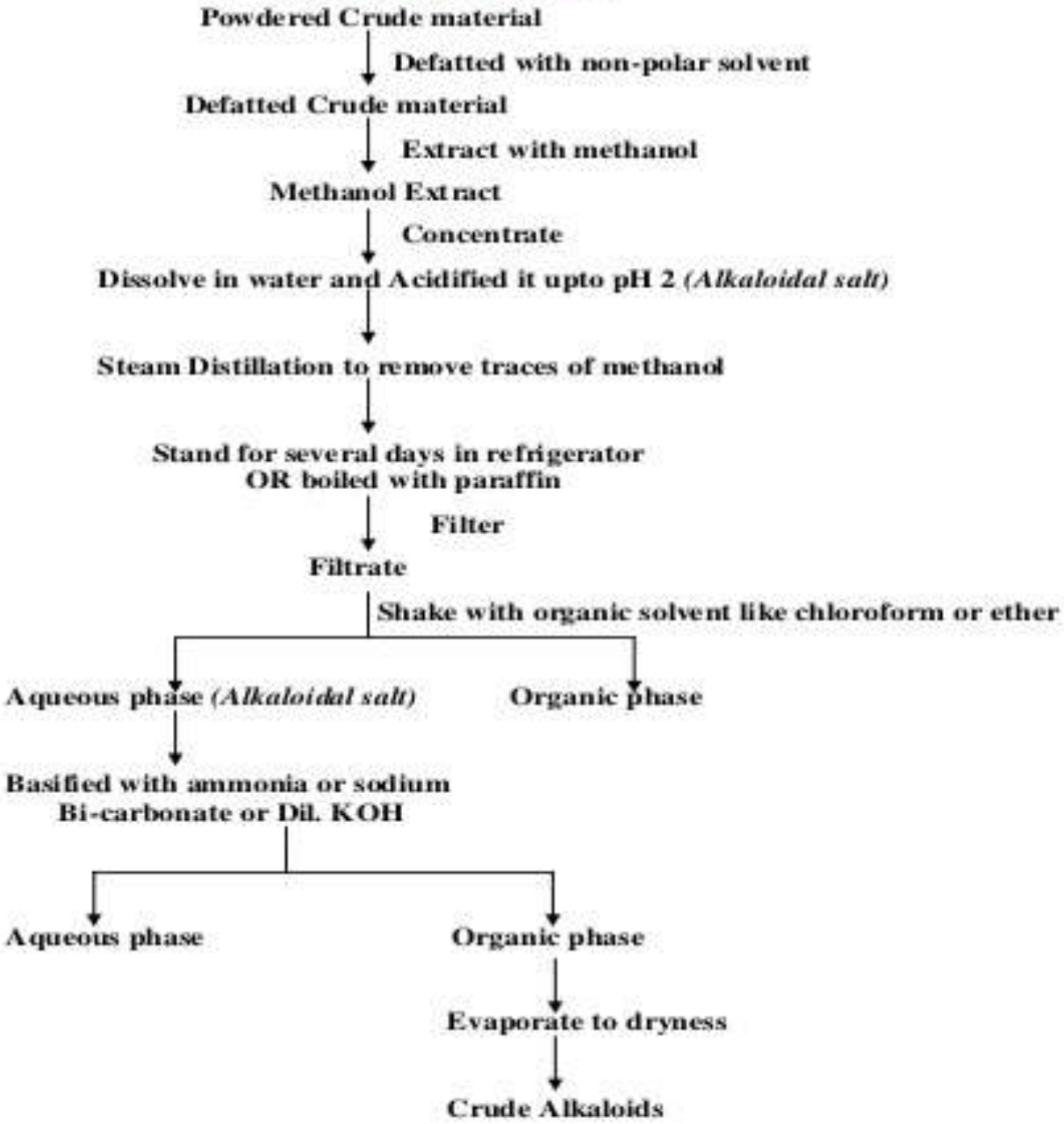
Extraction of alkaloids

1. Stass otto method



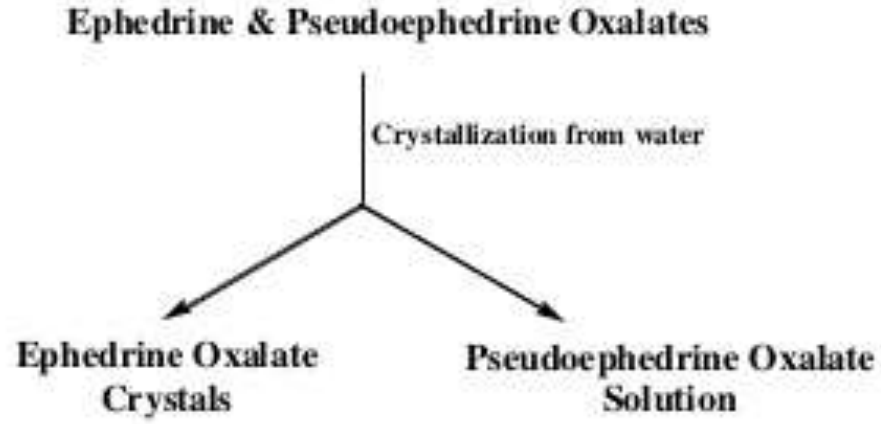
Extraction of alkaloids

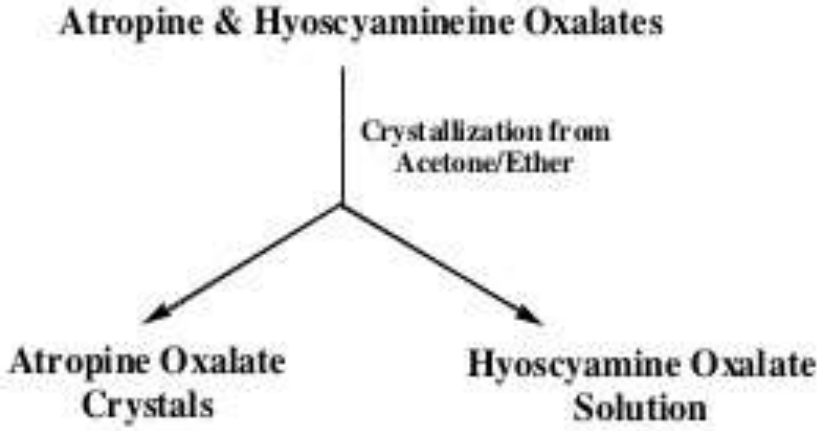
2. Manske's method



Purification of Alkaloids

1. Direct crystallization from solvent
2. Repeated acid base treatment
3. Fractional crystallization

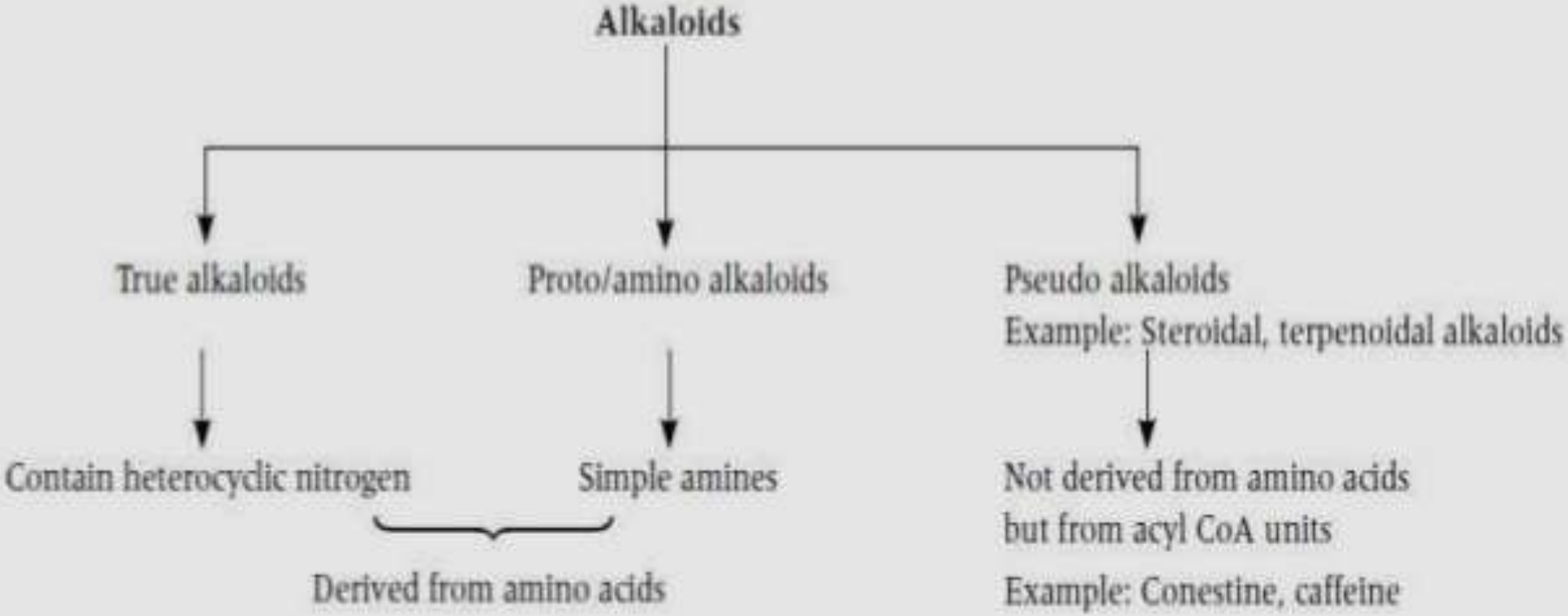




4. Chromatographic techniques
5. Gradient pH technique

Alkaloids

Definition: **the term "alkaloid" (alkali-like) is commonly used to designate basic heterocyclic nitrogenous compounds of plant origin that are physiologically active. Derived from amino acids.**



Classification of Alkaloids

❖**True (Typical) alkaloids –**

That are derived from amino acids and have nitrogen in a heterocyclic ring. Show wide range of physiological activity and give positive test with the general alkaloid reagent. e.g. Atropine

❖**Protoalkaloids –**

That are derived from amino acids and do not have nitrogen in a heterocyclic ring.

e.g. Ephedrine; mescaline

❖**Pseudo alkaloids-**

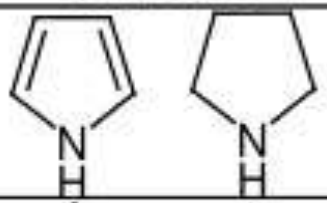
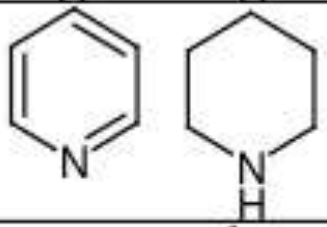
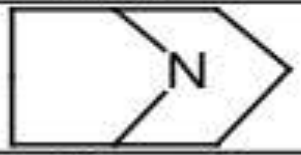
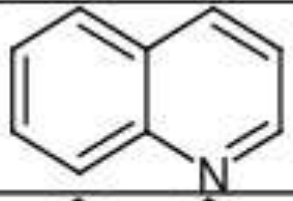
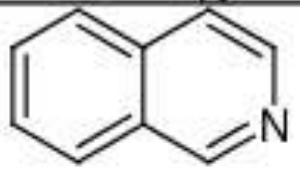
That are not derived from amino acids but have nitrogen in a heterocyclic ring. Do not show positive reaction with alkaloid reagents.

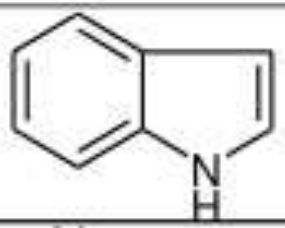
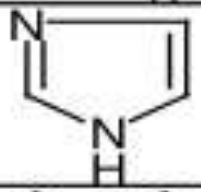
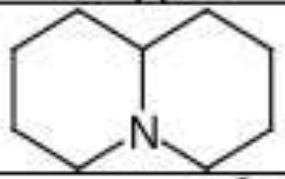
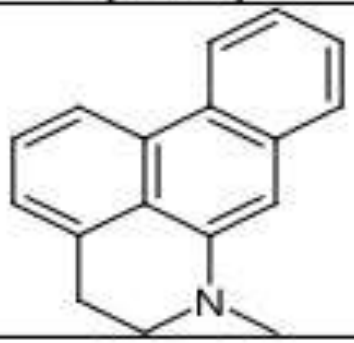
e.g. Purine alkaloids – Caffeine, steroidal alkaloids

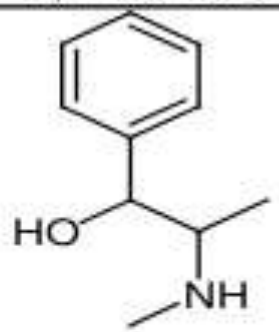
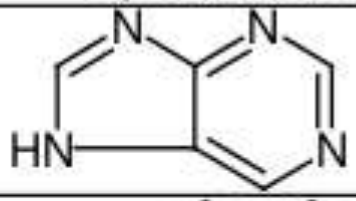
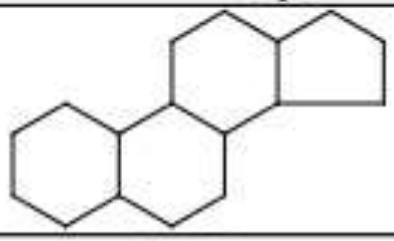
Classification of alkaloids

Chemical classification

A) True alkaloids

Sr. no.	Type	Structure	Examples
1.	Pyrrole and pyrrolidine		e.g. Hygrine, coca species
2.	Pyiridine and piperidine		e.g. Arecoline, anabasine, lobeline, conine, trigonelline
3.	Pyrrolizdine		e.g. Echimidine, senecionine, seneciphylline
4.	Tropane		e.g. Atropine, hyoscine, hyoscyamine, cocaine, pseudopelletirine
5.	Quinoline		e.g. Quinine, quinidine, cinchonine, cupreine, camptothecine
6.	Isoquinoline		e.g.Morphine, codeine, emetine, cephaline, narcotine, narceine, d-tubocurarine

7	Indole		e.g. Erotamine, ergotametriene, reserpine, vincristine, vinblastine, strychnine, brucine
8	Imidazole		e.g. Pilocrpine, isopilocarpine, pilosine
9	Norlupinane		e.g. Cystisine, laburinine
10	Piporphine (reduced isoquinoline napthalene)		e.g. Boldine

		B) PROTOALKALOID	
1.	Alkyalamine		Ephedrine, Pseudoephedrine
		C) Pseudoalkaloid	
1.	Purine		e.g. Caffeine, thophylline, theobromine
2.	Steroidal		e.g. Solanidine, conessine, protoveratrine
3.	Diterpene	$C_{20}H_{32}$	e.g. Aconitine, aconine, hypoaconine

Biosynthetic classification

1. Ornithine derived alkaloids

e.g. *Pyrrolidine alkaloid*-nicotine

Tropane alkaloid – Atropine, hyosyamine, coacaine

2. Lysine derived alkaloids

e.g. *Piperidine and pyridine alkaloid* – conine, lobaline, arecoline

Quinazolidine alkaloid- lupinine

3. Tyrosine derived alkaloids

e.g. *Isoquinoline alkaloid* – morphine, codeine, emetine, cephaline, berberine, d- tubocurine

Amino alkaloid- colchicine

4. Tryptophan derived alkaloids

e.g. *Indole alkaloid*- ergot alkaloid, vincristine, vinblastine, reserpine, strychnine, physostigmine, strychinine, brucine

Quinoline alkaloid – cinchonine, quinine, quinidine, camptothecin

5. Histidine derived alkaloids

e.g. *Imidazole alkaloid* – Pilocrpine

6. Phenylalanine derived alkaloids

e.g. **Amino alkaloid-** Ephedrine

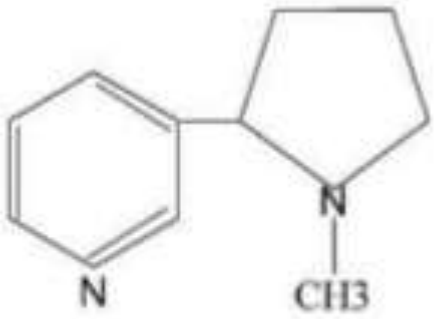
Pharmacological classification

1. Narcotic analgesic e.g.Morphine
2. Antimalerial e.g. Quinine
3. Reflux excitability e.g.Strychnine
4. Respiratory stimulant e.g.Lobeline
5. Neuralgia e.g. Aconitine
6. Oxytocic e.g. Erogotometrine
7. Bronchodilator e.g. Ephedrine, vasicine
8. Anticholinergic e.g. Atropine
9. CNS stimulant e.g. Caffeine
10. Antitussive e.g. Codeine
11. Antiarrythmic e.g.Quinidine
12. Antihypertensive e.g. Reserpine
13. Anticancer e.g. Vincristine
14. Antiglucoma e.g. Pilocarpine

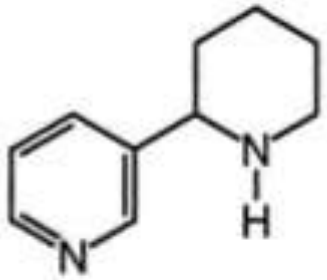
Pyridine – Piperidine Alkaloid: Tobacco

9. Tobacco

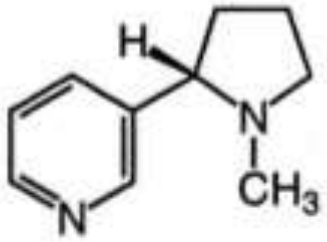
- Local name: Tambakhu
- Source: dried leaves of *Nicotiana tobacum, Turkish-N.rustica*
- Family: Solanaceae
- GS: indigenous to tropical america, China, US, India
- Constituents: varies Nicotine up to 1-10%, narcotine, anabasine
- Use: pesticidal, insecticidal.
  - Nicotine: stomach, contact, fumigant poison
- Could be used for plants & comparatively safer,



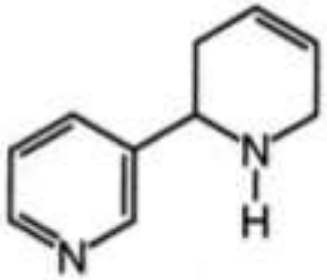
NICOTINE



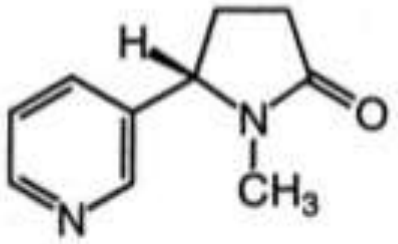
Anabasine



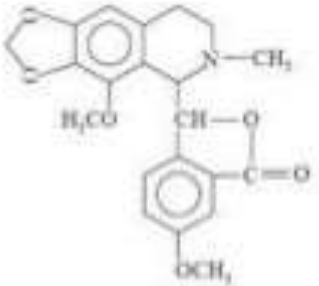
Nicotine



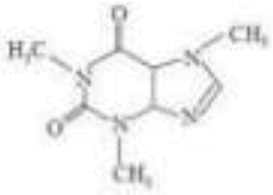
Anatabine



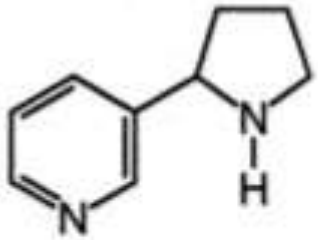
Cotinine



Narcotine



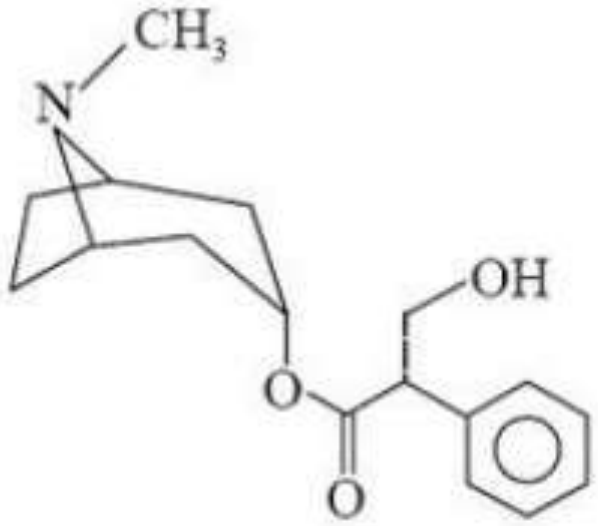
Caffeine



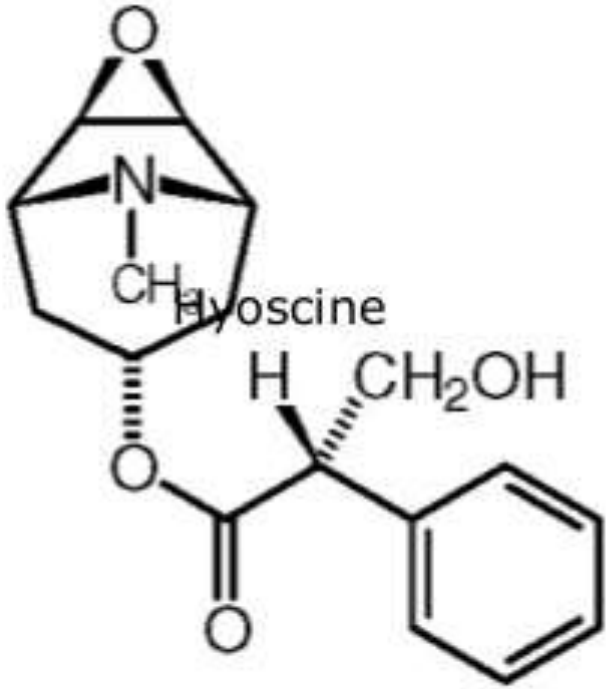
Nornicotine

Tropane Alkaloids

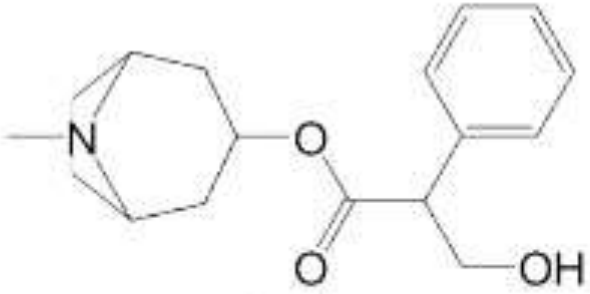
- Fusion od pyrrolidine & piperidine ring with common methylated nitrogen.
- Methylated nitrogen nuclues containing drugs are chemotaxonomic characters of family solanaceae.
- Hyoscyamine as main constituent: *Datura stramonium, Atropa belladonna, A.acuminata, Dubosia*
- Hyoscine as main constituent: D.metel, Dubosia
- Hyoscyamine & Hyoscine (both in low quantity): Hyoscyamus niger



Hyoscyamine



Hyoscine



atropine

10. Belladonna

- Syn: deadly night shade
- Source: dried leaves and other aerial parts of

European: ***Atropa belladonna,*** *Indian:* ***Atropa acuminata****-contains NLT 0.3% of l-hyoscyamine*

- Family: Solanaceae
- GS: Europe-England, India
- Constituents: roots-0.6%, stem-0.5%, leaves-0.4%, unripe-ripe berries-0.19-0.21%,seeds-0.33%
- Main: l-hyoscyamine, belladonin, pyridine and N-methyl pyrroline
- Use: parasympatholytic drug, anticholinergic, reduce the secretions such as sweat, saliva and gastric juice and also reduce spasm in case of intestinal gripping due to strong purgative.
- As an antitode in opium and chloral hydrate poisoning.

11. Datura stramonium

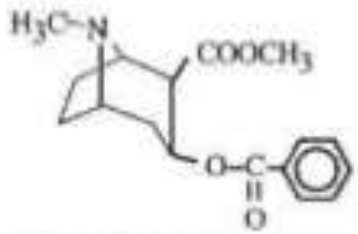
- Syn: Thornapple leaves, Jimson or Jamestown weed
- Source: dried leaves and flowering tops of *Datura stramonium (NLT 0.25% Hyoscyamine)*
- Family: Solanaceae
- GS: US, South america, France, Germany, Hungary
- Cultivation: seed propogation
  - Seeds sown by keeping a distance of 1 meter between two plants and 60 meter between 2 rows.
  - Calcarious soil, flowers in september
- Collection: twice, once before flowering and another during the flowering

- Morphology:
  - Color: greyish green leaves, white-purple flower
  - Odor: unpleasant
  - Taste: bitter
  - Shape: triangular ovate
- Microscopy:
  - Smooth cuticle
  - Covering & glandular trichome
  - Wavy epidermal cells
  - Palisade layer-upper side
  - Crystal layer
  - Vascular bundle

- Constituents: 0.2-0.5% alkaloid, l-hyoscyamine & hyoscine, small quantity of atropine
- CT: vitali morin test positive
  - Tropane alkaloid + Fuming HNO_3 → evaporation to dryness → addition of methanolic KOH → to an → acetone solution of nitrated residue → purple color due to tropane alkaloids.
  - Hyoscine hydrobromide + $AgNO_3$ → yellowish white ppt, soluble in ammonia.
- Use: in Asthma, control salivation, muscular rigidity, tremors in parkinsonism, motion sickness,
- Substitute & Adulternat:
  - Solanum nigrum: resemble stramonium small leaf but not contains calcium oxalate crystals.

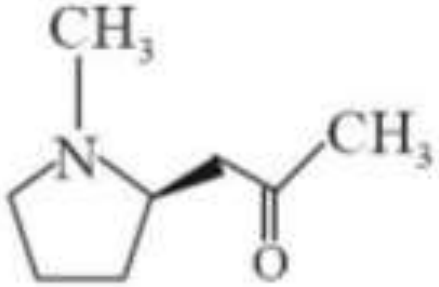
13. Coca

- Source: dried leaves of (bolivia-*Erythroxylon coca*), (*Peruvian-E. truxillense*), *E. novogranatense*
- Family: Erythroxylaceae
- GS: South america-peru, bolivia, now a days cultivated in India, Srilanka
- Constituents: 0.7-1.5% total alkaloid, lesser alkaloids, higher cocaine, cinnamyl cocaine, alpha-truxilline, tropocaine, benzoyl tropine, dihydroxytropine, benzoylecognine
  - Java coca has higher alkaloid & lesser cocaine.
  - Commercial price is determined by ecognine content because it is synthetically converted to cocaine.

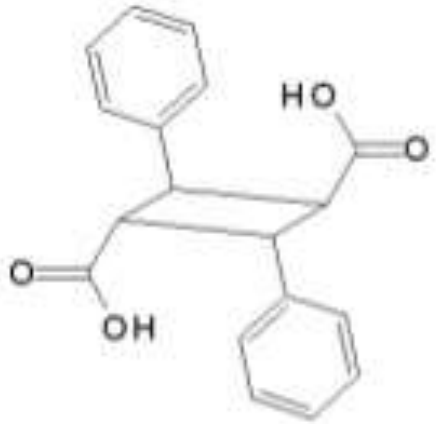


Structure 1. Cocaine

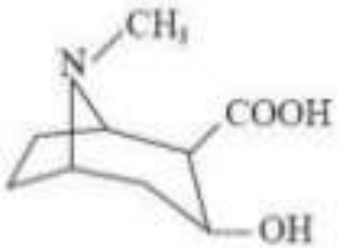
Cinnamylcocaine: . CH:CH.



Hygrine



Alpha-truxillic acid



Ecgonine

- Cinnamyl cocaine → cocaine + methyl alcohol + cinnamic acid
- Cocaine → ecgonine + methyl alcohol + benzoic acid
- Alpha-truxilline → ecgonine + methyl alcohol + alpha-truxillic acid
- CT: cocaine powder + H_2SO_4 → heated → water → characteristic smell of methyl benzoate.
- Use: local anaesthetic, stimulant, restorative, in colvulsion,
  - First local anaesthetic from which various other synthetic substitute with similar activity have been prepared.
  - Owing to hallucinogenic and addictive effects of cocaine, drug of abuse & limited to ophthalmic& ENT surgery

Quinoline Alkaloids
14. Cinchona

- Synonym: Jesuit's bark, Peruvian bark
- Biological source: It is the dried bark of the cultivated trees of *Cinchona calisaya*
- family Rubiaceae.
- Varities: *C. succirubra, C. calisaya, C. ledgeriana, C. officinalis*
- GS: India, Bolivia, Colombia Equador, Peru, Tanzania, Guatemala, Indonesia, and Sri lanka. In India Annamalai hills and Nilgiri hills, Tamilnadu and in Darjeeling.

- Cultivation:
- sub-tropical/tropical climates, height of about 1000-3000 m.
- If growing below this height, found to have less % of quinine.
- Rainfall: Uniform, 250-380 cm in a year. Atmospheric temp.: 16-24 degree C
- Soil: drained forest soil and rich in organic matter, acidic soil having pH of 4.2-5.6, small amount of N favorable for growth.
- Specification: Needs slopping situation, high humidity and protection from wind.
- Propagation: either seeds or budding or layering, in WB only budding is used and in TN budding or layering methods are applied.
- Seeds are very small and light in weight, about 1 gm seeds contain 3500 seeds so that seeds are admixed with soil during sowing
- Seeds immediately used for propagation , on storage they lose their viability
- Germination take place in 3-6 weeks.
- Seedlings with 2 pairs of leaves are transplanted and space of 6-10 cm is maintained in between 2 seedlings and 2 rows.
- Young seedlings are protected from direct sunlight.
- In forest soil, transplanted after 15 months of growth and before heavy rainfall. Distance of 2X2m is maintained between 2 plants.

- **Collection:**
- 4-20 yrs of age, plants, selected for harvesting,
- but the maximum alkaloid content is found in 6-10 yrs old plants.
- Bark is collected by coppicing method, vertical incisions are made on branches , trunk of tree and incisions are connected by horizontal circles, the bark is then stripped off.

- **Preparation:**
- Dried in sun light and further by artificial heat, drying is done below 80 degree C.
- During drying loses 70% of its wt.
- Avoid molding and fermentation during drying.

Morphology

- Odor: slight and characteristic odor
- Taste: astringent and bitter
- Form : quills and curved pieces.

Stem Bark	Root bark
1. Length about 30 cm 2. Thickness about 2-6 cm	Length about 2-7 cm
3. Outer surface is dull brown grey or grey color, inner surface is pale yellowish-brown to deep reddish in color.	Inner and outer surfaces are similar in color
4. Presence of mosses and lichens	no mosses and lichens
Bark is rough and has transverse fissures	Bark is scaly and shows depression.

Typical characters of 4 main species of Cinchona

Characters	*C. calisaya*	*C. ledgeriana*	*C. officinalis*	*C.succirubra*
Size	Diameter: 12-25mm Thickness: varies from 2-5 mm	Diameter: upto12mm Thickness: upto1.5 mm	Diameter: 12-25mm Thickness: 2-5 mm	Diameter: 20-40mm Thickness: 2-5 mm
Other features	Broad longitudinal fissures with transverse cracks	Broad longitudinal fissures and cracks more in numbers but less deep	Shows number of transverse cracks	Well marked longitudinal wrinkles, but less no. of transverse cracks.
Powder color	Brown	Brown	Yellow	Reddish brown

Microscopy:

1. Cork
2. Phellogen
3. Phelloderm
4. Cortex
5. Starch grains
6. Secretory cels
7. Medullary rays
8. Lignified Phloem fibres
9. Secondary Phloem

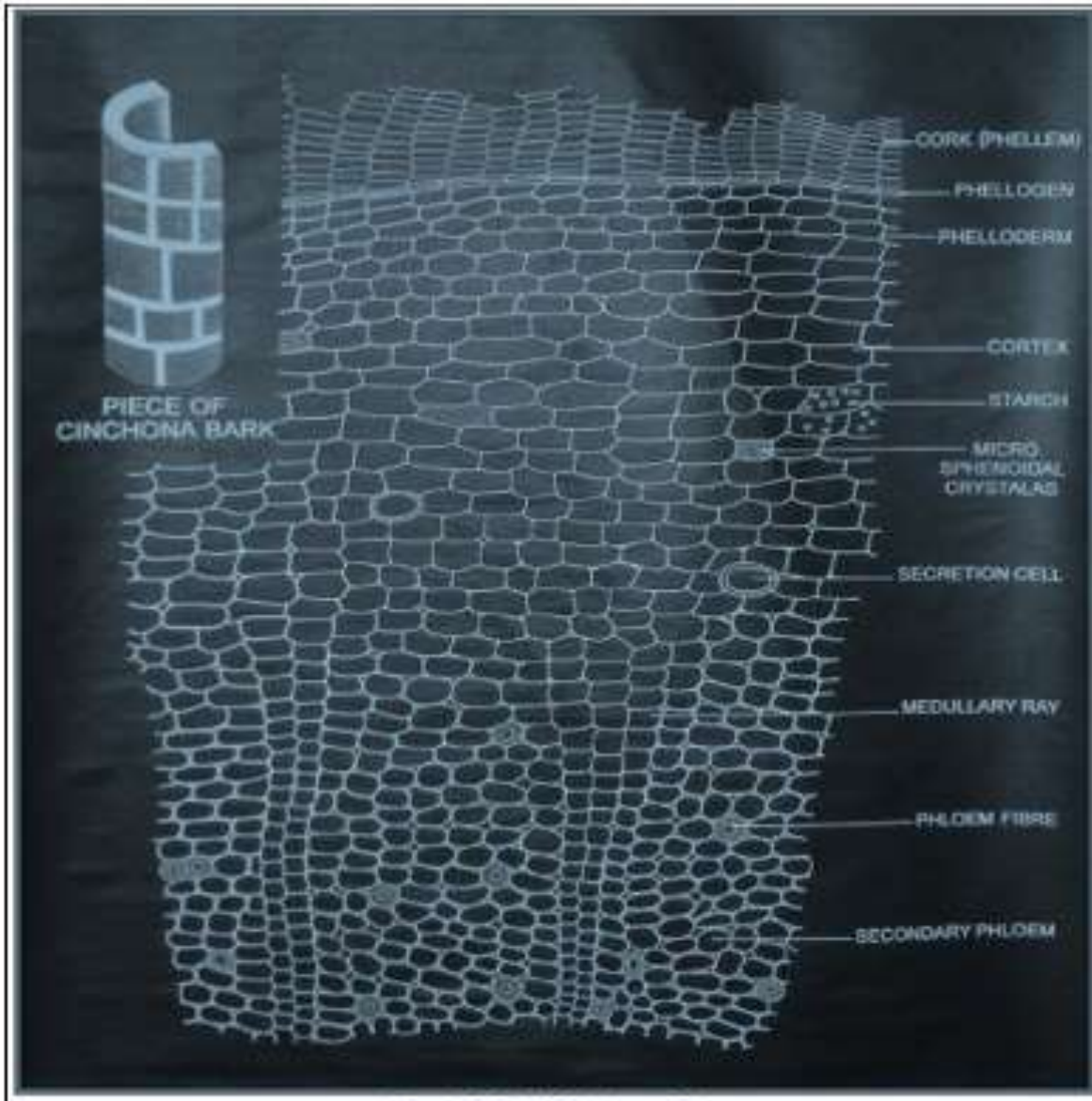


Fig. 4.2 T.S. of Cinchona Bark
(Refer colour diagrams given in Appendix-V)

Chemical Constituents:

- Quinoline Alkaloids: about 25 types
- Imp: quinine, quinidine, cinchonine and cinchonidine.
- Alkaloids of lesser importance are quinicine, cinchonocine hydroquinine, hydrocinchonidine and homocinchonidine.
- *C.succirubra:* 5-7% 0f total alkaloid of which 30% is quinine.
- *C. ledgeriana:* 6-10 % and in some cases upto 14% of which upto 75% is quinine.
- *C. calisaya:* 6-8% total alkaloids about 50% is quinine.
- Quinine and Quinidine are sterioisomers .
- Quinine and Quinidine form many salts but medicinally their sulphates are more significant.
- Cinchonine and cinchonidine are isomers.
- Also contains quinic and cinchotannic acid, also contains glycosides such as quinovin, tannins and bitter essential oil.
- Quinovin on Hydrolysis → quinovic acid +quinovose

Chemical Tests:

1.Powdered cinchona is slightly moistened with glacial acetic acid & heated in an ignition tube, purple vapours are produced at the upper part of tube.

2.Thalleoquin test: Powdered drug gives emrald green color with bromine water and dil. Ammonia solution.

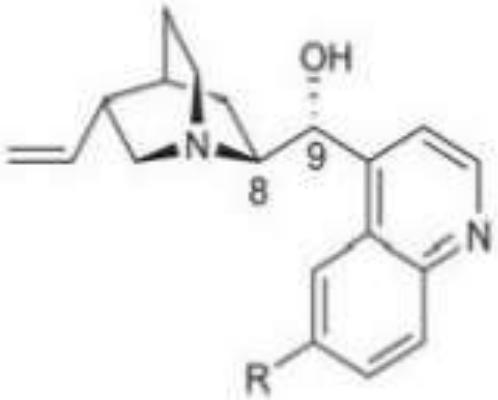
3.Quinidine solution gives a white ppt. with silver nitrate solution, which is soluble in nitric acid ,

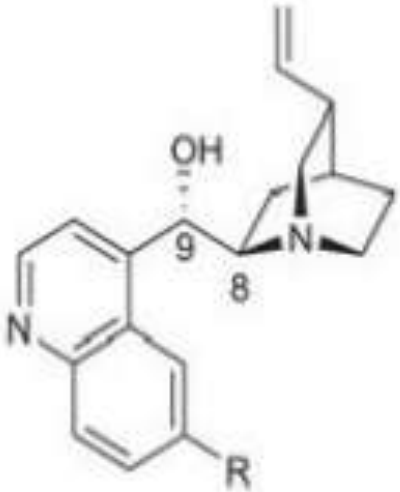
Uses:

- Quinine: Anti malarial, Skeletal muscles relaxant, night muscles spasm and abortifacient .
- Quinidine : Indicated for treatment of arrhythmia and cardiac depressant, atrial fibrillation
- Cinchonine : Has mild anti malarial activity.
- Cinchona extract and tincture: bitter stomachics and antipyretic.

Substituents:

- Cuprea Bark (*Remijia pedunculata*)
- Cuprea Bark (*Remijia purdiena*)
- *C. Colombia*





1a, R = OMe, (−)-Quinine (*QN*)
1b, R = H, (−)-Cinchonidine (*CD*)

2a, R = OMe, (+)-Quinidine (*QD*)
2b, R = H, (+)-Cinchonine (*CN*)

Isoquinoline alkaloid:

15. Opium

- Synonym: Raw opium
- Source: Opium (Raw Opium) is the dried latex obtained by incision from the unripe capsules of *Papaver somniferum*
- Family: Papaveraceae
- It is dried partially by spontaneous evaporation and partly by artificial heat.
- GS: India, Pakistan, Afganistan, Turkey, Russia, China and Iran.

Cultivation:

- Temperate/ subtropical climate in winter season.
- Cold weather gives a favorable effect on yield, but extreme cold conditions, including frost adversely affect the plant and yield of opium.
- Cool weather without freezing temperature , cloudiness and sufficient sunshine are the best climatic conditions for opium poppy.
- Grown from November to March.
- Propagation is done by sowing the seeds and 3-4 kg seeds per hectare are required and admixed with about 3-4 parts of sand are sown.
- Soil: highly fertile, well drained loamy soil with fine sand, should contain organic matter, N containing, pH around 7.
- Distance between two plants 25 cm and reaches maximum height of 1 m.
- Periodically, the thinning of plants is done for uniform and better development.
- Plants are kept free from weeds with the use of weedicides.
- Protected from various insect pests like cut worms, leaf minor and poppy borer.
- Nitrogen and phosphorus have remarkable effects on growth of plant.

Collection and Preparation:

- Plant bears flowers within 3-4 months, converted to capsules and attain maturity after 15-20 days.
- During maturity period , the capsules exudes maximum latex shows color change from dark green to light green.
- Such capsules are incised vertically in the afternoon with the help of specific needle like apparatus called 'nushtur'.
- It penetrates maximum into the capsule.
- Latex exudes out and thickens in night.
- Scrapped and collected next morning by an iron scoop called 'Charpala'.
- Process is repeated for about 4 times on the same capsule with 2 days interval

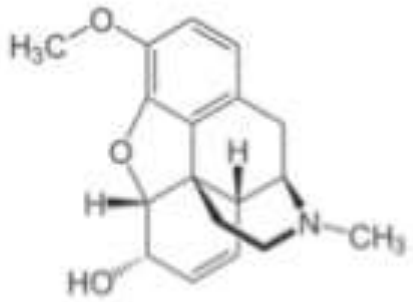
- To maintain the external exudation of latex superficial incisions are given.
- Latex is collected in plastic containers.
- Capsules are collected and dried and further the seeds are separated by beating

- **Morphology:**
- Odor: Strong characteristics & Taste: bitter
- 1. Indian Opium: Color- dark brown, Cubical pieces, Weight- 900 gm, enclosed in tissue paper and is brittle and plastic in nature, internally homogenous, powdered form is available in 5-10 kg pack.
- 2. Persian Opium: Color- dark brown, brick shaped, weight- 450 gm, hygroscopic in nature, granular or nearly smooth with brittle fracture.
- 3. Natural Turkish or European opium: color-brown or dark brown, conical or rounded and flattened, Weight- 250-1000 gm, become hard and brittle, covered with poppy leaves.
- 4. Manipulated Turkish opium: color- chocolate brown or dark brown internally, oval and flattened, weight- 2000 kg, plastic or even brittle, covered with poppy leaves.
- 5. Manipulated European opium: color- dark brown, elongated masses with rounded ends, weight- 150-500 gm, firm, plastic, and with brittle fracure, covered with poppy leaves.

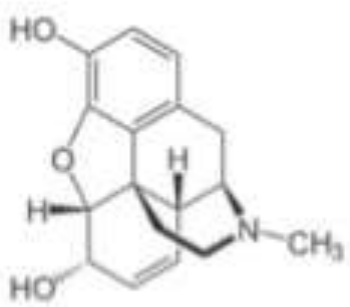
Chemical Constituents:

- More than 40 alkaloids usually combined with a specific acid (**Meconic acid**) or with other acids e.g. sulfuric and acetic acids.
- Derived from phenylalanine and tyrosine and are placed under benzylisoquinoline and phenanthrene types
- Opium alkaloids can be subclassified into 3 main groups with different basic nuclei:
- Phenanthrene alkaloids e.g. morphine and codeine.
- Benzylisoquinoline alkaloids e.g. papaverine and noscapine (narcotine).
- Phenylethylamine alkaloids e.g. narceine
- Opium alkaloids are present as salts of
- meconic acid.

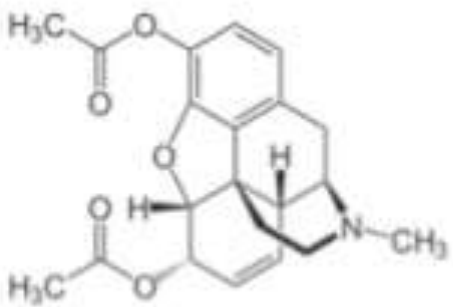
Meconic acid



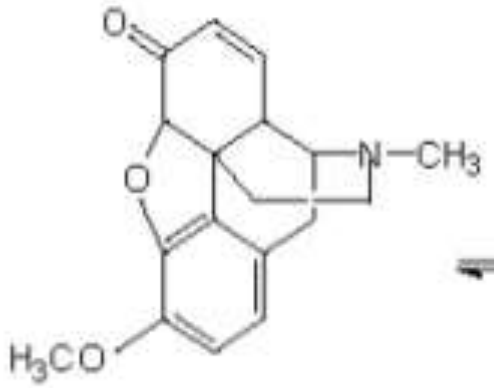
Codeine[4]



Morphine[5]



Heroin[6]



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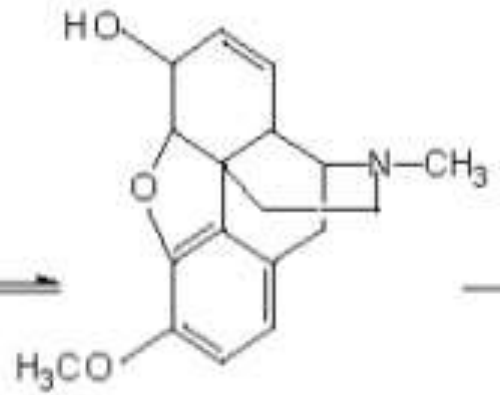
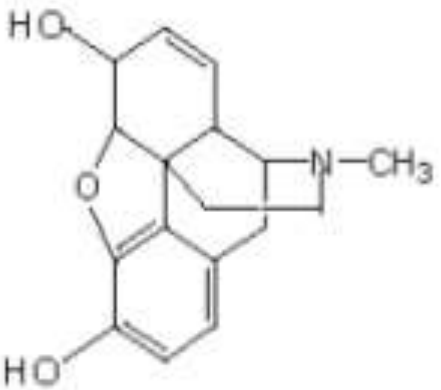


codeine



morphine

Morphine Properties:

- **Morphine** is **levorotatory**
- insoluble in water
- sparingly soluble
  in ethanol (1:250) and
  chloroform (1:1500)
- practically **insoluble in ether and benzene**.
- It contains **2 OH groups, one** is a **phenolic** at C-3 (gives a soluble phenate with alkali) and the other is a 2ry alcoholic at C-6.

R= H Morphine
R= CH_3 Codeine

- Chemical Test:
- As opium alkaloids are salts of meconic acid.. To check …
- 1. Test for meconic acid: opium is dissolved in water, filer it, to the filtrate add $FeCl_3$ → deep reddish purple color, which persists even after addition of HCl
- 2. Test for Morphine: Morphine powder, when sprinkled on HNO_3 → orange red color (codeine won't give)
- 3. Test for Morphine: Morphine solution + potassium ferricyanide + $FeCl_3$ → bluish green color (codeine won't)
- 4. Test for Papaverine: Papaverine solution in HCl → lemon yellow color with potassium ferricyanide solution.

USE

- Opium: Sedative-Hypnotic Class, Action is due to Morphine
- **Morphine**: potent analgesic, due to central narcotic effects, causes addiction. Severe pain or when patient does not show response to other narcotic
- Sedates cerebrum and medulla → respiration, emetic & cough reflex center
- Triggers Chemo receptor trigger zone → nausea, vomiting
- Respiratory depression ad constipation
- **Codeine:** relieves local irritation in bronchial tract, antitussive
- **Papaverine:** smooth muscle relaxant of bronchial, intestinal and blood vessels
- **Narcotine:** cough reflex depressant, cough linctuses

USE

- **Heroin:** diacetyl morphine: more narcotic, analgesic property than morphine
- Apomorphine: emetic & in poison
- Hydromorphine: narcotic analgesic habit forming tendency is less.
- Synthetic morphine compounds are called Opioids: non habit forming
- Morphine sulphate
- Codeine sulphate/phosphate
- Narcotine/Noscapine
- Papaverine hydrochloride

16. Ipecacuanha
(Tetra hydroxy-Iso-quinoline mono terpenoid)

- Brief: derived from condensation of dopamine with secologanin (a C10 monoterpene) to give two series of compounds.
- Syn: Ipecac
- Source: dried roots & rhizomes of *Cephalis ipecacuanha, C. acuminata*
- Family: Rubiaceae
- Specfication: should contain NLT 0.2% alkaloids, in that atleast 50% emetine
- GS: Brazil, India, Myanmar, Malaysia
- [*C.acuminata* from panama]

Cultivation:

- Propagation: seed, season: mid January to mid February
- Germination improvement: treatment with lime water or H2O2
- 2 month old seedling, transplanted at a spacing 10 X 10 cm, temp range: 23-28 degree C, rainfall: 300 cm
- Atmosphere: humid, facilitates plant growth
- Fertilizers: Nitrogenous, effect in increasing the quantity of emetine.
- **Collection & Preparation:**
- After 3 years of vegetative growth.
- Method: using pointed stick, levers
- Drying: roots are dried in the sun or by fires and transported down river to ports such as Rio de Janeiro

Morphology

Brazilian

- Roots & Rhizome
- Color: dark brick red to brown
- Odor: Faint
- Taste: bitter
- Size: up to 150 mm in length & 6 mm in thickness; rhizome 2 mm in diameter
- Shape: tortuous pieces for roots, cylindrical for rhizome
- Pith is present in rhizome not in root

Panama

- Color: reddish brown to greyish brown
- Odor: Faint
- Size: up to 9 mm in thickness
- Shape: cylindrical

Microscopic Characters (Fig.13.16)

Roots : Transverse section of root shows the presence of cork layer with brown contents, which is followed by phelloderm composed of thin walled parenchyma. It is full of starch grains and acicular crystals of calcium oxalate. Xylem consists of tracheids and small vessels. Secondary medullary rays with starch grains are also present. Size of the starch grains is upto 15 microns.

Rhizomes : Pericycle is with thick walled sclereids, protoxylem and spiral vessels. Columbian variety differs only in one aspect i.e. the starch grains are larger in size and upto 22 microns.

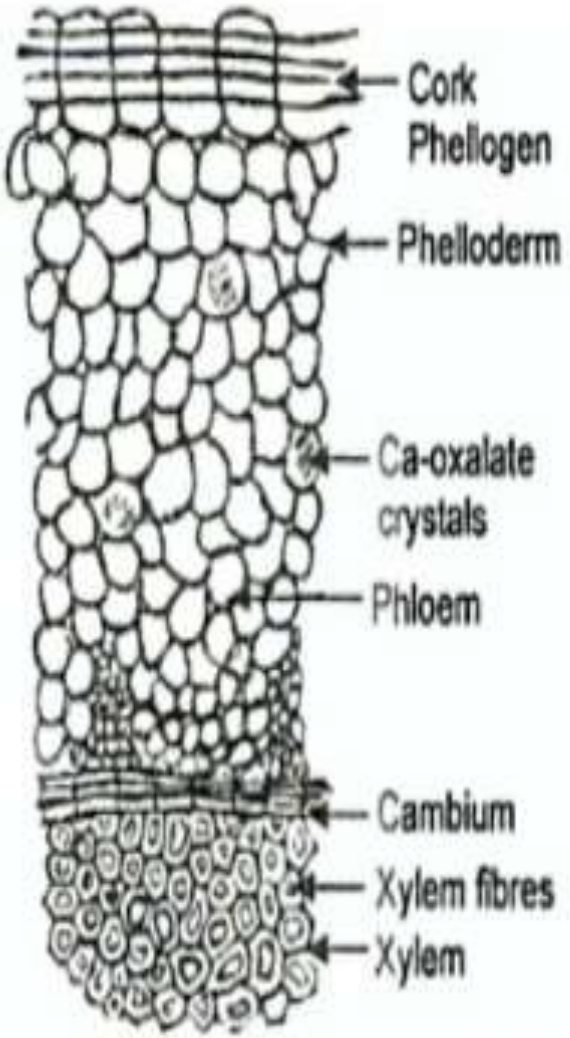
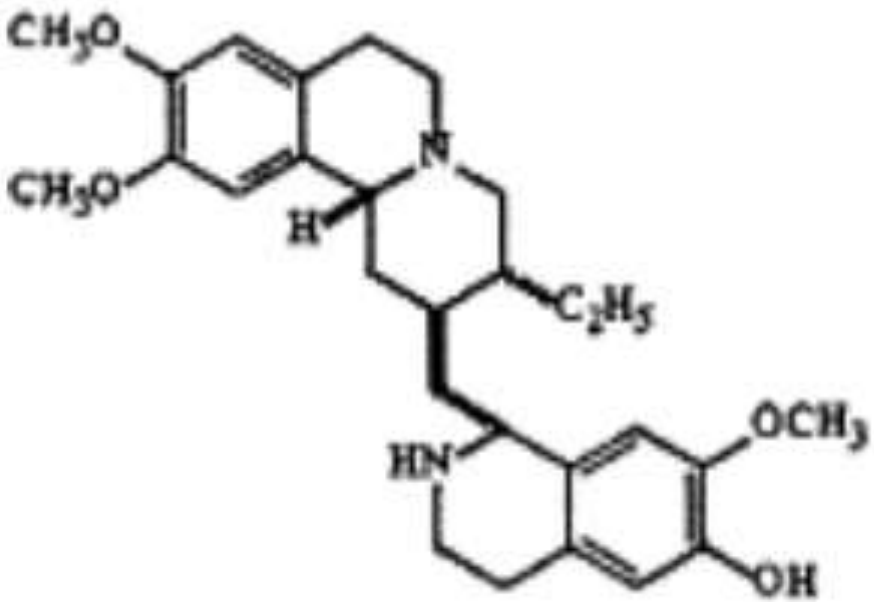


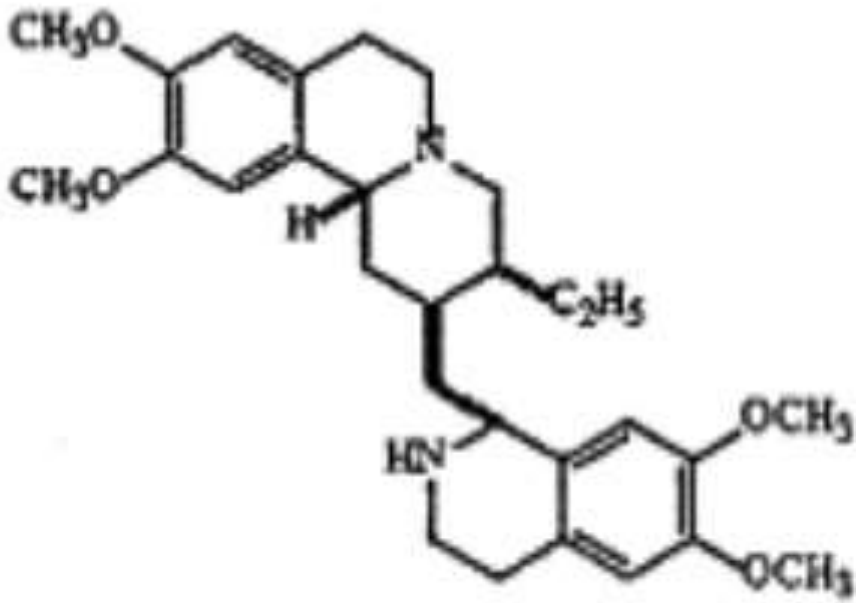
Fig. 13.16 : T. S. of Ipecacuanha Root

Chemical Constituents:

- Isoquinoline alkaloid of phenolic & non phenolic group.
- Alkaloid: Rio- up to 2% while Panama- about 2.2%
- Imp: emetine, cephaeline, psychotrione, o-methyl psychotrine and emetamine
- Proportion of emetine: cephaeline (4:1-rio, 1:1-panama, 2.5:1-johore
- Cephaeline→ emetine by methylation of phenolic C(6) OH group.
- phenolic: cephaeline, psychotrine
- Non-phenolic: emetine,o-methyl psychotrine
- Misc: ipecacuanhic acid, starch, Cal Ox, glycoside: ipecacuanhin



Cephaeline



Emetine

Chemical Test

- Powdered drug + HCl + water → shake & filter → filtrate → Potassium chlorate → presence of yellow color gradually changes to red, after standing due to emetine.
- Emetine + Frohde's reagent (H_2SO_4 + sodium molybdate) → bright green color
- USE:
- Expectorant in small doses, emetic in higher doses
- Cephaeline is more emetic less expectorant than emetine
- Emetine HCl → antiprotozoal
- Anti-tumor

Indole alkaloids

20. Rauwolfia

- **Synonyms:** Rauwolfia root, Serpentina root, Chhotachand.
- **Source:** Dried roots of *Rauwolfia serpentina*
- *Family:* Apocynaceae.
- It contains NLT 0.15% of reserpine and ajmalcine.
- **GS:** Tropical regions of Asia, America and Africa. Commercially, In India, Sri lanka, Myanmar, Thailand and America. In India UP, Bihar, Orissa, Tamil Nadu, West Bengal, Karnataka, Maharashtra and Gujarat.

Cultivation

- Climate: wide range of, best suited humid, shade
- Soil: wide range of with large amount of sand → makes plants more susceptible for disease
- Clay: loamy soil, pH: acidic up to 4
- Temperature: 10-38 degree C, Rainfall: 250-500 cm
- Propagation: **seeds**, roots, cutting, root stumps
- Initially seeds are sown into the nursery beds.
- Sowing period: May or breakdown of monsoon
- Implanting seedling: August, at a distance of 16-30 cm
- Various fertilizer, manures: ammonium sulphate, urea, bone meal.
- **Collection:**
- when plants are 3-4 year old.
- Uprooting, roots are cut properly, washed as to remove earthy matter & dried in air.

Morphology

- Color: bark: greyish yellow, Wood: pale yellow
- Odor: odorless
- Taste: bitter
- Size: 10-18 cm long; 1-3 cm in diameter
- Shape: sub-cylindrical, slightly tapering & tortuous
- **Microscopy:**
- Cork: striated
- Phelloderm (few layers of parenchyma)
- Narrow phloem: small scattered sieve tubes
- Secondary phloem: Calcium oxalate crystal:
- Xylem: Vessels, tracheids, wood parenchym wood parenchyma & wood fibre

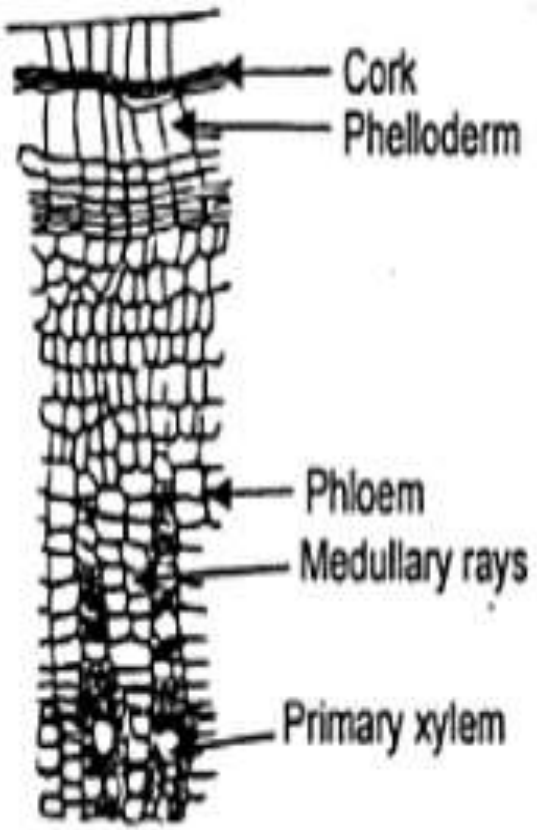
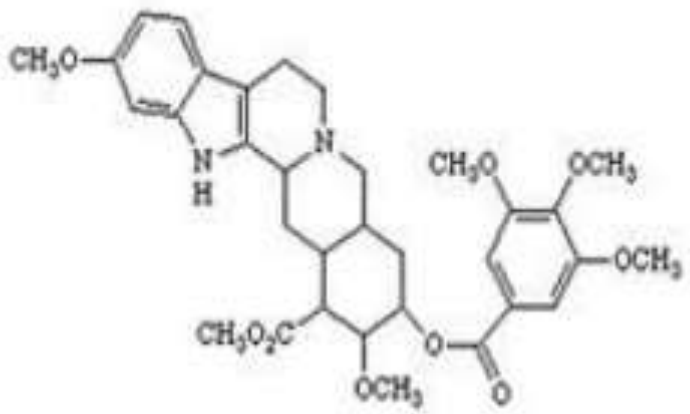


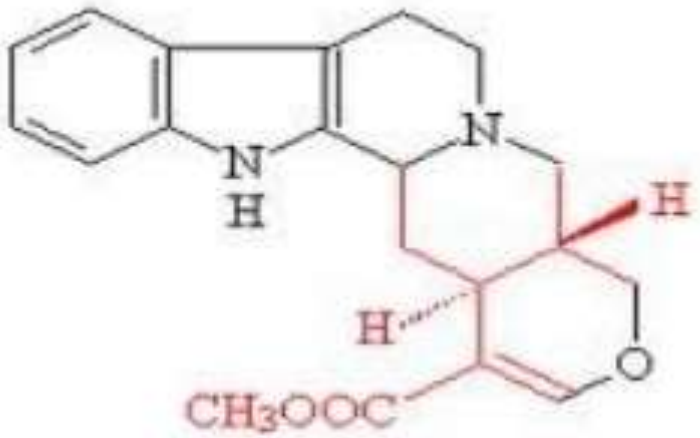
Fig.13.11 : T.S. of Rauwolfia Root

Constituents:

- About 30 indole alkaloids; range from 0.7-3% on GS
- Mostly in bark, root
- Indole, Indoline, Indolenine ,Oxyindole, Pseudo indoxyl alkaloids
- Reserpine
- Oleo-resin, phytosterol, fatty acid, alcohol, sugar
- Ajmalicine, ajmaline, rauwolfinine, rescinnamine, reserpinine, yohimbine, serpentine, serpentinine
- Reserpine: ester of trimethoxy benzoic acid
- Rescinnamine: ester of trimethoxycinnamic acid



Reserpine



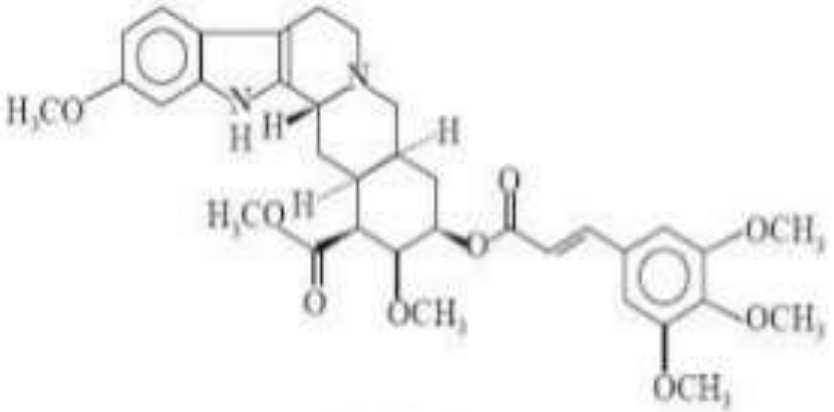
Ajmalicine

Allied Species:

Rauwolfia vomitoria

R. obscura

R. Rosea



Rescinnamine

Chemical test

- With conc. HNO_3 → red coloration of medullary rays
- Reserpine: with vanillin in acetic acid → violet red color
- Modle alkaloids: powdered rauwolfia + H_2SO_4 + p-dimethyl amino benzaldehyde → violet-red color
- **USE:**
- Reserpine: lowers BP by depleting store of catecholamine at nerve ending, prevent re-uptake of nor-epinephrine at storage sites, allowing enzymatic destruction of neuronal transmitter.
- Neuropsychiatric disorder
- Rescinnamine: anti hypertensive but Higher dose → **depression**
- Dereserpine: anti hypertensive & tranquiliser
- Ajmalicine: treatment of circulatory diseases, relief of obstruction of normal cerebral blood flow.
- Syrosingopine: same as above but milder

21. Vinca

- **Syn:** Catharanthus, Periwinkle
- **Source:** dried whole plant of *Catharanthus roseus* or *Vinca rosea*
- Family: Apocynaceae.
- **GS:** Indigenous to Madagascar. Cultivated in South Africa, India, U.S.A., Europe, Australia and Caribbean islands.

Chemical Constituents:

20 dimeric indole-dihydroindole (indoline) alkaloids possess oncolytic activity.

Vincristine and Vinblastine are most significant.

Indole: Vinblastine termed as Catharanthine

Dihydroindole alkaloid: Vindoline

Other: ajmallcine, lochnerine, serpentine and tetrahydroalstonine.

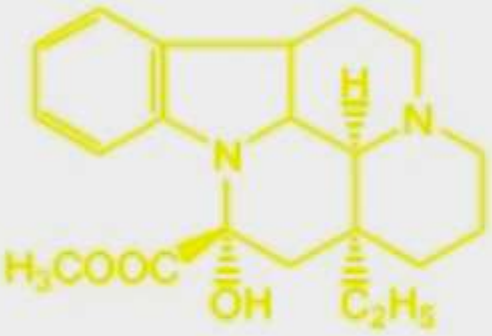
USE:

Vincristine sulphate: antineoplastic: arrest mitosis at the metaphase, IV: acute acute leukemia for children, Hodgkin's disease, reticulum cell sarcoma, lymphosarcoma, myosarcoma

Vinblastine sulphate: either above mechanism or by interfering with amino acid metabolism, suppress immune response

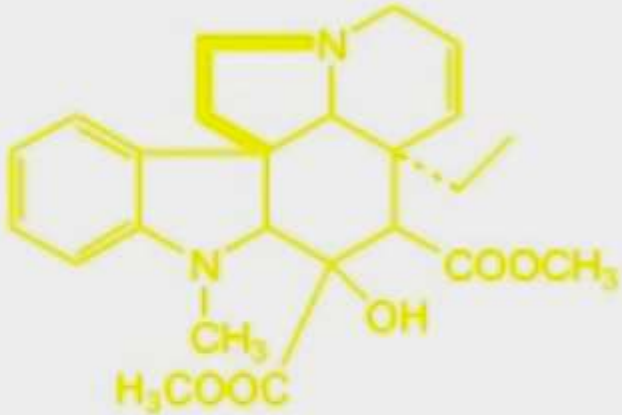
Hypotensive & Antidiabetic

Structure



Vincamine

Catharanthine



Vindoline

22. Nux-Vomica:

Syn Crow fig, Semen Strychni

Source: Dried ripe seeds of *Strychnous nuxvomica* Linn.

Family: Loganiaceae.

Contain NLT 1.2 % of total alkaloids calculated as strychinine.

GS: East India, Sri lanka, Northern Australia, and India. Abundantly in South India i.e. Tamil nadu, Kerala and on Malabar coast. Also available in Bihar, Orrisa, Konkan, Mysore and Gorakhpur.

Collection & Preparation

-Found throughout the tropical area, 1300 m above sea level.

-Plant height: 10-12 m, crooked trunk, several branches.

-Leaves are orange, oppositely arrange, oval shape, entire margin and acute apex.

-Flowers are greenish white and the bark is greyish to yellow.

-Fruit are orange yellow, consists a berry about the size of a small orange.

-When ripe it has a hard orange-yellow pericarp and a white, pulpy interior – containing 4-5 seeds & Seeds are washed to remove pulp and dried.

-Unripen seeds are separated by the floating test in water.

-Seeds are dried on mat and packed in gunny bags for marketing.

-Collection of fruit and seeds is carried out from November-February.

-Exported in small sacks known as pockets.

- **Morphology:**

Hilum, ridge on testa and micropyle

Color: greenish brown

Taste: bitter

- **Microscopic Characters:**

Lignified Trichomes, layer of collapsed cells and endosperm

Chemical Constituents:

1.5-5% bitter Indole alkaloids, Strychnine and Brucine

-Vomicine, α-colubrine, pseudostrychnine,

Isostrychnine, N-oxystrychnine, protostrychnine, β-colubrine and novacine.

Glycosides: loganin, chlorogenic acid and fixed oil

Allied specids: *Strychnos nuxblanda, S. potatorum*

Properties:

- Brucine is the dimethoxy derivative of Strychnine.
- Both alkaloids contains 2 Nitrogen atoms.
- Hemitoxiferine is a degradation product of strychnine. Dimerization of hemitoxiferine produces a valuable skeletal muscle relaxant Toxiferine

R = H Strychnine

Chemical test

Thin sections of nux vomica seeds are defatted and following test are performed.

1. Stain the TS of nux vomica with conc. HNO_3 → endospermic cells take yellow color due to presence of **brucine.**
2. Stain the TS of nux vomica with ammoium vandate & H_2SO_4 → Manddin's reagent -- endospermic cells become purple due to **strychine**
3. Strychnine + H_2SO_4 + Potassium dichromate → violet color → red → finally yellow

USE

-Bitter stomachic, tonic, CNS stimulant, increases BP, certain forms of cardiac failure, stimulant for respiratory & CVS

-Brucine: very less physiological action, 1/6th potency as compared to strychine, 4 times bitter than strychine.

Brucine: denaturinng alcohol, inedible fats, dog poison.

23. Ergot

Syn: Ergot of rye, Ergot.

Source: It is the dried sclerotium of a fungus, *Claviceps purpurea* (Clavicipitaceae or Hypocreaceae) developed in ovary of rye plant,

Secale cereale Linne (Graminae). It contains not less than 0.19 % of the total alkaloids of ergot, calculated as ergotoxine.

GS: Switzerland, Yugoslavia, Hungary and Czechoslovakia.

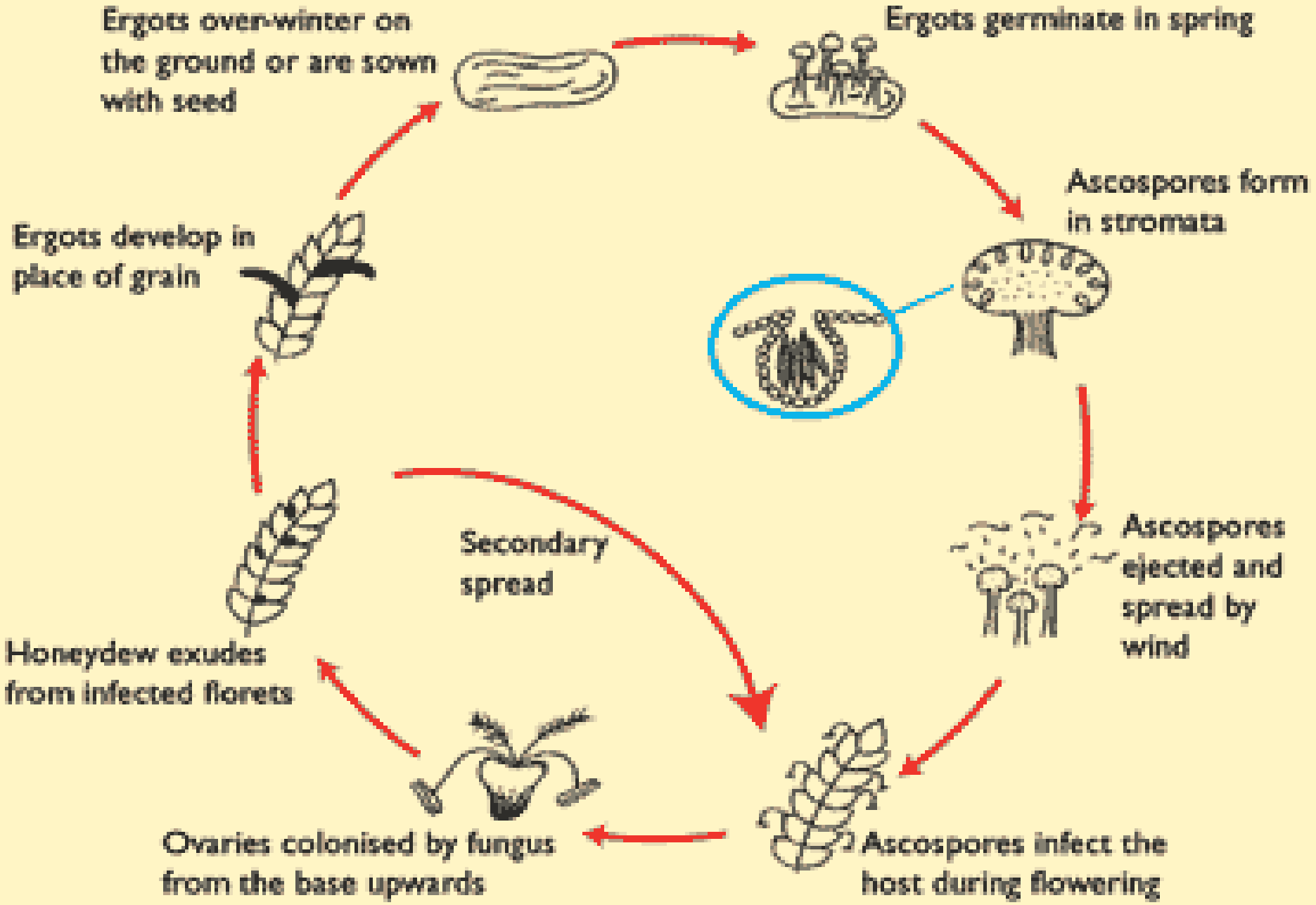
History and Source

- Origin: french word, Argot, which means fur and indicates the shape & attachment of the sclerotia to the infected rye spikes, like the fur which is attached to the body of birds.
- Even in old days, ergot fungus was known to be a pathogen, infecting the rye fields in European countries and Russia.
- It is known that the toxic effects were observed owing to its contamination of ergot with rye grains.
- The toxic symptoms were gangrene in the extremities and convulsions.
- In the middle ages, such symptoms were reported and called St. Antony's fire.
- After knowing the cause, it was called as ergotism which had severly occurred in 16th century in Germany.
- Further it was discovered that the ergot has specific uses in obstestrics and came into wide use from 19th century onwards.
- In 1836, it was introduced in London Pharmacopoeia.

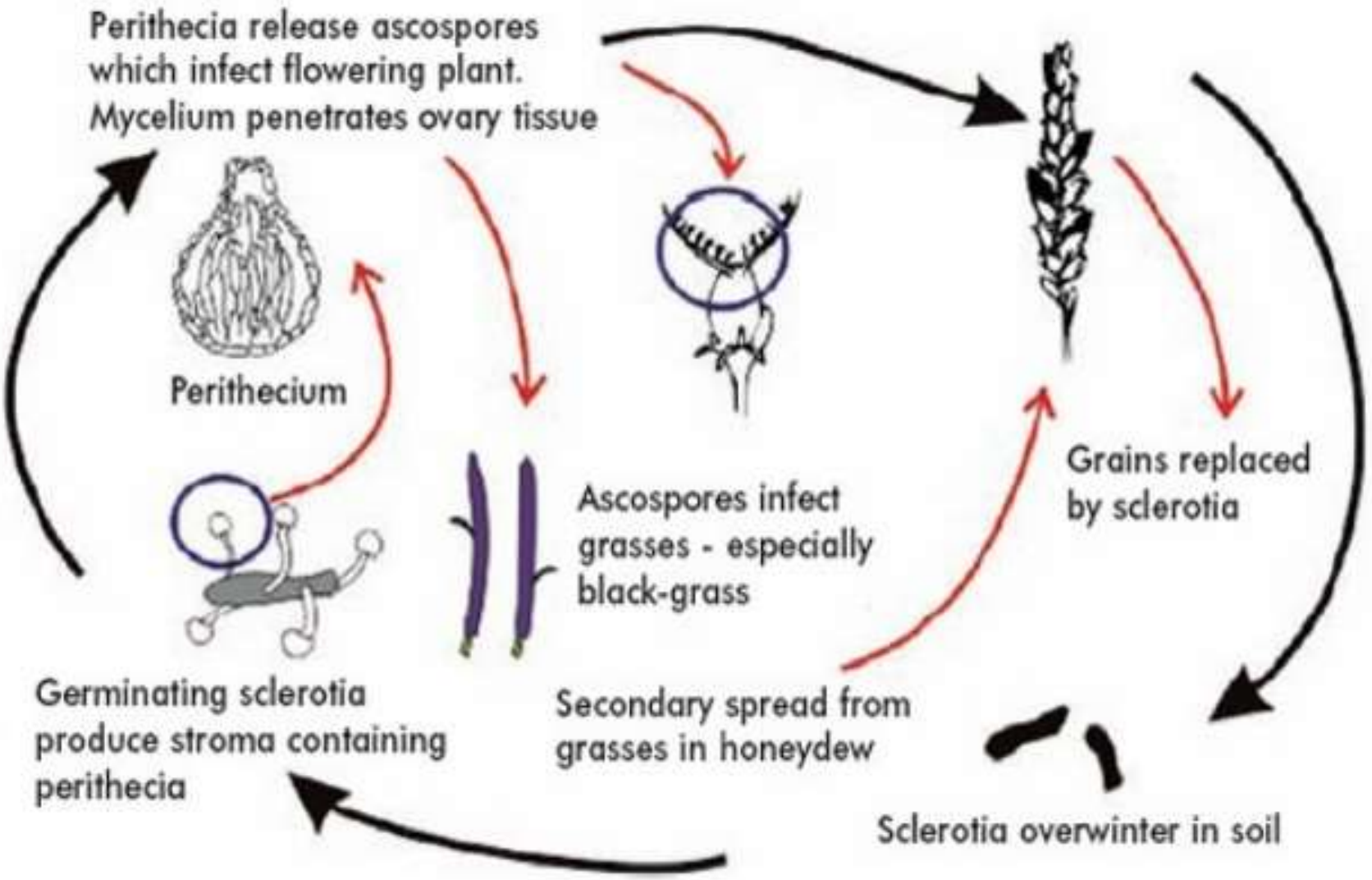
Collection and Preparation:

- Two ways : Natural and Artificial i.e. saprophytic production
- Ergot is produced by natural way i.e. cultivation of rye plants and subsequently infecting with this fungus.
- For natural way of production, rye plant is host and ergot is parasite.
- More than 600 plants from different families of wild and cultivated grasses are act as hosts
- other fungus are *C. microcephala*, *C. nigricans* and *C. paspali*
- Rye is the better host for the large scale production of ergot by way of quality and quantity.
- Among the various stages of development of this fungus, sclerotial stage or a dormant stage contains the maximum amount of drug.

Figure 1. The life cycle of ergot



Life cycle of Ergot



- The ovary of the rye plant at its base, gets infected by ascopores to ovaries is influenced by wind and insects.
- After infecting, the ascopores germinate in the favorable conditions like moisture and damp climate.
- The germination of ascopores leads to formation of hyphal strands which go on invading the wall of ovary with the help of an enzyme.
- By this way, the hyphe from soft, white mass of tissue over the surface of the ovary which is called as mycelium.
- The mycelium secrets a viscous and sugary fluid called honey-dew.
- At this time, the hyphal strands produces asexual spores called conidiospores, which remain in a suspended form in honey-dew.
- Due to the sugary fluid i.e. honey-dew, the insects and ants are attracted which further help in the spread of the fungus to other host plants.
- The development stage is the sexual stage and called as Sphacelia stage.

- The hyphae further invade into the deeper parts of ovary and slowly replace the entire tissue of ovary by a compact tissue called pseudoparenchyma which is hard and dark purple.
- It is called as sclerotium stage and is considered as resting or dormant stage of the fungus and contain maximum amount of ergot alkaloid.
- If this sclerotium is left uncollected, it eventually falls on the ground and in the favourable season, i.e. spring gives out stromata which are in the elongated form.
- Each stromatum has globular head and stalk.
- The head portion contains a large number of perithecia and every perithecium is like a flask shaped structure which contains a number of sacs, each sac containing the ascopores which are thread like in appearance.
- Ascopores are the sexual spores capable of inducing fresh life cycle of fungus by infecting the ovary of rye plant.

- Selection of correct strain of fungus (Claviceps purpurea), appropriate containers for preparing large scale ergot inoculum and an idea nutrient medium are important requirements for commercial production of ergot.
- The various chemical races of fungus can produce only specific ergot alkaloids like ergotamine, ergometrine and ergotoxin in appreciable quantity from their sclerotia.
- The ascopores of this species with the specific chemical race are germinated on nutritive medium and by this way large bulk of conidiospores are formed.
- The suspension of this strain of fungus is sprayed on rye plants in large cultivated areas.

Saprophytic production is much practised now-a-days, because mycelial dry weight gives even more than 20% of alkaloids, while natural sclerotia contains less than 1% of alkaloids. The process of fermentation is properly regulated or controlled for optiumum bioproduction of useful metabolites.

- Morphology
- Color: externally dark violet, internally whitish or pinkish white
- Odor: disagreeable or faint
- Taste: unpleasant
- Size: sclerotia 1-3 cm in length & 1-5 cm in width
- Shape: sclerotium is fusiform, triangular and usually tapering on both ends
- Fracture: brittle with short fracture

Microscopy

1. Few thin, flattened, polygonal cells of purple to dark brown color
2. Inner part made up of dense pseudo parenchymatous cells composed of chitin
3. Mycelial cells-central region are round or oval, thick and with high refractive walls.
4. Contain cells with fixed oil
5. Sclerotium does not contain starch, calcium oxalate or any of lignified tissue.

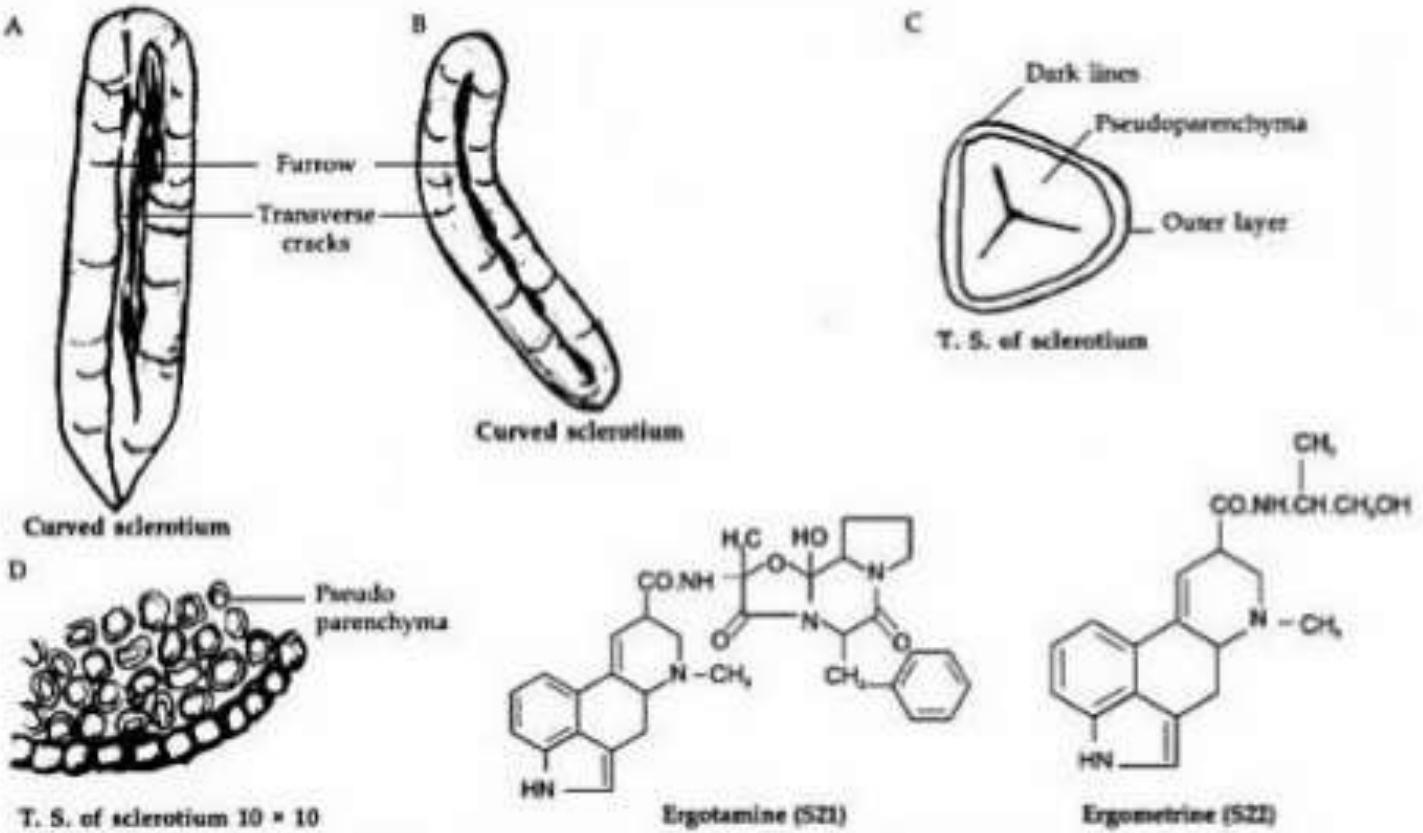
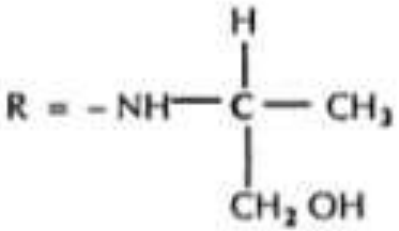

A
Furrow
Transverse cracks
Curved sclerotium
B
Curved sclerotium
C
Dark lines
Pseudoparenchyma
Outer layer
T. S. of sclerotium
D
Pseudo parenchyma
T. S. of sclerotium 10 × 10
Ergotamine (S2I)
Ergometrine (S2I)

Fig. 9.3 Ergot

Constituents

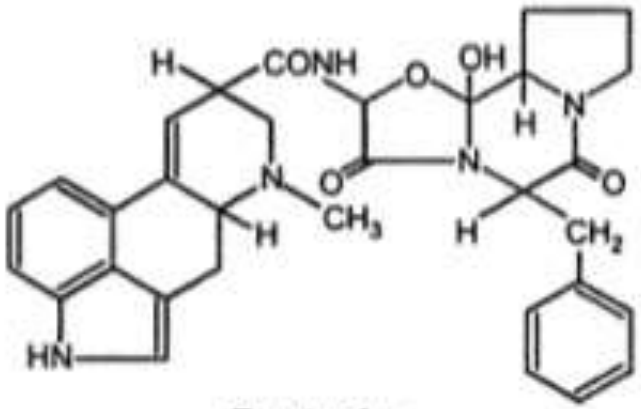
- Indole alkaloids (1-0.25%)
- Derivatives of lysergic acid
- Lysergic acid: peptide derivative form, peptide alkaloids
- Water soluble and insoluble categories.
- Laevo form medicinally active and dextro form inert in action
- Water soluble: laevo-ergometrine, dextro-ergometrinine
- Water insoluble: ergotamine, ergosine, & ergotoxin
- Pigment, ergosterol, fungisterol, histamine, tyramine, amino acids, acetyl choline, chitin, up to 30% fixed oil, 8% moisture.

R = – OH	Lysergic acid	Isolysergic acid
R = –NH_2	Lysergic acid amide	Isolysergic acid amide
	(Ergine)	**(Erginine)**

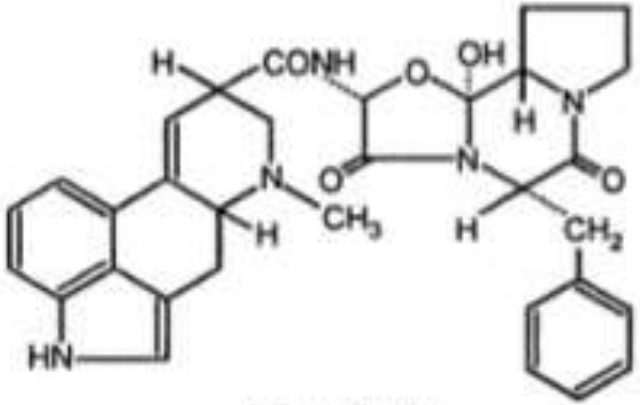


Ergometrine	Ergometrinine

R = – N (C_2H_5)$_2$ Lysergic acid diethylamide (LSD)



Ergotamine



Ergocristine

Tests for identification:

1. Van-Urk's Reagent (p-dimethyl aminobenzaldehyde (PDAB) in 15% H_2SO_4, containing traces of $FeCl_3$) + Alkaloid → Deep blue color.
2. Ergot + solvent ether + H2SO4 → filter → filtrate → red-violet color in aqueous layer, when treated with saturated solution of NaHCO3.
3. Little quantity of ergotamine is dissolved in glacial acetic acid and ethyl acetate → small portion of this is treated with H2SO4 → shaken well by which blue color with red tinge appears, by addition of FeCl3, blue color deepens, while red tinge becomes faint.
4. Ergometrine gives a blue fluorescence in water.

- **Uses:**

- Ergonovine: oxytocic, sometimes used to enhance labor pain in delivery cases, prevent post partum heamprrhage.
- Ergotamine tartarate: analgesic, migraine treatment, given along with caffeine
- Ergotoxin methanesulphonates (mesylates): geriatric patients
- Lysergic acid derivative LSD: psychomimetic (controlled under narcotic)

25. Pilocarpus

Imidazole Alkaloids

- Syn: Jaborandi
- Source: leaves of closely related plants of the genus *Pilocarpus spp*
- Family: Rutaceae
- Genus: *P. jaborandi, P. pennatifolius, P. microphyllus*
- *P.microphyllus* → Maranham jaborandi is the main source of the drug.
- GS: South america: Brazil, Venezuela, Carribean island, Central america

Constituents

- Imidazole alkaloid: **pilocarpine**

Isopilocarpine, pilocarpidine, pilosine, pseudopilocarpine and isopilosine.

Test:

Pilocarpine solution + dil. H_2SO_4 + H_2O_2 + benzene + Potassium chromate → on shaking organic layer → bluish violet color , aq. Layer yellow color

USE:

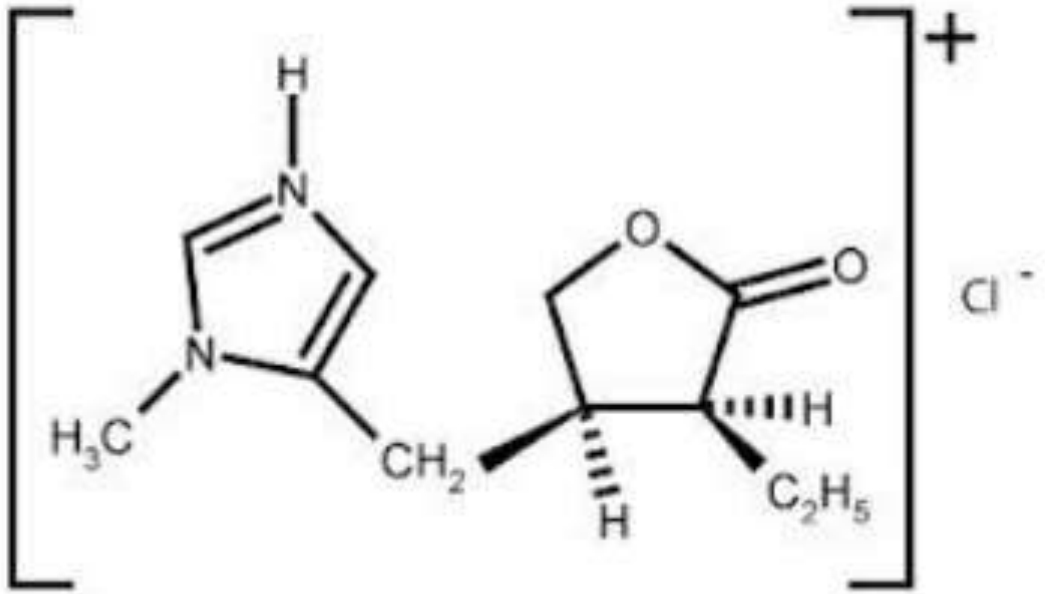
In the form of pilocarpine hydrochloride

Physiological antagonist of Atropine

Acts directly on ANS by post-ganlionic cholinergic nerves.

Contraction of pupil of the eye, Increase sweating & salivation.

Use in ophthalmology in Glaucoma.


H
N
N
H3C
CH2
O
O
H
H
C2H5
+
Cl -

Steroidal Alkaloids

- Arise by inclusion of a basic nitrogen at some point in the steroid molecule.
- C27 compounds having cyclopenteno phenanthrene moiety.
- Could act as a precursor for synthesis of various other steroids

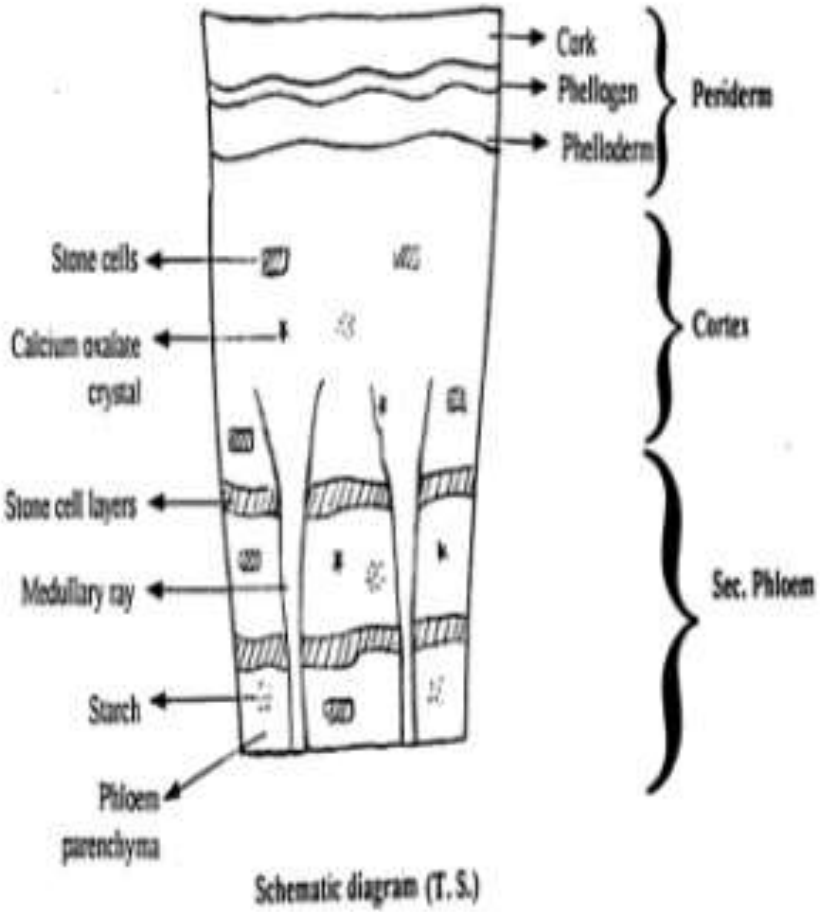
- Steroidal alkaloids are pseudo alkaloids, they have steroidal nucleus with heterocyclic nitrogen
- Example: Kurchi, Veratrum

19. Kurchi

- Syn: holarrhena
- Source: dried stem bark of *Holarrhena antidysenterica*
- Family: Apocynaceae
- Specification: should contain NLT 2% of total alkaloid.
- GS: thorough out India-OS,AS,UP,MH
- Collection:
- Plants which are 8-10 years old are selected; LS, TS are made on the trunks from July to september.
- After detachment, the bark is separated from the wood and dried.
- The recurved pieces of the bark are marked.

Morphology

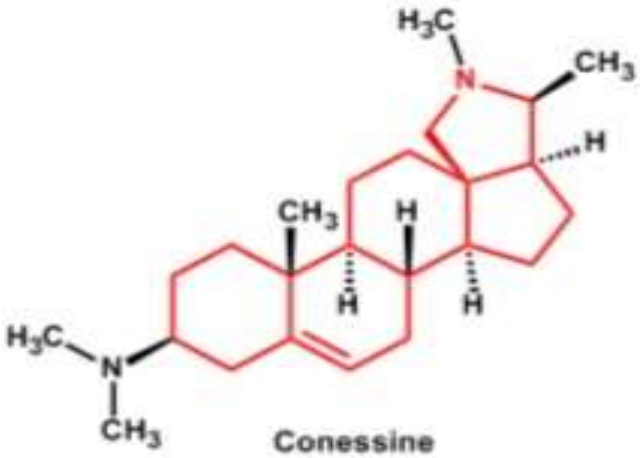
- Color: buff to pale brown or outer surface, slightly brown in inner surface
- Outer surface: longitudinally wrinkled and bears horizontal lenticels.
- Pieces are recurved with varying size and thickness.
- The drug shows a short and granular fracture.
- Odor: odorless
- Taste: bitter & acrid



Schematic diagram (T.S.)

Constituents

- Contains total 25 alkaloids (1.5-3%).
- C21 group steroidal alkaloids.
- Conessine (kurchicine), norconessine, isoconessine, dioxyconessine, holarrhimine, holarrhidine
- Use:
- Antiprotozoal and used to treat amoebic dysentery
- Conessine is highly active against Entamoeba histolytica.
- Formulation kutajarisht



Conessine

- Bark is useful in skin diseases; osteoarthritis and other gastric disordes

Veratrum

- **Synonyms: White hellebore, European white hellebore, white hellebore**
- **Biological source: It consist of dried rhizomes of *Veratrum album* Linn,**
- **Synonyms: Indian poke, green false hellebore, False hellebore**
- **Biological source: It consist of dried rhizomes of *Veratrum viride* var**
- **Family: Liliaceae**

Chemical constituents:

1. *Veratrum album* Linn- alkaloids like: ceveratrum, proveratrin A and proveratrine B, germidine, germitrine,

starch, resin and calcium oxalate.

Uses:

first aid for intestinal problems

useful for treatment of constipation, dizziness, intestinal cramps, menstrual disorders, hypertension in pregnancy

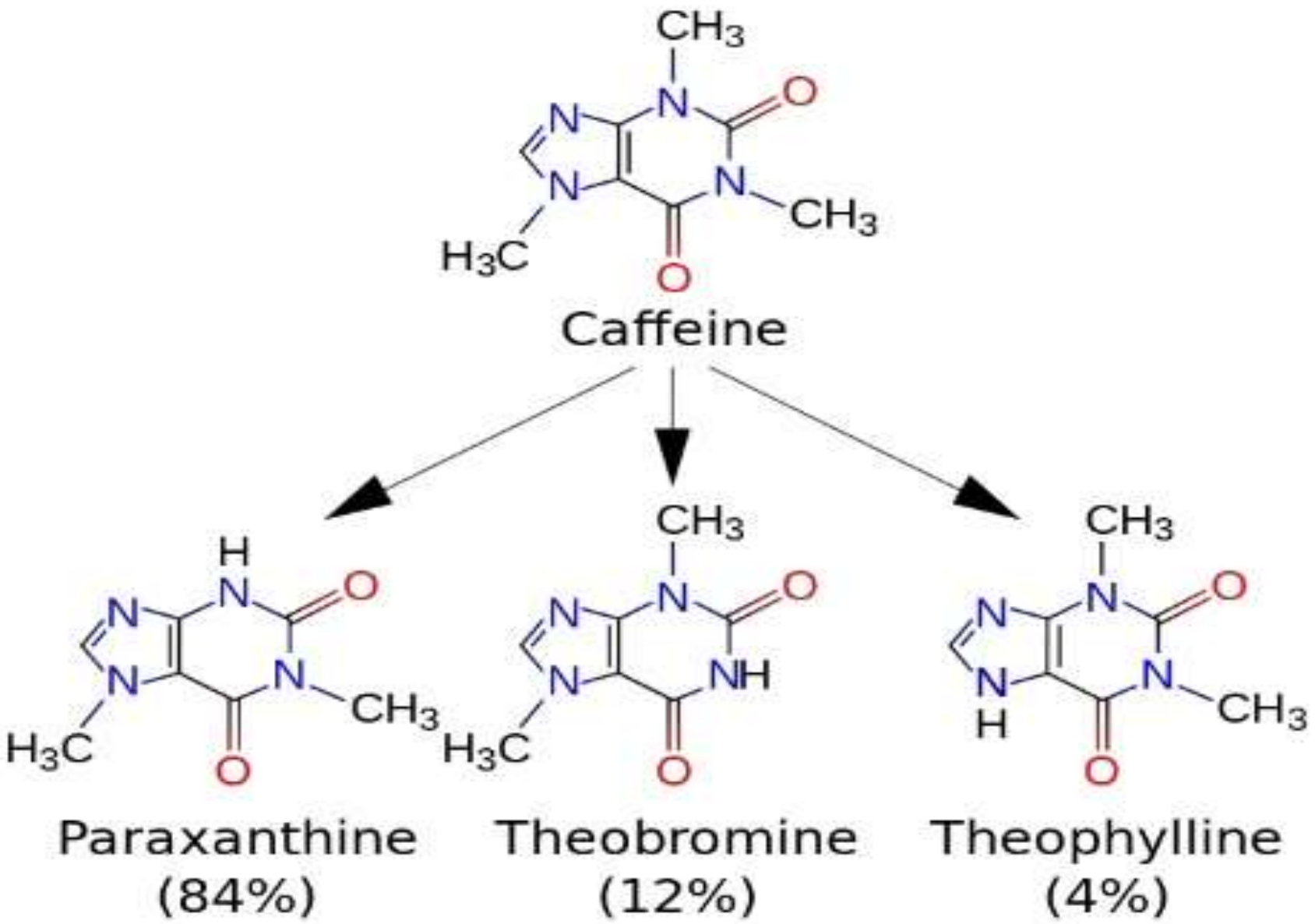
***2. Veratrum viride* var –** steroidal alkaloids like O-acetyljervine, cevadine, cyclopamine, veratramine and veratridine

Uses:

hypotensive agent used during hypertension due to renal dysfunction

used to induce renal vasodialation for treatment of chronic glomerulonephritis

PURINE ALKALOID


Caffeine
Paraxanthine
(84%)
Theobromine
(12%)
Theophylline
(4%)

4. Tea

- Syn: Thea
- Prepared leaves & leaf buds of *Thea sinesis*
- Family: Theaceae
- GS: India, Srilanka, China, Indonesia, Japan
- Morphology:
- Small shrub height 1-1.5 m, much branched and bears grey bark
- Leaves: dark green,lanceolate or elliptical, blunt at apex, base tapering
- Flowers: solitary and in groups
- Odor: characterisitc & Bitter taste

Preparation of Green tea:

1. freshly collected leaves, exposed to air until moisture is removed.
2. roasted and stirred continuously until leaves become moist & flaccid.
3. passed to rolling table and rolled into balls and subjected to pressure which removes moisture.
4. leaves are shaken out to the copper pans and roasted again till the leaves assume dull green color.
5. leaves are winnowed, screened and graded into various varieties.

- CC: caffeine, theobromine, theophylline
- Color: gallotannin
- Odor: volatile oil
- Use: CNS stimulant, beverages, diuretic

5. Coffee

- Syn: coffee beans
- Source: dried ripe fruits of *Coffee arabica & C. liberica*
- Family: Rubiaceae
- GS: Brazil, India
- Constituents: Caffeine, tannin, fixed oil, protein, chlorogenic acid.
- During roasting process, the agreeable smell of coffee is developed due to oil called caffeol composed of mainly furfural along with minor quantities of phenol, pyridine & valerianic acid.
- Use: source of caffeine, CNS stimulant & diuretic

Alkaloidal Amine

1. EPHERDA
DERIVED FROM PHENYL ALANINE

- Do not contain heterocyclic nitrogen atoms (protoalkaloids)
- Mostly are simple derivatives of phenylethylamine
- **Synonym:** Ma-Huang "ma"=astringent, "huang"=yellow
- **Biological Source:** Dried young stems of *Ephedra gerardiana* (wall.) Stapf. And *E. nebrodensis*
- Family: Ephedraceae
- Contain not less than 1 % of total alkaloids, calculated as ephedrine.
- **Varities:** Indian: *E. gerardiana, E. intermedia*

Chinese: *E. sinica and E. equisetina*.

- **Geographical Source**: China, Pakistan, North-west part of India, Australia, Kenya, Spain and Yugoslavia.
- **Cultivation :**

  Altitude: 2500-3000m, Annual rainfall should not exceed 50cm, Propagated by seeds or by layers or divisions of the root stock. Seeds are sown early in the spring at a distance of 5 cm, distance of one meter between two rows. Requires proper irrigation and weeding. The alkaloidal content varies from season to season and found to be maximum in autumn when plants and twigs are dark in color
- **Collection:** After attaining 4 yrs plants are collected for the extraction of alkaloid. Twigs are generally dried in sun or even by artificial ways. After drying stored in dry and well closed containers, away from light.
- **Macroscopic Characters:**

  Gymnospermous plant, thin woody stems, cylindrical and grey to greenish in color (5mm diameter). Internodes at a about 3-3.5 cm, bears scaly leaves from the nodes in a whorl of 2.

- **Microscopy:**

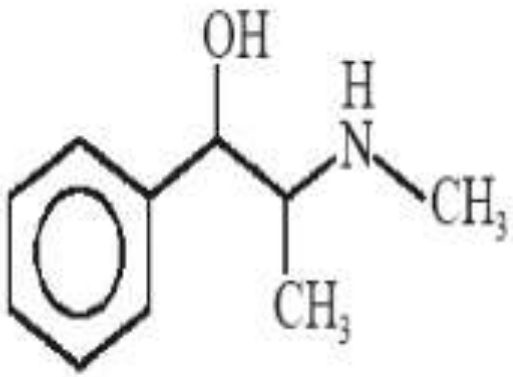
i. Unicellular epidermis made up of quadrangular cells along with thick walled cuticle.

ii. Non-lignified , hypodermal fibers

iii. Chlorenchymatus cortex

iv. Lignified pericyclic fibers.

v. Parenchymatous dark brown colored pith



Ephedrine

- **Chemical Constituents:**
- Contains amino alkaloids
- Ephedrine, nor-ephedrine, n-methyl ephedrine, pseudo-ephedrine, etc.
- Ephedrine ($C_{10}H_{15}NO$) is 1-phenyl-1-hydroxy-2-methyl aminopropane, soluble in water, alcohol, organic solvents and oils

- **Chemical test:** Ephedrine+ water and HCL and then treated with $CUSO_4$ and NaOH, the solution gives violet color and when shaken with solvent ether the organic layer shows purple and aq. Layer shows blue color.
- Uses:
- Sympathomimetic effects
- Bronchodilator in Asthma, colds, flu and Hay fever medications
- Allopathic: Sinumed (dries a runny nose)
- Used as an anti-inflammatory.
- Weight loss (increases metabolism, decreases appetite – CNS stimulant – acts on adrenergic receptors)
- Also used to correct low B.P. conditions.
- Ephedradines have hypotensive effects
- Above ground parts are normally used, although the roots are better to use (less alkaloids)

2. COLCHICUM
DIHYDROXY PHENYL ALANINE
(& DOPAMINE

- **Synonyms:** Meadow saffron seeds, Autumn crocus.
- **Biological source:** Dried ripe seeds of *Colchicum luteum* Baker and *Colchicum autumnale* Linn.
- Family: Liliaceae.
- Colchicum corm is also used medicinally.
- **Geographical Source** :Various parts of Europe, like England, Holland, Czechslovakia, Poland and Yugoslavia. Also cultivated in India.

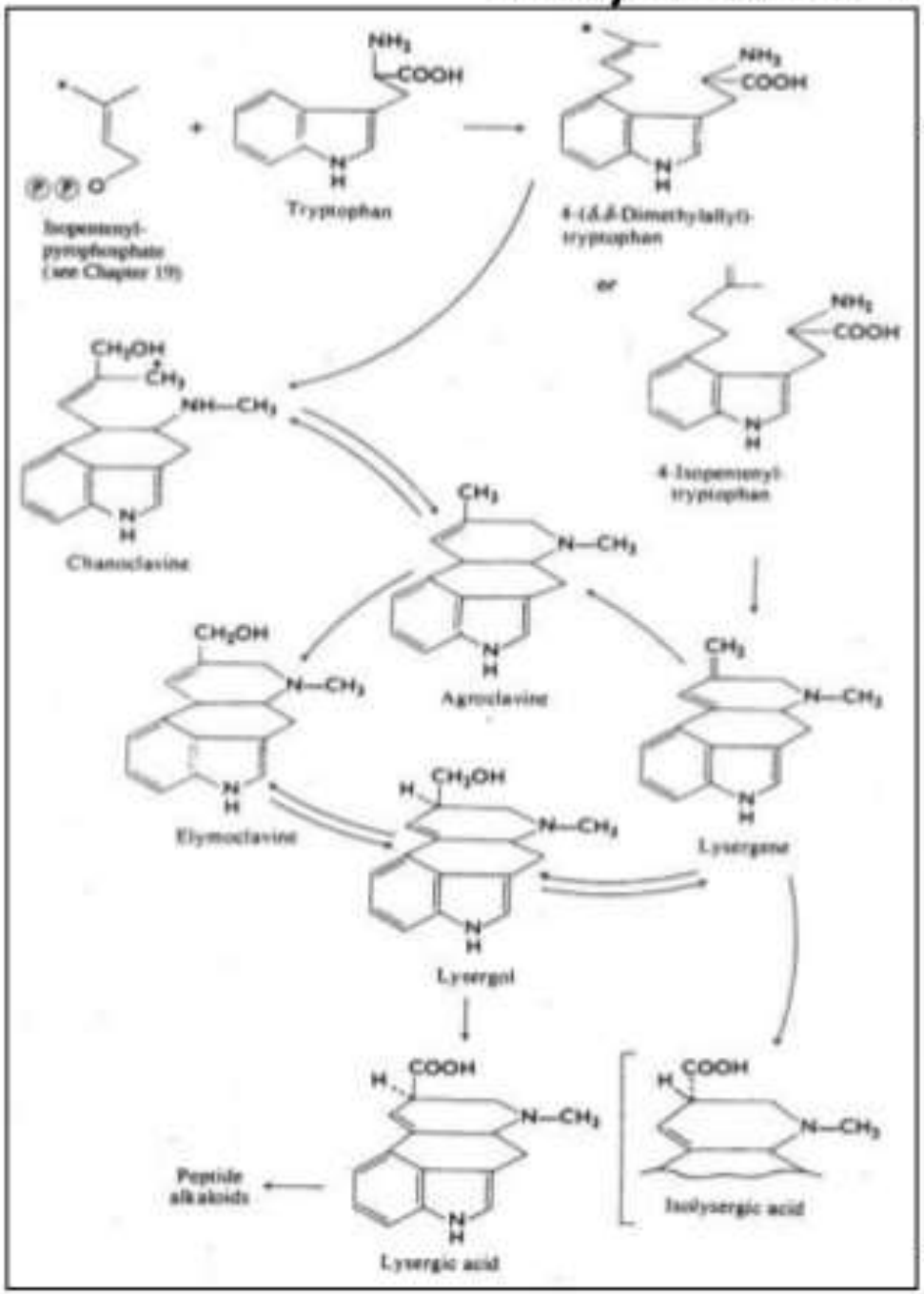
- **Chemical Constituents:** 0.2-1% of amino alkaloids, colchicine is the main constituent, seeds contain upto 0.8% of colchicine and in corms upto 0.6%, also contains demecolcine. Both contain tropolane or cycloheptatrien-ol-one ring structure.
- Constituent in colchicum seeds and corm.
- Amorphous, yellow-white alkaloid (darkens on exposure to light).
- Gives a strong yellow coloration with strong mineral acids.
- Dissolves readily in water, alcohol and chloroform, but only slightly in ether or petroleum spirit.

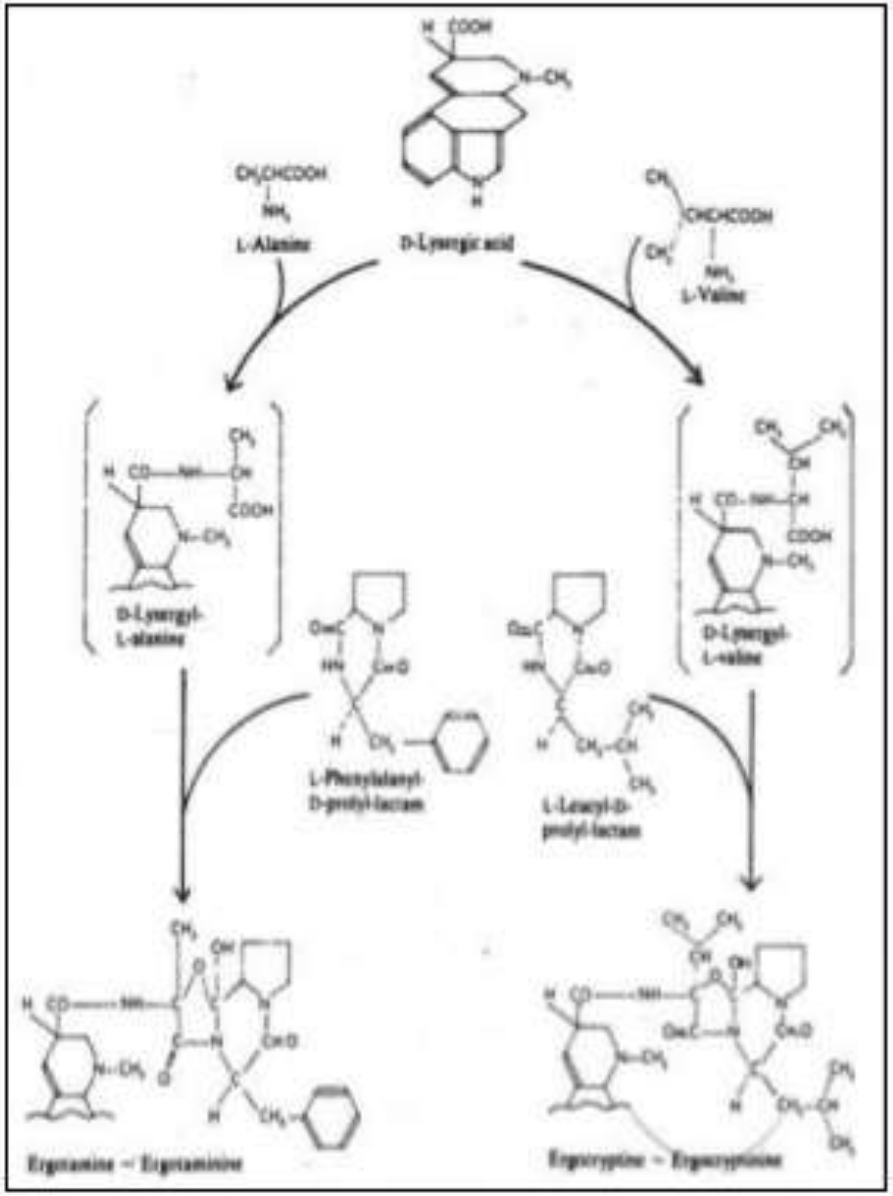
- **Chemical test**:

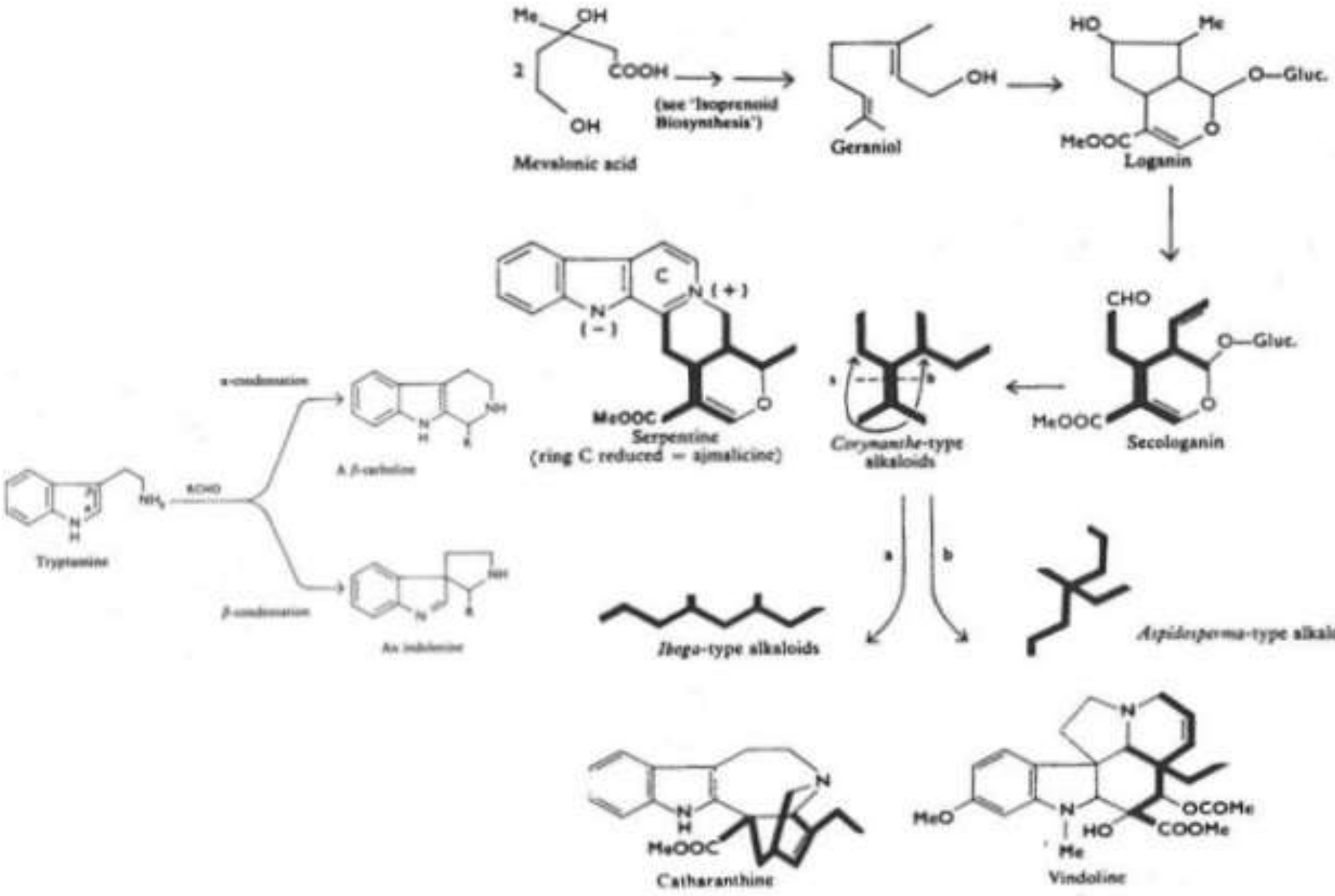
1. Gives yellow color with 70% sulphuric acid.
2. Alcoholic solution when treated with $FeCl_3$ gives red color.

- **Uses**: Antitumor activity.
- Relieve gout (used with caution – professional supervision)and rheumatism.
- Also used in biological experiments to produce polyploidy (multiplication of the chromosomes in a cell nucleus), hence used in horticulture and cultivation of medicinal plants

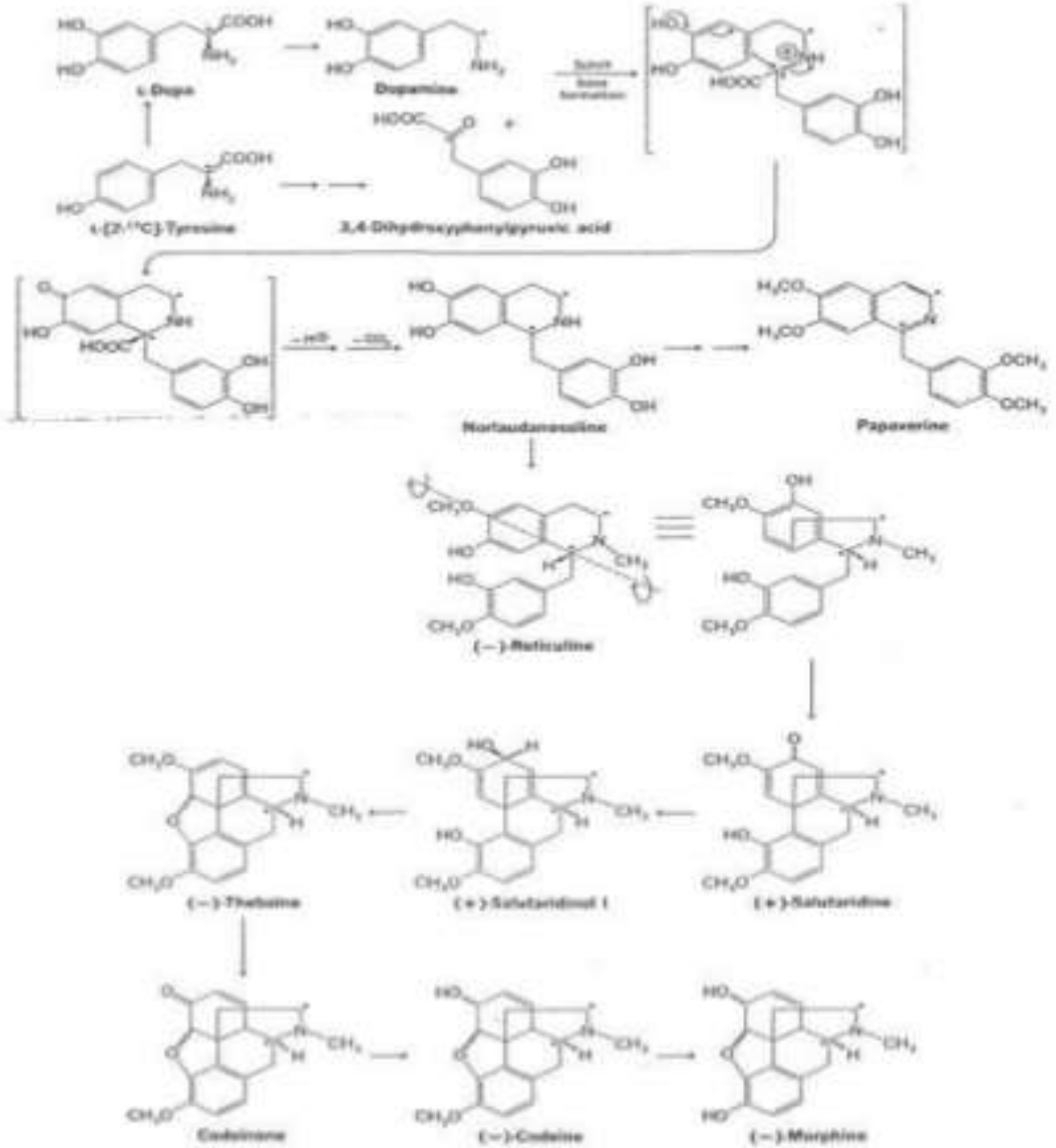
Biosynthesis of indole alkaloids



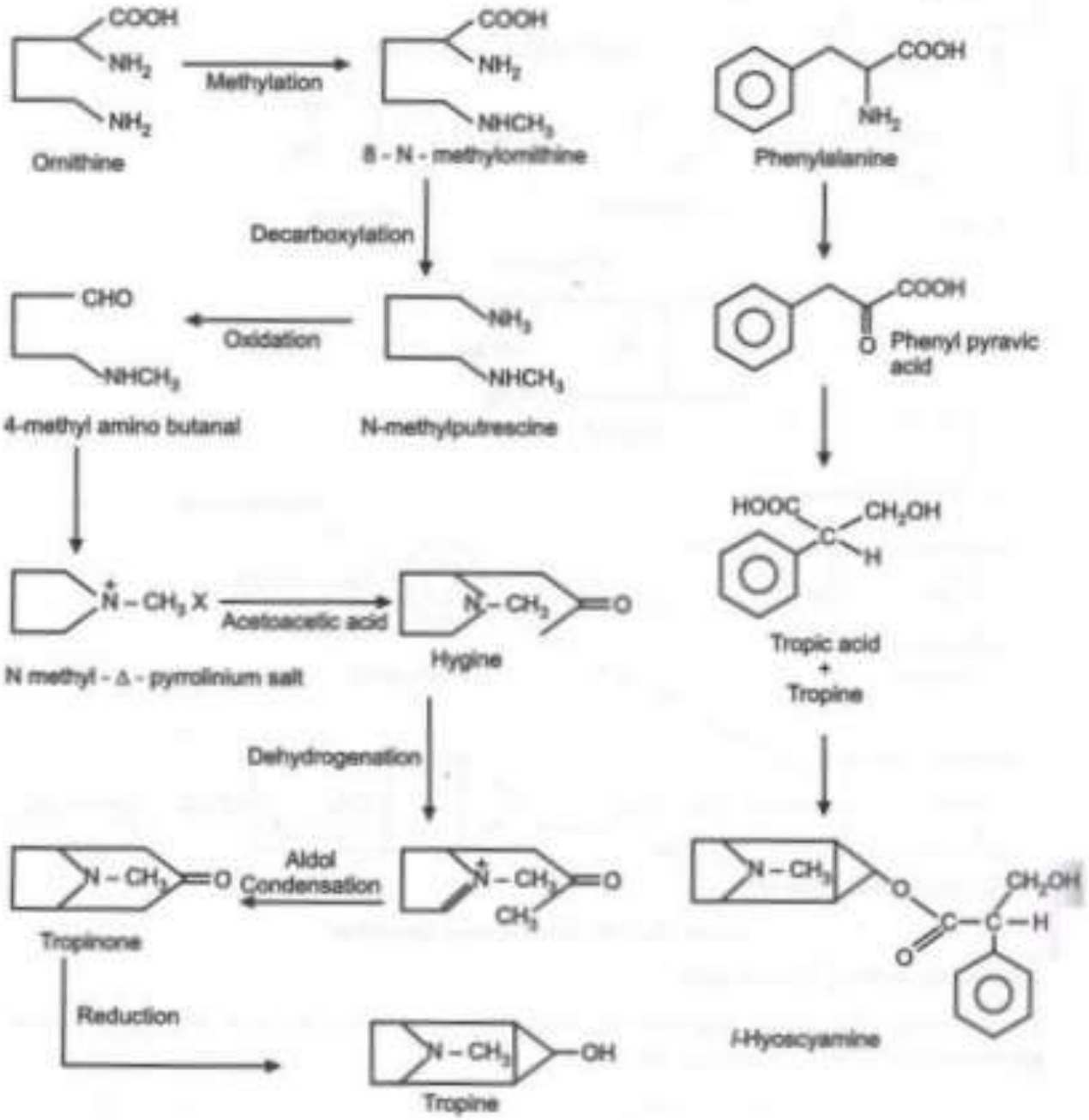



Me
OH
3
COOH
OH
(see 'Isoprenoid Biosynthesis')
Mevalonic acid
Geraniol
HO
Me
O—Gluc.
MeOOC
Loganin
CHO
O—Gluc.
MeOOC
Secologanin
C
N (+)
N (−)
MeOOC
Serpentine
(ring C reduced = ajmalicine)
Corynanthe-type alkaloids
a
b
Tryptamine
RCHO
α-condensation
β-condensation
A β-carboline
An indolenine
Iboga-type alkaloids
Aspidosperma-type alkaloids
Catharanthine
MeO
OCOMe
COOMe
HO
Me
Vindoline

Biosynthesis of Isoquinoline alkaloids



Biosynthesis of Tropane alkaloids



ALKALOIDS

- THIRD YEAR B.PHARM (SEM-V)

- Pepared by

Dr . R.Y.Patil
Pune district education Association's
Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre Kharadi Pune.

- **UNIT-II**
- General introduction, composition, chemistry & chemical classes, bio sources, methods of extraction, therapeutic uses and commercial applications of following secondary metabolites:
- **Alkaloids:** Vinca, Rauwolfia, Belladonna, Opium,
- **Phenylpropanoids and Flavonoids:** Lignans, Tea, Ruta
- **Steroids, Cardiac Glycosides & Triterpenoids**: Liquorice, Dioscorea, Digitalis
- **Volatile oils**: Mentha, Clove, Cinnamon, Fennel, Coriander,
- **Tannins:** Catechu, Pterocarpus
- **Resins:** Benzoin, Guggul, Ginger, Asafoetida, Myrrh, Colophony
- **Glycosides:** Senna, Aloes, Bitter Almond
- **Iridoids, Other terpenoids & Naphthaquinones:** Gentian, Artemisia, taxus, carotenoids

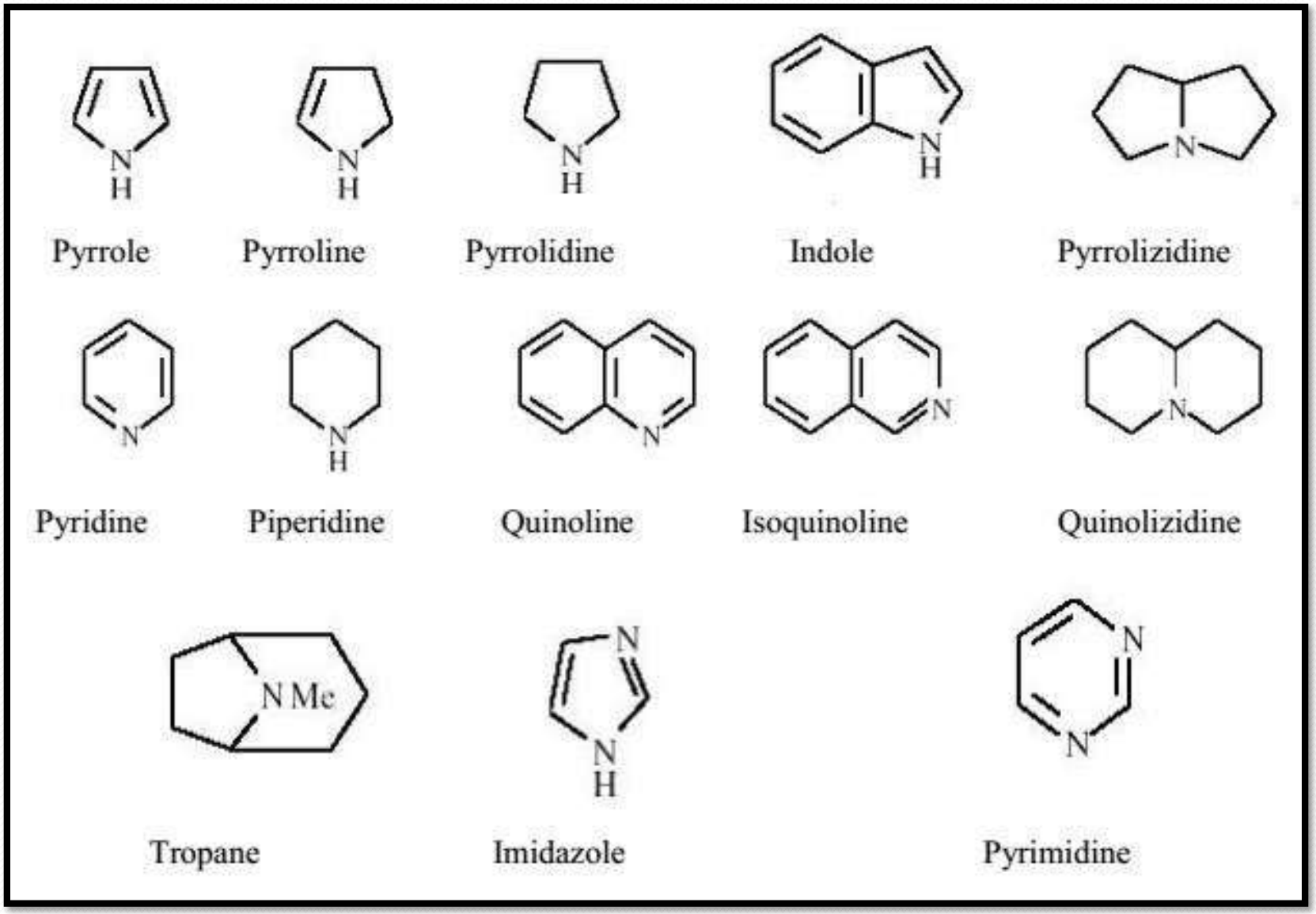
Learning outcome

- After completion of Unit II students should be able to

1. Explain bio sources, chemistry, chemical classes, methods of extraction, therapeutic uses and commercial applications of Alkaloids
2. Explain bio sources, chemistry, chemical classes, methods of extraction, therapeutic uses and commercial applications of Phenylpropanoids and Flavonoids
3. Explain bio sources, chemistry, chemical classes, methods of extraction, therapeutic uses and commercial applications of Steroids, Cardiac Glycosides & Triterpenoids
4. Explain bio sources, chemistry, chemical classes, methods of extraction, therapeutic uses and commercial applications of Volatile oils
5. Explain bio sources, chemistry, chemical classes, methods of extraction, therapeutic uses and commercial applications of Tannins

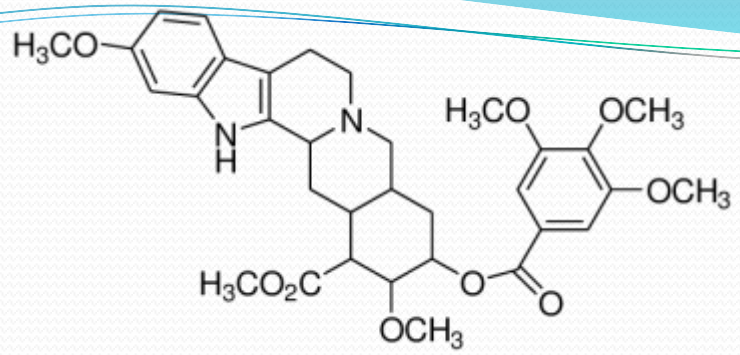
ALKALOIDS

- Term alkaloids was coined by German Pharmacist Meissner in 1819.
- Term alkaloids are derived from “alkali like” they are basic in nature, they contain one or more nitrogen atom (usually in heterocyclic ring).
- Alkaloids are basic nitrogenous organic compounds of plant origin having marked physiological action when administered internally.
- Occurs most commonly in plant families like Apocynaceae, Leguminosae, Papaveraceae,Ranunculaceae, Rubiaceae, Solanaceae etc.
- Among monocotyledons Lilliaceae and Amarylidaceae are two most promising families.

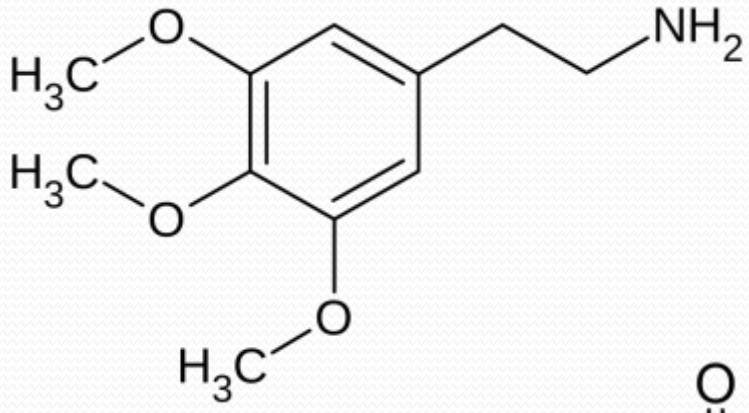

Pyrrole
Pyrroline
Pyrrolidine
Indole
Pyrrolizidine
Pyridine
Piperidine
Quinoline
Isoquinoline
Quinolizidine
N Me
Tropane
Imidazole
Pyrimidine

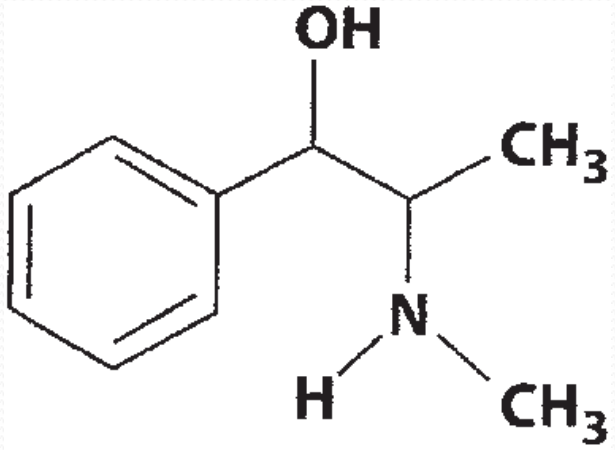
Types

- True alkaloids
  - Contain heterocyclic nitrogen
  - Derived from amino acids
- Proto alkaloids
  - Amino alkaloids
  - Nitrogen is not in heterocyclic ring
  - eg.- Mescaline, Ephedrine
- Pseudo alkaloids
  - Not derived from amino acids
  - Do not show many typical characteristics of alkaloids but give standard chemical test for alkaloids
  - eg.- Caffeine, conessine

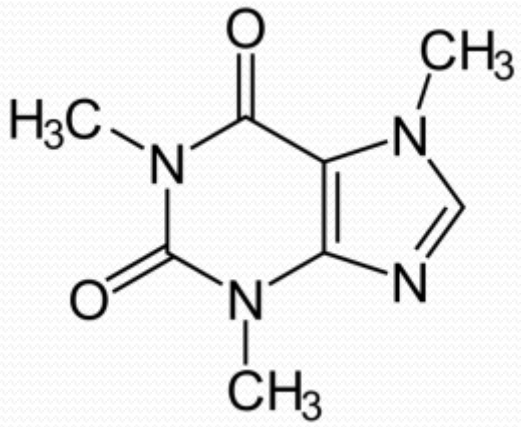


Reserpine





ephedrine



Properties

- Colorless, bitter, crystalline solids with sharp melting point
- Free bases of alkaloids are soluble in organic non polar solvent, salts are soluble in water
- Alkaloids are generally optically active, among them levo isomers are more active.
- Most of alkaloids are basic in reaction due to the availability of lone pair of electron on nitrogen.
- The alkaloids turn to be neutral or acidic when adjacent functional groups are electron withdrawing.
- The alkaloid contain one or more number of nitrogen and it may exist in the form as primary, secondary, tertairy and quaternary.

Chemical Tests

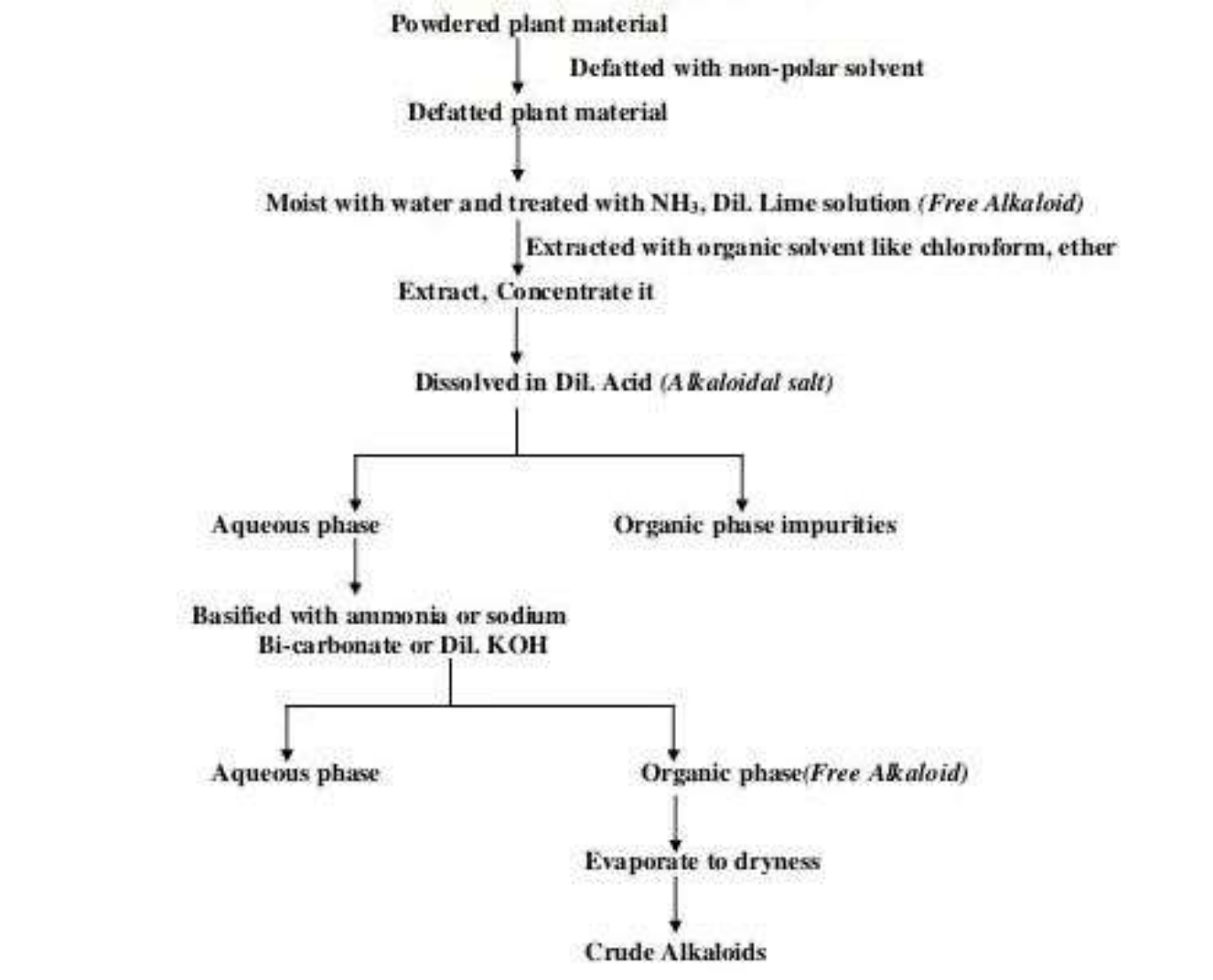
- Mayer's Reagent (Potassium mercuric iodide solution) gives cream coloured precipitate.
- Wagner's reagent (Iodine-Potassium iodide solution) gives reddish brown coloured precipitate.
- Dragendorff's reagent (Potassium bismuth iodide solution) gives reddish brown coloured precipitate.
- Hager's reagent(Picric acid) gives yellow coloured precipitate.
- Scheibler's reagent (Phospotungstic acid) gives precipitate

CLASSIFICATION

- Pharmacological classification
- Taxonomic classification
- Biosynthetic classification
- Chemical classification

Extraction of Alkaloids

Stas Otto method



Role of Alkaloids in Plants

- Alkaloids are reserve substance with ability to supply nitrogen
- They might be the defensive mechanisms, for plants to protect from grazing animals, herbivores and insects.
- They might have possible role as growth regulatory factors in the plants.
- Alkaloids are present normally in conjugation with plant acids, like meconic acid, cinchotannic acid. Therefore alkaloids could be acting as carrier within plants for transportation of such acids.
- It is possible that they are the end products of detoxification mechanism in plants.

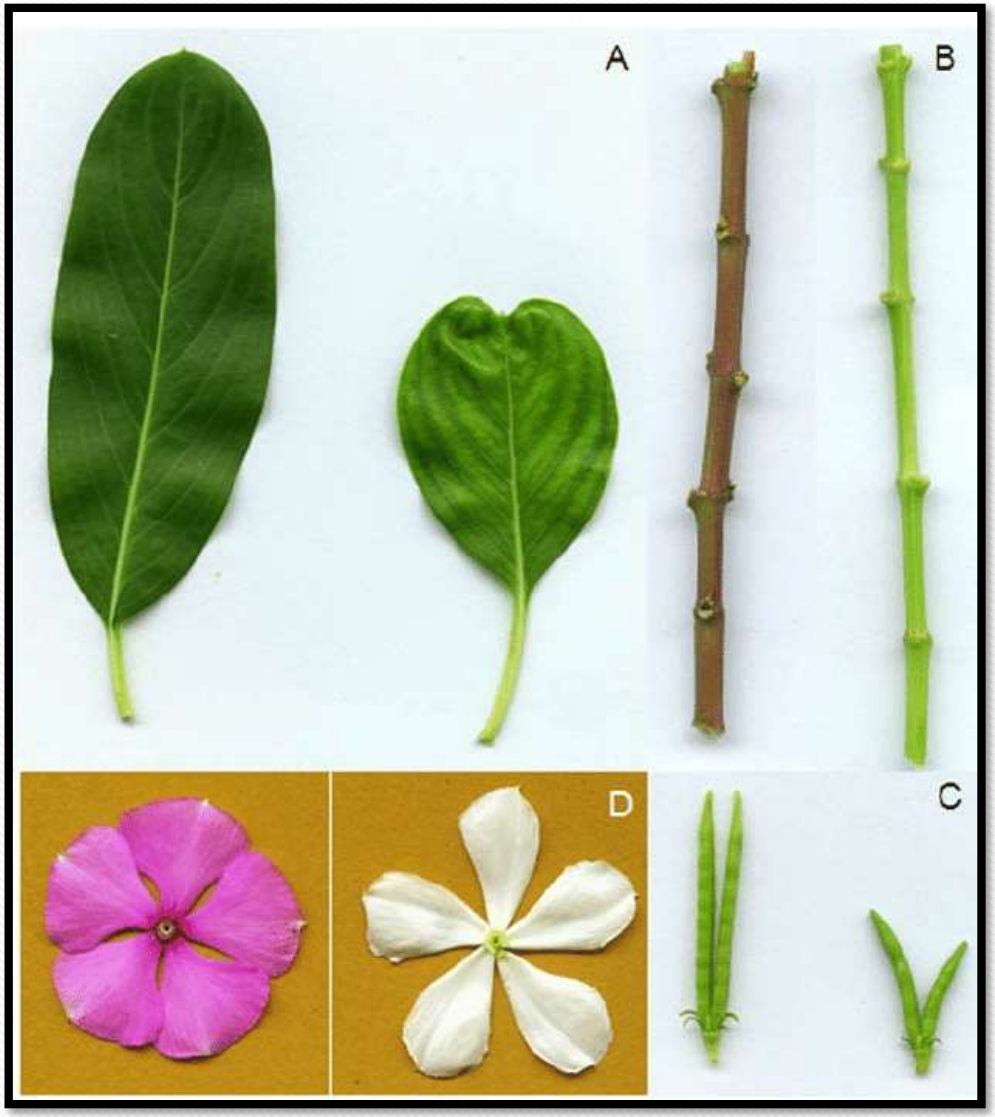
Vinca

- Synonym : Periwinkle, Sadabahar
- Biological source : Vinca is obtained from dried whole plant of *Catharanthus roseus.*

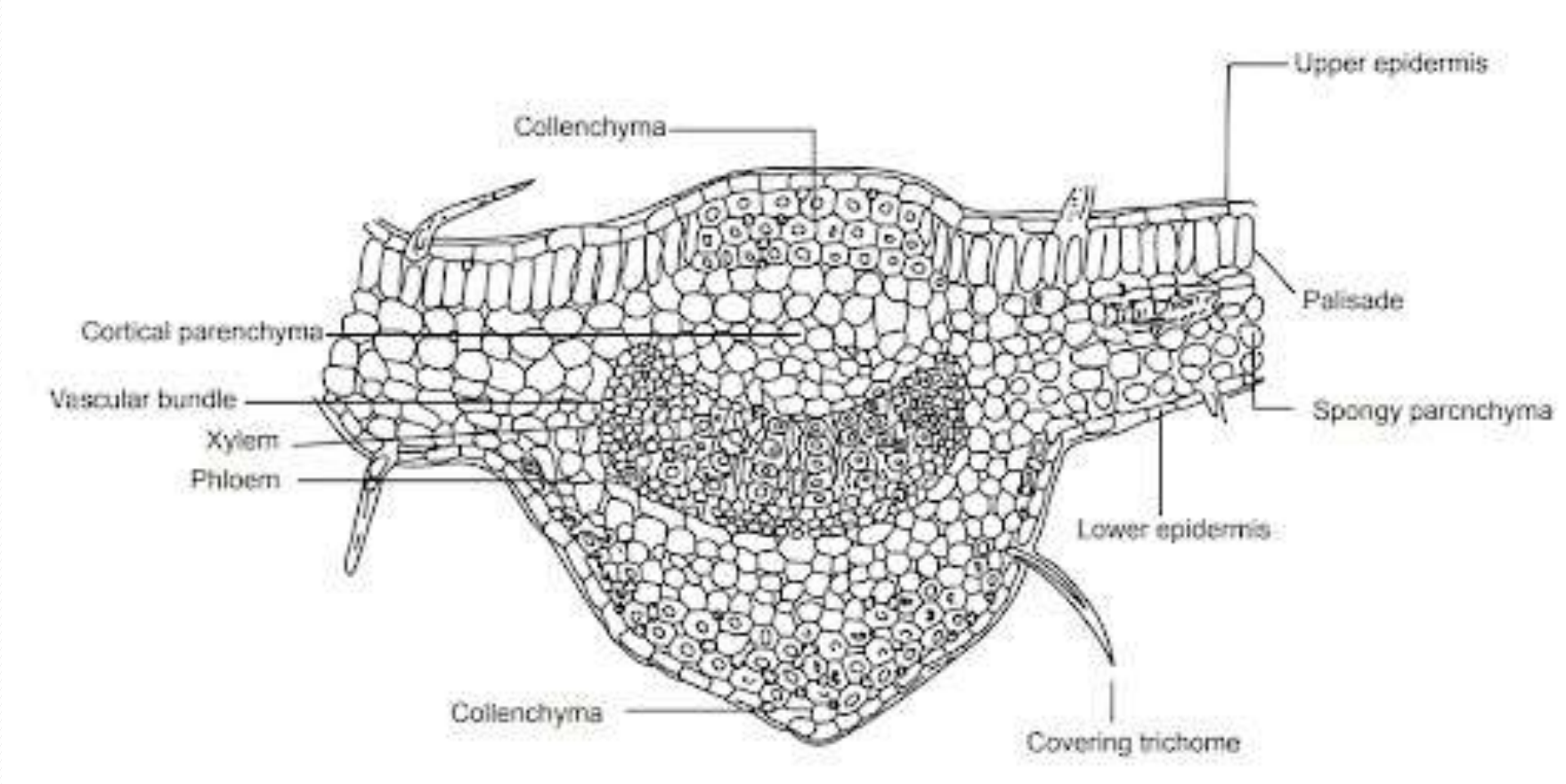
  It is also known as Vinca rosea.
- Family : Apocynaceae
- Geographical source- cultivated in South Africa, India, U.S.A., Europe, Australia as an ornamental plant as well as for its medicinal properties.

Macroscopic characters

- Vinca is an erect, pubescent herb with branched tap root
- Leaves are simple, petiolate, ovate, reticulate, entire, with acute apex and glossy appearance, green in colour
- Flowers are violet, pink- white, or carmine- red in colour.
- Roots are pale grey in colour
- Fruits are follicles with several black seeds


A
B
D
C

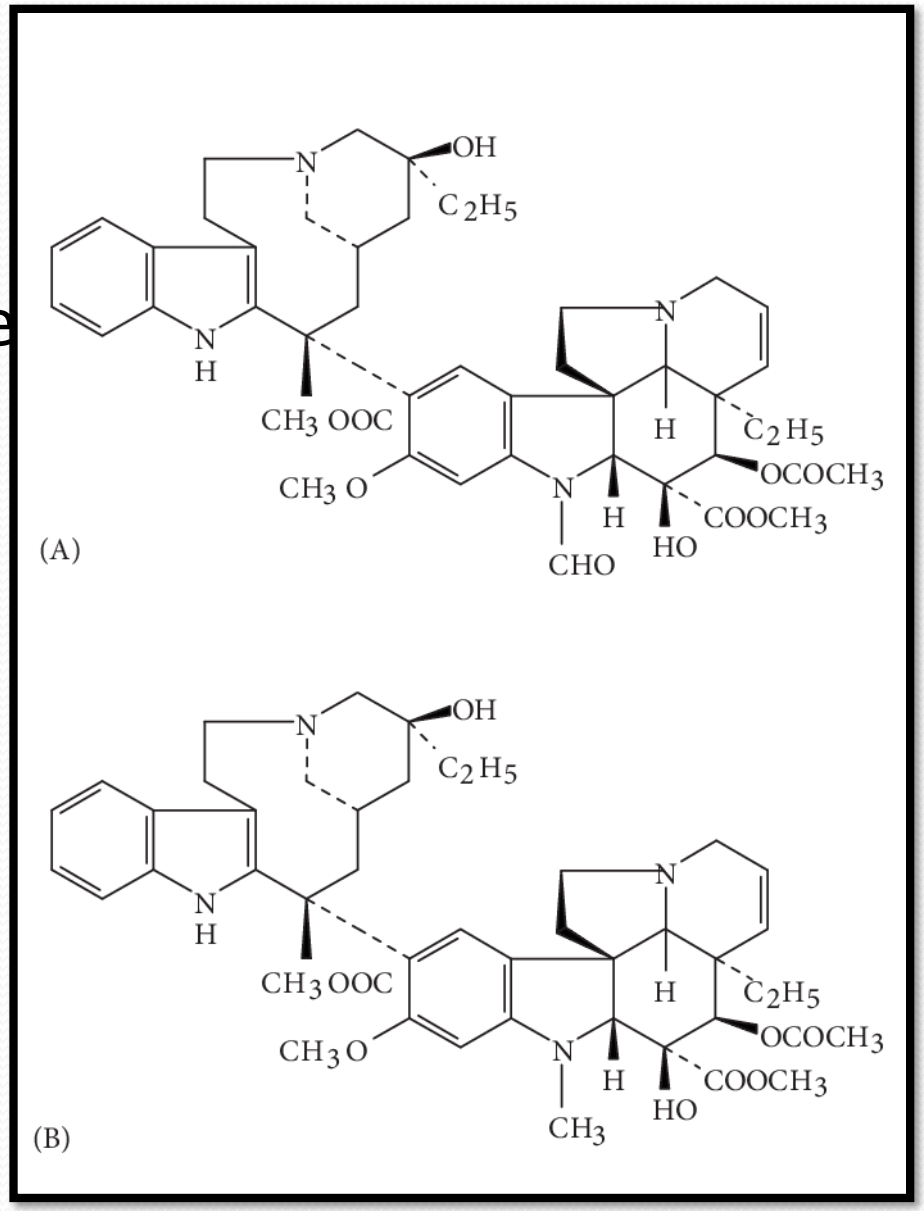
Microscopic characters



Chemical Constituents

Indole alkaloids

Vincristine and Vinblastine



- 500 kg crude drug contain 01 gm vincristine
- Other alkaloids are

  Ajmalicine

  Lochnerine

  Serpentine

  Tetrahydroalstonine.

Uses

- Used as a antineoplastic agent.

  Acts by arresting mitosis at metaphase

RAUWOLFIA

- Rauwolfia is known to Indian system of medicine since last many centuries.
- Because of snake like shape of the drug it has been known as 'Sarpgandha'
- Rauwolfia refers to the name of scientist Dr. Rauwolf.
- It has found its place as important drug in treatment of insanity and snake bite since traditional times.
- Drug came into limelight after the isolation of Reserpine, its most significant alkaloid, in 1952 by Mueller.

RAUWOLFIA

- Synonym : Chhotachand, Sarpagandha
- Biological source : Rauwolfia is obtained from dried roots and rhizomes of plant *Rauwolfia serpentina.*
- Family : Apocynaceae
- It should contain NLT 0.14% of alkaloids
- Geographical Source- Asia, America, Africa

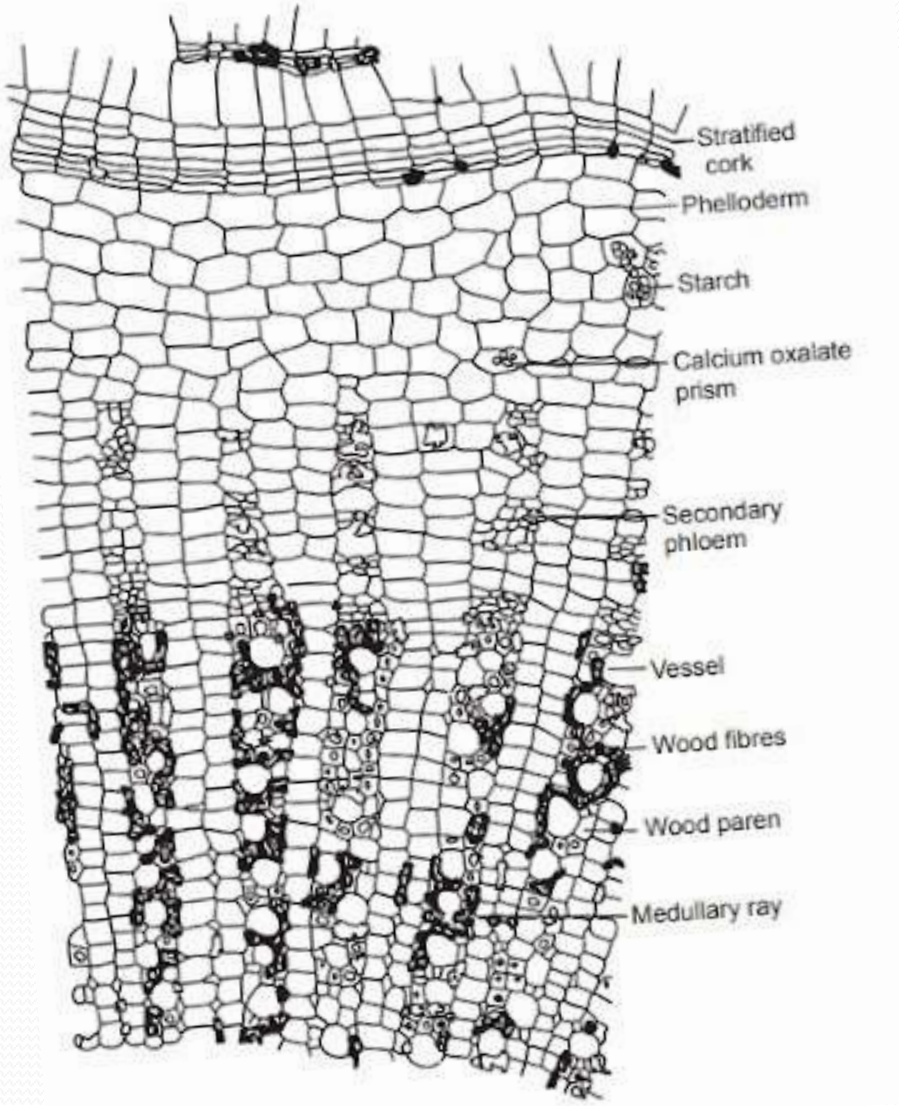
  Commercially produced in India, Srilanka, Myanmar, Thialand

Rauwolfia Plant

Macroscopic characters - Rauwolfia Root

- Colour- root bark is greyish yellow to brown and wood is pale yellow
- Odour- odourless
- Taste- Bitter
- Size- 10 to 18 cm long, 01 to 03 cm diameter
- Shape- roots are subcylindrical slightly tapering
- Fractures are short and irregular
- Roots are rough with longitudinal marking and slightly wrinkled surface

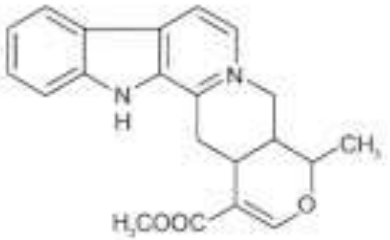
Microscopy-


Stratified cork
Phelloderm
Starch
Calcium oxalate prism
Secondary phloem
Vessel
Wood fibres
Wood paren
Medullary ray

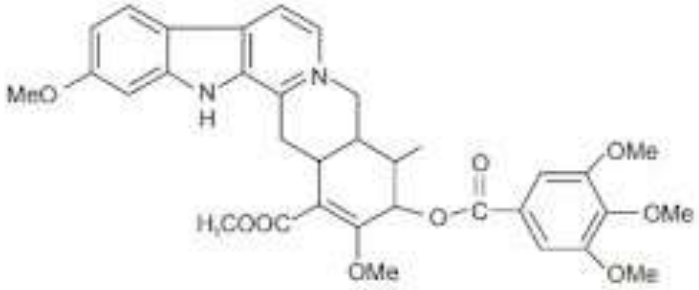
Chemical Constituents

- About 30 Indole alkaloids have been reported in drug
- Alkaloids are mostly concentrated in bark of root
- Rauwolfia alkaloids are broadly classified in following types
  1. Indole alkaloids
  2. Indoline alkaloids
  3. Indolinine alkaloids
  4. Oxyindole alkaloids
  5. Pseudo Indoxyl alkaloids

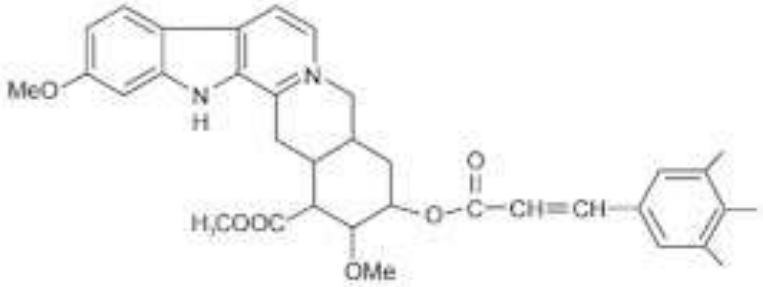
The important alkaloid of Rauwolfia is Reserpine



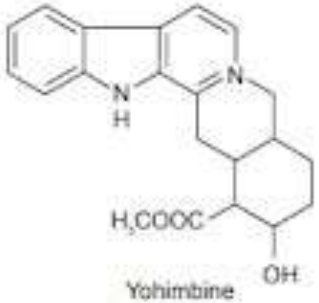
Serpentine



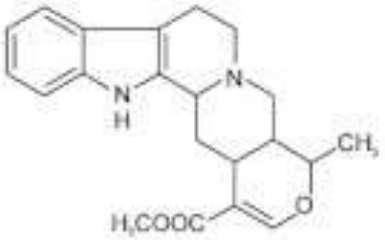
Reserpine



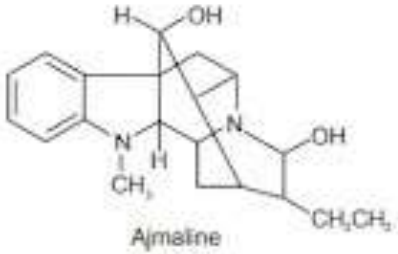
Rescinnamine



Yohimbine



Ajmalicine



Ajmaline

Chemical tests

- 1.A red coloration along the medullary rays is observed when freshly fractured surface is treated with conc. nitric acid
- 2.Reserpine shows violet colour when treated with Vannilin in acetic acid

Uses

- Reserpine is used as Antihypertensive, it lowers the blood pressure by depleting store of catecholamines at nerve ending, it prevents reuptake of nor epinephrine at storage site, allowing enzymatic destruction of neuronal transmitters.
- Recinnamine is also used as antihypertensive, but it causes mental depression in higher doses.
- Desperidine is used as antihypertensive and tranquilliser
- Ajmalicine used in the treatment of circulatory diseases, in relief of obstruction of normal cerebral blood flow

Allied Drugs/ substitutes

- *Rauwolfia vomitoria* (African Rauwolfia)

  Five discontinued bands of sclerenchyma

  Large vessels
- *Rauwolfia densiflora*

  contains sclerenchyma
- *Rauwolfia tetraphylla*

  Uniform cork

  Abundant sclerides and fibres

  Absence of Rescinnamine

BELLADONNA

- In ancient times, juice of belladonna was used as a cosmetics, because of its dilatory effect on the pupil of the eye
- Drug was first introduced in London Pharmacopoeia in 1809
- Synonym-Deadly night shade leaf
- Biological source : Belladona is obtained from dried leaves and other aerial parts of Atropa acuminata (Indian belladonna), Atropa belladonna (European belladonna).
- Family : Solanaceae
- It should contain NLT 0.3% of alkaloids calculated as l-hyoscyamine

- Geographical source- England,

  In India it is found in Western Himalayas from Shimla to Kashmir

Macroscopic characters

Colour- Leaves- Green to brownish green

Flowers-Purple to yellowish brown

Fruits- Green to Brown

Odour- Slight and characteristics

Taste- Bitter and acrid

Size- Leaves- 5to 25 cm long & 2.5 to 12 cm wide

Flowers-Corolla 2.5 cm long & 1.5 cm wide

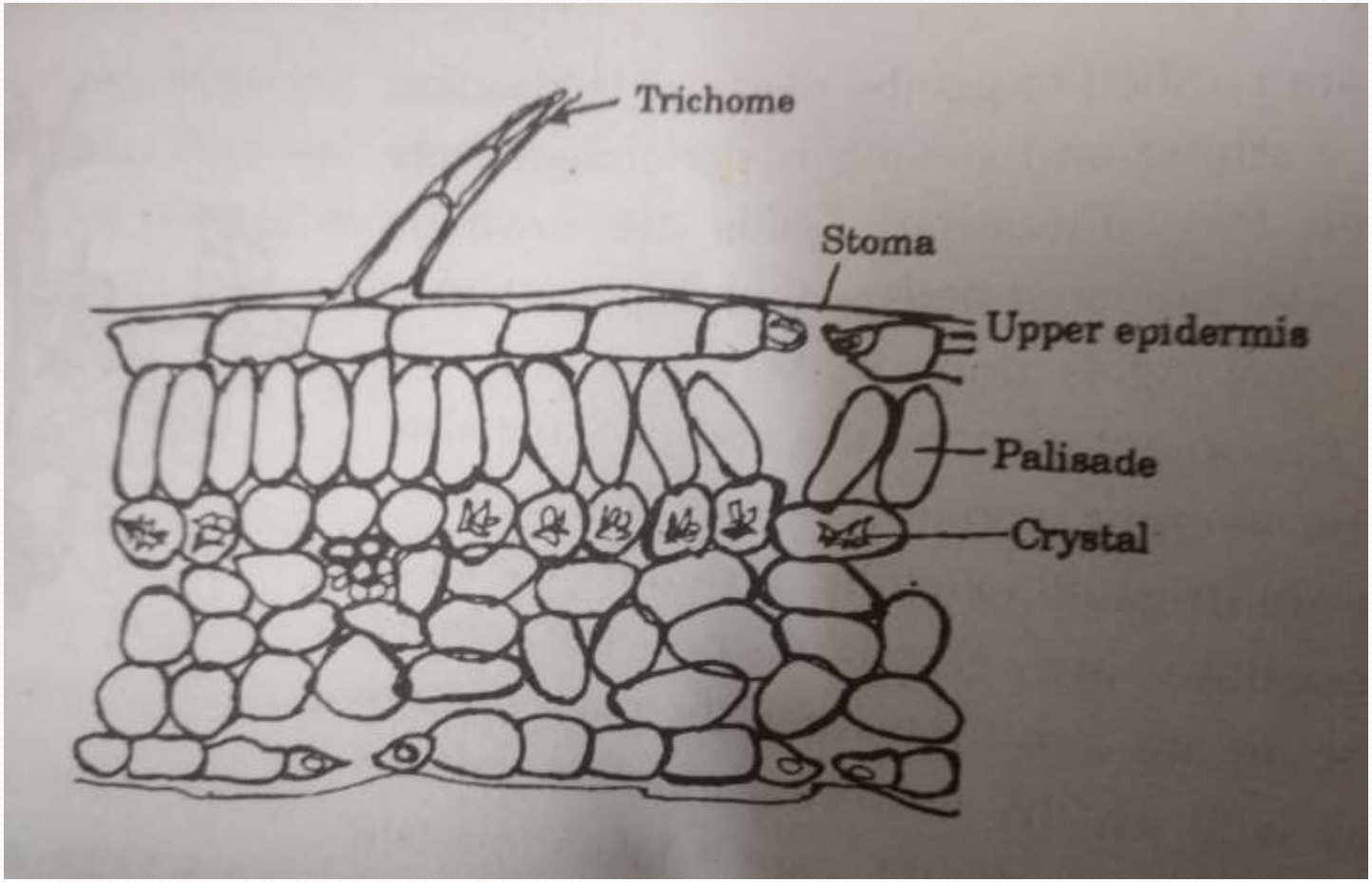
Fruits- about 10 cm in diameter

Shape-Leaves Ovate, lanceolate, acuminate apex, entire margin

Flowers campanulate (bell shaped)

Fruits Berries sub globular, numerous flat seeds

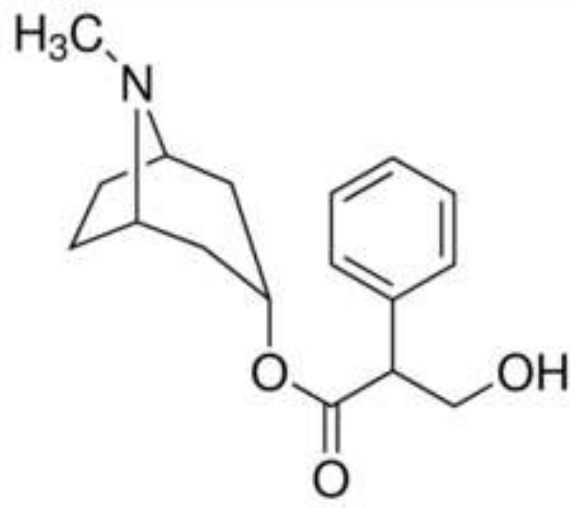
Microscopy



Chemical Constituents

Total alkaloidal content of drug is 0.4 to 1% and varies in different parts roots (0.6%), Stems (0.05%), Leaves (0.4%) Berries (0.2%) and seeds (0.33%)

Main alkaloid is *l*- hyoscyamine and its recemic form atropine



- Drug also contains

  Belladonine
  Scopoletin
  hyoscine
  n-methyl pyrroline

USES-

Parasympatholytic drug with anticholinergic property

Used to reduce secretions such as sweat, saliva and gastric juice

Used as antidote in opium and chloral hydrate poisoning

OPIUM

- Synonym : Raw opium, Afim.
- Biological source : It is dried latex obtained by incision from the unripe capsules of *Papaver somnifarum*
- Family- Papaveraceae
- The genus Papaver has 50 different species out of which 6 species are found in India
- *P. somniferum(Opium poppy), P. nudicaule (Iceland poppy), P. rhoeas (Corn poppy), P. orientale, P. argemone, P. dubium*
- Geographical source- India, Pakistan, Afganistan, Turkey, Russia and Iran

- Poppy is an erect plant attaining 60-120 cm height
- Rarely branched
- Leaves are linear, oblong/ovate and have serrate margin
- Flowers are large, bluish white, purple/ violet
- Indian poppy is considered as the only legal source of opium to many countries including USA and Britain
- In India about 54 thousand hectares of land is under opium cultivation. It is under government control and cultivation is restricted to few states.

- Propagation is done by seeds
- After sowing, within 3-4 months, the plants bear flower which is converted to capsules within few days
- After 15-20 days capsules attains maturity
- During, maturity period the capsule exudes maximum latex which shows a colour change from dark green to light green
- Such capsules are incised vertically in the afternoon with the help of specific needle like apparatus called as ‘Nushtur’

- Nushtur penetrates maximum upto 2mm in to the capsule
- Because of incisons latex exudes out and thickens due to cold weather in night
- Such latex is scrapped off with iron scoop called as ‘Charpala’
- The opium collected by this way is further processed at Government Opium Factory at Ghazipur

OPIUM FACTORY
GHAZIPUR

Govt Opium factory -Neemuch (MP)

Macroscopic characters

Colour- Dark brown

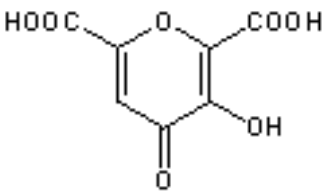
Odour- Strong characteristics

Taste- Bitter

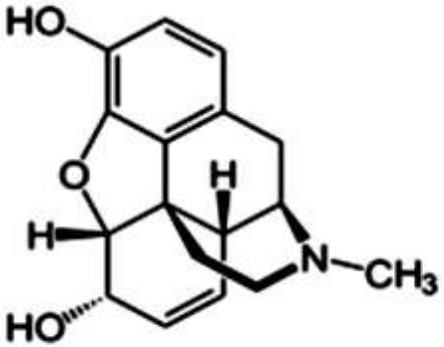
Found in the form of cubical pieces weighing about 900 gm

Chemical Constituents

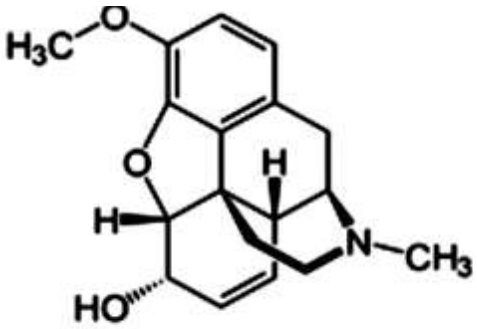
- Latex contains mainly the alkaloids derived from amino acids phenylalanine and tyrosine
- Chemically they are placed under benzylisoquinoline and phenanthrene types
- Narcotine (Noscapine), narceine and papaverine belongs to benzylisoquinoline type
- Morphine, codeine and thebaine belongs to phenanthrene types
- Opium alkaloids are present as salt of Meconic acid



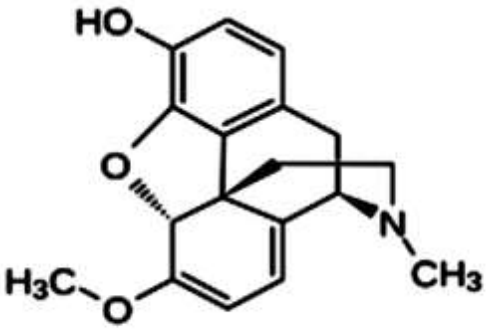
Meconic acid



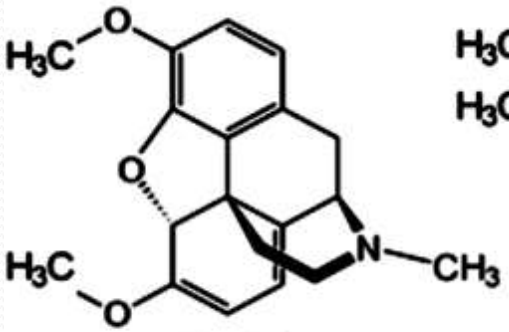
Morphine



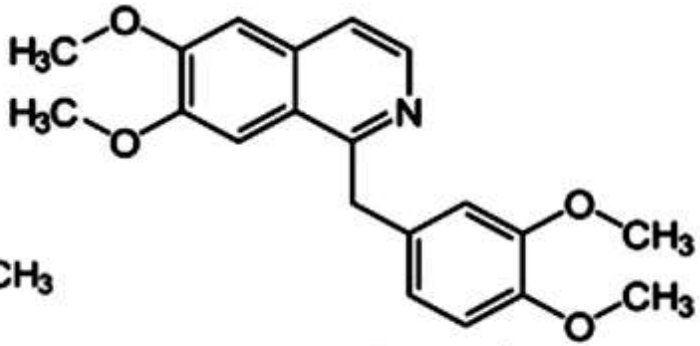
Codeine



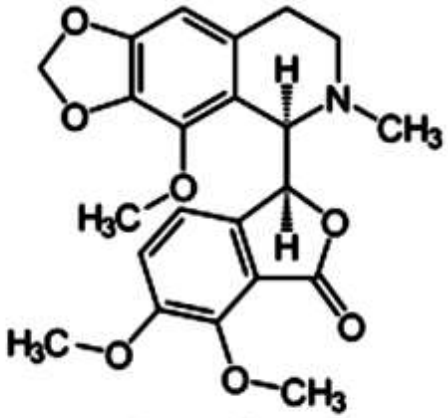
Oripavine



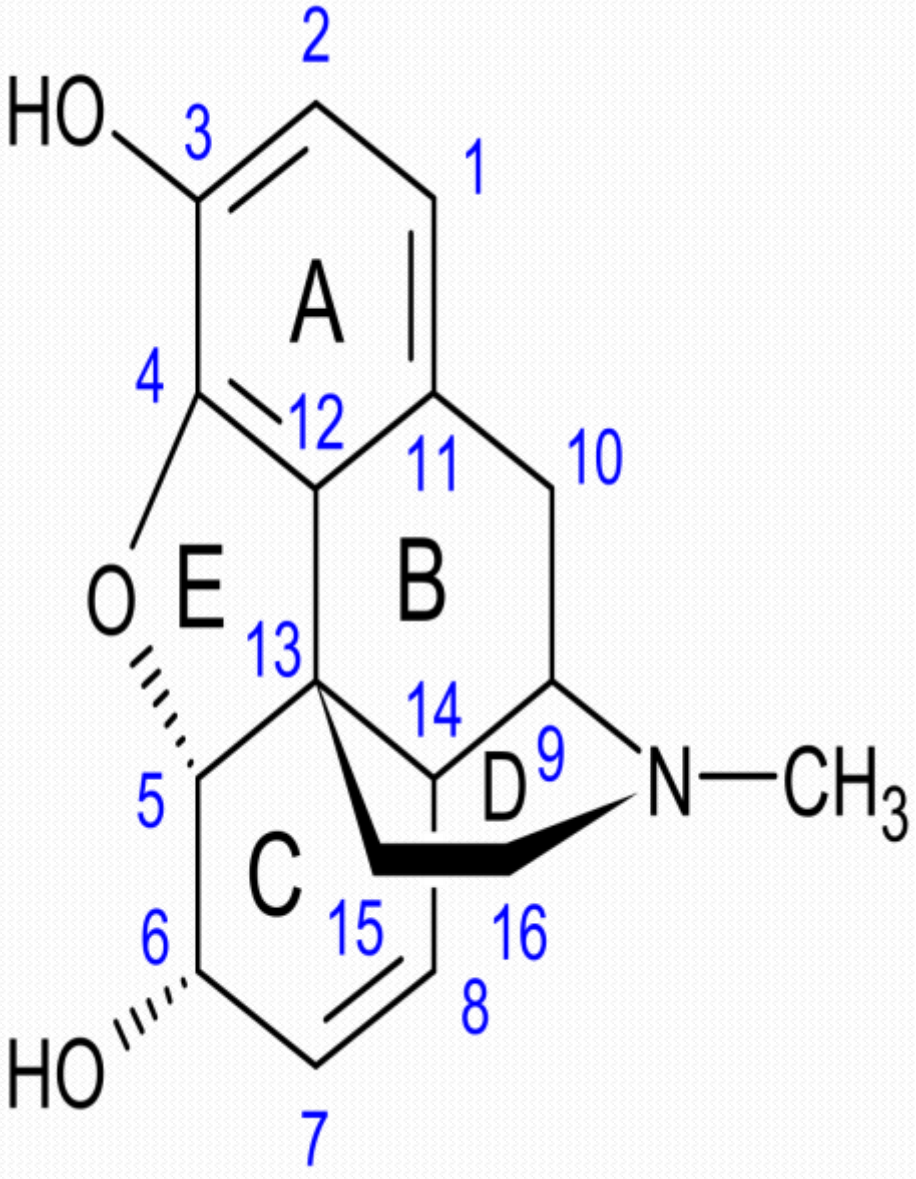
Thebaine



Papaverine



Noscapine


HO
2
3
1
A
4
12
11
10
O
E
B
13
14
5
D
9
N—CH3
C
6
15
16
8
HO
7

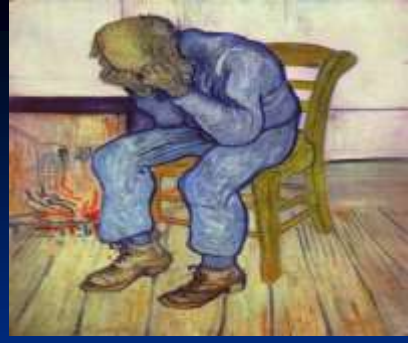
- **Morphine-**
  - Monoacidic, laevorotatory, phenolic alkaloid, contain an alcoholic hydroxyl group at C(6) position.
  - Due to presence of phenolic hydroxyl group, it is soluble in alkali hydroxide.
  - Diacetyl derivative of morphine is heroin
- **Codeine (Methyl morphine)**
  - Strong monoacidic, laevorotatory, soluble in water and organic solvent
- **Papaverine**
  - Weak monoacidic base, optically inactive,insoluble in water, slightly soluble in organic solvent.

Chemical Tests

- Marquis test – Morphine + Conc. H_2SO_4 + HCHO → Dark violet color
- Meconic acid + $FeCl_3$ → Deep reddish purple color
- Morphine + SiO_2 → Blue color
- Papaverine solution in HCl and potassium ferricyanide develops a lemon yellow color.
- Morphine, when sprinkled with concentrated HNO_3, shows an orange red color, codeine does not respond to this test.
- Morphine solution when treated with ferric chloride and potassium ferricyanide gives a bluish-green color.

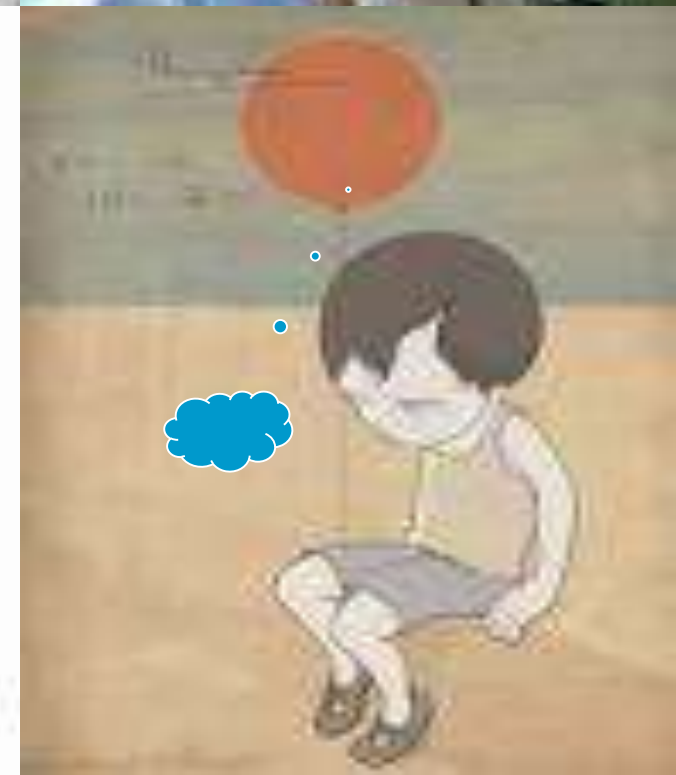
Uses

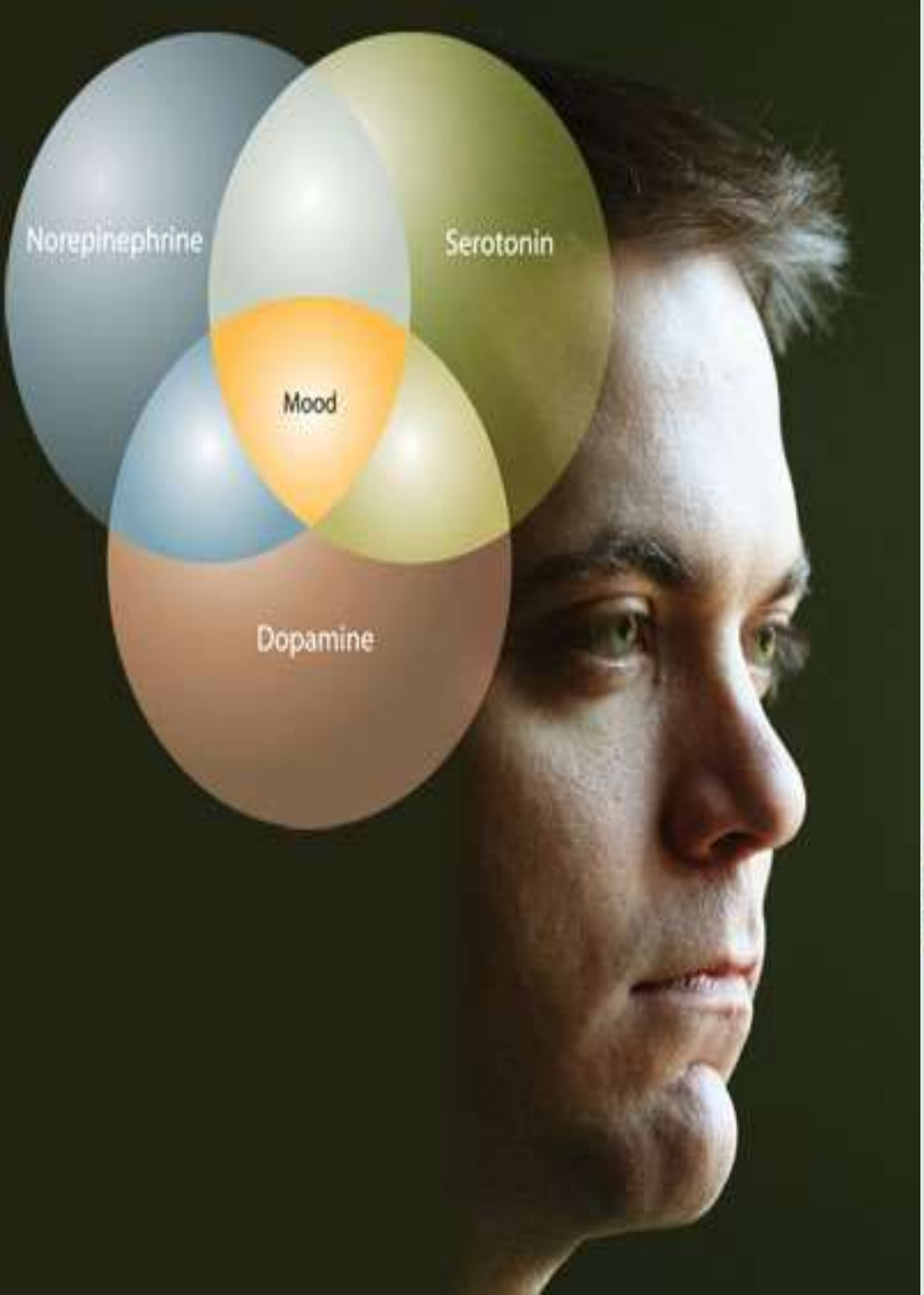
- Morphine is used as a Analgesic.
- Sedatives and hypnotics
- Morphine also produces respiratory depression and constipation.
- Codeine –Antitussive so used in cough syrup preparation.
- Papaverine – smooth muscle relaxants.
- Apomorphine – Emetic (for treating poisoning cases).

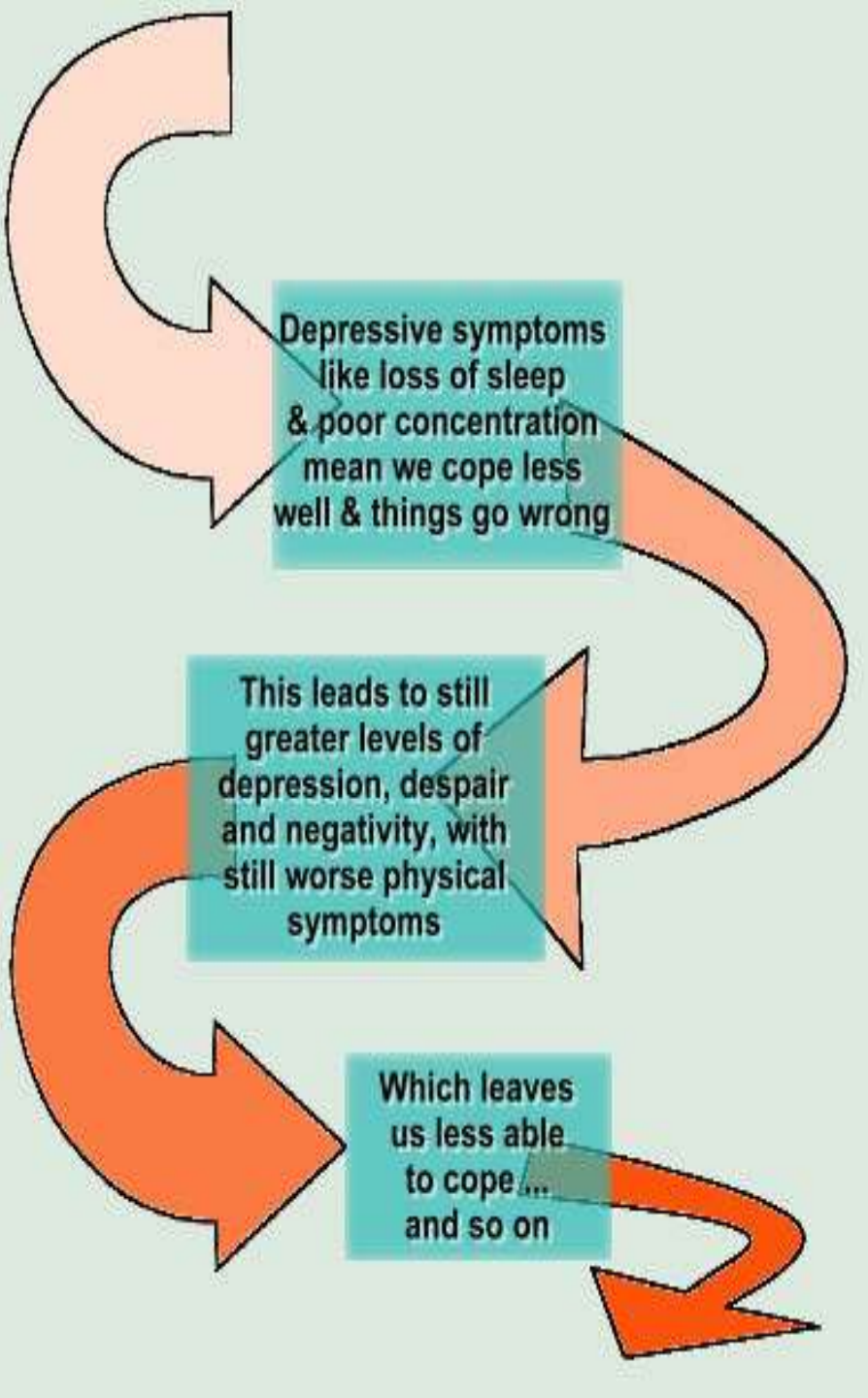


Antidepressant, Anti-anxiety Drugs

Presented By
Mr. Krunal Kanase
HOD , Department of Pharmacology
PDEA's Shankarrao Ursal College of Pharmaceutical Sciences &
Research Center, Kharadi.




Norepinephrine
Serotonin
Mood
Dopamine


Depressive symptoms like loss of sleep & poor concentration mean we cope less well & things go wrong
This leads to still greater levels of depression, despair and negativity, with still worse physical symptoms
Which leaves us less able to cope ... and so on

Classification of Major Affective Disorders

- Major Affective Disorders
  - Episodal Depression
  - Seasonal Affective Disorder
  - Atypical Depression
  - Major/ Endogenous Depression
  - Mania
  - Bipolar depression

Episodal (reactive) Depression

- Adverse life events.
- Physical illness.
- Drugs.
- Other psychiatric disorders.

Reactive (episodal) Depression

- More than 60% of all depressions.
- Core depressive syndrome: feelings of misery, apathy, inadequacy, pessimism, anxiety, tension, guilt. Ugliness, Low self –esteem,
- Bodily complaints

- Withdrawn.
- Loss of interest in pleasurable activities.
- Indecisiveness, loss of motivation.
- Retardation of thought and action.
- Sleep disturbance

- In severe cases, it is accompanied by hallucinations and delusions.
- Recurrent suicidal ideation, a suicide attempt or a specific suicide plan.

- **significant weight change (without dieting)**
- **Psychomotor agitation or retardation.**

1. Has a genetic component.
2. Depression can be drug-induced.
3. Depression can be drug-repressed.
4. Depression can be treated with drugs.
5. Depression can be treated with Electroconvulsive Therapy (ECT).

Mania

Mania alone is rare (10%) and most frequently cycles with Major/endogenous depression

(Manic-Depressive Disease, Bipolar Disorder).

Core Symptoms:

- **Characterized by an elevated "high" mood.**
- **Talkative, go on-and-on about the things they will do.**
- **Increased self-esteem.**
- **Auditory hallucinations.**
- **Decrease need to sleep. Expensiveness, unnecess buying.**
- **Lack judgment, Supermen**

- The precise cause of affective disorders remains elusive.
- Evidence implicates alterations in the firing patterns of a subset of biogenic amines in the CNS,

Norepinephrine (NE) and Serotonin (5-HT).

↓ Activity of NE and 5 -HT systems?.

Almost all NE pathways in the brain originate from the cell bodies of neuronal cells in the locus coereleus in the midbrain, which send their axons diffusely to the cortex, cerebellum and limbic areas (hippocampus, amygdala, hypothalamus, thalamus).

- **Mood:** -- higher functions performed by the cortex.
- **Cognitive function:** -- function of cortex.
- **Drive and motivation:** -- function of brainstem
- **Memory and emotion:** -- function of the hippocampus and amygdala.
- **Endocrine response:** -- function of hypothalamus.

Serotonin System

As with the NE system, serotonin neurons located in the pons and midbrain

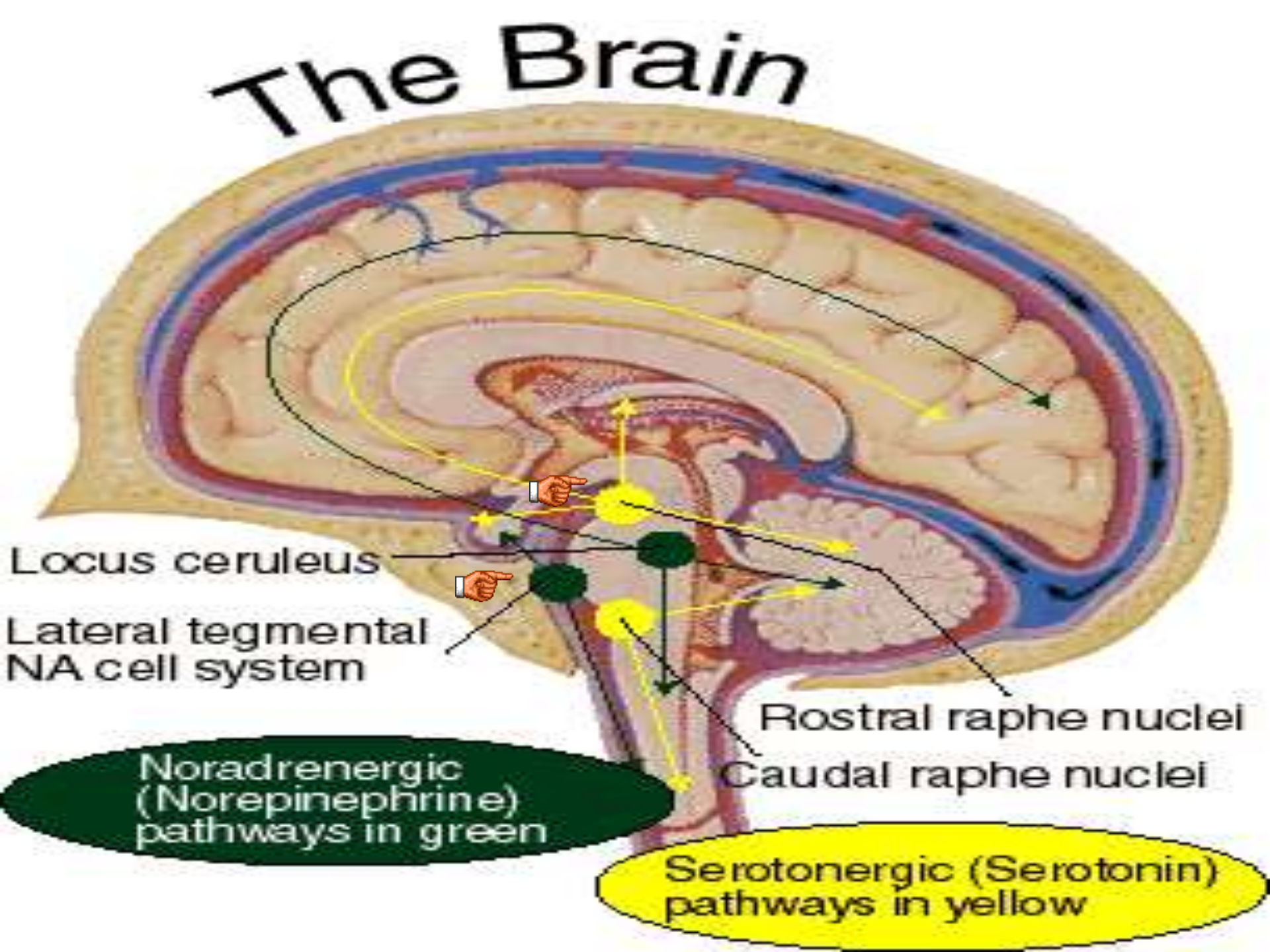
(in groups known as raphe nuclei)

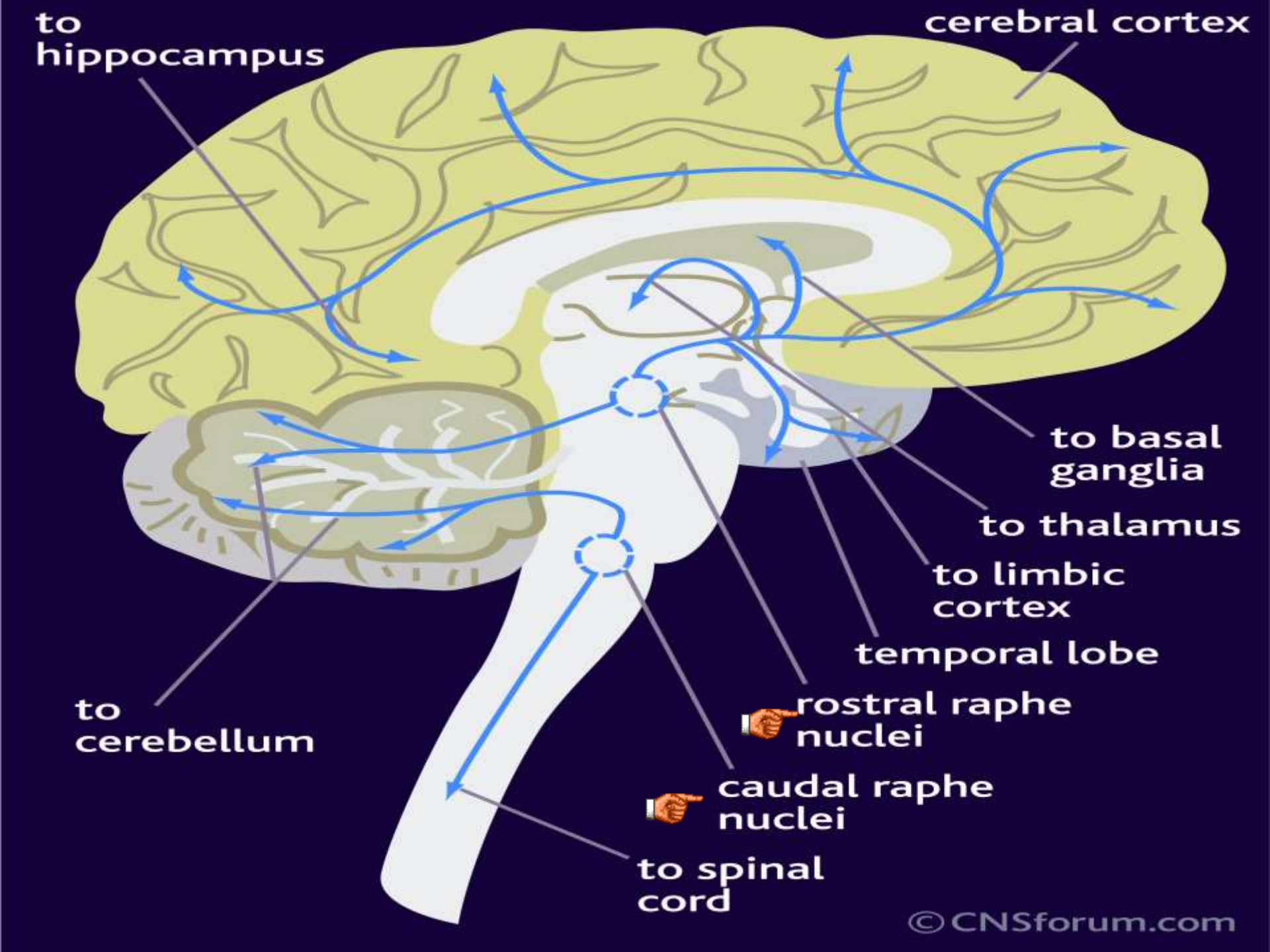
send their projections diffusely to the cortex, hippocampus, amygdala, hypothalamus, thalamus, etc. --same areas implicated in depression.

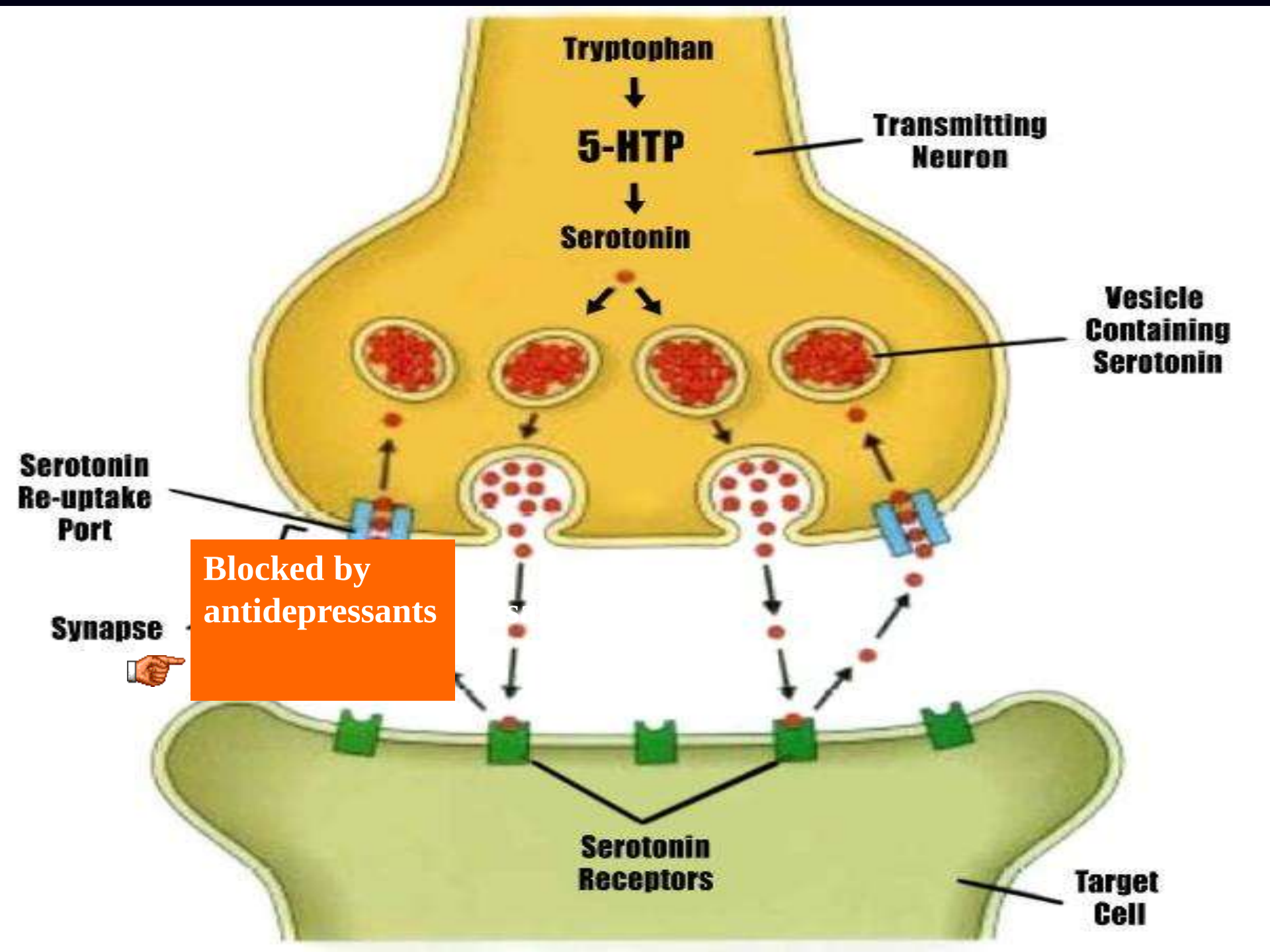
This system is also involved in:

- **Anxiety.**
- **Sleep.**
- **Sexual behavior.**
- **Temperature regulation.**
- **CSF production.**

The Brain

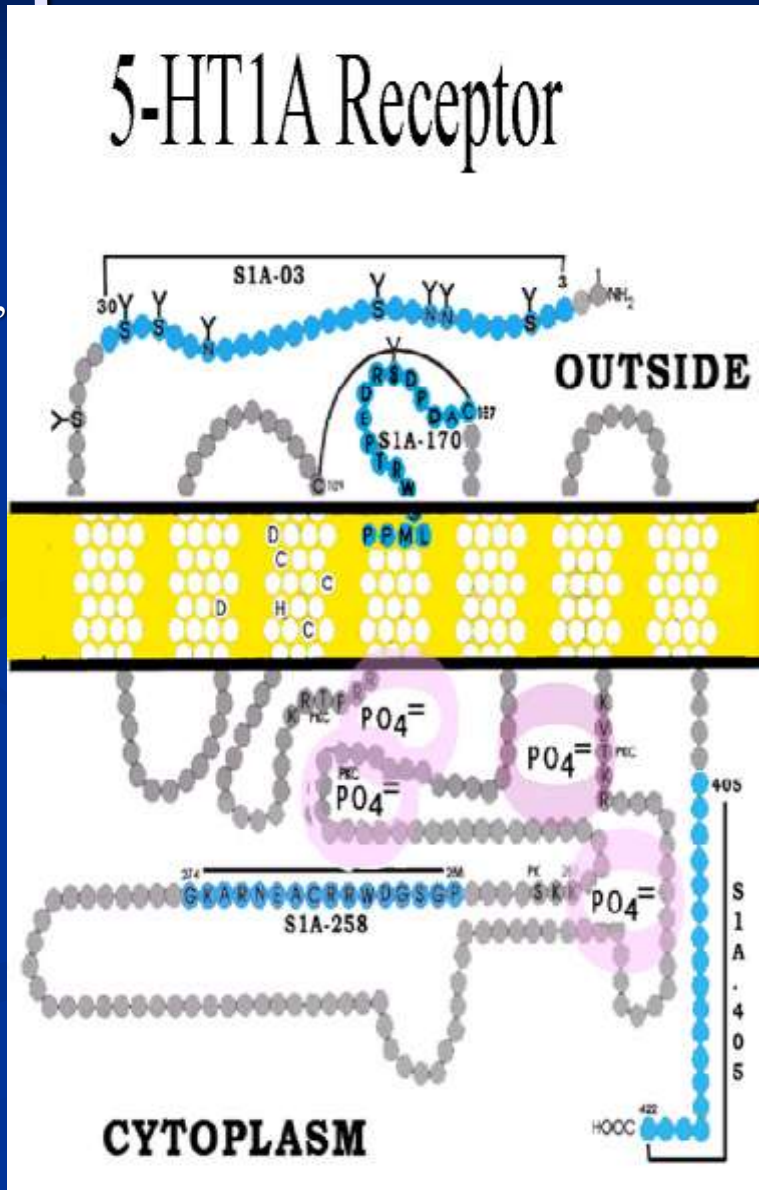

Locus ceruleus
Lateral tegmental
NA cell system
Rostral raphe nuclei
Caudal raphe nuclei
Noradrenergic
(Norepinephrine)
pathways in green
Serotonergic (Serotonin)
pathways in yellow


to hippocampus
cerebral cortex
to basal ganglia
to thalamus
to limbic cortex
temporal lobe
rostral raphe nuclei
caudal raphe nuclei
to spinal cord
to cerebellum
© CNSforum.com


Tryptophan
5-HTP
Serotonin
Transmitting Neuron
Vesicle Containing Serotonin
Serotonin Re-uptake Port
Blocked by antidepressants
Synapse
Serotonin Receptors
Target Cell

Serotonin receptors

- $5–HT_1$
  - subtypes
    - **$5–HT_{1A}$**, $5–HT_{1B}$, $5–HT_{1D}$, $5–HT_{1E}$, $5–HT_{1F}$
  - primarily responsible for the ***therapeutic*** (antidepressant) effects of increased intrasynaptic serotonin
- $5–HT_2$
  - subtypes
    - **$5–HT_{2A}$**, $5–HT_{2B}$, $5–HT_{2C}$
  - primarily responsible for the ***toxic*** effects of increased intrasynaptic serotonin



Serotonin receptors

- Over all 14 types divided in to 1, 2, 3, and 4-7 family
- All are G-protein coupled receptors except 3
- 1- decreases cAMP while 4-7 increase
- 3- ligand gated cation channel

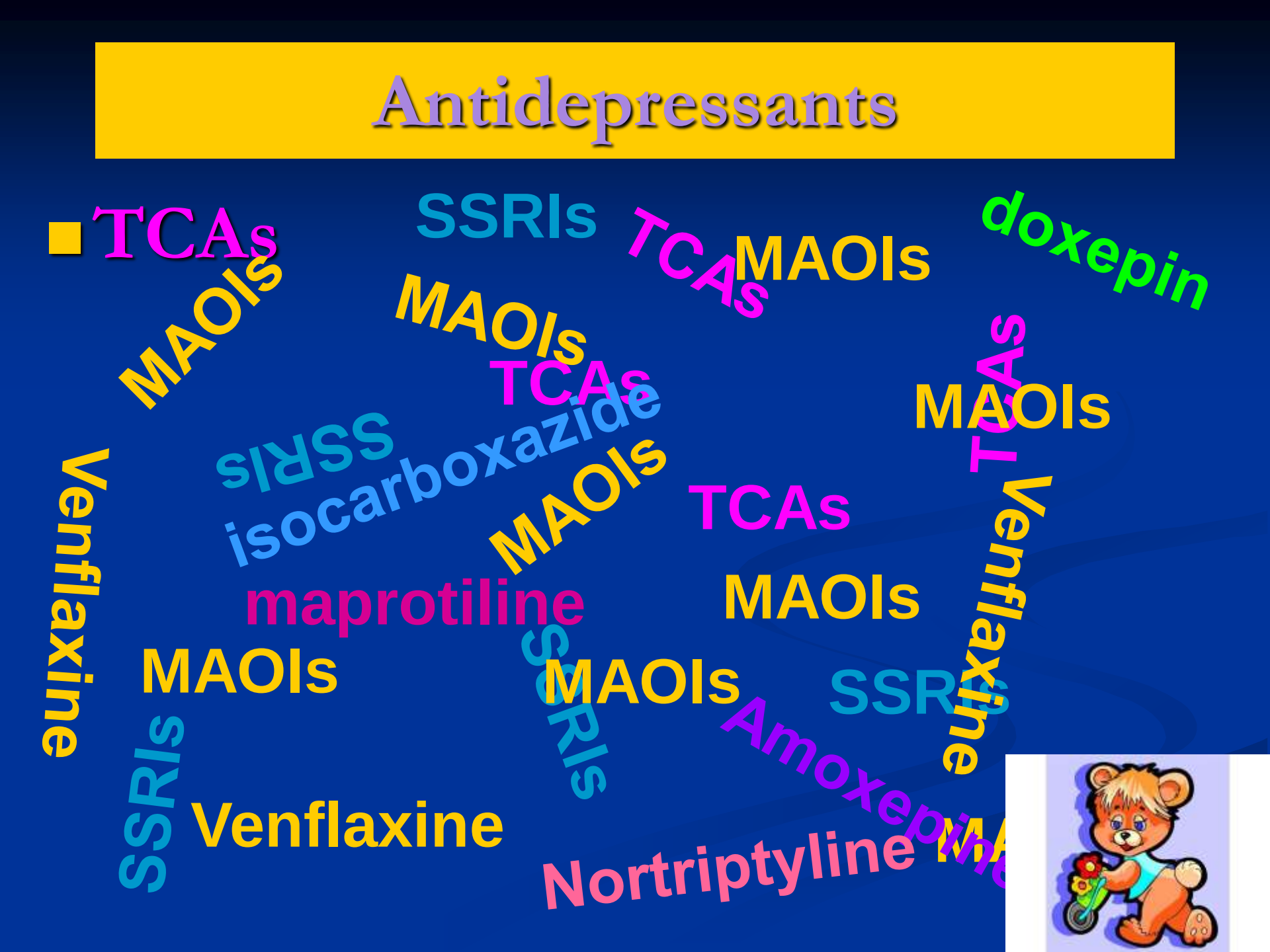
Alternative Therapies

No way of *a priori* knowing which therapy will be best for a patient.

- **Light Therapy**
- **Psychological Treatment**
- **ECT** (patients with suicidal tendency and for quick action)
- **St. John's Wort (Plant)**

Antidepressants

- TCAs



Reversible inhibitor of MAO-A (RIMAs)

Moclobemide ,Clorgyline

- (Isocarboxacid, phenelzine, tranylcypromine.)

- CM
- RITA-Don't- Copy
- Exams-For-PCS
- MAD-Boy-turned-violent

ANTIDEPRESSANTS

Tricyclic antideprssants (TCAs)

NA + 5 HT reuptake inhibitor

Imipramine, Amitriptyline

Trimipramine, Doxepin

Dothiepin, Clomipramine

Predominantly NA reuptake inhibitor

Desipramine, Nortriptyline

Amoxapine, Reboxetine

Selective serotonin reuptake inhibitors (SSRIs)

Fluoxetine, Fluvoxamine

Paroxetine, Sertraline

Citalopram, Escitalopram

Atypical antidepressants

Trazodone, Mianserine

Mirtazapine, Venlafaxine

Duloxetine,Tianeptine

Amineptine, Bupropion

Mechanism of Action

1. Inhibition of MAO enzymes.
   (MAOIs).
2. Inhibition of NE and 5-HT reuptake.
   (TCAs, SSRIs, Newer TCAs).

ATYPICAL

3. Prominent alpha blocking and weak 5-HT antagonists.
   (Nefazodone, trazodone,)
4. Serotonin and noradrenalin reuptake inhibitor (SNRIs)
   (venlafaxine, duloxetine)
5. Noradrenergic and specific serotonergic antidepressants (NaSSA)
   (Mirtazapine)
6. Inhibitor of Dopamine and Noradrenalin
   (Bupropion)
7. Blockade of pre-synaptic alpha 2 receptors
   (Mianserin)
8. Increases rather than inhibiting 5-HT uptake
   (Tianeptine, Amineptine)

MAO (monoamine oxidase) an enzyme Two types

MAO – A

-Peripheral adrenergic nerve endings

-Intestinal mucosa

-Human placenta

-Liver

-Serotonin , Noradrenalin and dopamine

-Inhibited by ***moclobemide*** and clorgyline

MAO-B

-brain (basal ganglia)

-Platelets

-Liver

-Deaminates dopamine

-Inhibeted by selegiline (deprenyl)

A-B

C-D

Isoniazide, iproniazide, phenelzine, isocarboxazide,tranylcypromine were non selective and irreversible inhibitors (Hit and run drugs) used previously but not used now due to drug drug and drug food interactions. Linezolide (new drug against MRSA) Cheese and serotonin syndrome

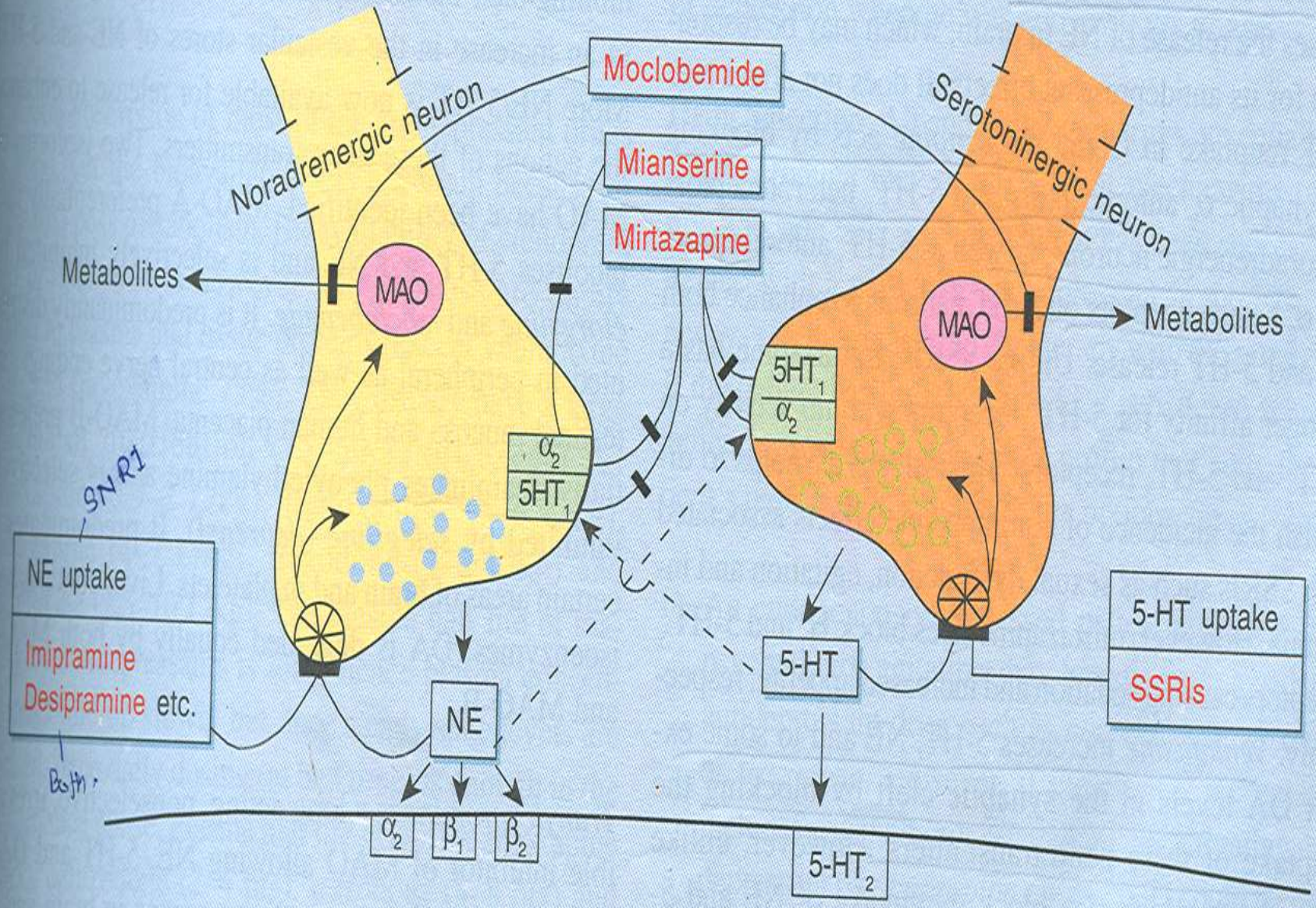


Fig. 36.1 Potential Sites of Action of Some Antidepressant Drugs.

Nonselective MAOIs not favorable Cheese Reaction

Cheese, beer, wine,
meat, fish, yeast,
(contain large amount of
tyrammine and other
indirectly acting amines)

Due to irreversible block of MAO These escape degradation in intestinal wall and liver

Reach to circulation Displace large amount of noradrenalin from loaded nerves

- *Hypertensive crises*, CVA
- Medical Emergency

Tt. I.V. Phentolamine, Prazosin

Nonselective MAOIs not favorable

- **Cold and Cough medicines contain Ephedrine**

(Same result as cheese reaction)

- Levodopa- excitement and hypertension
- Tricyclic antidepressants- excitement, rise in BP, temperature

Reversible inhibitor of MAO-A (RIMAs)

- Moclobemide-
  - **Reversible** and selective MAO-A inhibitor
  - Short duration of action
  - Competitive enzyme inhibition
  - Tyramine is able to displace it
  - Cheese reaction is less likely
  - **Devoid of anticholenergic, sedative, cognitive, cardiovascular effects**
  - Good for **elderly** with heart diseases

Tricyclic Antidepressants (TCAs)

- **Imipramine** represents the class (Prototype)
- Inhibit monoamine reuptake
  (serotonin and noradrenalin)
- Increase the concentration of ***Serotonin and NA*** at synapse and potentiate the action (therapeutic effects)
- Other receptors acted (Adverse effects)
  - **Muscarinic- Anticholinergic side effects (dryness etc.) #**
  - **Alpha- alpha blocking actions (postural hypotension etc.) #**
  - **Histamine-Antihistaminic (sedation) #**
  - ***Dopamine- antipsychotic (amoxapine, maprotiline)***

TCAs actions (CNS)

- In Normal person
  - Tiredness
  - Light-headedness
  - Sleepiness
  - Difficulty in thinking
  - Difficulty in concentration,
  - Gait disturbances
  - Provoke anxiety
  - Unpleasant

- In Depressed

-Sedation immediately

-Elevation of mood (2-4Weeks)

-Suppresses REM prolongs total sleep duration

Lower seizure threshold and produce convulsions in overdose

Don't carry abuse potential, Development of dependence is less

TCAs uptake blockade is *not* directly responsible for antidepressant action?

- Uptake blockade occurs quickly but antidepressant action occurs after months
- Initially

Pre synaptic alpha 2 and 5-HT1 auto receptors are activated by increased amount of NA and Serotonin in synaptic cleft resulting in decreased firing

- But on long term

desensitize and down regulation of these receptors and induce adaptive changes in the number and sensitivity of receptors and amine turnover leading to enhanced NA and Serotonin transmission required for antidepressant action.

TCAs on other systems

- **ANS**
  - ***Potent anticholinergic***

    (dry mouth, blurring of vision,, constipation, urinary hesitancy)
  - Weak alpha 1 blocking

    (postural hypotension, impairment of ejaculation,)
  - H1 antihistaminic

    (sedation)
- **CVS**
  - Tachycardia
  - Postural hypotension
  - Cardiac *arrhythmias* (T wave suppression or inversion) due to intra ventricular conduction interference due to NA and Anti cholinergic actions

Tolerance to Anticholinergic and hypotensive actions develop latter on

TCAs (Pharmacokinetics)

- Good oral absorption
- Highly bound to Proteins (plasma and tissue)
- Metabolized in liver (oxidation, glucuronide conjugation and CYP2D6, CYP3A4, CYP1A2
- Many active metabolites may be produced
- Mostly can be given once a day (at bed)
- Have *Therapeutic Window* phenomenon (50-200ng/ml of imipramin)

TCAs Adverse effects

- ***Anticholinergic***- dry moth, bad taste, constipation, epigastric fullness, urinary retention (more common in elderly male), blurred vision, palpitation
- **Sedation**, mental confusion, weakness
- Increased appetite and weight
- Sweating, fine tremors
- Precipitation of seizures
- **Postural hypotension**
- **Cardiac arrhythmias**
- Rashes and jaundice

TCAs (Acute Poisoning)

- Usually suicidal attempt
- Presents as
  - Excitement
  - delirium,
  - Anticholinergic symptoms like atropine poisoning
  - Muscle spasm
  - Convulsions
  - Respiratory depression
  - Coma
- Treatment
  - Gastric lavage
  - I.V. line
  - Oxygen
  - Maintenance of BP and Temperature
  - Diazepam iv
  - Propranolol / lignocain

TCAs (Interactions)

- Potentiation of sympathomimetics (direct acting)
- Reduce action of sympathomimetics (indirect acting)
- Reduce antihypertensive action of guanethidine and clonidine (by preventing their transport in to neurons)
- Potentiate other CNS sedatives
- SSRIs inhibit metabolism of TCAs
- With MAO inhibitors dangerous hypertensive crisis with excitement and hallucinations
- Retard the absorption of other drugs
- Phenytoin, phenylbutazone, chlorpromazine, aspirin, displace TCAs and produce toxicity
- Phenobarbitone induce metabolism and inhibit the effect of the drug

Miscellaneous

- **Amoxapine**
  - Tetra cyclic compound
  - *Blocks D2* reuptake also
  - Has mixed antidepressant and neuroleptic effects
  - Good for psychotic depression
- **Reboxetine**
  - Selective NA reuptake blocker
  - Weak action on 5-HT mechanism
  - Anticholinergic effects are minimal

Selective Serotonin Reuptake Inhibitors (SSRIs)

- **Limitations of TCAs**
  - Anticholinergic effects
  - Alpha blocking action
  - Cardio toxicity
  - Sedation, seizures ppt
  - Low safety margin
  - Weight gain
  - Therapeutic window
  - Overdose poisoning common
  - Lag of 1 month period
  - Incomplete response to Tt

- Answers may be given by SSRIs
  - **Selectively inhibit membrane associated SERT (serotonin transporter)**
  - **More tolerability and better acceptability**
  - **Used in depression as well as in OCD, phobias**
  - **No sedation, No seizure ppt**
  - **No alpha blocking action**
  - **Less chances of arrhythmia**
  - **No weight gain**
  - **Now 1st choice for OCD, Panic disorders, Social Phobia, Eating disorders, Premenstrual syndrome, Post traumatic stress**

Important points

- TCAs have slightly more efficacy
- Some patients not responding to TCAs may respond to SSRIs,
- SSRIs preferred in prophylaxis of recurrent depression
- In severe depression TCAs appear to be more efficacious

Individual compounds

- **Fluoxetine**
  - Prototype of SSRIs
  - Longest acting
- **Paroxetine**
  - Short acting
  - More GI side effects
- **Fluvoxamine**
  - Short acting
  - Commonly used in indoor patients
- **Sertraline**
  - Less chances of drug interactions due to low potency to cause cytochrome enzyme depression

❑Citalopram

•Similar to sertraline but should be avoided in patients attempting suicide

❑Escitalopram

•Active enantiomer of citalopram side effects are less

SSRIs

- **Side effects**
  - **Gastric upset**
  - **Nausea**
  - **Interfere with ejaculation**
  - **Nervousness**
  - **Restlessness**
  - **Insomnia**
  - **Anorexia**
  - **Headache**
  - **Diarrhea**
  - **Epistaxis**
  - **Ecchymosis**
- **Others**
  - ***Inhibit*** **cytochrome enzymes** and elevate the plasma level of other drugs
  - Other serotonergic drug (MAOIs) is taken may precipitate **Serotonin Syndrome** manifesting as agitation, restlessness, sweating, twitching, convulsions

Atypical Antidepressants

- Trazodone
  - Blocks 5-HT uptake
  - Has prominenent alpha blocking
  - Weak 5-HT2 antagonistic
  - No anticholinergic effect
  - Bradycardia
  - Has anxiolytic action also
  - Prolonged and painful penile erection (priaprism)

- Mianserin
  - **Unique** not inhibit NA and 5-HT uptake
  - Blocks pre-synaptic alpha 2 receptors increases release and turnover of NA
  - Antagonist at serotonin 2, 1c, and H1 receptors
  - Has sedative effect
  - Damages liver and bone marrow (Reserve drug)

Atypical Antidepressants

- Tianeptine / and Amineptine
  - ***Increases*** rather inhibiting 5-HT uptake
  - Neither sedative nor stimulant
  - Effective in anxiodepressive states
- Mirtazapine (NaSSA)
  - Noradrenergic and specific serotonergic antidepressant
  - Blocks alpha 2 auto receptor (on NA neuron) and hetero- (on 5-HT neuron) receptors increasing both NA and serotonin release.
- Venlafaxine / Duloxetine
  - SNRI selective in action
  - Faster onset of action
  - Increases BP
  - Duloxetine increases uretheral tone used in urinary incontinence (over active bladder)
- Bupropion
  - Inhibits DA and NA uptake has excitant effect
  - Used to reduce smoking

Antidepressant uses

- Depression (ECT may be needed in severely depressed and patients having suicidal tendency)
- Bipolar affective disorders TCAs and lithium or SSRIs with lithium or valporate/ lamotrigine
- SSRIs with atypical antipsychotic in psychotic depression
- Obsessive compulsive disorders (SSRI and Clomipramine)
- Eating disorders

- Anxiety disorders
- ***Neuropathic pain***
- Attention deficit hyperactivity disorder in children
- Enuresis- (Imipramine 25mg at night)
- Overactive bladder (stress incontinence)
- Migraine prophylaxis
- Pruritus (Topical doxepin)

Antianxiety Drugs

- Anxiety - emotional state
  - Unpleasant
  - Associated with uneasiness
  - Discomfort
  - Fear
  - Undefined threat
  - Fear about future

Some amount of anxiety is must for progress

When it becomes excessive, disproportionate, hampers performance

then only

needs treatment

Antianxiety Drugs

- Drugs producing restful state of mind without interfering with normal mental or physical functions.
- Have no effect on thought control
- Don't produce extra pyramidal side effects
- Can Produce physical dependence
- May Have abuse potential
- Don't selectively block conditioned avoidance response in animals
- ***Have anticonvulsant activity***

Antianxiety Drugs

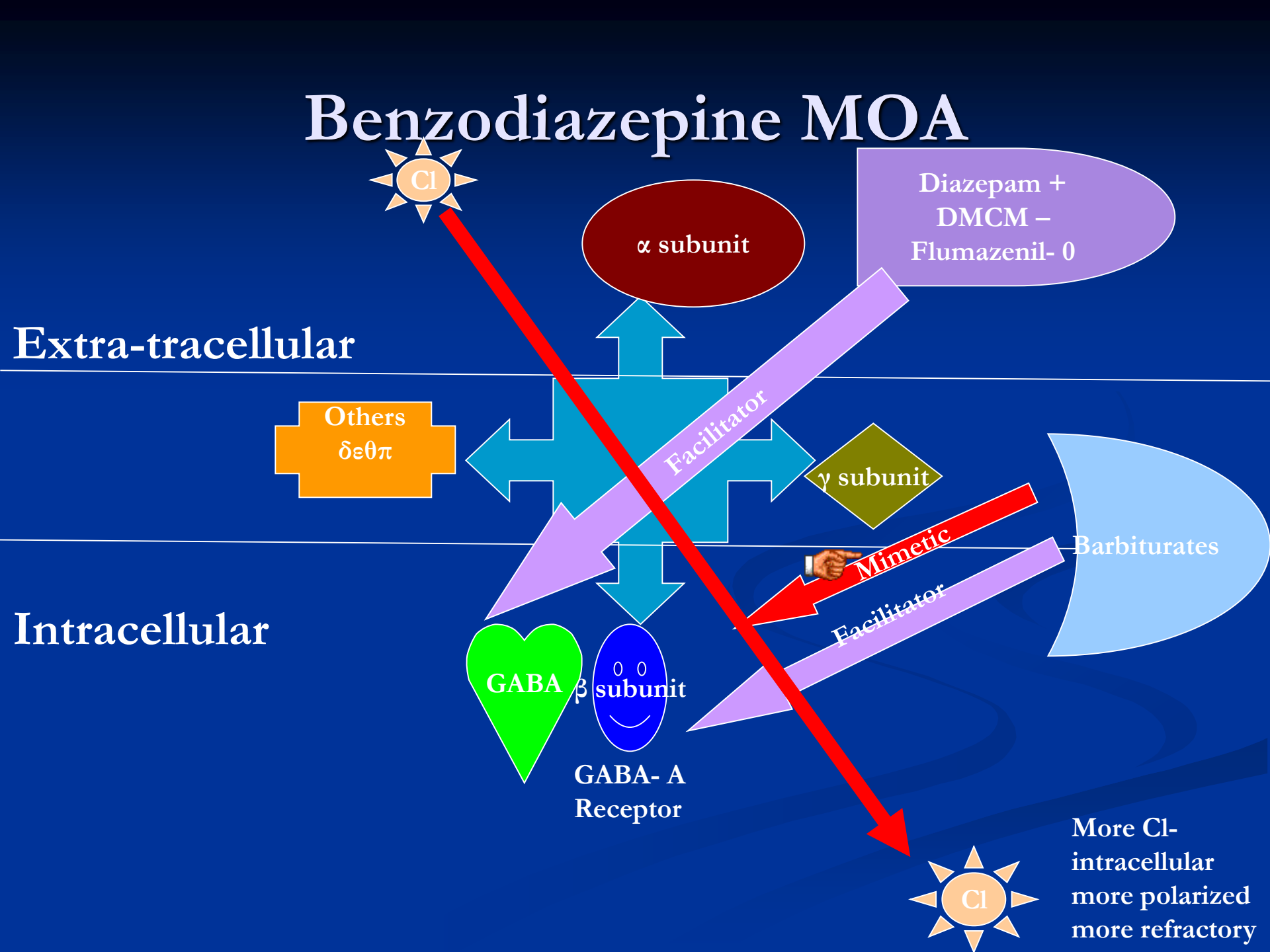
- Benzodiazepine
  - Diazepam
  - Chlordiazepoxide
  - Oxazepam
  - Lorazepam
  - Alprazolam
- Azapirones
  - Buspirone
  - Gepirone
  - Ispapirone
- Others
  - Beta blocker- **Propranolol**
  - Antihistaminics- Hydroxyzine
  - SSRIs and other antidepressant drugs

PHO/BIG/DOCLA

Benzodiazepines

- Relieve anxiety at low dose (higher dose induce sleep and impair performance)
- Selective taming effect
- More selective to limbic system
- Have low side effects in Antianxiety dose
- Lorazapam and clonazepam IM for psychotic and manic patients
- Act by ***facilitating*** GABAergic transmission

Benzodiazepine MOA



Benzodiazepines

- Adverse effects
  - Sedation
  - Light headedness
  - Psychomotor impairment
  - Cognitive impairment
  - Vertigo
  - Confusional state
  - Increased weight
  - Impaired sexual functions
  - Potential to produce dependence
  - All are almost similar selection is empirical

Benzodiazepines (Individual drugs)

- **Chlordiazepoxide**
  - First BZD
  - Long lasting effect
  - Chronic anxiety
- **Diazepam**
  - Has two phase of metabolism
  - Broken in to active metabolites
  - Long duration of action
- **Oxazepam**
  - Polar compound
  - Penetration In brain is slow
  - No active metabolite
  - Used in short lasting anxiety state
- **Lorazepam**
  - Less lipid soluble
  - Slow entry in brain
  - No active metabolite
  - IM

Alprazolam-

high potency, mood elevating in depressed pt. less drowsiness

Buspirone

- Does not produce sedation, cognitive impairment,
- Does not interact with BZD receptor or modify GABAergic transmission
- No tolerance
- No physical dependence
- No muscle relaxant
- No anticonvulsant property

Buspirone

- Relieves mild to moderate generalized anxiety
- Effects develop slowly (not used for acute)
- ***Partial agonist on 5HT1A (pre-synaptic) and antagonist on 5HT postsynaptic receptors***
- Presynaptic auto-receptors stimulated leading to reduced activity of dorsal raphe serotonergic neurones
- Also has weak D2 blocking effect

- Hydroxazine
  - H1 antihistaminic
  - Sedative, anti -emetic and spasmolytic
  - Anti - Pruritus

- Propranolol
  - Reduces sympathetic symptoms like rise in BP, Tremors, sweating etc.
  - Performance or situational anxiety

  (like examination fear, social phobia, public lecture)

Questions

- Classify antipsychotic drugs
- Classify antidepressant drugs
- Pharmacological actions of chlorpromazine
- Pharmacological actions of amitriptyline
- Drug indued parkinsonism
- MOA of antipsychotic
- MOA of antidepressants
- Lithium

Questions

- Drug of choice of cheese reaction-Phentolamine
- Moclobemide is reversible and selective MAO-A inhibitor
- All antidepressants don't inhibit DA uptake except amoxapine, maprotiline, Bupropion
- Antidepressants don't carry abuse potential
- SSRIs are inhibitor of CYP enzymes
- Serotonin syndrome
- Trazodone – may produce priaprism due to high α1 blocking property
- Mianserin unique not inhibiting NA or 5HT but blocks Pre-synaptic α2 receptors
- Tianeptine, Amineptine - unique increase 5-HT uptake
- Venlafaxine, Duloxetine – SNRI
- Mirtazapine- NaSSA- blocks α2 auto and hetro receptors and enhance NA and 5HT release
- Nocturnal enuresis- Imipramine
- Benzodiazepines- GABA facilitatory
- Buspirone- partial agonist at 5HT1A autoreceptors,antagonist at 5HT1A postsynaptic
- DOC of performance or situational anxiety- B-blockers

Thanks

Antipsychotic drugs

Presented By
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Phenothiazines

- Aliphatic side chain:
  -Chlorpromazine
  -Trifluprомazine

- Piperidine side chain:
  -Thioridazine

- Piperazine side chain:
  -Trifluoperazine
  -Fluphenazine

- Butyrophenones
  -Haloperidol
  -Trifluperidol
  -Penfluridol

- Thioxanthenes
  -Flupenthixol

- Other heterocyclics
  -Pimozide, Loxapine

- Atypical antipsychotics

  -Clozapine

  -Risperidone

  -Olanzapine

  -Quetiapine

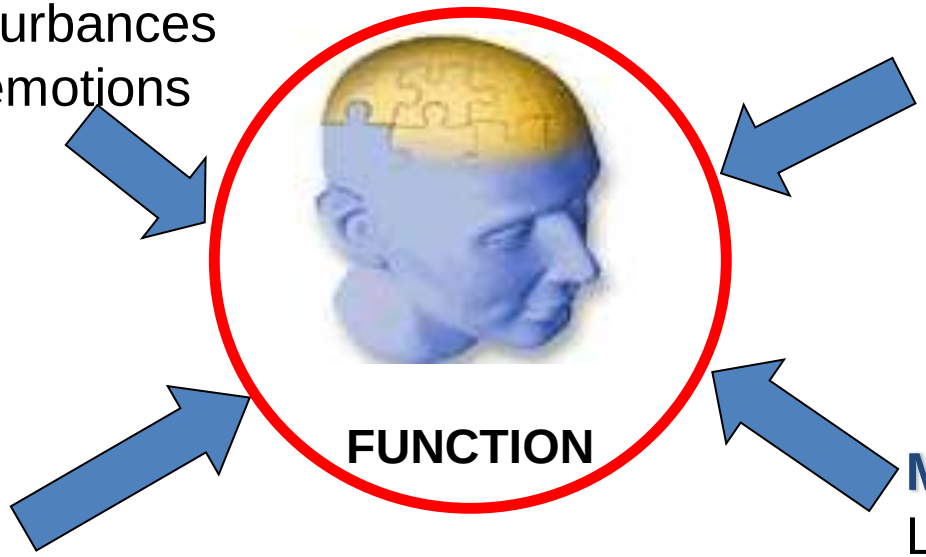
  -Aripiprazole

  -Ziprasidone

Schizophrenia - symptoms

Positive Symptoms(↑↑DA)
Hallucinations
Delusions (bizarre, persecutory)
Disorganized Thought
Perception disturbances
Inappropriate emotions

Negative Symptoms(↓↓NMDA)
Blunted emotions
Anhedonia
Lack of feeling



Cognition
New Learning
Memory

Mood Symptoms
Loss of motivation
Social withdrawal
Insight
Demoralization
Suicide

- **Positive/active symptoms** include thought disturbances, delusions, hallucinations

- **Negative/passive symptoms** include social withdrawal, loss of drive, diminished affect, paucity of speech. impaired personal hygiene

Prognosis of Schizophrenia

- 10% continuous hospitalization
- ≤ 30% recovery = symptom-free for 5 years
- 60% continued problems in living/episodic periods

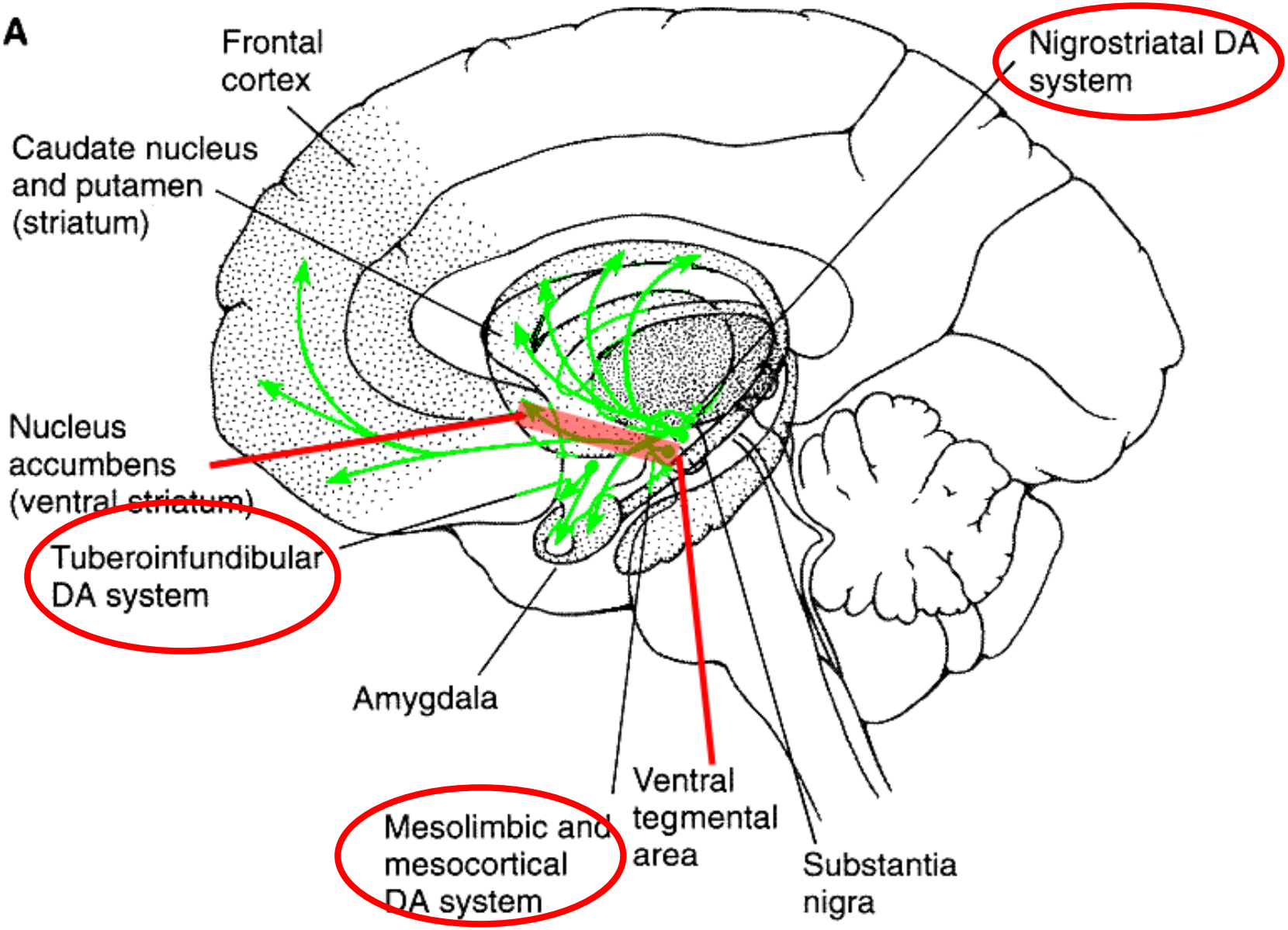
Etiology

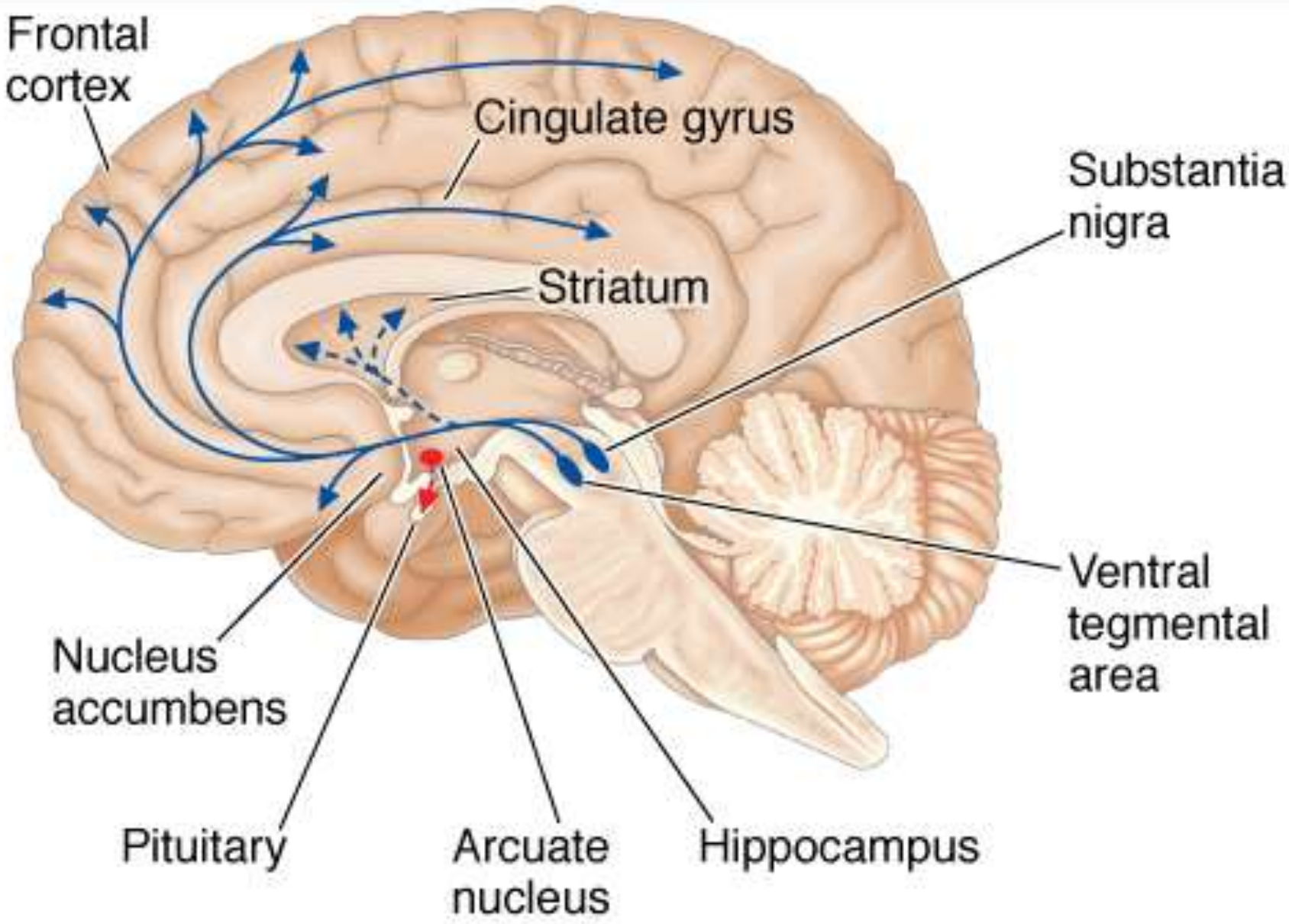
- A gene encodes for neuregulin-1 has been associated with schizophrenia.
- Hereditary Influences may account for 10% of schizophrenia cases
- Prenatal Biological Trauma 5-10% cases of schizophrenia
- Perinatal biological trauma

Schizophrenia Pathophysiology

	Schizophrenia Pathophysiology	Pharmacologic Profile of APDs
Past	Excess dopaminergic activity	Dopamine D_2-receptor antagonists
Present		
	-Renewed interest in the role of serotonin (5-HT)	Combined 5-HT_2/D_2 antagonists
Future		
	-NMDA	NMDA agonists
	Imbalance in cortical communication and cortical-midbrain integration, involving multiple neurotransmitters	More selective antagonists Mixed agonist/antagonists Neuropeptide analogs

Dopaminergic Pathways and Innervation




Frontal cortex
Cingulate gyrus
Substantia nigra
Striatum
Ventral tegmental area
Nucleus accumbens
Pituitary
Arcuate nucleus
Hippocampus

Schizophrenia - Dopamine Hypothesis

- Repeated administration of stimulants like **amphetamines and cocaine**, which enhance central dopaminergic neurotransmission, can cause a psychosis that resembles the positive symptoms of schizophrenia.

- Low doses of amphetamine can induce a psychotic reaction in schizophrenics in remission.

- Some early studies with postmortem tissue revealed increased numbers of DA receptors (in particular D2-like) in schizophrenic patients

Serotonin Hypothesis of Schizophrenia

- Hallucinogens such as **LSD** (lysergic acid diethylamide) and **mescaline** are serotonin (5-HT) agonists
- 5-HT_{2A}-receptor blockade is a key factor in the mechanism of action of the main class of atypical antipsychotic drugs such as clozapine and quetiapine.
- 5-HT_{2A}-receptor modulate the release of dopamine in the cortex, limbic region, and striatum.

Schizophrenia - Glutamate Hypothesis

- Preclinical as well as clinical studies provide evidence of **hypofunction of NMDA receptors** as a primary, or at least, a contributory process in the pathophysiology of schizophrenia
- Several clinical trials with agents that act at the glycine modulatory site on the NMDA receptor have revealed consistent reductions in negative symptoms and variable effects of cognitive and positive symptoms
- These studies also provide evidence that suggests the effects of **clozapine** on negative symptoms and cognition may be through **activation of the glycine modulatory site on the NMDA receptor**.

ANTIPSYCHOTICS

- Pre-90's
  - "Typical", conventional, traditional neuroleptics, major tranquilizors
  - Modeled on D_2 antagonism
  - EPS/TD
- Post-90's
  - "Atypical", novel, 2^{nd} generation
  - Modeled on 5-HT2/D2 antagonism
  - Less EPS, prolactin effects
  - Weight gain, sedation, diabetes

Adverse Effects

- **Sedation** - initially considerable; tolerance usually develops after a few weeks of therapy; dysphoria
- **Postural hypotension** - results primarily from adrenergic blockade; tolerance can develop
- **Anticholinergic effects** - include blurred vision, dry mouth, constipation, urinary retention; results from muscarinic cholinergic blockade
- **Endocrine effects** - increased prolactin secretion can cause galactorhea; results from antidopamine effect
- **Hypersensitivity reactions** - jaundice, photosensitivity, rashes, agranulocytosis can occur
- **Idiosyncratic reactions** - malignant neuroleptic syndrome
- **Weight gain**
- Neurological side effects -

Neurological Side Effects of antipsychotics

REACTION	FEATURES	TIME OF MAXIMAL RISK	PROPOSED MECHANISM	TREATMENT
Acute dystonia	Spasm of muscles of tongue, face, neck, back; may mimic seizures; *not* hysteria	1 to 5 days	Unknown	Diphenhydramine, promethazine
Akathisia	Motor restlessness; *not* anxiety or "agitation"	5 to 60 days	Unknown	Reduce dose or change drug: antiparkinsonian agents, benzodiazepines or propranololc may help
Parkinsonism	Bradykinesia, rigidity, variable tremor, mask facies, shuffling gait	5 to 30 days	Antagonism of dopamine	Antiparkinsonian agents helpful-trihexyphenidyl, procyclidine, benztropines

a. Many drugs have been claimed to be helpful for acute dystonia. Among the most commonly employed treatments are diphenhydramine hydrochloride, 25 or 50 mg intramuscularly, or benztropine mesylate, 1 or 2 mg intramuscularly or slowly intravenously, followed by oral medication with the same agent for a period of days to perhaps several weeks thereafter. **b.** For details regarding the use of oral antiparkinsonian agents, *see* the rest of slides **c.** Propranolol often is effective in relatively low doses (20-80 mg per day). Selective beta1-adrenergic receptor antagonists are less effective. **d.** Despite the response to dantrolene, there is no evidence of an abnormality of Ca2+ transport in skeletal muscle; with lingering neuroleptic effects, bromocriptine may be tolerated in large doses (10-40 mg per day).

REACTION	FEATURES	TIME OF MAXIMAL RISK	PROPOSED MECHANISM	TREATMENT
Neuroleptic malignant syndrome	Catatonia, stupor, fever, unstable blood pressure, myoglobinemia; can be fatal	Weeks; can persist for days after stopping neuroleptic	Antagonism of dopamine may contribute	Stop neuroleptic immediately: dantrolene or bromocriptine may help: antiparkinsonian agents not effective
Perioral tremor ("rabbit" syndrome)	Perioral tremor (may be a late variant of parkinsonism)	After months or years of treatment	Unknown	Antiparkinsonian agents often help
Tardive dyskinesia	Oral-facial dyskinesia; widespread choreoathetosis or dystonia	After months or years of treatment (worse on withdrawal)	Excess function of dopamine hypothesized	Stop neuroleptics, then Diazepam, Change to clozapine/olanzapine,

Adverse Effects - EPS

Details on two main extrapyramidal disturbances (EPS):

- Parkinson-like symptoms
  - tremor, rigidity
  - direct consequence of block of nigrostriatal DA_2 R
  - reversible upon cessation of antipsychotics
- Tardive dyskinesia
  - involuntary movement of face and limbs
  - less likely with atypical antipsychotics (AP)
  - appears months or years after start of AP
  - ? result of proliferation of DA R in striatum
    - » presynaptic?
  - treatment is generally unsuccessful

Weight gain – 40% - weight gain now attributed to ratio of binding to D2 and 5-HT2 receptors; possibly also histamine (for newer antipsychotics anyway)

Sexual dysfunction

- result from NE and SE blockade
- erectile dysfunction in 23-54% of men
- retrograde ejaculation in
- loss of libido and anorgasmia in men and women

Seizures - <1% for generalized grand mal

Neuroleptic malignant syndrome (1-2% early in trt)

- combination of motor rigidity, hyperthermia, and autonomic dysregulation of blood pressure and heart rate (both go up)
- can be fatal in 5-20% of cases if untreated
- treatment – discontinue meds; give trts for fever and cardiac problems

Sensitivity to sun

- some phenothiazines collect in skin (chlorpromazine)
- sunlight causes pigmentation changes – grayish-purple (look bruised)
- in eye, brown cornea, brownish cloud to vision and possibly permanent impairment

Agranulocytosis - <1% (with clozapine)

- reduced white blood cell count
- lowered resistance to infection
- can be fatal

Jaundice – elevated bilirubin in liver - < ½%

Limitations Of Conventional Antipsychotics

- Approximately one-third of patients with schizophrenia fail to respond
- Limited efficacy against
  - Negative symptoms
  - Affective symptoms
  - Cognitive deficits
- High proportion of patients relapse
- Side effects and compliance issues

Antipsychotic Drugs – New Generations “atypical”

About 40-60% do not respond to phenothiazines or cannot handle side effects

- Questions remain about the efficacy of phenothiazines and haloperidole for negative symptoms
- Drugs needed that are low in extrapyramidal side effects and at least equal in efficacy for positive symptoms, perhaps better for negative

Antipsychotic Drugs – New Generations "atypical"

- clozapine
- risperidone
- olanzapine
- sertindole
- Quetiapine
- Aripiprazole
- Ziprasidone

Clozapine (1989)

- Selectively blocks dopamine D2 receptors, avoiding nigrostriatal pathway
- **α**-blockade
- Also blocks **H_1**
- More strongly blocks **5-HT2** receptors in cortex which then acts to modulate some dopamine activity
- Among non-responders to first generation meds or those who cannot tolerate side effects, about 30% do respond to Clozapine

Clozapine

- Extrapyramidal side effects are minimal
- May help treat tarditive dyskinesia
- S/E- orthostatic hypotension effects, sedation, weight gain, increased heart rate
- Increased risk for **seizures** (2-3%)
- **Agranulocytosis in 1%**
- Agranulocytosis risks increase when co-administered with carbamazepine

Risperidone (1994)

- Fewer side effects than Clozapine
- Marketed as first line approach to treatment
- Blocks selective D2, norepinephrine, and 5-HT2
- effective for positive and negative symptoms
- Extrapyramidal side effects low (but are shown at high doses)
- Shares sedation, weight gain, rapid heart beat, orthostatic hypotension, and elevated prolactin
- No agranulocytosis risks
- Increased risk of stroke in elderly
- May cause anxiety/agitation (possible OCD)

Olanzipine – 1996

- Improved negative symptom reduction
- Argued to be better than risperidone in extrapyramidal issues
- Does not cause prolactin elevation
- reduced agranulocytosis risks

Sertindole – 1995

- Improved negative symptom reduction
- Low risk for extrapyramidal side effects – major advantage
- No sedation and very mild prolactin elevation– major advantages
- Shares orthostatic hypotension, tachycardia, and weight gain
- Common side effects are rhinitis and reduced ejaculatory volume (not associated with disturbed function)
- concern about sudden cardiac death or episodes due to cardiac arrhythmia led to its voluntary removal in 1998

Quetiapine - 1997

- No increased risks for extrapyramidal symptoms
- Shares **sedation (sleepiness),** orthostatic hypotension, weight gain
- Does cause anticholinergic side effects (like older and Clozapine) – dry mouth, constipation
- Does not elevate prolactin

Ziprasidone - 2001

- Similar to advantages of others, but argued not to cause weight gain
- May induce cardiac arrhythmias
- Also has anxiolytic and antidepressant activity

Non- psychiatric Indications

- As Antiemetics
  -chlorpromazine, prochlorperazine, haloperidol

- Anaesthesia
  -droperidol (with fentanyl)

- Intractable Hiccups
  -chlorpromazine
  -haloperidol

MCQs

Q1. A female suffering from psychosis, taking fluphenazine now complains of sudden onset of high grade fever, muscle rigidity and altered sensorium. The diagnosis is:

A. Malignant hyperthermia

B. Tardive dyskinesia

C. Akathisia

D. Neuroleptic malignant syndrome

- Ans- D - Neuroleptic malignant syndrome

Q2. Antipsychotic drug induced parkinsonism is treated by:

A. Levodopa
B. Selegiline
C. Amantadine
D. Central anticholinergic drugs

- Ans- D (Trihexyphenidyl
  Procyclidine
  Biperiden)

Q3. Least extrapyramidal side effects are seen with:

A. Clozapine
B. Haloperidol
C. Trifluoperazine
D. Chlorpromazine

- Ans- A- Clozapine

Q4. Risperidone is associated with the risk of:

A. Cerebrovascular accidents
B. Agranulocytosis
C. Diabetes Insipidus
D. Gout

- Ans- A - Cerebrovascular accidents

Q5. The antipsychotic drug that can also be used as antiemetics:

A. chlorpromazine
B. Clozapine
C. Aripiprazole
D. Loxapine

- Ans- A - chlorpromazine

Q6. The antipsychotics that can also be used as anaesthetic drug:

A. Chlorpromazine
B. Penfluridol
C. Clozapine
D. Droperidol

- **Ans- D -** Droperidol

Q7. The antipsychotic drug that can also be used to treat Intractable Hiccups:

A. Clozapine
B. Chlorpromazine
C. Haloperidol
D. Ziprasidone

- Ans- B, C - Chlorpromazine, Haloperidol

Thank you

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FLAME EMISSION SPECTROSCOPY AND ATOMIC ABSORPTION SPECTROSCOPY

Presented by:- Dr. Vijaya.U. Barge
(Vice Principal & Professor)

Learning Objectives

- After completing this sub-unit the students should be able:
- To learn principle and steps involved in flame photometry.
- To study instrumentation of flame photometry.
- To learn various components used in flame photometry.To study experimental procedure for quantitative analysis.To study interferences in flame photometry.
- To study applications and limitations of flame emission photometry.

CONTENTS

- ATOMIC ABSORPTION SPECTROPHOTOMETRY
- FLAME EMISSION SPECTROPHOTOMETRY

Atomic Absorption Flame Spectrophotometry (AAS)

AAS : is an analytical technique that measures the concentrations of elements. It makes use of the absorption of light by these elements in order to measure their concentration.

- Atomic absorption is a very common techniquefor detecting metals and metalloids in environmental samples like aluminum, Cu, lead, Li, Mg, Zn etc.

Atomic Absorption Flame Spectrophotometry (AAS)

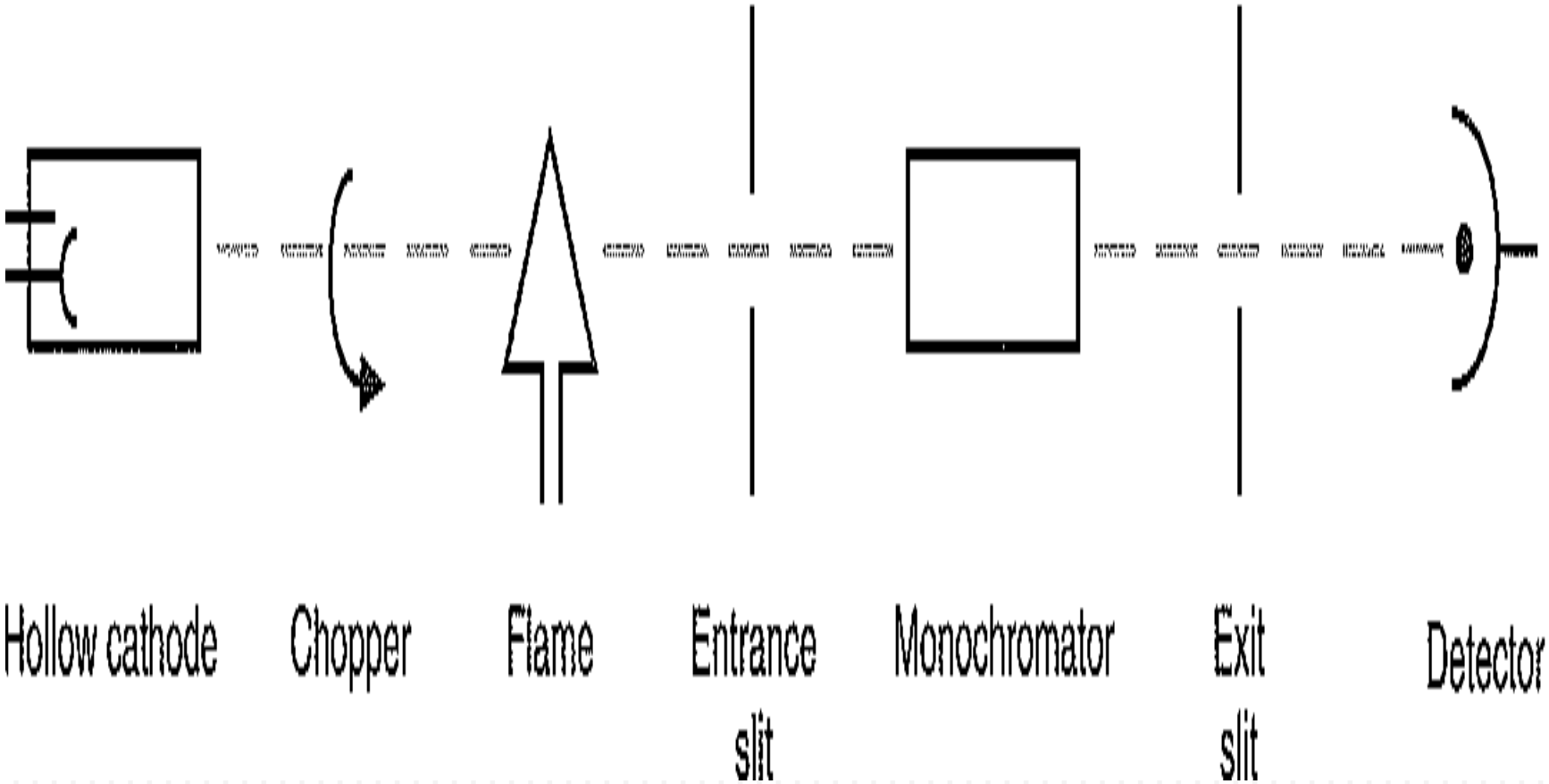
- **Basic principle**: Atomic absorption in which the element is not excited in the flame, but is merely dissociated from its chemical bond & placed in an unexcited (ground) state.

- Thus the ground state atoms capable of absorbing radiation in the flame, resulting in net ↓ in intensity of the beam from the lamp, The analyte concentration is determined from the amount of absorption.

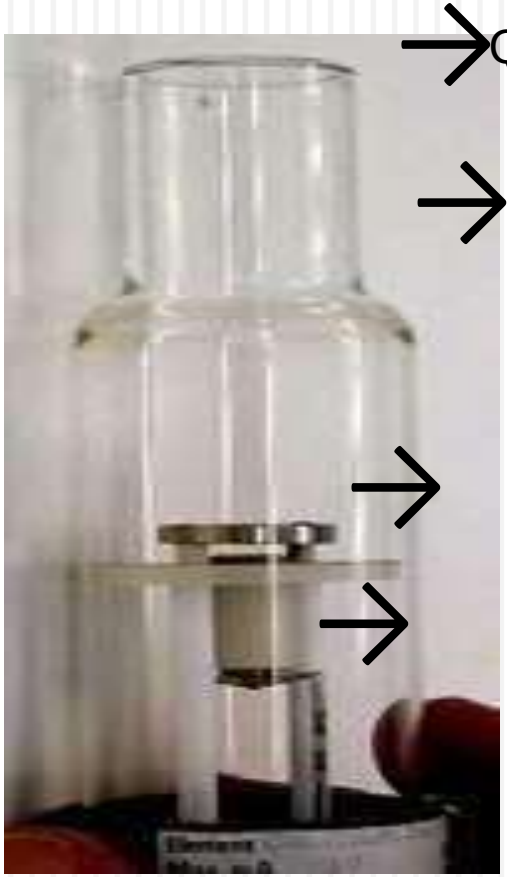
Atomic Absorption Flame Spectrophotometry (AAS)

- Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.
- Absorption bands- .001 - .01 nm.
- Entire absorption spectrum of atoms – line spectrum.

Parts Of AAS


Hollow cathode
Chopper
Flame
Entrance slit
Monochromator
Exit slit
Detector

Light Source



Hollow Cathode Lamp

- The light source is usually a hollow cathode lamp.
- It contains a tungsten anode and a hollow cylindrical cathode made of the element to be determined.
- These are sealed in a glass tube filled with an inert gas (neon or argon).
- Neon: iron & lead(reddish-orange)
- Argon: lithium(blue to purple glow)
- Each element has its own unique lamp which must be used for that analysis.

How It Works

- Applying a potential difference between the anode and the cathode leads to the ionization of some gas atoms .

- These gaseous ions bombard the cathode and eject metal atoms from the cathode in a process called sputtering. Some sputtered atoms are in excited states and emit radiation characteristic of the metal as they fall back to the ground state .

- The shape of the cathode which is hollow cylindrical concentrates the emitted radiation into a beam which passes through a quartz window all the way for absorbtion by ground state atoms in the flame.

Burner

- **Elements** to be analyzed needs to be in atomic sate.
  - Nebulization :Sample converted to aerosol

Atomization: flame, electrothermal (graphite tube) atomizers

- Flame: it is oldest and most commonly used atomizers in AAS, principally the air-acetylene flame with a temperature of about 2300 °C and the nitrous oxide (N2O)-acetylene flame with a temperature of about 2700 °C.

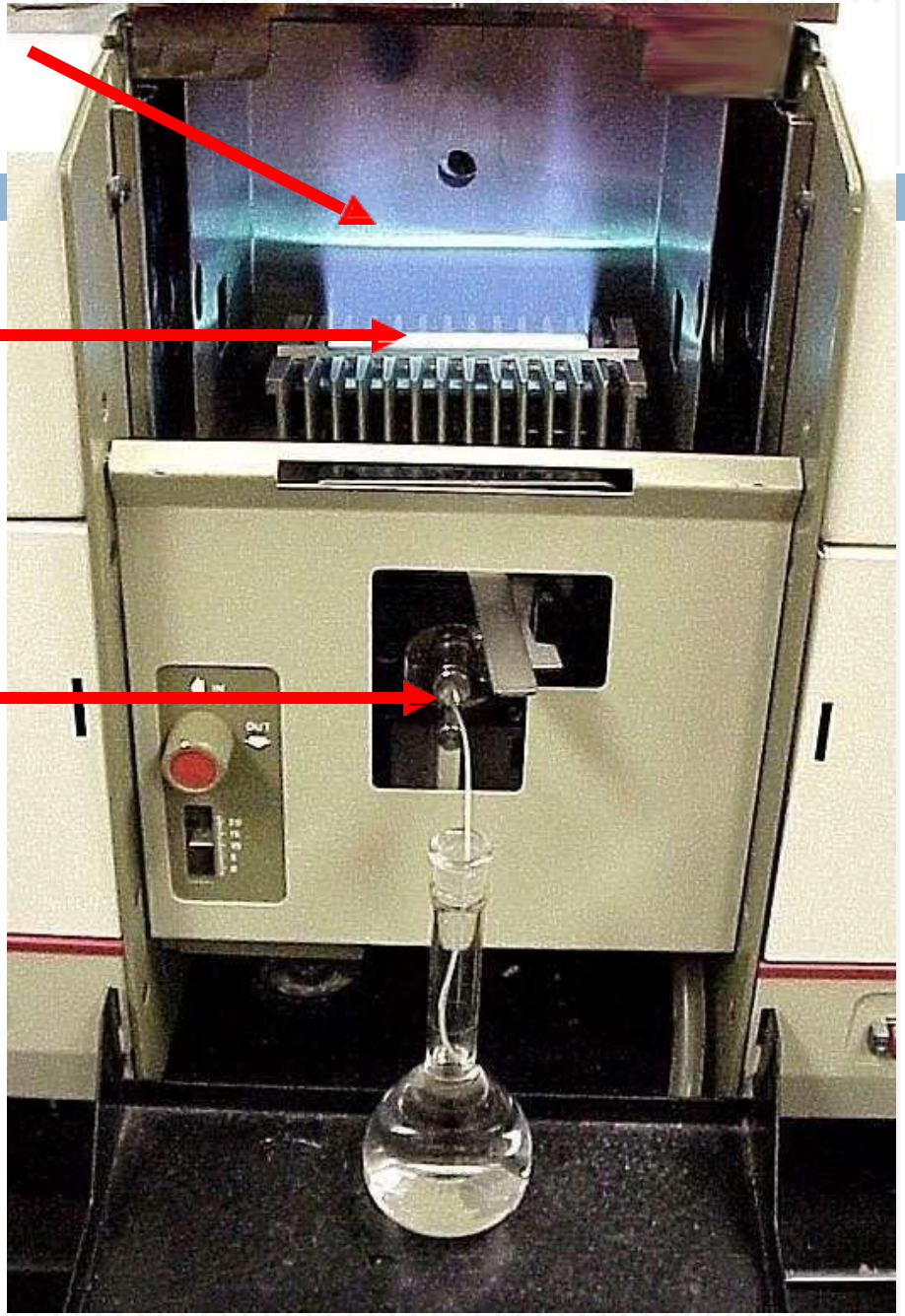
Stages In Flame

- Desolvation (drying) – the solvent is evaporated and the dry sample nano-particles remain;
- Vaporization(transfer to the gaseous phase) – the solid particles are converted into gaseous molecules;
- Atomization – the molecules are dissociated into free atoms.

Light beam

Sample is vaporized in the flame.

Aspirator tube sucks the sample into the flame in the sample compartment.



Types Of Burner

I. Total Consumption burner

- Mixing of gas and sample within flame.
- Flame is hot enough for molecular dissociations needed for some chemical systems

II. Premix long path burner/ Laminar flow burner

- Gases are mixed and sample is atomized before being burned.

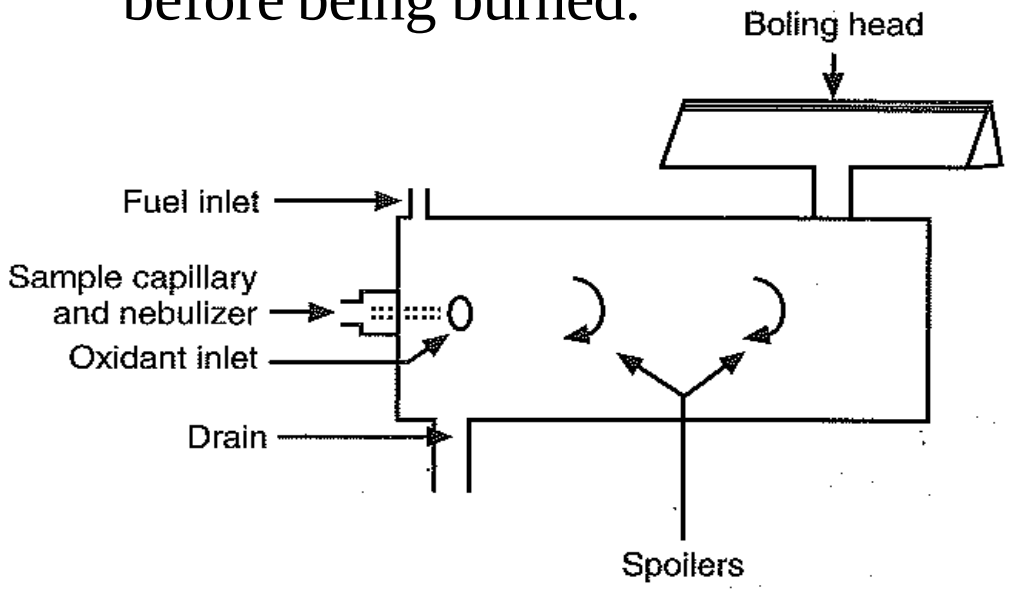


Figure 3-13 Laminar flow burner.

Advantages of long path burner

- Larger droplets go waste and only the fine mist enters the flame thus produces less noisy signal
- Path length through the flame of the burner is longer then the total consumption burner – greater absorption and increases sensitivity of measurement.

Disadvantages of long path burner

- Flame is not as hot as that of total consumption burner - cant dissociate certain metal complexes in flame- Ca- phosphate complexes.

Monochromator

- The monochromater in AAS is placed between flame and detector
- Used to select the specific wavelength of light which is absorbed by the sample, and to exclude other wavelengths.
- To allow the single line in the spectrum of analyte.
- To minimize the emission from the flame itself because detector detects photons over a wide wavelength range.

Detector And Read Out Device

- The light selected by the monochromator is directed onto a detector that is typically a photomultiplier tube whose function is to convert the light signal into an electrical signal proportional to the light intensity.

- The signal could be displayed for readout, or further fed into a data station for printout by the requested format.

AAS Applications

The are many applications for atomic absorption:

- **Clinical analysis** (blood samples: whole blood, plasma, serum; Ca, Mg, Li, Na, K, Fe)

- Environmental analysis : Monitoring our environment – *e g* finding out the levels of various elements in rivers, seawater, drinking water, air, and petrol.

- Mining: By using AAS the amount of metals such as gold in rocks can be determined to see whether it is worth mining the rocks to extract the gold .

Advantages of flame AAS	Disadvantages of flame AAS
•Inexpensive •Easy to use •High precision	-Only solution can be used -Large samples are needed 1-2ml -Less sensitive than graphite furnaces standards are not to achieve due to -flame instability - Variation in composition & temperature.

Flame Emission Spectroscopy

- Flame emission spectroscopy is also an analytical technique that is used to measure the concentrations of elements in samples

- **Principle**: atoms of some metals,

  when given sufficient heat energy (hot flame) become excited & reemit this energy at wavelengths characteristic of the element.

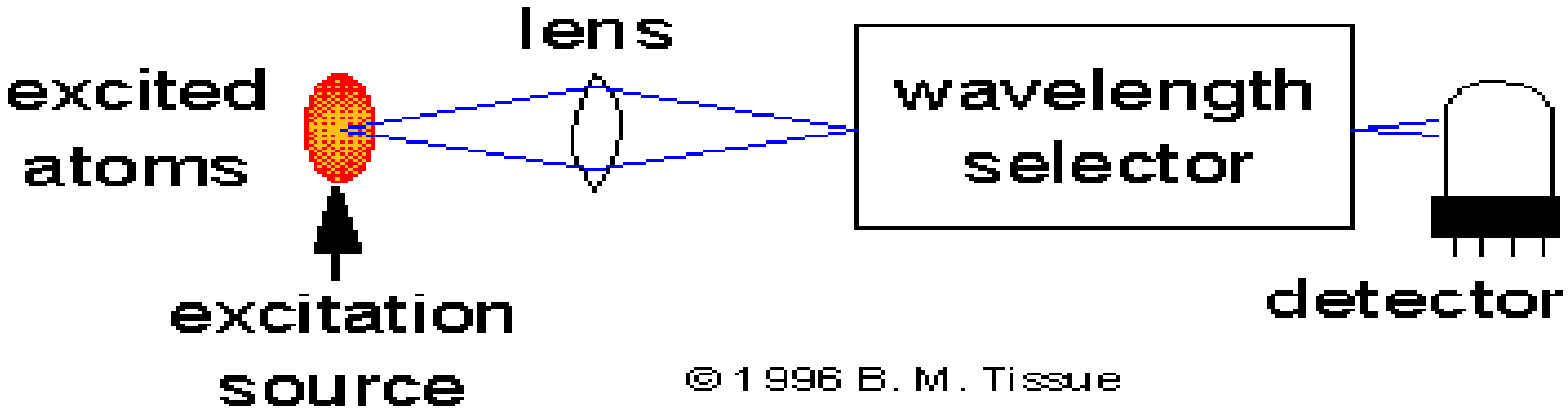
The intensity of radiant energy of characteristic wavelength produced by the atoms in the flame is directly proportional to the number of atoms excited in the flame ,which in turn is directly proportional to the concentration of the alkali metal in the sample

Flame Emission Spectroscopy

- The excited atoms decay back to lower levels by emitting light . Emissions are passed through monochromators or filters prior to detection by photomultiplier tubes.
- Alkali metals are easy to excite by flame
- Li- red emission
- Na – yellow emission
- K- red violet emission
- Rubidium- red emission
- Mg- blue emission

Flame Emission Spectroscopy

The instrumentation of flame emission spectroscopy is the same as that of atomic absorption, but without the presence of a radiation source .



In flame emission the sample is atomized and the analyte atoms are excited to higher energy levels, all in the atomizer

Flame Emission Spectroscopy

- The source of energy inAtomic Emission could be a flame like the one used in atomic absorption, or an inductively coupled plasma (ICP) .
- The flame (1700 – 3150 oC) is most useful for elements with relatively low excitation energies like sodium, potassium and calcium.
- The ICP (6000 – 8000 oC) has a very high temperature and is useful for elements of high excitation energies.

Application Of Flame Emission Spectroscopy

1. Electrons of alkali metals like sodium, potassium, lithium become easily excited hence preferentially analyzed by flame photometry.

2. Used in clinical laboratory to determine concentrations of sodium and potassium in biological fluids like serum, urine and sweat.

3. Serum lithium levels – therapeutic monitoring.

Comparison Between Atomic Absorption and Emission Spectroscopy

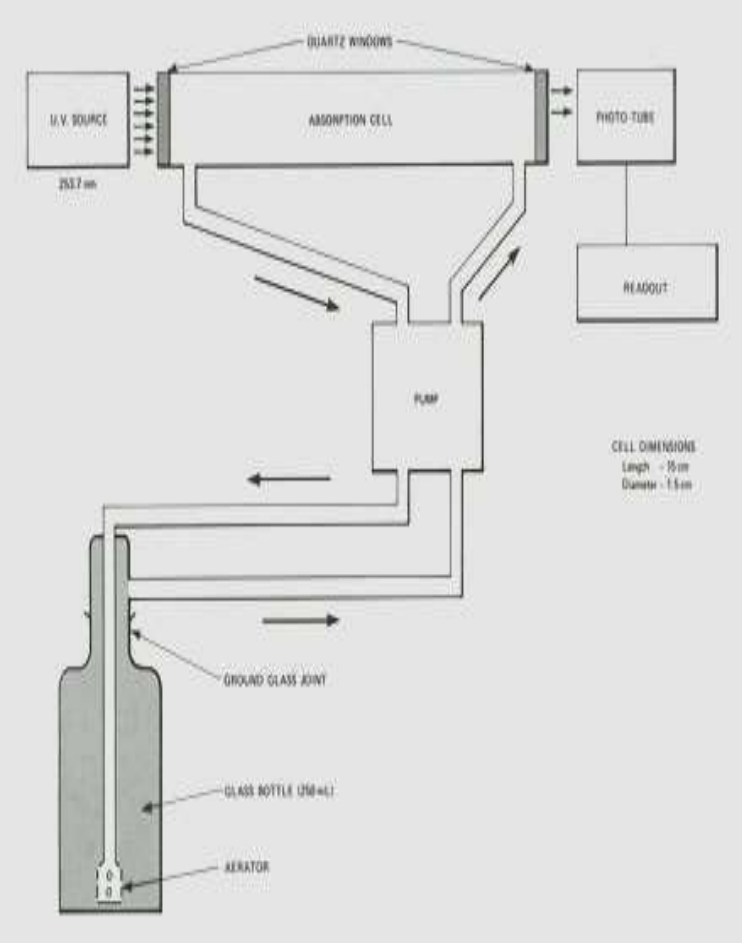
Absorption	Emission
- Measure trace metal concentrations in complex matrices .	- Measure trace metal concentrations in complex matrices .
- Atomic absorption depends upon the number of ground state atoms	-Atomic emission depends upon the number of excited atoms .

Comparison Between Atomic Absorption and Emission Spectroscopy

- It measures the radiation absorbed by the ground state atoms.
- Presence of a light source (HCL).
- The temperature in the atomizer is adjusted to atomize the analyte atoms in the ground state only.

- It measures the radiation emitted by the excited atoms.
- Absence of the light source.
- The temperature in the atomizer is big enough to atomize the analyte atoms and excite them to a higher energy level.

Flameless Atomic Absorption



- Here flame is not used, but high temperature achieved by carbon rod.
- temp -2700C
- 100 times more sensitive than flame methods and are highly specific for the element measured.

Flameless Atomic Absorption

- Atomization techniques- electrothermal

1. Drying : Sample is dried on carbon support by removing solvent.
2. Pyrolysis- majority of matrix constituents are removed
3. Atomization: the analyte element is released to the gaseous phase
4. Cleaning- residues in the graphite tube removed by high temperatures

Flameless Atomic Absorption

Advantages

- Solution and solid samples can be used
- Efficient atomization
- Greater sensitivity
- Small sample size 5 – 50 microlitres
- Provides reducing environment for easily oxidised elements

Disadvantages

- Expensive
- Low precision
- High operator skill

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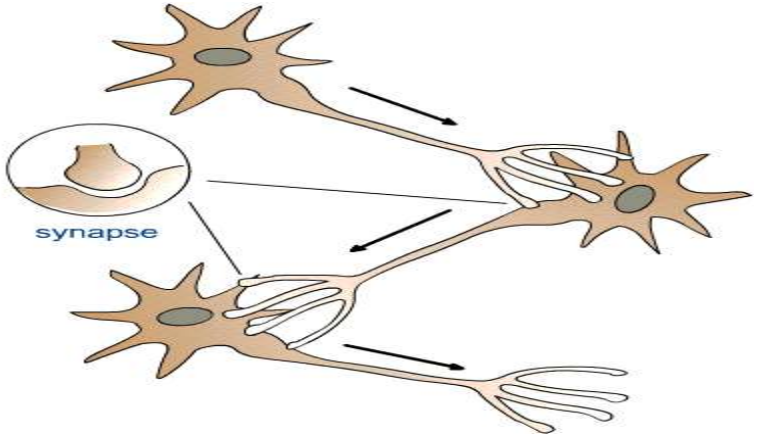
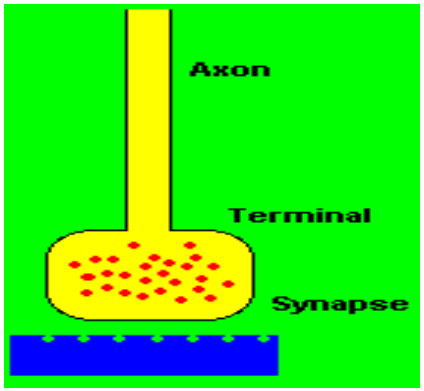
THANK-YOU

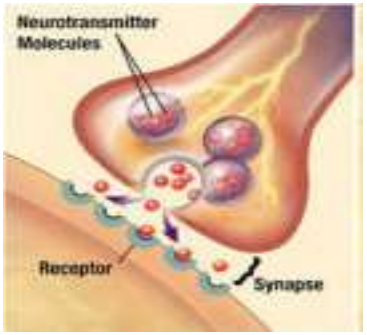


Autonomic Nervous System



By

Mr. Krunal G. Kanase



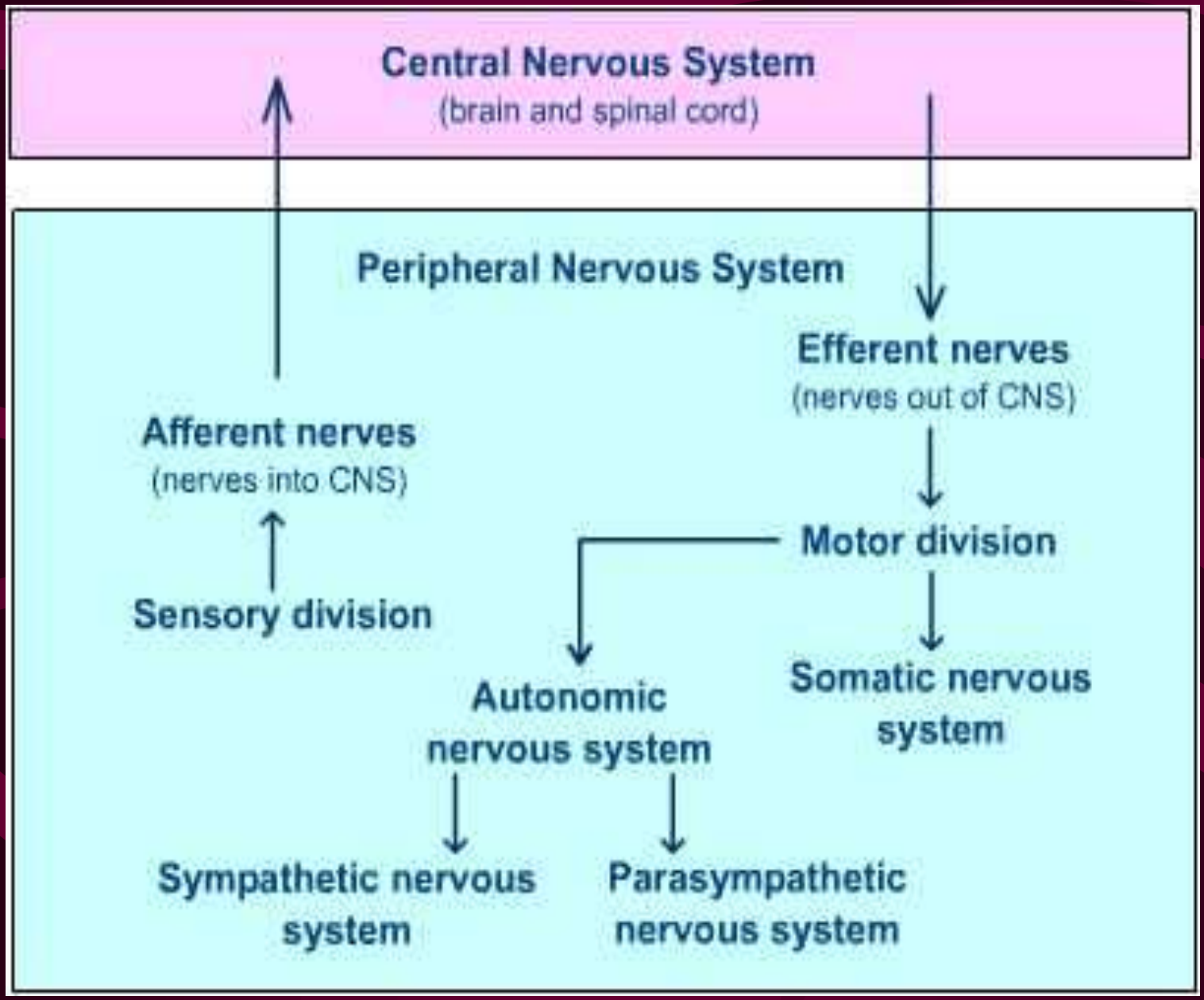
NERVOUS SYSTEM DIVISIONS

- The **central nervous system (CNS) consists of the brain and** spinal cord.
- The **peripheral nervous system (PNS)** consists of cranial nerves and spinal nerves. The PNS includes the autonomic nervous system (ANS).

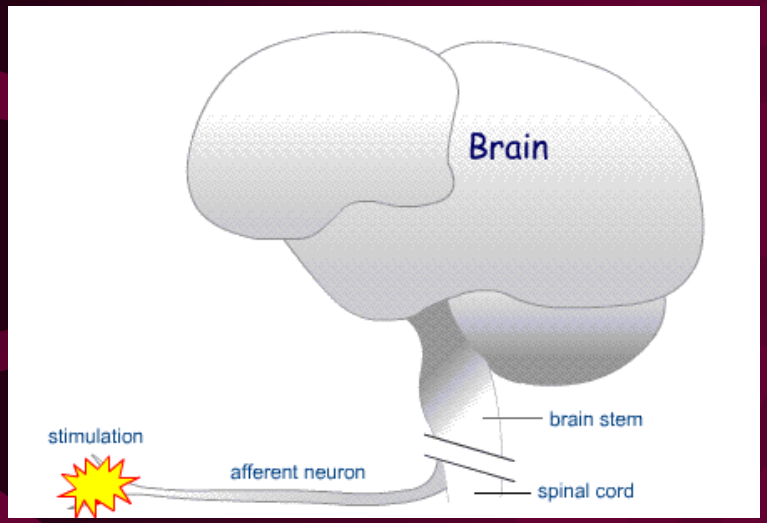
a) autonomic nervous system, which includes the enteric nervous system.

b) somatic efferent nerves, innervating skeletal muscle.

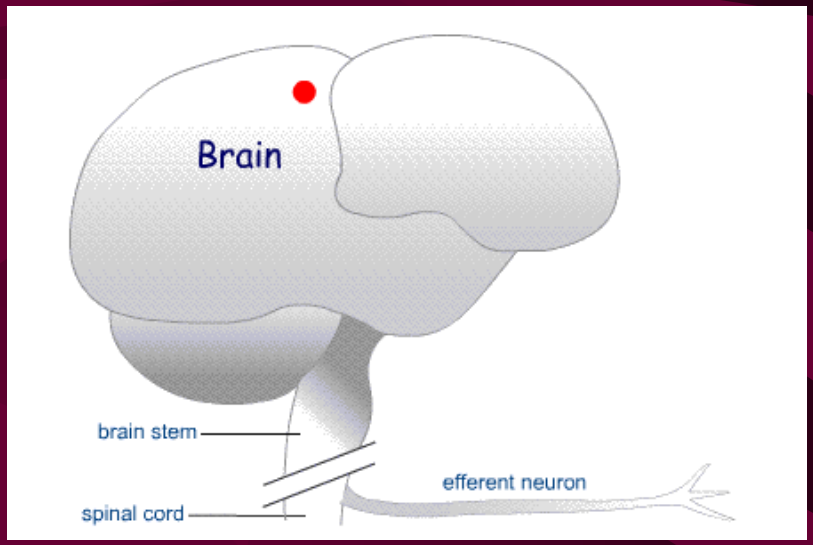
c) somatic and visceral afferent nerves.


Central Nervous System
(brain and spinal cord)
Peripheral Nervous System
Efferent nerves
(nerves out of CNS)
Afferent nerves
(nerves into CNS)
Motor division
Sensory division
Somatic nervous system
Autonomic nervous system
Sympathetic nervous system
Parasympathetic nervous system

Sensory Division



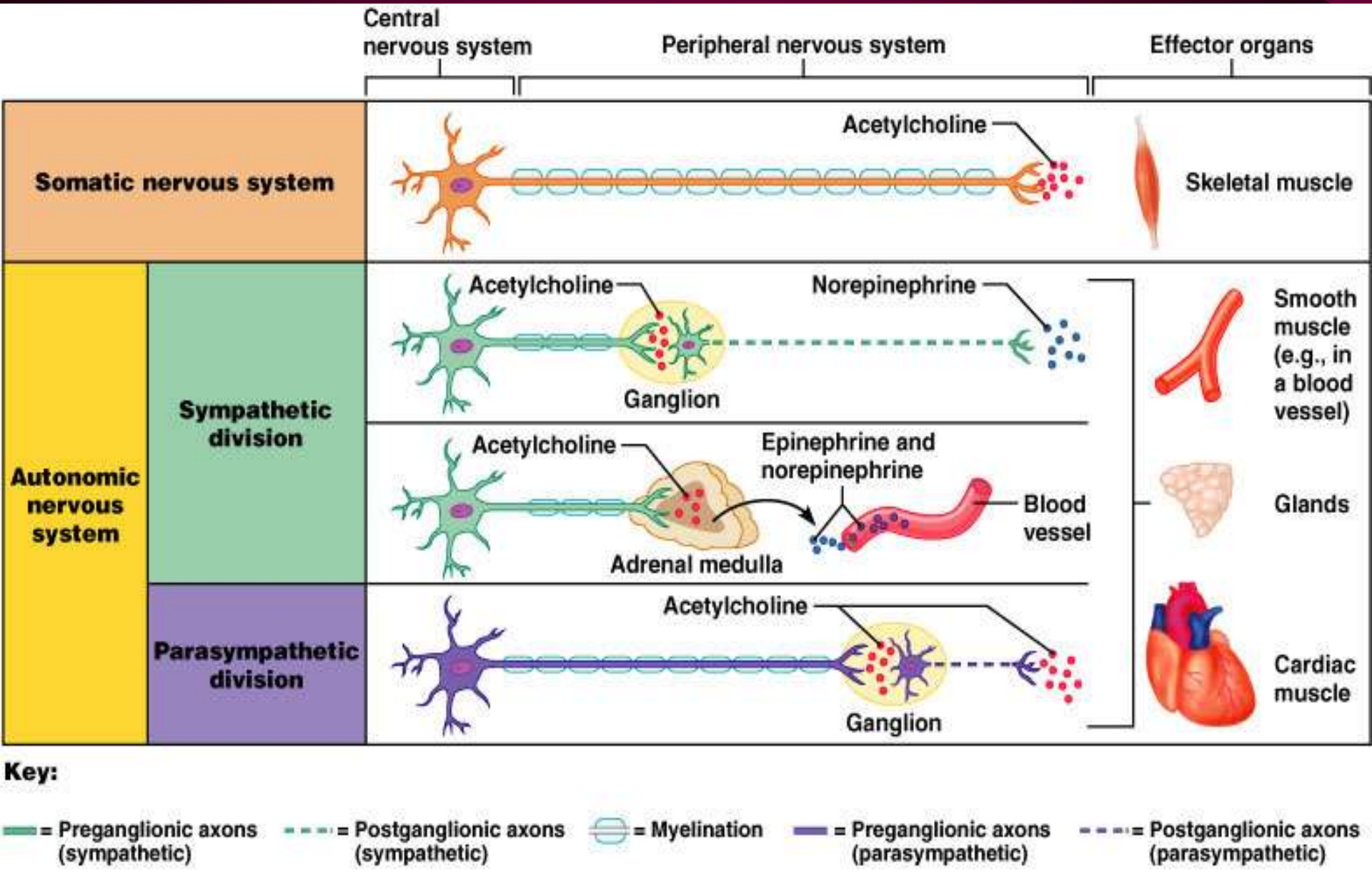
Motor Division

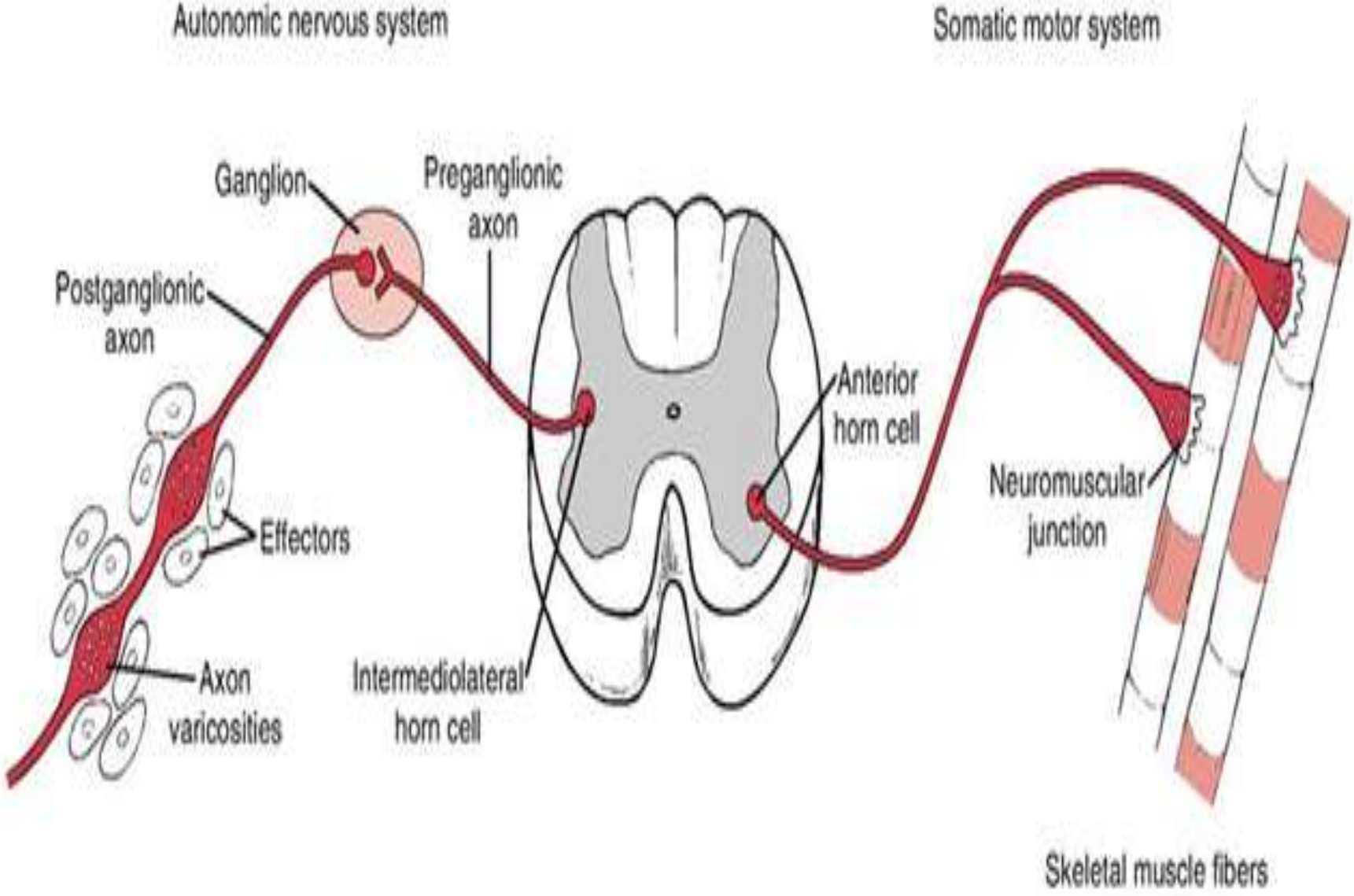


Diffrence Between Somatic and autonomic system

	Somatic	Autonomic
1. Organ supplied	Skeletal muscles	All other organs
2. Distal most synapse	Within CNS	Outside CNS (in ganglia)
3. Nerve fibres	Myelinated	Pregang.—myelinated Postgang.—non-myelinated
4. Peripheral plexus formation	Absent	Present
5. Efferent transmitter	Acetylcholine	Acetylcholine, Noradrenaline
6. Effect of nerve section on organ supplied	Paralysis and atrophy	Activity maintained, no atrophy

Comparison of Somatic and Autonomic Systems




Autonomic nervous system
Somatic motor system
Ganglion
Preganglionic axon
Postganglionic axon
Anterior horn cell
Neuromuscular junction
Effectors
Axon varicosities
Intermediolateral horn cell
Skeletal muscle fibers

BASIC ANATOMY AND PHYSIOLOGY OF THE AUTONOMIC NERVOUS SYSTEM

1. The autonomic nervous system consists of three main anatomical divisions: *sympathetic* and *parasympathetic* and the *enteric* nervous system, consisting of the intrinsic nerve plexuses of the gastrointestinal tract, which are closely interconnected with the sympathetic and parasympathetic systems.
2. conveys all the outputs from the central nervous system to the rest of the body, except for the motor innervation of skeletal muscle.
3. The main processes that it regulates are:

a) contraction and relaxation of vascular and visceral smooth muscle

b) all exocrine and certain endocrine secretions

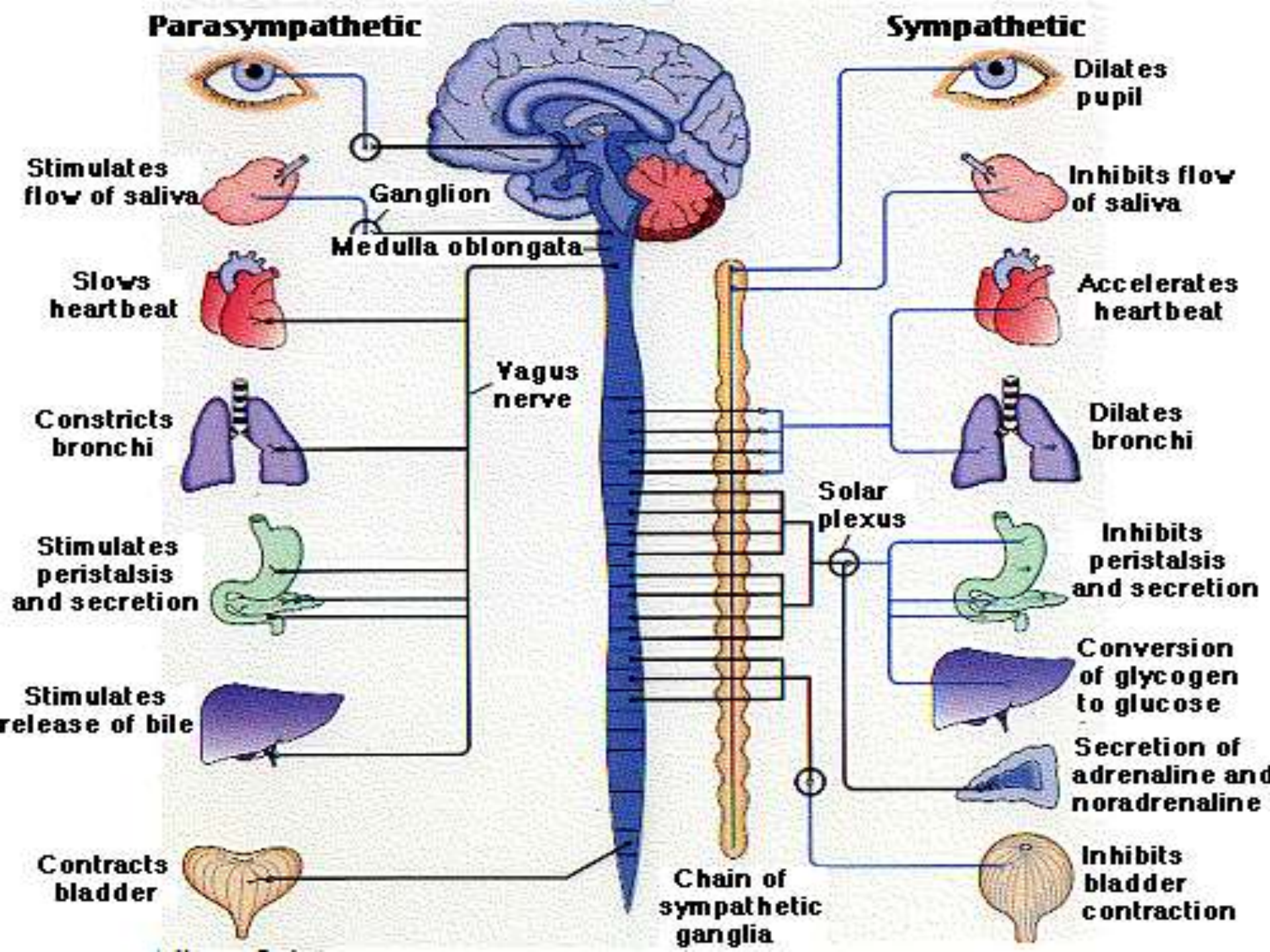
c) the heartbeat

d) energy metabolism, particularly in liver and skeletal muscle

- The main difference between the autonomic and the somatic efferent pathways is that the former consists of two neurons arranged in series, whereas in the latter a single motor neuron connects the central nervous system to the skeletal muscle fibre.
- The two neurons in the autonomic pathway are known, respectively, as *preganglionic* and *postganglionic*.
- In the sympathetic nervous system, the intervening synapses lie in *autonomic ganglia*, which are outside the central nervous system, and contain the nerve endings of preganglionic fibres and the cell bodies of postganglionic neurons. In parasympathetic pathways, the postganglionic cells are mainly found in the target organs, discrete parasympathetic ganglia (e.g. the ciliary ganglion) being found only in the head and neck.

Diffrence Between Sympathetic and parasympathetic system

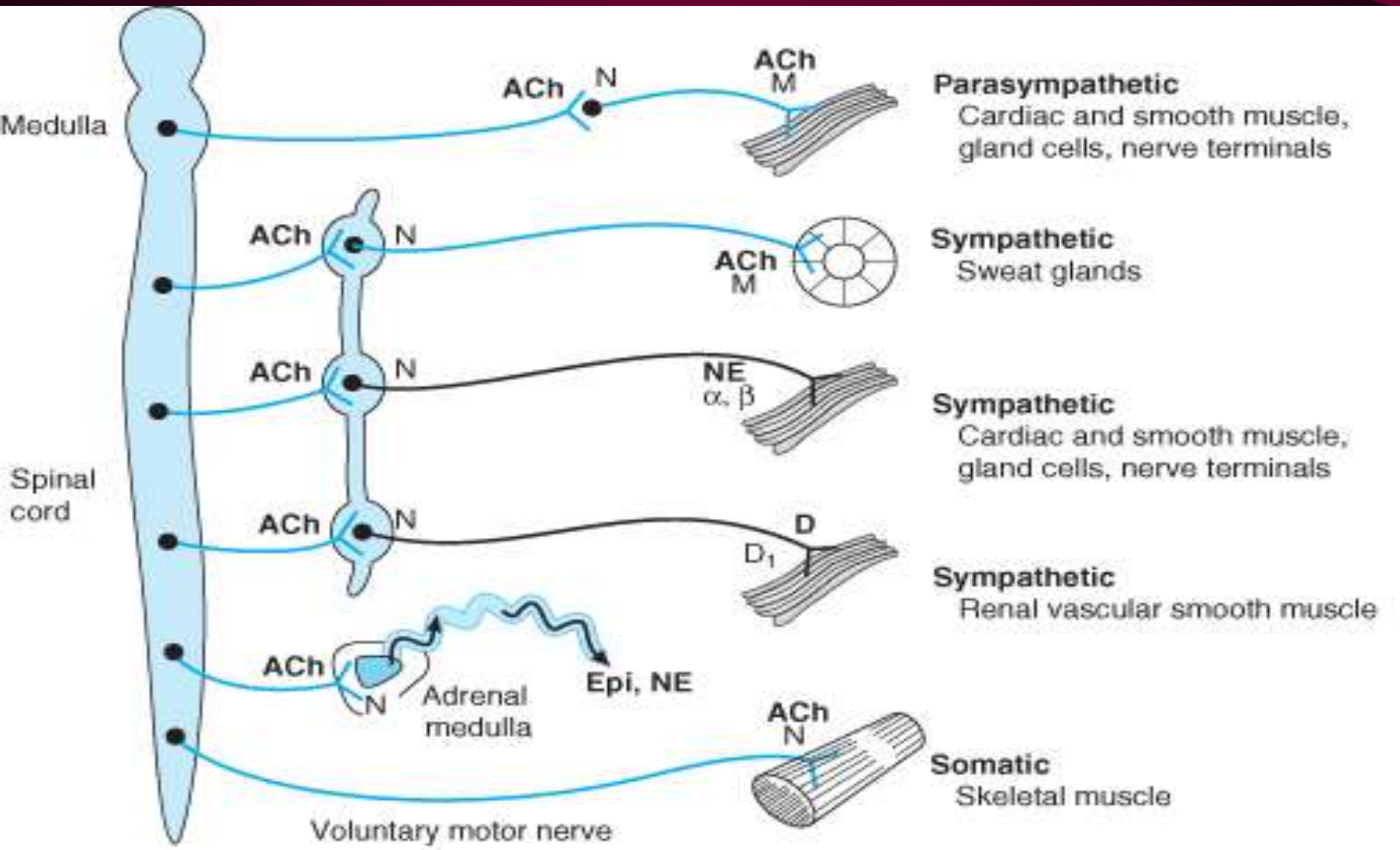
	Sympathetic	Parasympathetic
1. Origin	Dorso-lumbar (T_1 to L_2 or L_3)	Cranio-sacral (III, VII, IX, X; S_2–S_4)
2. Distribution	Wide	Limited to head, neck and trunk
3. Ganglia	Away from organs	On or close to the organ
4. Postgang. fibre	Long	Short
5. Pre: post ganglionic fibre ratio	1: 20 to 1: 100	1: 1 to 1: 2 (except in enteric plexuses)
6. Transmitter (neuroeffector)	Noradrenaline (major) Acetylcholine (minor)	Acetylcholine
7. Stability of transmitter	NA stable, diffuses for wider actions	ACh—rapidly destroyed locally
8. Important function	Tackling stress and emergency	Assimilation of food, conservation of energy


Parasympathetic
Sympathetic
Dilates pupil
Stimulates flow of saliva
Ganglion
Medulla oblongata
Inhibits flow of saliva
Slows heartbeat
Accelerates heartbeat
Vagus nerve
Constricts bronchi
Dilates bronchi
Solar plexus
Stimulates peristalsis and secretion
Inhibits peristalsis and secretion
Conversion of glycogen to glucose
Stimulates release of bile
Secretion of adrenaline and noradrenaline
Contracts bladder
Chain of sympathetic ganglia
Inhibits bladder contraction

Functions…….

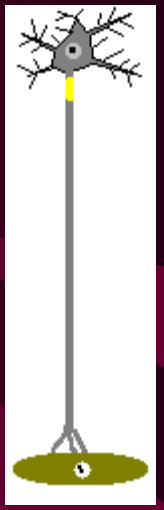
Organ	Sympathetic Response	Parasympathetic Response
Heart (cardiac muscle)	• Increase rate	• Decrease rate (to normal)
Bronchioles (smooth muscle)	• Dilate	• Constrict (to normal)
Iris (smooth muscle)	• Pupil dilates	• Pupil constricts (to normal)
Salivary glands	• Decrease secretion	• Increase secretion (to normal)
Stomach and intestines (smooth muscle)	• Decrease peristalsis	• Increase peristalsis for normal digestion
Stomach and intestines (glands)	• Decrease secretion	• Increase secretion for normal digestion
Internal anal sphincter	• Contracts to prevent defecation	• Relaxes to permit defecation
Urinary bladder (smooth muscle)	• Relaxes to prevent urination	• Contracts for normal urination
Internal urethral sphincter	• Contracts to prevent urination	• Relaxes to permit urination
Liver	• Changes glycogen to glucose	• None
Pancreas	• Secretes glucagon	• Secretes insulin and digestive enzymes
Sweat glands	• Increase secretion	• None
Blood vessels in skin and viscera (smooth muscle)	• Constrict	• None
Blood vessels in skeletal muscle (smooth muscle)	• Dilate	• None
Adrenal glands	• Increase secretion of epinephrine and norepinephrine	• None

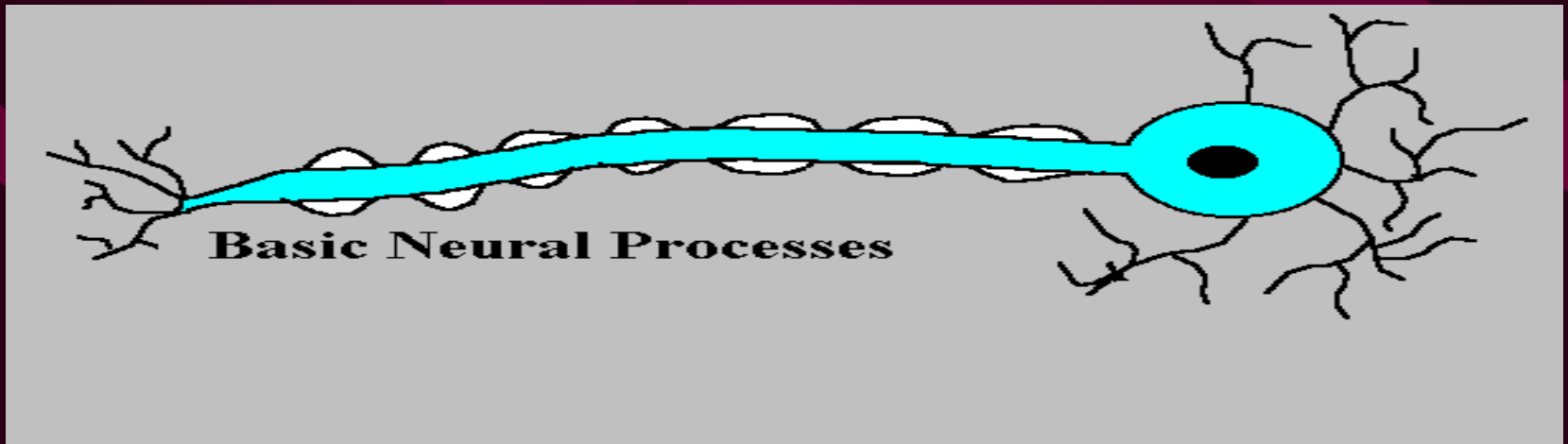
Transmitters



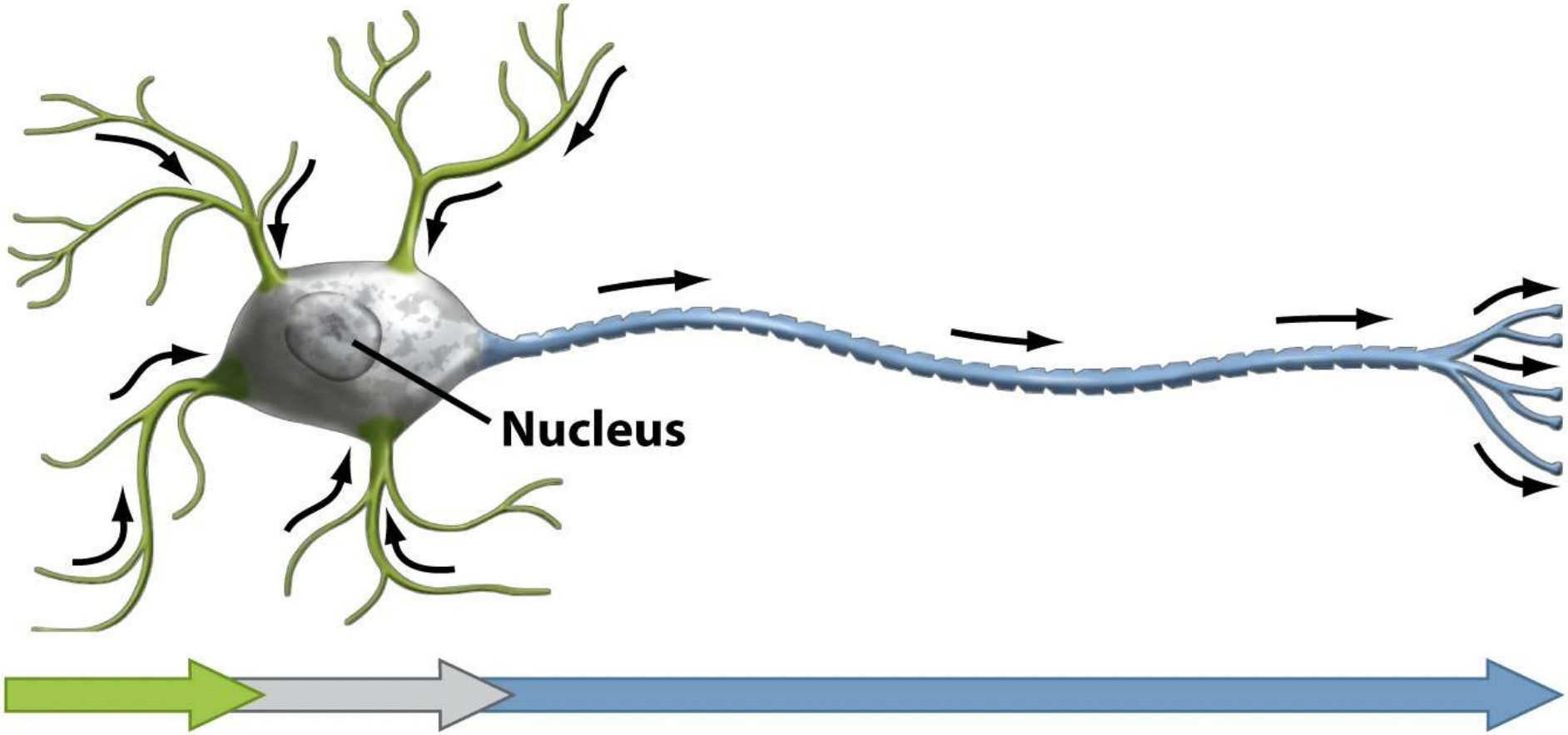
Steps in Neurohumoral Transmission

- Impulse conduction
- Transmitter release
- Transmitter acion on postjunctional membrane
- Postjunctional activity
- Termination of transmitter action





Information flow through neurons



Dendrites
Collect electrical signals

Cell body
Integrates incoming signals and generates outgoing signal to axon

Axon
Passes electrical signals to dendrites of another cell or to an effector cell

Figure 45-2b Biological Science, 2/e
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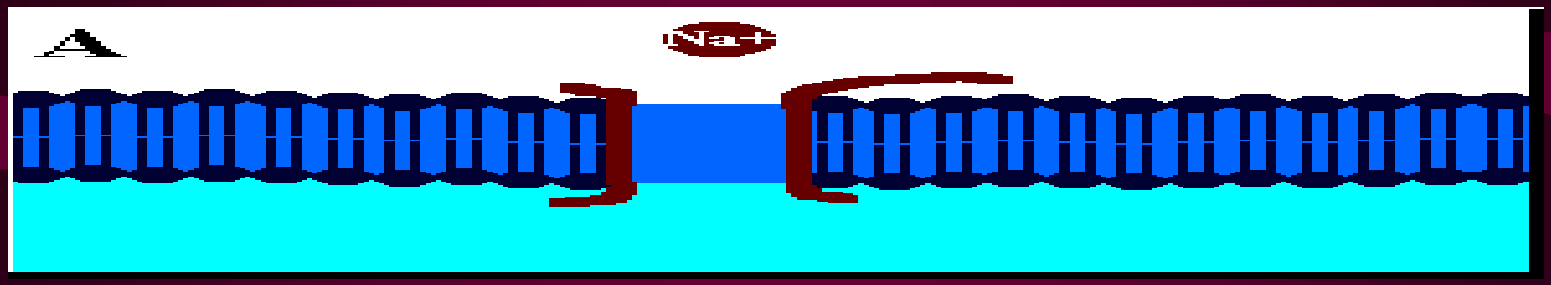
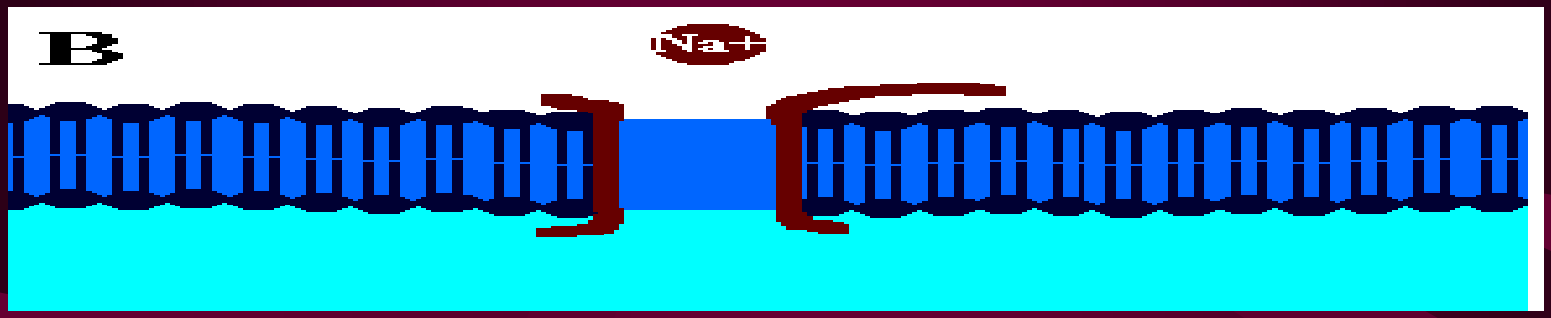
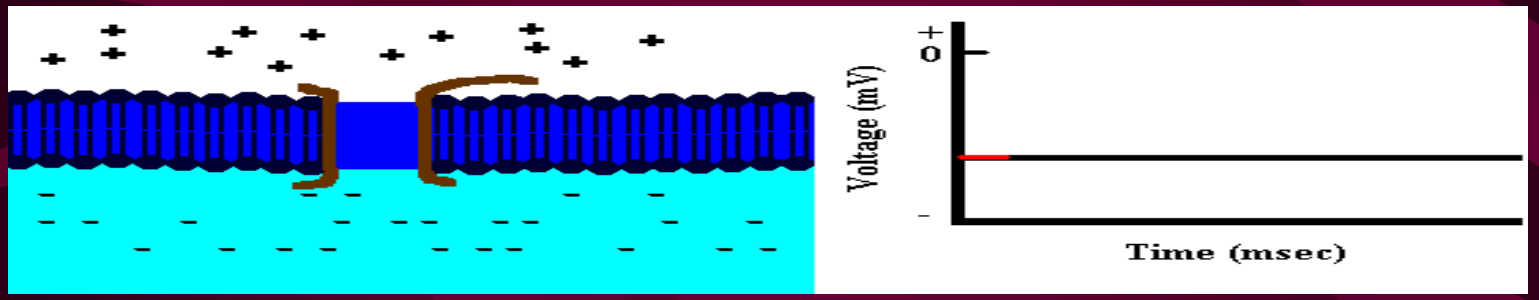
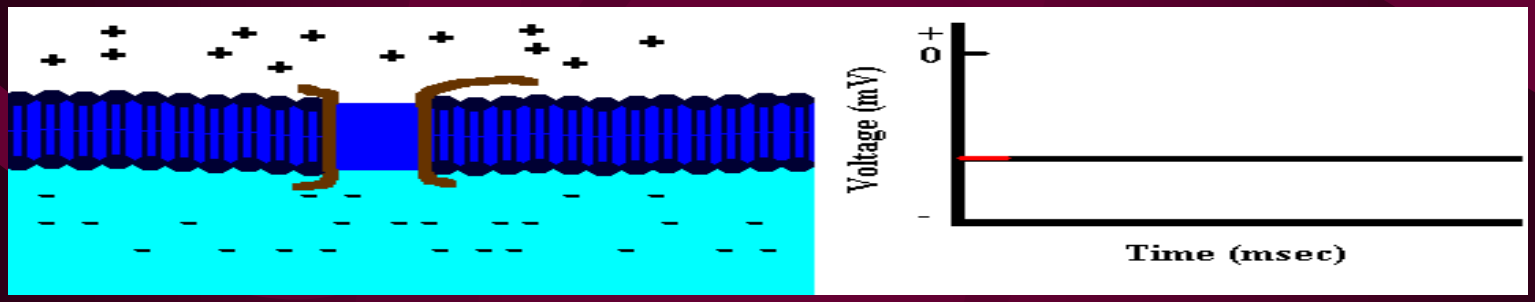

A
Na+


B
Na+


+
0
-
Voltage (mV)
Time (msec)


+
0
-
Voltage (mV)
Time (msec)

PROPAGATION OF ACTION POTENTIAL

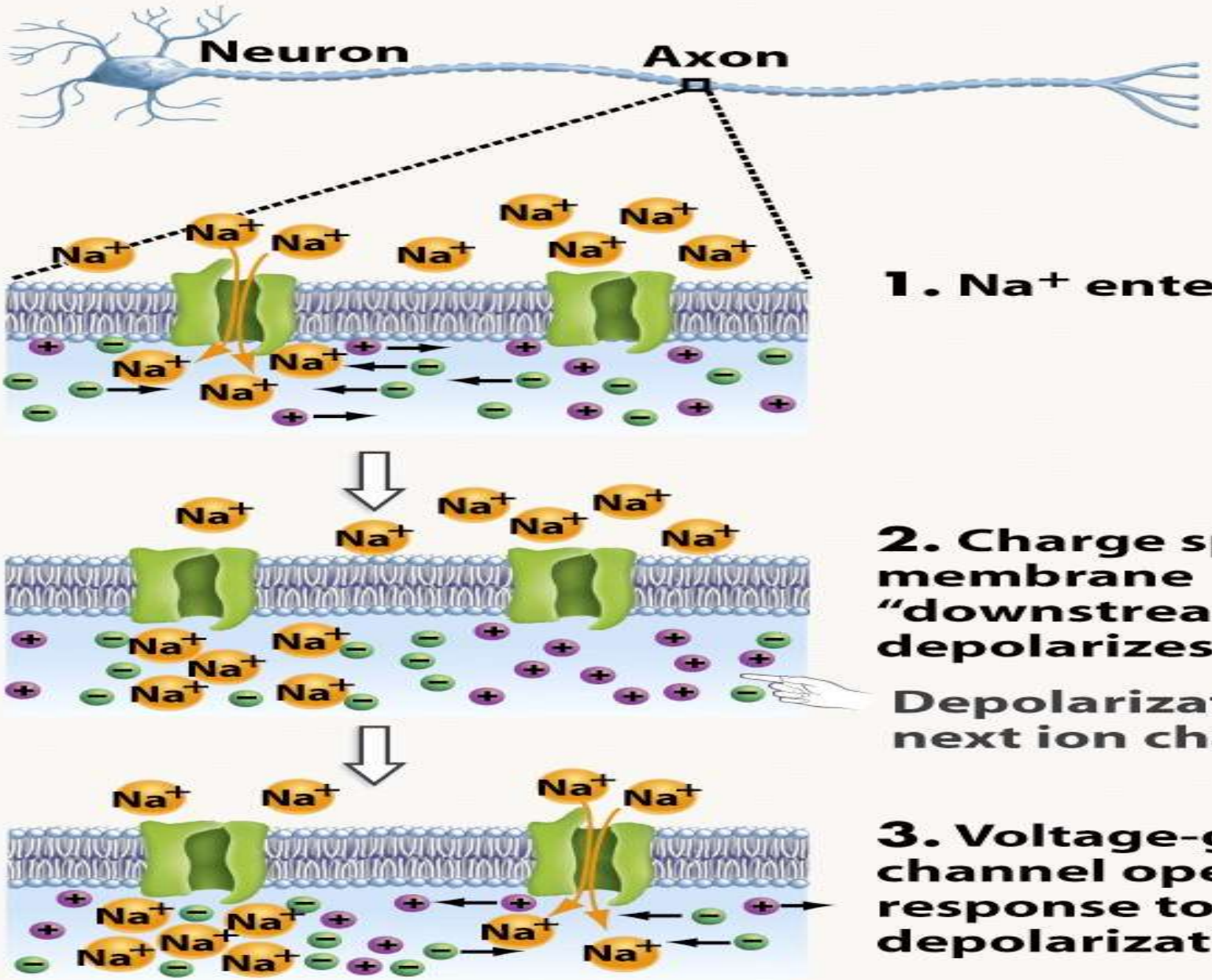


Figure 45-11a Biological Science, 2/e
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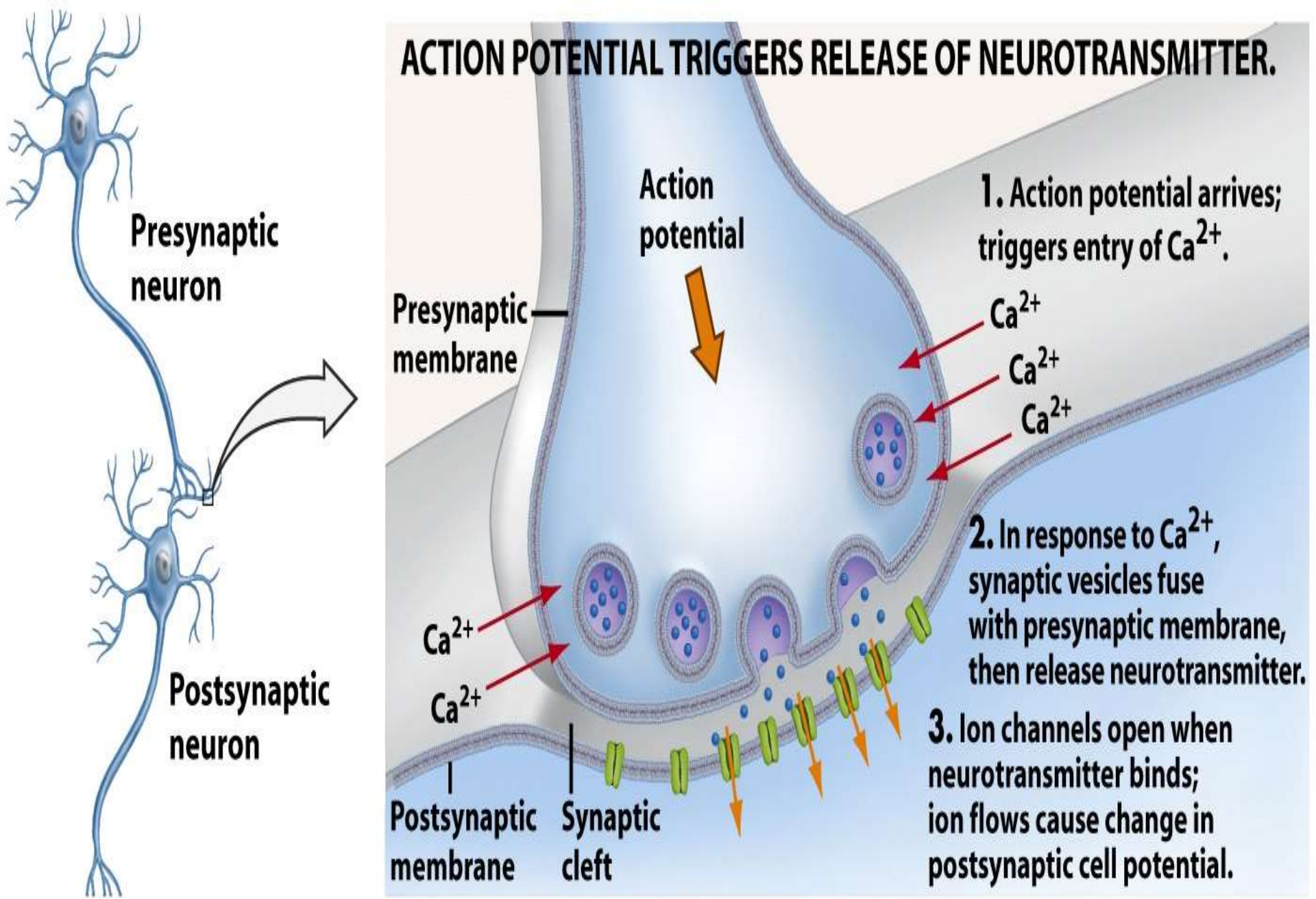

Figure 45-15 Biological Science, 2/e

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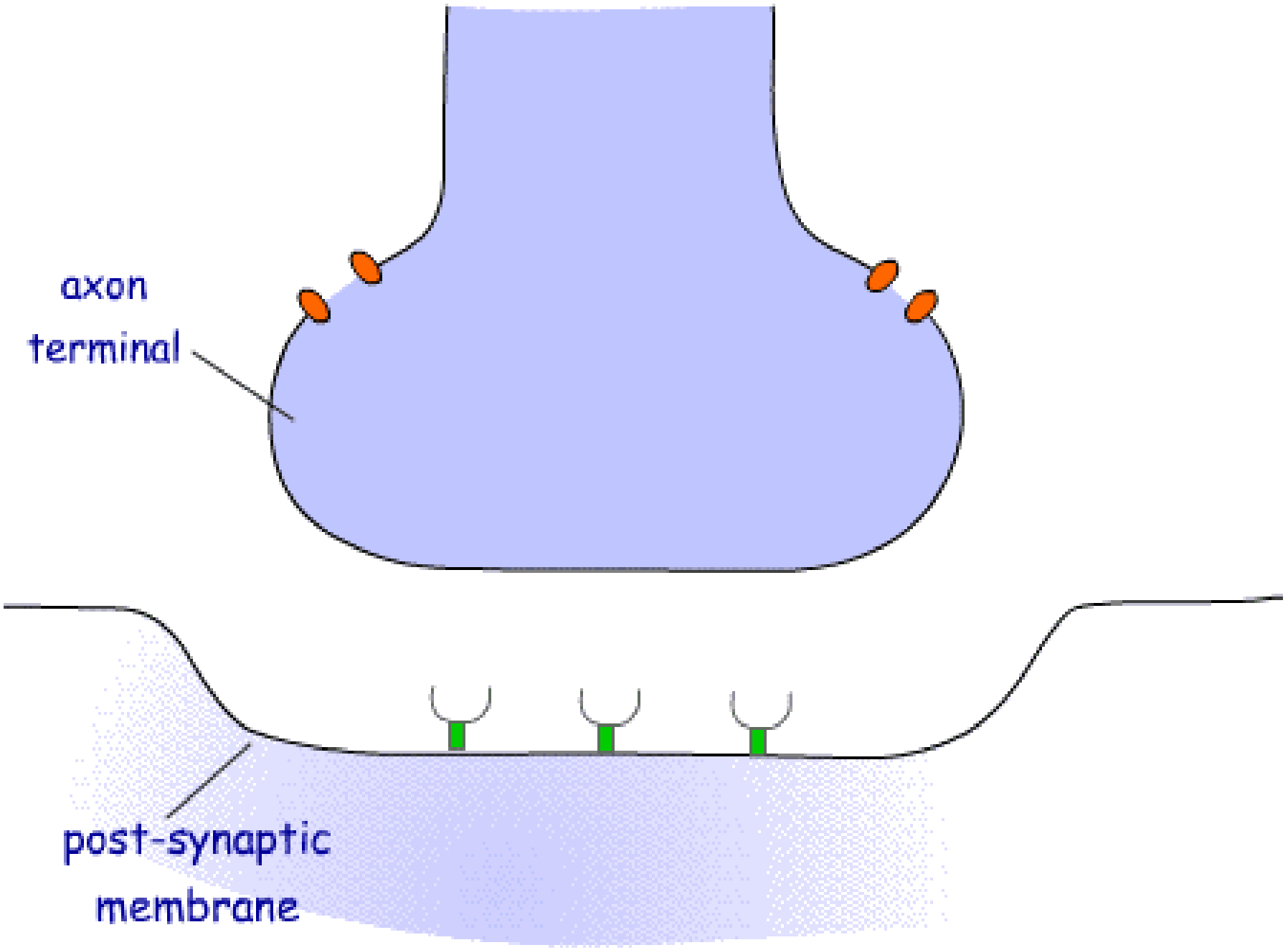
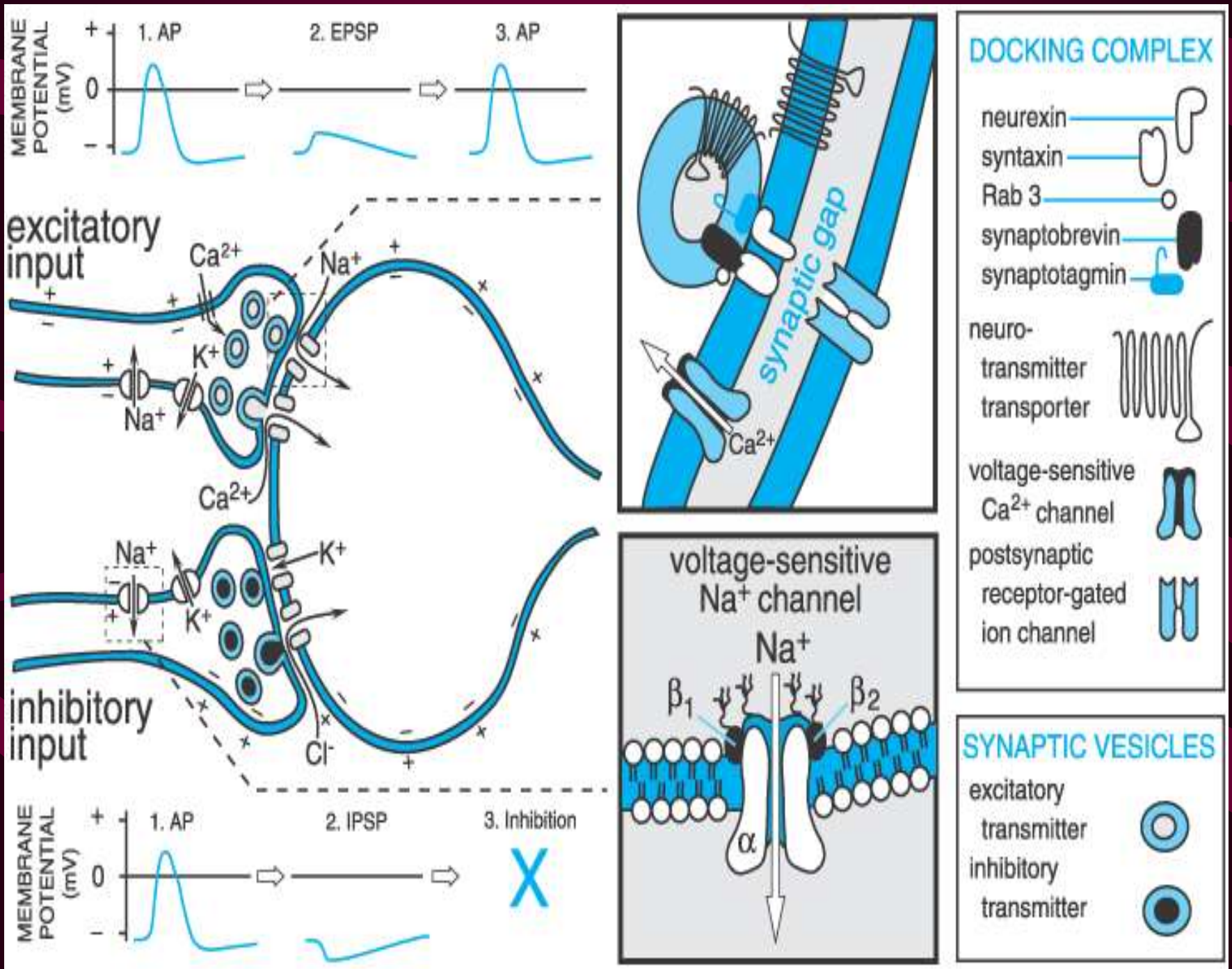

axon
terminal
post-synaptic
membrane


MEMBRANE POTENTIAL (mV)
+
0
–
1. AP
2. EPSP
3. AP
excitatory input
Ca2+
Na+
K+
Na+
Ca2+
Na+
K+
K+
inhibitory input
Cl-
MEMBRANE POTENTIAL (mV)
+
0
–
1. AP
2. IPSP
3. Inhibition
X
synaptic gap
Ca2+
voltage-sensitive Na+ channel
Na+
β1
β2
α
DOCKING COMPLEX
neurexin
syntaxin
Rab 3
synaptobrevin
synaptotagmin
neuro-transmitter transporter
voltage-sensitive Ca2+ channel
postsynaptic receptor-gated ion channel
SYNAPTIC VESICLES
excitatory transmitter
inhibitory transmitter

Cotransmitter

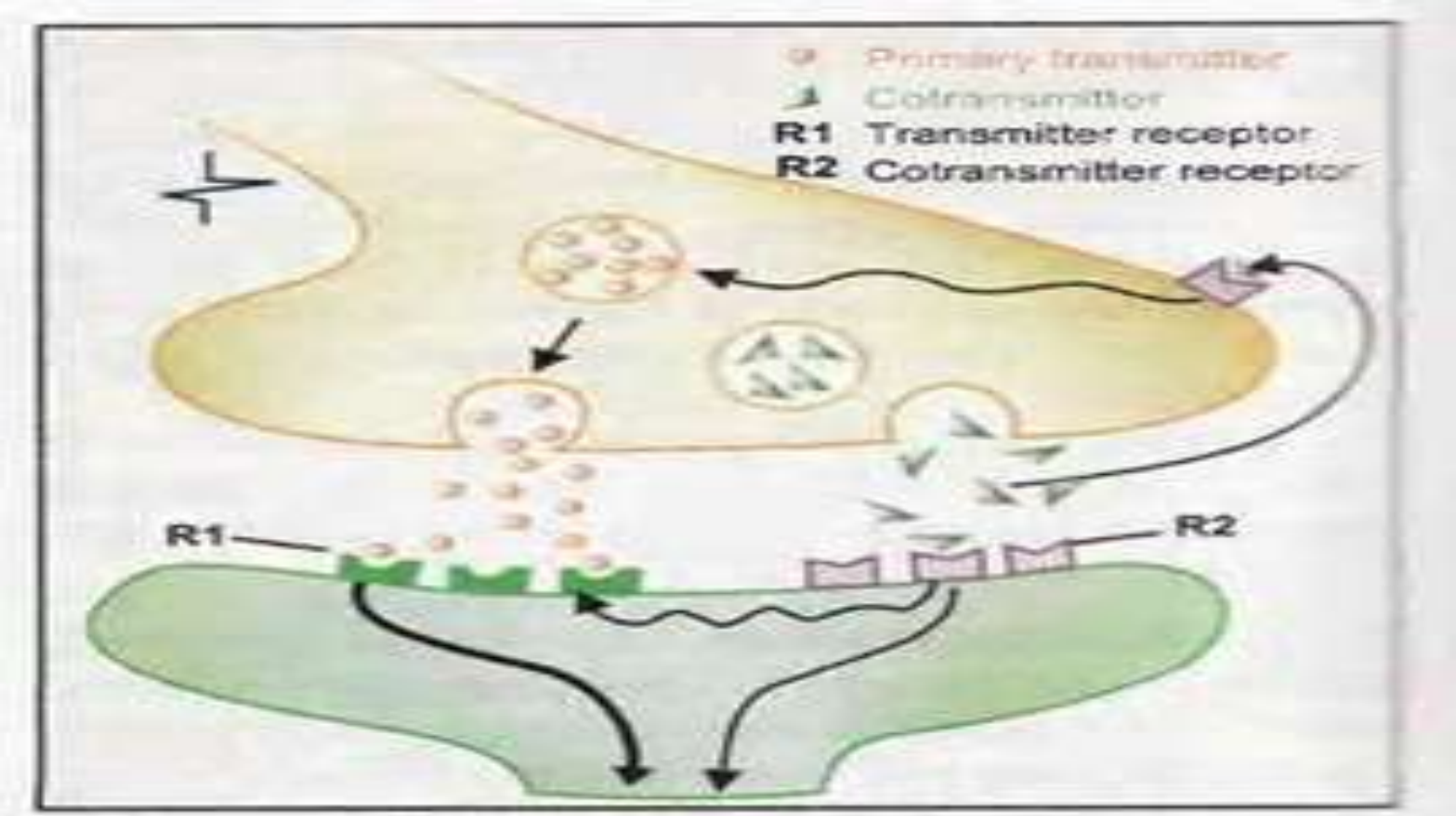
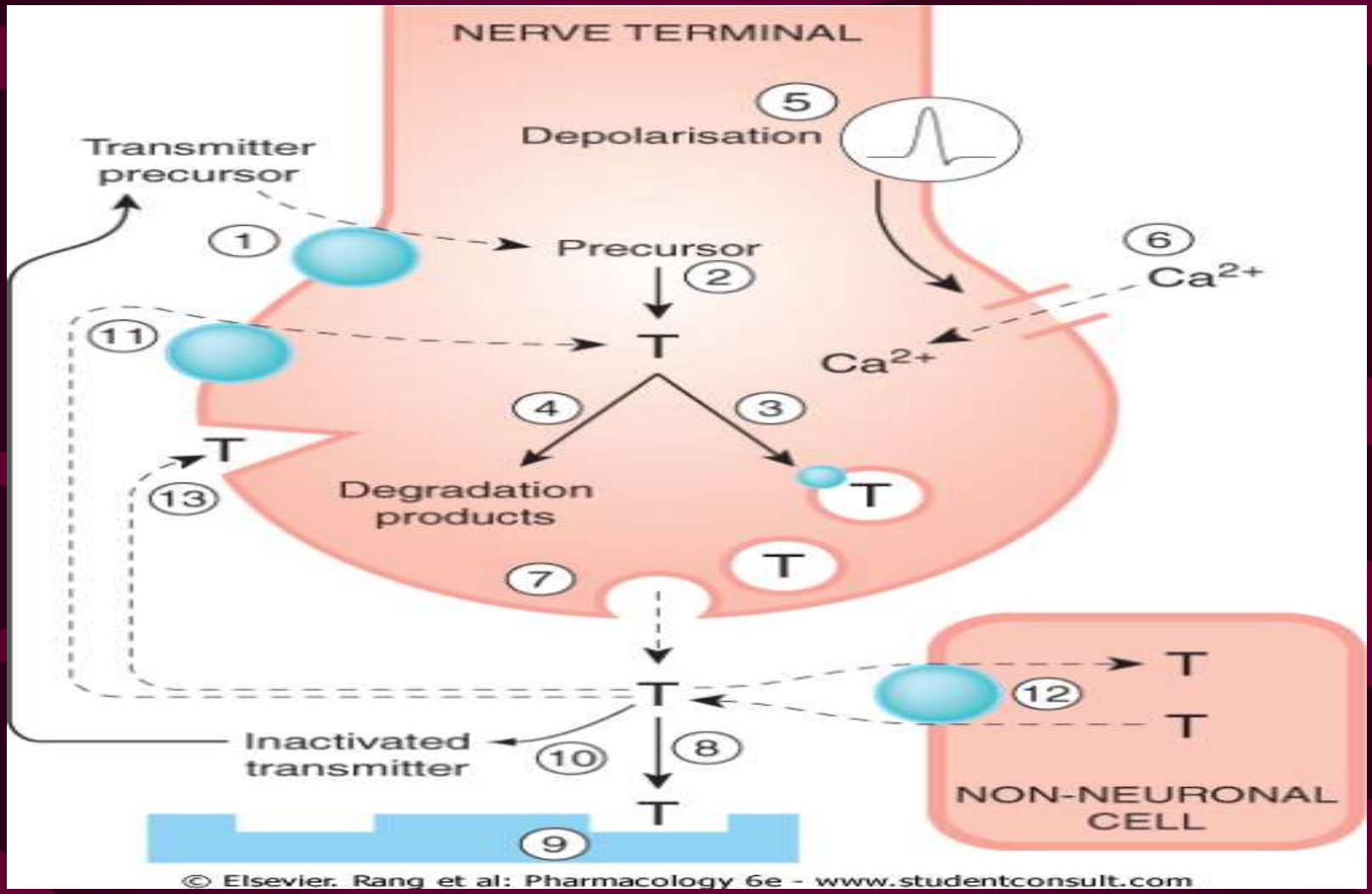


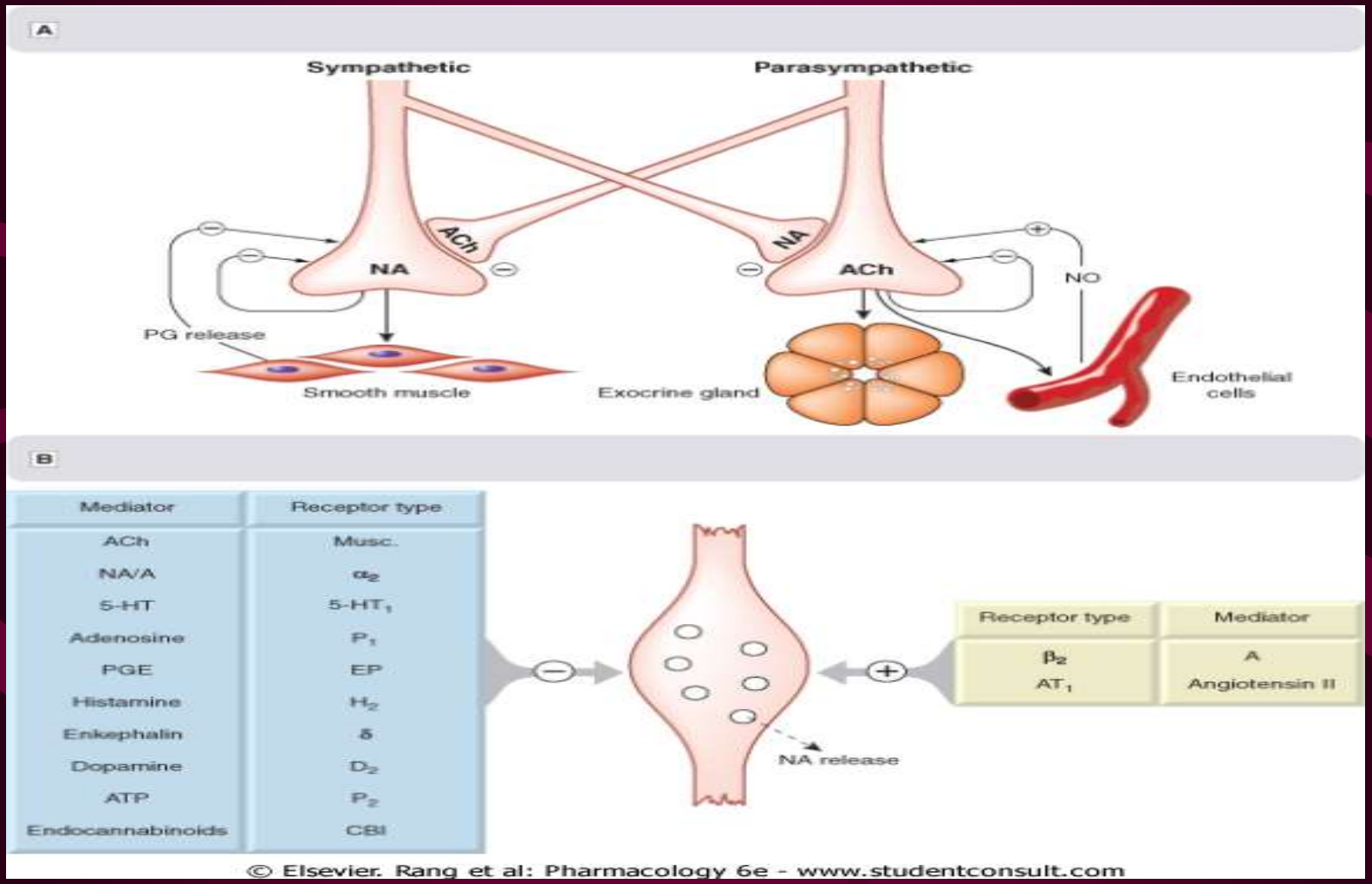
Fig. II.3: Cotransmission

The main processes involved in synthesis, storage and release of amine and amino acid transmitters



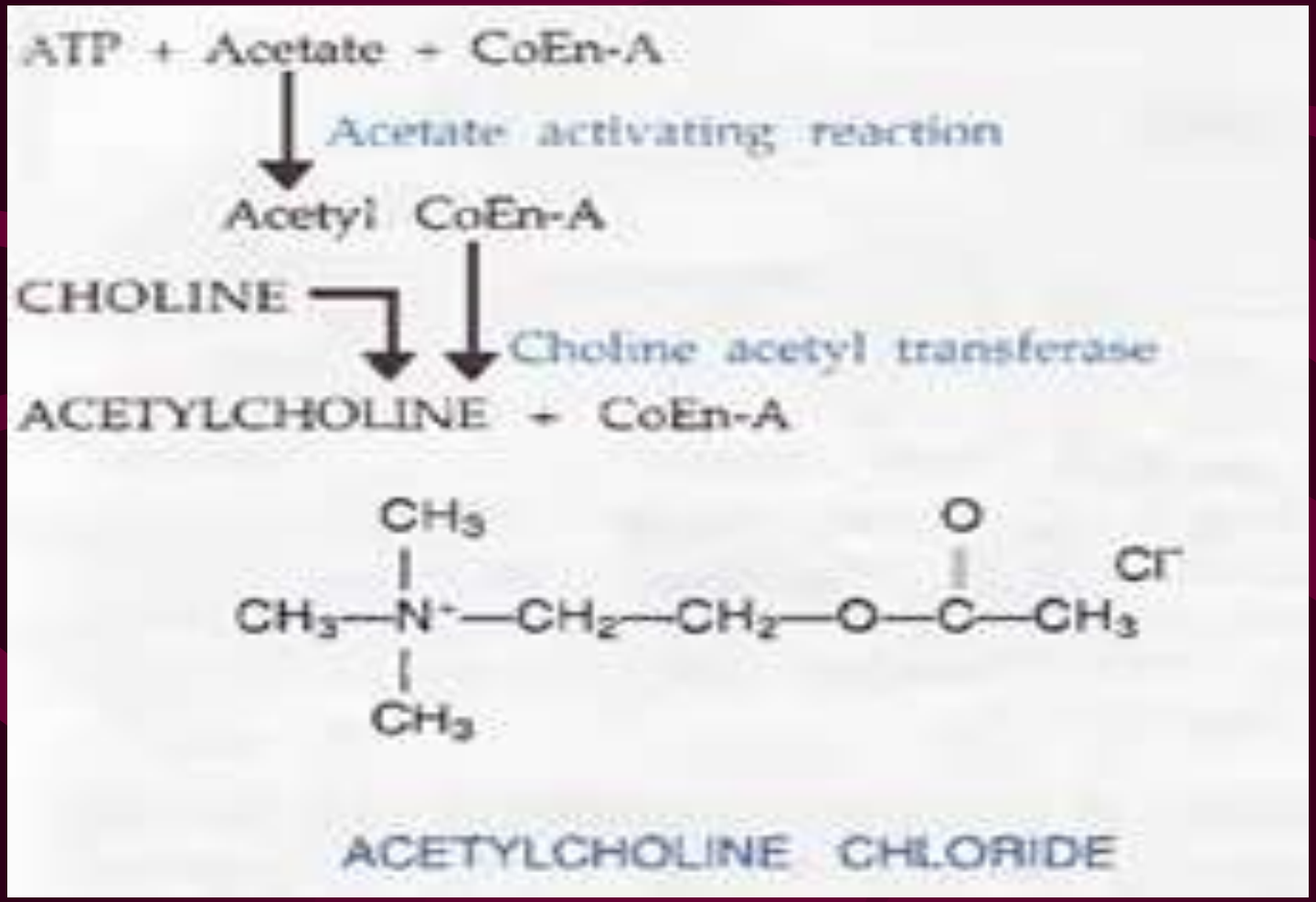
1, Uptake of precursors; 2, synthesis of transmitter; 3, uptake/transport of transmitter into vesicles; 4, degradation of surplus transmitter; 5, depolarisation by propagated action potential; 6, influx of Ca^{2+} in response to depolarisation; 7, release of transmitter by exocytosis; 8, diffusion to postsynaptic membrane; 9, interaction with postsynaptic receptors; 10, inactivation of transmitter; 11, reuptake of transmitter or degradation products by nerve terminals; 12, uptake of transmitter by non-neuronal cells; and 13, interaction with presynaptic receptors. The transporters (11 and 12) can release transmitter under certain conditions by working in reverse.

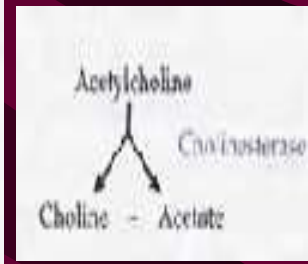
Presynaptic regulation of transmitter release from noradrenergic and cholinergic nerve terminals.

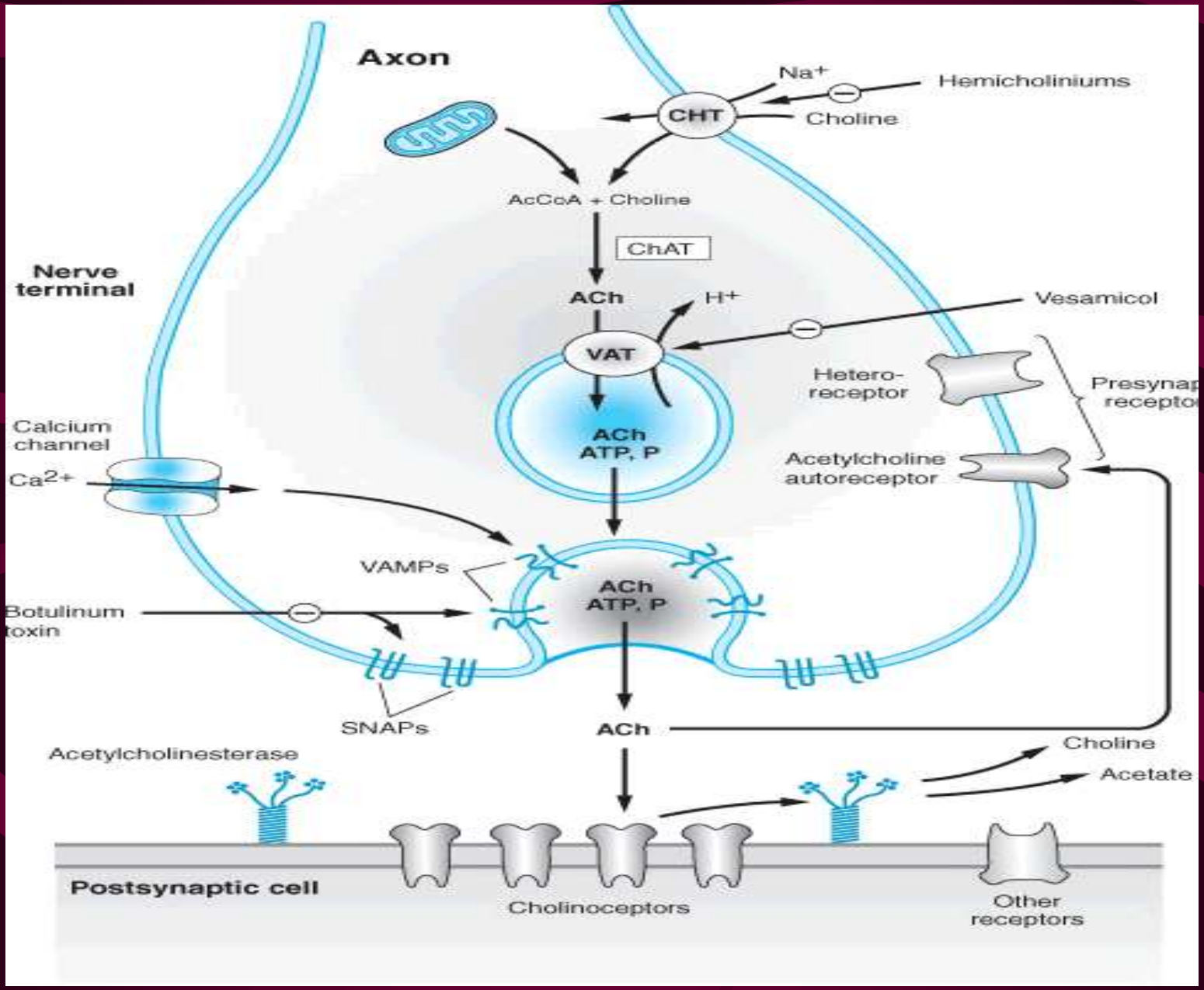


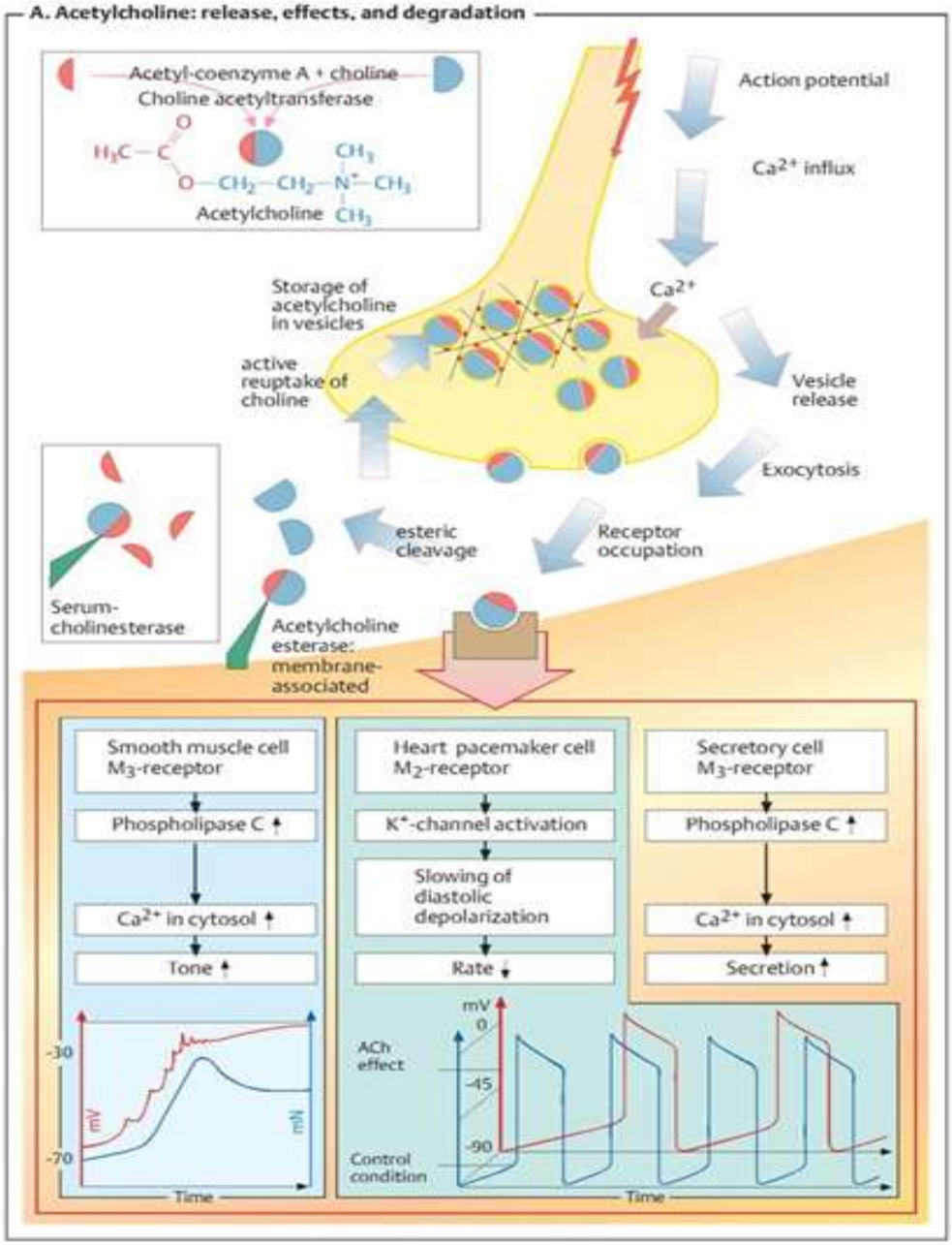
Cholinergic System and Drug

Synthesis of Acetylcholine

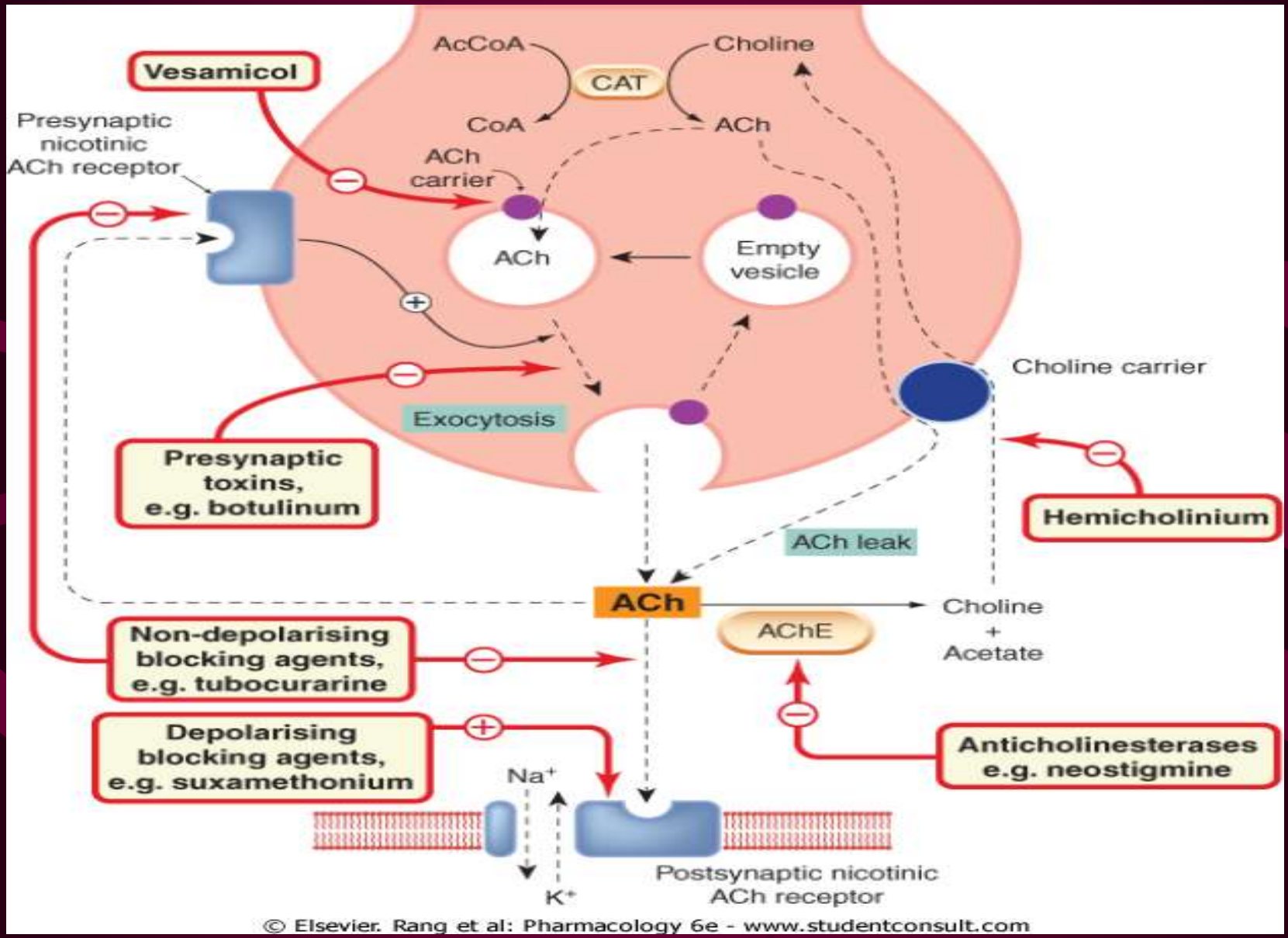





Axon
Na+
Hemicholiniums
CHT
Choline
AcCoA + Choline
ChAT
Nerve terminal
ACh
H+
Vesamicol
VAT
Hetero-receptor
Presynap
recepto
ACh
ATP, P
Calcium channel
Ca2+
Acetylcholine autoreceptor
VAMPs
ACh
ATP, P
Botulinum toxin
SNAPs
ACh
Choline
Acetylcholinesterase
Acetate
Postsynaptic cell
Cholinoceptors
Other receptors


A. Acetylcholine: release, effects, and degradation
Acetyl-coenzyme A + choline
Choline acetyltransferase
Acetylcholine
Action potential
Ca^{2+} influx
Ca^{2+}
Storage of acetylcholine in vesicles
active reuptake of choline
Vesicle release
Exocytosis
esteric cleavage
Receptor occupation
Serum-cholinesterase
Acetylcholine esterase: membrane-associated
Smooth muscle cell M_3-receptor
Phospholipase C ↑
Ca^{2+} in cytosol ↑
Tone ↑
Heart pacemaker cell M_2-receptor
K^+-channel activation
Slowing of diastolic depolarization
Rate ↓
Secretory cell M_3-receptor
Phospholipase C ↑
Ca^{2+} in cytosol ↑
Secretion ↑
-30
-70
mV
mN
Time
ACh effect
Control condition
mV
0
-45
-90
Time

ELECTRICAL EVENTS IN TRANSMISSION AT FAST CHOLINERGIC SYNAPSES



Cholinergic Receptors

- Muscarinic -

  M_1- Neuronal receptor

  M_2 – Cardiac Muscarinic

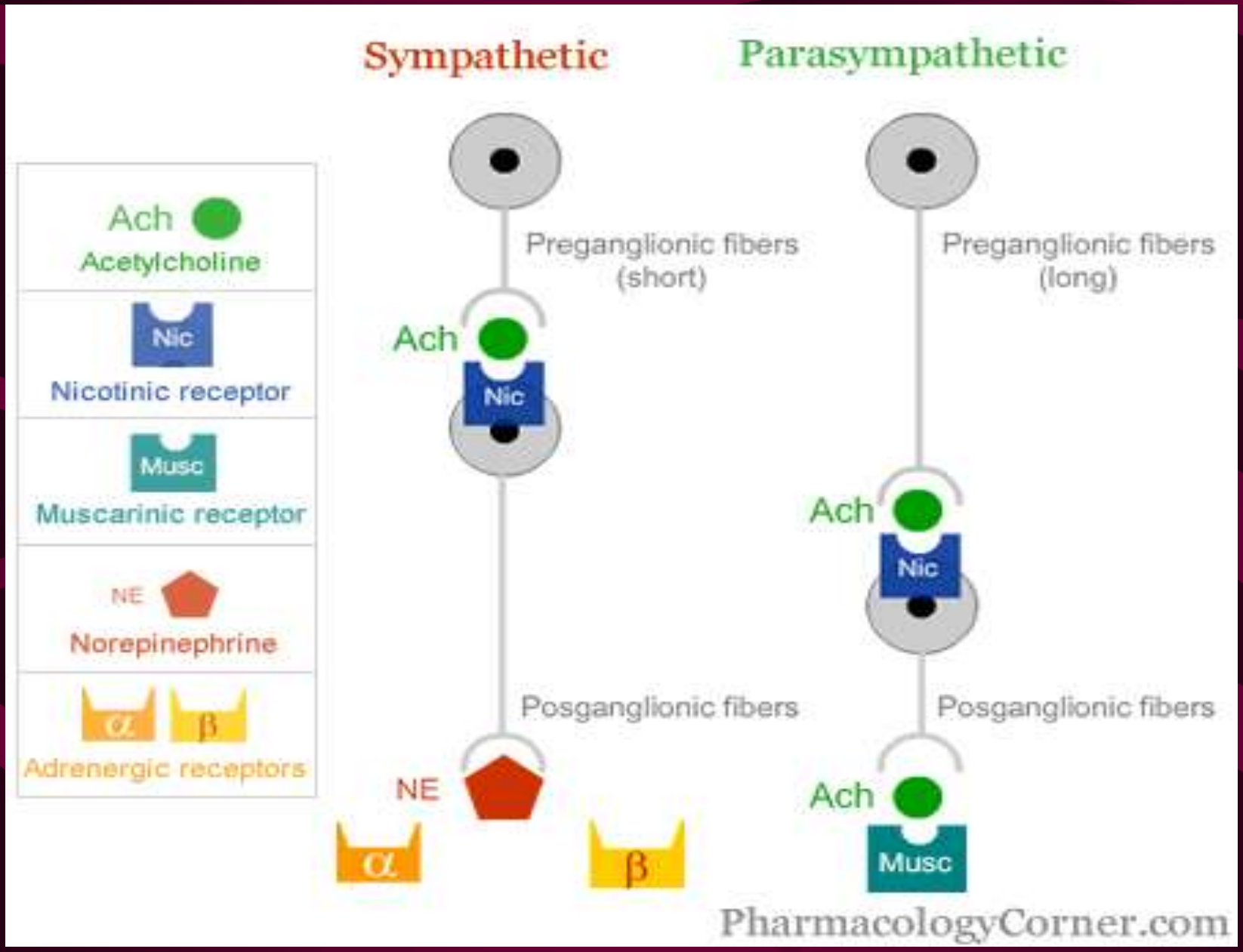
  M_3 –Visceral smooth muscle contraction and glandular secretion

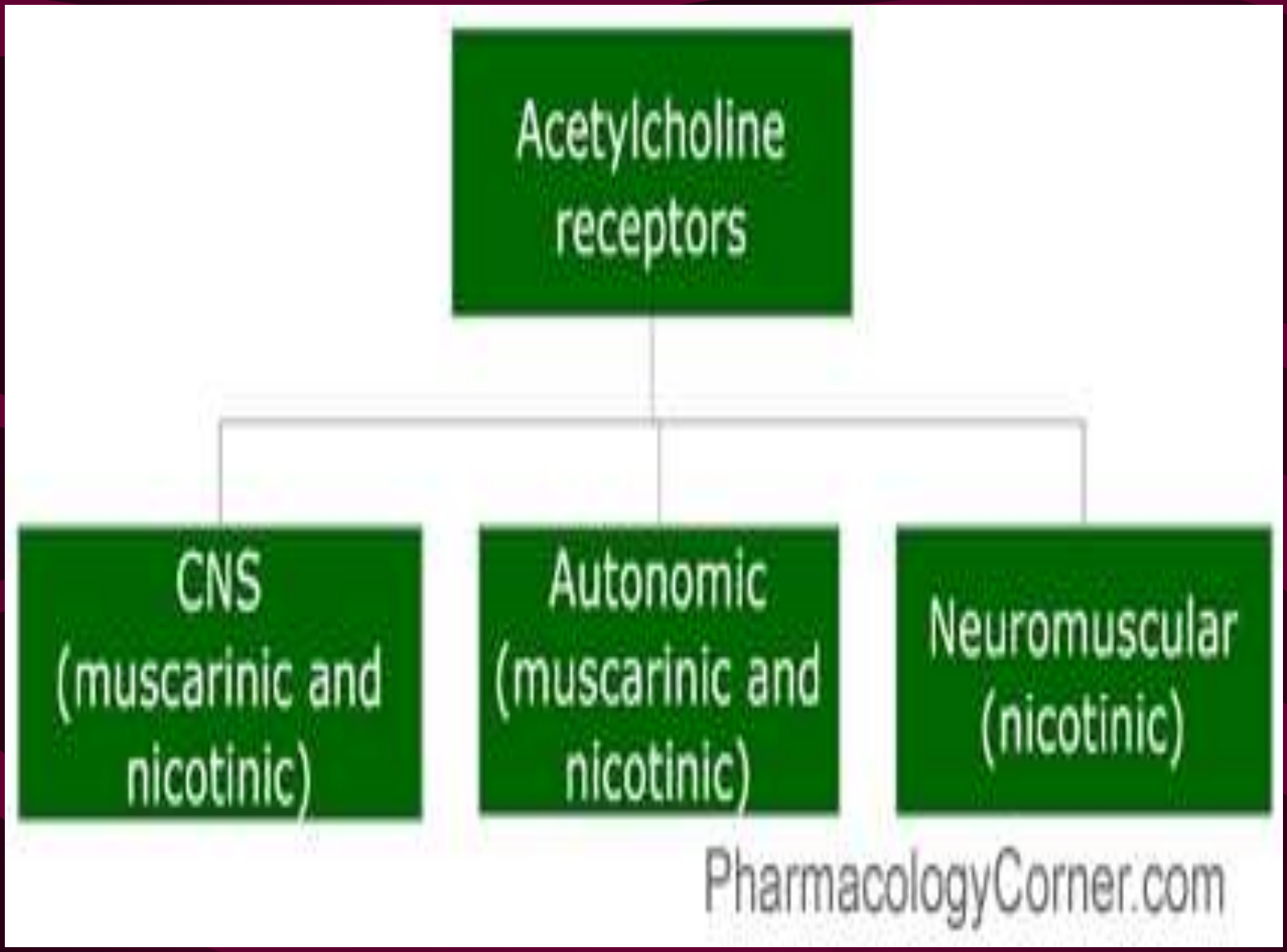
  M_4, M_5 – Nerve ending of in certain areas of brain regulate the release of other neurotransmitters

- Nicotinic - $N_{M,}$ $N_{N.}$

G-protein couple Receptor

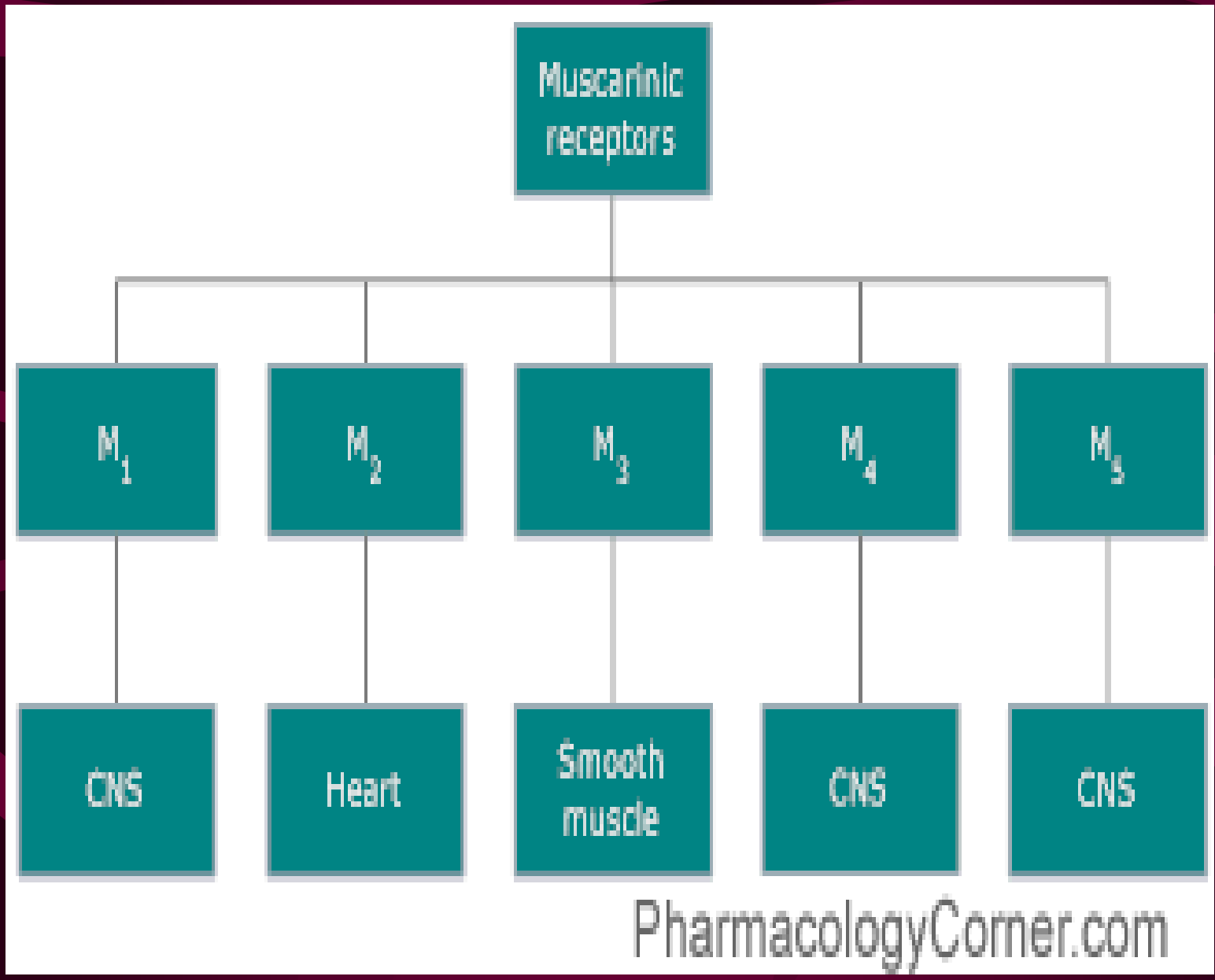
Ligand gated ion channel receptor

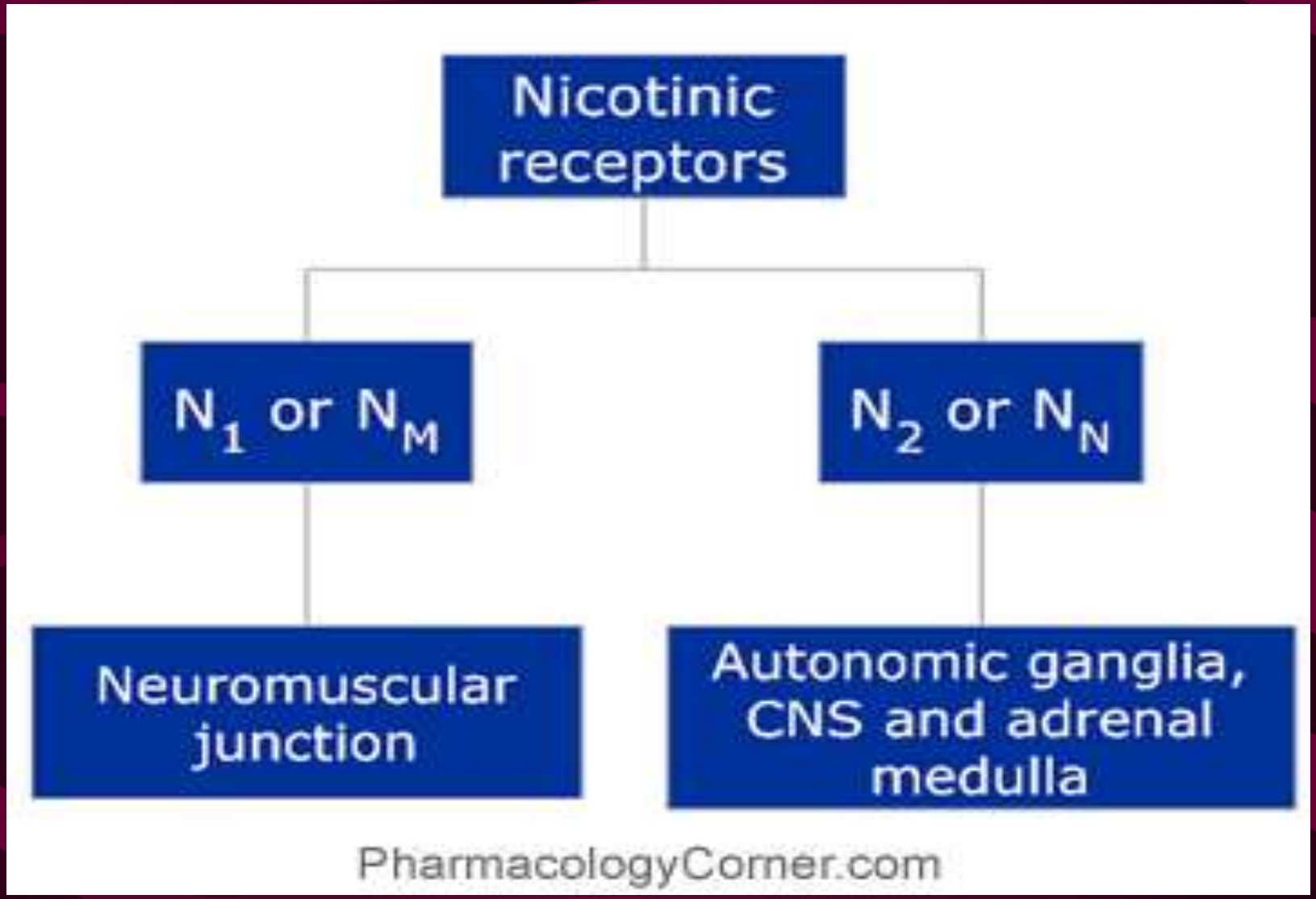

Sympathetic
Parasympathetic
Ach
Acetylcholine
Nic
Nicotinic receptor
Musc
Muscarinic receptor
NE
Norepinephrine
α
β
Adrenergic receptors
Preganglionic fibers (short)
Preganglionic fibers (long)
Ach
Nic
Posganglionic fibers
Posganglionic fibers
NE
Ach
Musc
PharmacologyCorner.com


Acetylcholine receptors
CNS (muscarinic and nicotinic)
Autonomic (muscarinic and nicotinic)
Neuromuscular (nicotinic)
PharmacologyCorner.com

Receptors….

	M_1		M_2	M_3
1. Location and function subserved	*Autonomic ganglia:* Depolarization (late EPSP) *Gastric glands:* Hist. release, acid secretion *CNS:* Learning, memory, motor functions		*SA node:* Hyperpolarization, ↓ rate of impulse generation *AV node:* ↓ velocity of conduction *Atrium:* shortening of APD, ↓ contractility *Ventricle:* ↓ contractility (slight) (receptors sparse) *Cholinergic nerve endings:* ↓ ACh release *CNS:* tremor, analgesia *Visceral smooth muscle:* contraction	*Visceral smooth muscle:* contraction *Iris:* constriction of pupil *Ciliary muscle:* contraction *Exocrine glands:* secretion *Vascular endothelium:* release of NO→ vasodilatation
2. Nature	G-protein coupled, 7-TM		G-protein coupled, 7-TM	G-protein coupled, 7-TM
3. Transducer mechanism	IP_3/DAG—↑ cytosolic Ca^{2+}, PLA_2↑—PG synthesis		K^+ channel opening, ↓ cAMP	IP_3/DAG—↑ cytosolic Ca^{2+} PLA_2↑—PG synthesis
4. Agonists*	MCN-343A, Oxotremorine		Methacholine	Bethanechol
5. Antagonists*	Pirenzepine, Telenzepine		Methoctramine, Tripitramine	Hexahydrosiladifenidol, Darifenacin


Muscarinic receptors
M1
M2
M3
M4
M5
CNS
Heart
Smooth muscle
CNS
CNS
PharmacologyCorner.com


Nicotinic receptors
N_1 or N_M
N_2 or N_N
Neuromuscular junction
Autonomic ganglia, CNS and adrenal medulla
PharmacologyCorner.com

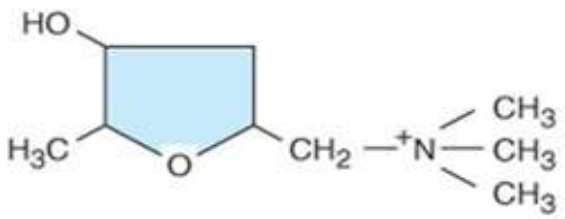
Cholinergic Drugs
(Cholinomimetics, Parasympathomimetics)

- Cholinergic Agonist

1. Choline esters- Acetylcholine, Methacholine, Carbachol, Bethanechol
2. Alkaloids- Muscarine, Pilocarpine, Arecholine.

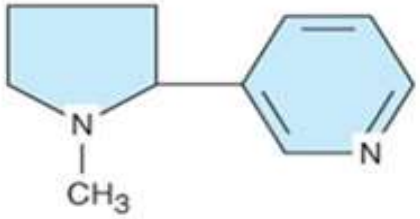
Structure of cholinomimetics drug……..

ACTION CHIEFLY MUSCARINIC

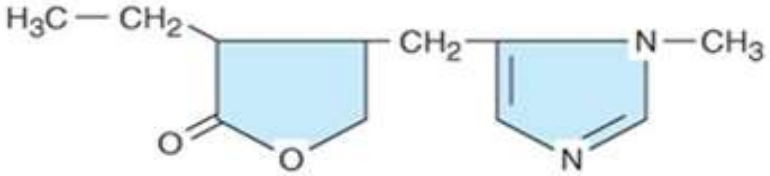


Muscarine

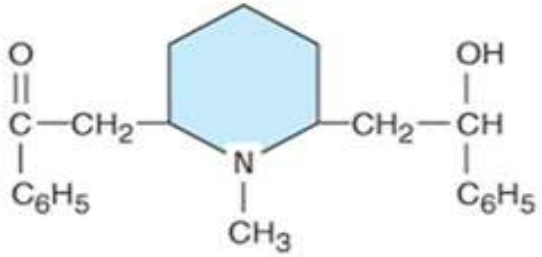
ACTION CHIEFLY NICOTINIC



Nicotine

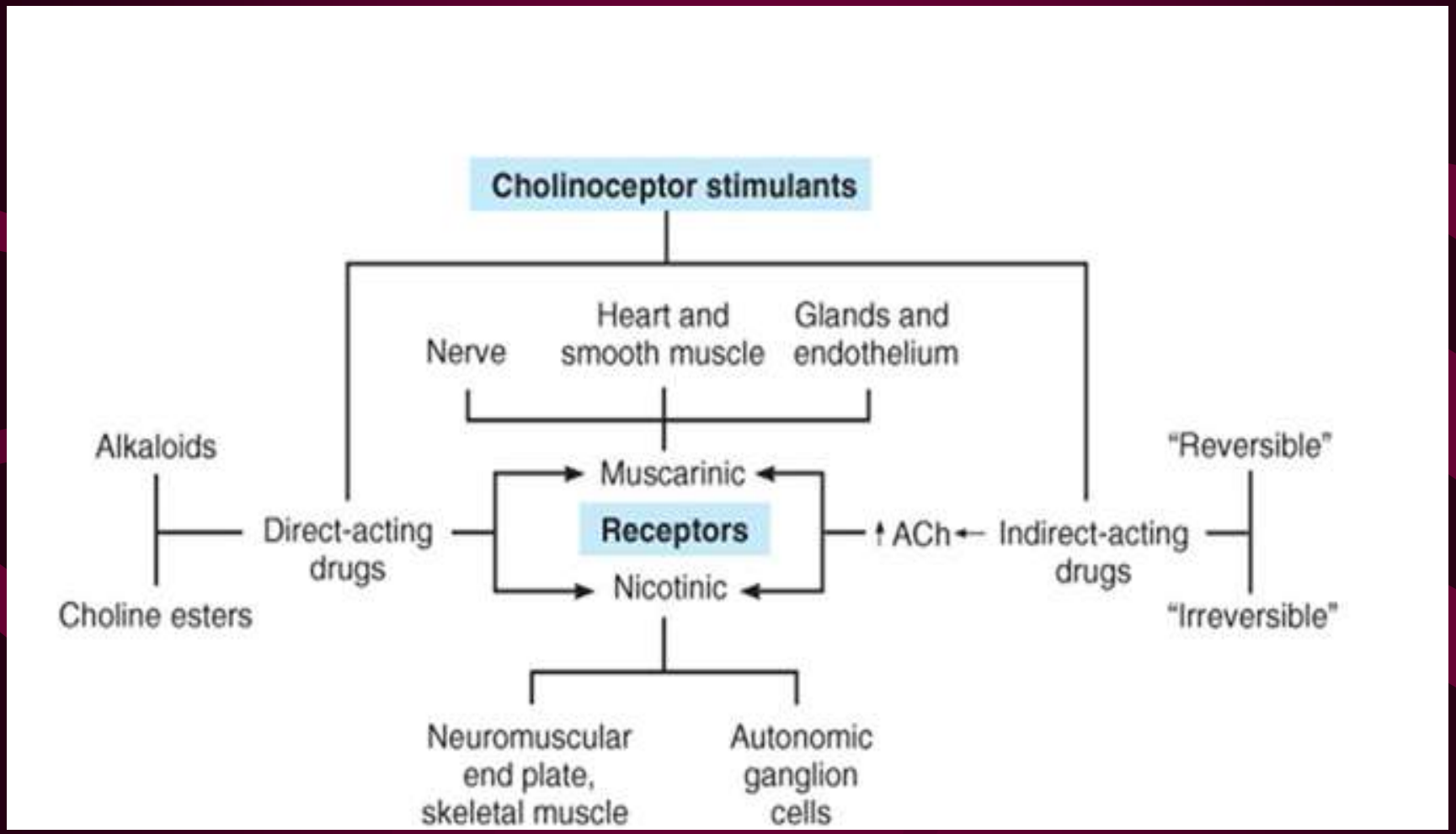


Pilocarpine



Lobeline

Major group of cholinoreceptor activating drugs, receptors and target tissues



Propeties of cholinesters

Choline Ester	Susceptibility to Cholinesterase	Muscarinic Action	Nicotinic Action
Acetylcholine chloride	++++	+++	+++
Methacholine chloride	+	++++	None
Carbachol chloride	Negligible	++	+++
Bethanechol chloride	Negligible	++	None

Pharmacological actions-

A) Muscarinic

1. Heart
2. Blood vessels
3. Smooth muscle
4. Glands
5. Eye

B) Nicotinic

1. Autonomic Ganglia
2. Skeletal Muscles
3. CNS

Effect of direct acting cholinoreceptors stimulats

Organ	Response
Eye	
Sphincter muscle of iris	Contraction (miosis)
Ciliary muscle	Contraction for near vision
Heart	
Sinoatrial node	Decrease in rate (negative chronotropy)
Atria	Decrease in contractile strength (negative inotropy). Decrease in refractory period
Atrioventricular node	Decrease in conduction velocity (negative dromotropy). Increase in refractory period
Ventricles	Small decrease in contractile strength
Blood vessels	
Arteries	Dilation (via EDRF). Constriction (high-dose direct effect)
Veins	Dilation (via EDRF). Constriction (high-dose direct effect)

Cont……

Lung	
Bronchial muscle	Contraction (bronchoconstriction)
Bronchial glands	Stimulation
Gastrointestinal tract	
Motility	Increase
Sphincters	Relaxation
Secretion	Stimulation
Urinary bladder	
Detrusor	Contraction
Trigone and sphincter	Relaxation
Glands	
Sweat, salivary, lacrimal, nasopharyngeal	Secretion

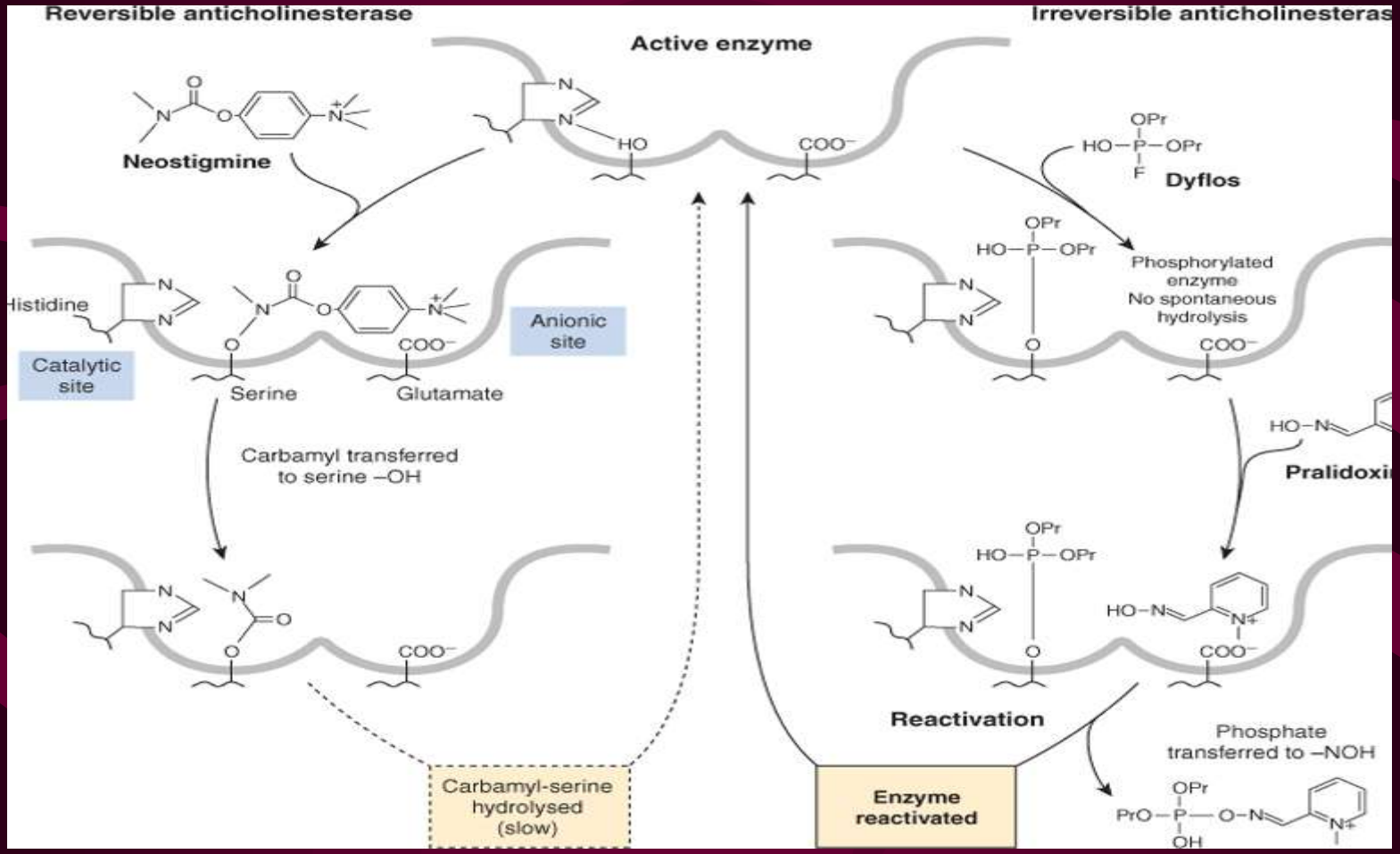
Organ or Tissue Function	Predominant Adrenoceptor Type	Adrenergic Response	Cholinergic Response[a]
Heart[b]			
Rate (chronotropic effect)	β_1	Increase	Decrease
Contractile force (inotropic effect)	β_1	Increase	None
Conduction velocity (dromotropic effect)	β_1	Increase	Decrease
Eye			
Pupil size	α_1	Constriction of radial muscle causing dilation (mydriasis)	Contraction of circular muscle (miosis)
Accommodation		No innervation	Contraction of ciliary muscle producing accommodation for near vision
Bronchial smooth muscle	β_2	Relaxation	Contraction
Blood vessels (arteries and arterioles)[c]			
Cutaneous	α_1	Constriction	No innervation[e]
Visceral	α_1	Constriction	No innervation[e]
Pulmonary	α_1	Constriction	No innervation[e]
Skeletal muscle	α_1, β_2	Constriction[d]	No innervation[e]
Coronary	α_1, β	Constriction, dilation[f]	No innervation[e]
Cerebral	α_1	Constriction	
Veins	α_1	Constriction	No innervation
Gastrointestinal tract (tone, motility, and secretory activity)	α_2, β_2	Decrease[g]	Increase
Sphincters	α	Contraction	Relaxation
Splenic capsule	α_1	Contraction	No innervation
Urinary bladder			
Detrusor muscle	β	Relaxation	Contraction
Trigone-sphincter muscle	α_1	Contraction	Relaxation
Uterus	α_1, β_2	Contraction-relaxation[h]	Contraction-relaxation
Glycogenolysis			
Skeletal muscle	β_2	Increase	None
Liver	α_1, β_2	Increase	None

DRUGS THAT INHIBIT CHOLINESTERASE

- **Short-acting anticholinesterases ---edrophonium,** a quaternary ammonium compound that binds to the anionic site of the enzyme only.
- Medium-duration anticholinesterases ----**neostigmine** and **pyridostigmine**, which are quaternary ammonium compounds of clinical importance, and **physostigmine** (eserine), a tertiary amine, all carbamyl, as opposed to acetyl, esters, and all possess basic groups that bind to the anionic site. Transfer of the carbamyl group to the serine hydroxyl group of the esteratic site occurs as with Ach
- Irreversible anticholinesterases-----fluoride (in **dyflos**), organic group (in **parathion** and **ecothiopate**

Effects of anticholinesterase drugs

- ***Effects on autonomic cholinergic synapses--***increased secretions from salivary, lacrimal, bronchial and gastrointestinal glands; increased peristaltic activity; bronchoconstriction; bradycardia and hypotension; pupillary constriction; fixation of accommodation for near vision; fall in intraocular pressure
- ***Effects on the neuromuscular junction---***twitching of muscles
- ***Effects on the CNS----***
- ***Neurotoxicity of organophosphates-----***


Reversible anticholinesterase
Irreversible anticholinesteras
Active enzyme
Neostigmine
Dyflos
Histidine
Catalytic site
Anionic site
Serine
Glutamate
Phosphorylated enzyme
No spontaneous hydrolysis
Carbamyl transferred to serine –OH
Pralidoxi
Reactivation
Phosphate transferred to –NOH
Carbamyl-serine hydrolysed (slow)
Enzyme reactivated

Clinical uses of anticholinesterase drugs Body

- To treat *myasthenia gravis* (**neostigmine** or **pyridostigmine**).
- As a test for myasthenia gravis and to distinguish weakness caused by anticholinesterase overdosage ('cholinergic crisis') from the weakness of myasthenia itself ('myasthenic crisis'): **edrophonium**, a short-acting drug given intravenously.
- *Alzheimer's dieases* (e.g. **donepezil**
- *Glaucoma* (**ecothiopate** eye drops).

Anticholinergic Drug and Drug acting on Autonomic Ganglia

1. Natural alkaloids- Atropine, Hyoscine (Scopalamine)
2. Semisynthetic Derivatives- Homatropine, Atropine methonitrates, Ipratropium bromide, Tiotropium bromide.
3. Synthetic compounds

i) Myadritic- Cyclopentolate, Tropicamide

ii) Antisecretory –Antispasmodics-

- Quaternary compounds- Propanthelin, Oxyphenonium, Clidium, Glycopyrolate
- Tertiary compounds- Dicyclomine, Pirenzepine

iii) Vasicoselective- Oxybutynin,

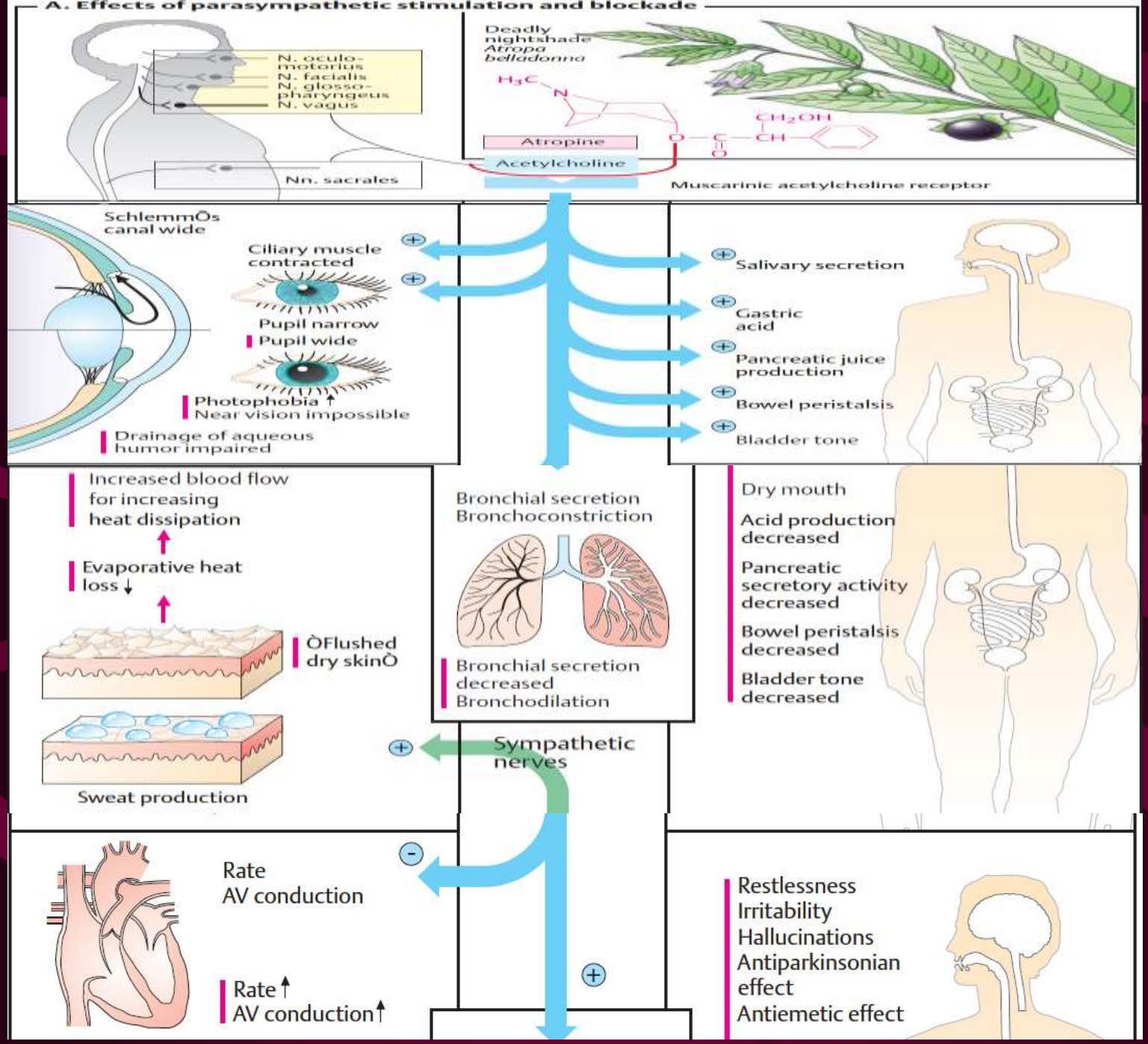
iv) Antiparkinsonian- Trihexyphenidyl, Procyclidine, Biperiden

Parasympatholytics Agents

Pharmacological Actions

- ***Inhibition of secretions-*** Salivary, lacrimal, bronchial and sweat glands are inhibited by very low doses of atropine, producing an uncomfortably dry mouth and skin. Gastric secretion is only slightly reduced. Mucociliary clearance in the bronchi is inhibited, so that residual secretions tend to accumulate in the lungs. Ipratropium lacks this effect.
- ***Effects on heart rate-*** Atropine causes tachycardia through block of cardiac mAChRs. The tachycardia is modest, up to 80-90 beats/min in humans. At very low doses, atropine causes a paradoxical bradycardia, possibly due to a central action
- ***Effects on the eye-***The pupil is dilated (*mydriasis*) by atropine administration, and becomes unresponsive to light. Relaxation of the ciliary muscle causes paralysis of accommodation (*cycloplegia*), so that near vision is impaired. Intraocular pressure may rise; although this is unimportant in normal individuals, it can be dangerous in patients suffering from narrow-angle glaucoma.

- ***Effects on the gastrointestinal tract-***Gastrointestinal motility is inhibited by atropine, although this requires larger doses than the other effects listed, and is not complete. This is because excitatory transmitters other than ACh are important in normal function of the myenteric plexus .
- ***Effects on other smooth muscle-***Bronchial, biliary and urinary tract smooth muscle are all relaxed by atropine
- ***Effects on the CNS-***Atropine produces mainly excitatory effects on the CNS. At low doses, this causes mild restlessness; higher doses cause agitation and disorientation. In atropine poisoning, which occurs mainly in young children who eat deadly nightshade berries, marked excitement and irritability result in hyperactivity and a considerable rise in body temperature, which is accentuated by the loss of sweating.
- Local anaesthetic- Mild anaesthetic action


A. Effects of parasympathetic stimulation and blockade
N. oculo-motorius
N. facialis
N. glosso-pharyngeus
N. vagus
Nn. sacrales
Deadly nightshade
Atropa belladonna
Atropine
Acetylcholine
Muscarinic acetylcholine receptor
Schlemm's canal wide
Ciliary muscle contracted
Pupil narrow
Pupil wide
Photophobia
Near vision impossible
Drainage of aqueous humor impaired
Salivary secretion
Gastric acid
Pancreatic juice production
Bowel peristalsis
Bladder tone
Increased blood flow for increasing heat dissipation
Evaporative heat loss
'Flushed dry skin'
Sweat production
Bronchial secretion
Bronchoconstriction
Bronchial secretion decreased
Bronchodilation
Dry mouth
Acid production decreased
Pancreatic secretory activity decreased
Bowel peristalsis decreased
Bladder tone decreased
Sympathetic nerves
Rate
AV conduction
Rate
AV conduction
Restlessness
Irritability
Hallucinations
Antiparkinsonian effect
Antiemetic effect

Clinical uses of muscarinic antagonists

1. **Cardiovascular** Treatment of *sinus bradycardia* (e.g. after myocardial infarction): atropine.
2. **Ophthalmic** : To *dilate the pupil*: for example tropicamide or cyclopentolate eye drops.
3. **Neurological** Prevention of *motion sickness*: for example scopolamine (orally or transdermally).
4. *Parkinsonism* (especially to counteract movement disorders caused by antipsychotic drugs): for example **benzhexol**, **benztropine**.
5. **Respiratory** : *Asthma* and *chronic obstructive pulmonary disease* : ipratropium or tiotropium by inhalation.
6. **Anaesthetic premedication:** To dry secretions: for example atropine, scopolamine. (Current anaesthetics are relatively non-irritant, so this use is now less important.)
7. **Gastrointestinal** Body : To facilitate endoscopy and gastrointestinal radiology by relaxing gastrointestinal smooth muscle (*antispasmodic* action): for example scopolamine.
8. As an antispasmodic in irritable bowel syndrome or colonic diverticular disease: for example **dicycloverine** (dicyclomine).
9. To treat peptic ulcer disease by suppressing gastric acid secretion : **pirenzepine** (M_1-selective antagonist). This is used less since the introduction of histamine H_2 antagonists and proton pump inhibitors.

BELLADONA ALKALOIDS

ATROPINE FROM ATROPA BELLADONNA

HYOSCINE FROM HYOSCYAMUS NIGER

Mechanism of action

- Antimuscarinic drugs block the muscarinic receptors, which can be reversed by increasing the concentration of muscarinic agonist.
- Antimuscarinic drugs prevents:
  - the release of inositol triphosphate (IP_3)
  - the inhibition of adenylcyclase

(That are caused by muscarinic agonist)

Pharmacokinetics

- Tertiary amines will absorbed from GIT
- Quaternary amines 10-30% absorbed from GIT
- Tertiary amines are widely distributed in the body
- Quaternary amines limited in their distribution
- Atropine is excreted largely as unchanged drug in urine
- Only about 1% of the oral dose of scopolamine is excreted in urine as unchanged
- Atropine effects in body remain only for a few hours but in the eye its effects persist for about 72 hours

COMPARISON BETWEEN ATROPINE AND HYOSCINE

	ATROPINE	HYOSCINE
Source	Atropa belladonna and Dhatura stramonium	hyoscyamus niger and Scopolia - cariolica
Chemistry	Ester of Tropic Acid and Tropine	Ester of Tropic Acid and Scopine
Mechanism of Action	Antimuscarinic, competitive antagonist of ACH	Antimuscarinic, competitive antagonist of ACH
Duration of Action	Prolonged	Short
Peripheral Antimuscarinic Action	More prominent on heart, G . I tract and bronchial muscle	More prominent on eyes, salivary and bronchial secretions and sweat

(Contd)

Actions on C.N.S	**Stimulation followed by depression**	**Depression from the beginning (in presence of pain, there may be excitement)**
Loss of memory	**--------**	**Amnesia to recent events**
Motion Sickness	**Less useful**	**More useful**
Parkinsonism	**Less useful**	**More useful**
Toxicity	**Restlessness, excitement, mania,delerium**	**Drowsiness**
Dose	**0.25- 2mg**	**0.3- .0.6mg**

ANTICHOLINERGIC DRUGS/MUSCARINIC RECEPTOR ANTAGONISTS

PHARMACOLOGICAL PROPERTIES OF ANTICHOLINERGICS

ATROPINE IS A COMPETITIVE ANTAGONISTS OF ACH AND OTHER MUSCARINIC AGONISTS ON MUSCARINIC RECEPTORS SELECTIVELY REDUCE OR ABOLISH THE MUSCARINIC EFFECTS OF ACH. ACTIONS MORE MARKED IN ORGANS WITH HIGH PARASYMPATHETIC INNERVATION.

EFFECTS ON CNS

- Scopolamine causes drowsiness, amnesia, fatigue, and dreamless sleep. It is effective in motion sickness

Toxic doses of both alkaloids produces CNS excitation- restlessness irritability, disorientation, hallucination or delirium. Stimulation followed by depression, coma, medullary paralysis and death.

EFFECTS ON EYE

✓Mydriasis due to blockade of the cholinergic stimulation of sphincter ,this will allow adrenergic
✓Action on the radial muscle to dominate.

✓Photophobia – due to mydriasis. Light reflex is lost.

✓Cycloplegia – paralysis of ciliary muscle.

✓Reduction of lacrimal secretion – patient complains of dry and sandy eye when receiving large doses of anticholinergic drugs.

✓Intraocular pressure is increased in patients having narrow angle glaucoma.

EFFECTS ON GIT

Therapeutic dose of atropine produce a decrease in Tone, amplitude and frequency of peristalsis, and cause constipation

Large doses decrease secretion of (HCL) mucin and proteolytic enzymes in gastric juice.

EFFECTS ON RESPIRATORY & GENITOURINARY TRACT

Respiratory tract:

Inhibition of secretions of upper respiratory tract, prevent laryngospasm, induced by excessive secretions due to Certain general anaesthetic

Inhibition of bronchoconstriction produced by cholinergic drugs, histamines, kinin & PGF_2

Genitourinary Tract: No Effect on uterus.

Urinary Tract: Decrease in tone and contraction of ureter, and bladder

EFFECTS ON CVS

Heart

Small dose – bradycardia due to blockade of M_1 receptors

Larger dose – tachycardia due to blockade of M_2 receptors

Blood Vessels

Majority are not affected.

In toxic doses vasomotorparalysis occurs leading to fall in blood pressure.

Dilatation of cutaneous blood vessels may occur causing flushed skin

EXOCRINE SECRETIONS

Salivary and bronchial secretions are inhibited – dry mouth

Sweating is inhibited – hot skin.

Adverse Effects

- Atropine and other antimuscarinic drugs are especially dangerous in infants and children. Dangerous degree of hyperthermia can occur,
- Poisoning with these drugs can cause the following effects
  - **Dryness of mouth and skin, due to inhibition of salivary and sweat secretion.**
  - **Dilatation of pupil and cycloplegia, which produce photophobia and blurring of vision for near objects.**

(Cont..)

- **Tachycardia and palpitations due to blockade of vagal effect on sinoatrial node of heart**

- flushed (red) and hot skin due to cutaneous vasodilatation and absence of sweating.

- Hallucinations, agitation, and delirium can occur due to their effects on CNS

- Severe cases of poisoning with these drugs can occur.

ANTAGONIST FOR THREE TYPES OF MUSCARINIC RECEPTORS

M1. Pirenzepine, telenzepine

M2. AF-DX116, methoctramine, himbacine, tripitramine.
Tripitramine is used to block cholinergic bradycardia.

M3. Hexahydrosiladifenidiol and darifenacin.
Darifenacin is used for overactive bladder

THERAPEUTIC CLASSIFICATION OF ANTICHOLINERGICS

MYDRIATICS

Tertiary amines: Homatropine, hydrobromide, eucatropine, cyclopentolate, tropicamide.

There advantages over atropine are: They are short acting and produce less cycloplegia.

ANTI SPASMODIC

Quaternary ammonium compounds: Propantheline, methantheline, oxyphenonium, glycopyrrolate

Tertiary amines: Dicyclomine, oxyphencyclimine, piperidolate amprotropine, oxybutynin chloride

ANTI-PARKINSONIAN AGENT

These are tertiary amines, e.g. trihexyphenidyl, benztropine, biperidine, procyclidine, cycrimine, ethopropazine

SELECTIVE ANTI-MUSCARINIC DRUGS

Ipratropium
Oxitropium

→ Relives bronchospasm in asthma/COPD

Oxybutynin

Falvoxate HCl

→
1. Relieves bladder spasm after surgery
2. Urteral spasm due to ureolithiasis

CLINICAL USES OF ANTICHOLINERGICS

USE IN GIT

- **Pirenzepine acts synergistically with H2 blockers in the treatment of peptic ulcer**
- **Increased tone and motility of GIT**
- **Irritable bowel syndrome**
- **To reduce salivary secretion in heavy metal poisoning, Parkinsonism and Oesophageal stricture.**

EYE

- **Topical use of mydriatic for funduscopic examination**

- **Topical use of cycloplegic for iritis, iridocyclitis, choroiditis**
- **Alternating with miotic to prevent or break the adhesions between iris and lens**

RESPIRATORY TRACT

- Ipratropium inhalation in bronchial asthma and COPD

CVS

- To antagonize reflex cardiac slowing
- In hyperactive carotid sinus reflex
- In patients with inferior or posterior wall infarction having decreased cardiac output, sinus or nodal bradycardia.
- In AV block due to digitalis toxicity.

CNS

- Benztropine for treating extrapyramidal disorder due to antipsychotic drugs.
- Scopolamine (oral transdermal) for prevention and treatment of motion sickness

GENERAL ANAESTHESIA

- To inhibit excessive salivation and secretion of respiratory tract and to prevent reflex vagal stimulation of the heart

- Atropine given with neostigmine to counter its muscarinic effect when given to end the effect of competitive type of neuromuscular blocking agent.

GENITOURINARY TRACT

- Atropine with an opioid in the treatment of renal colic
- To relieve urteral spasm and irritability of bladder (urinary urgency) and after urologic surgery (e.g, prostatectomy) and also reducing involuntary voiding in patients with neurological diseases oxybutynin is used.

ANTICHOLINESTERASES AND MUSHROOM POISONING

- Antidote for organophosphate poisoning

- To antagonize muscarinic effect of neostigmine in myasthenia gravis

- Rapid type of muscarinic (inocybe) poisoning

CONTRAINDICATIONS OF ATROPINE

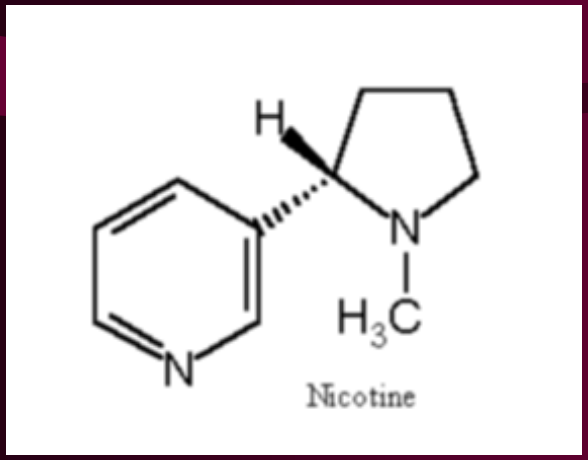
- Narrow angle glaucoma
- Enlarged prostate
- Delayed type of mushroom poisoning
- Pyloric stenosis
- Congestive heart failure with tachycardia
- Patients over the age of 40 years as it may precipitate an acute attack of congestive glaucoma.

Chronic lung disease as this reduces respiratory tract secretions.

Antimuscarinics in Dentistry

- Atropine is the drug of choice for use in dentistry as Anti sialogogue to control salivation
- Atropine sulphate tablets administered one hour before appointment in a dose of 0.4mg to achieve dry field and to enhance visibility of tooth surface.
- Minimize interruption during procedure to remove pooled saliva
- As an adjunct to orthodontic bounding, prosthetics fixing, impression taking, to reduce moisture contamination
- Radiographic procedure when a dry field is important

GANGLIONIC STIMULATING AND BLOCKING AGENTS



Nicotine

History of Tobacco

By 17th century

- In Western Europe, used as treatment for migraines
- Japan & China stop enforcing prohibition of use
- Russia opens door to West

Nicotine Forms

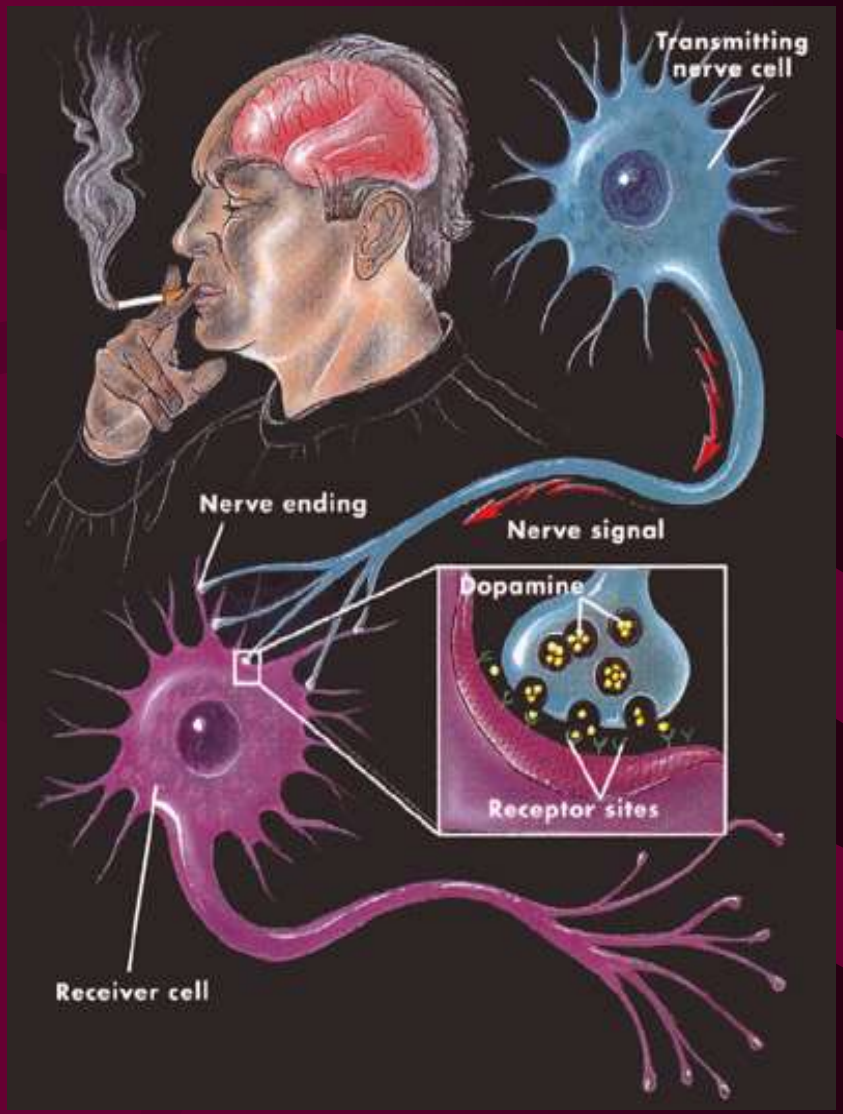
- Tobacco
  - Smokeable
    - Cigarette
    - Pipe
    - Cigar
  - Leaf (Chewing)
  - Snuff (powdered)
- Transdermal Patch

Ganglionic stimulating drugs

- Nicotine
  - Alkaloid isolated from leaves of tobacco.
  - Volatile liquid
  - Tertiary amine (lobeline also)
  - Dimethylphenyl-piperazimium (DMPP)
  - Succinylcholine

Nicotine Pharmacology

- Biphasic action- nicotinic acetylcholine receptors
  - Agonist – low doses
  - Antagonist – high doses
- Works in CNS and PNS
- One of the most toxic dependence-producing psychoactive compounds overall
  - Nicotine acts to stimulate dopamine release in mesolimbic dopamine pathway (reward center).



Effect of nicotine on GIT

- ↑ tone & ↑ motor activity of bowel.
- Nausea
- Vomiting
- Diarrhea (occasionally)

Effect of nicotine on exocrine glands

- Initially stimulation of salivary secretion followed by inhibition.

Effect of nicotine on CNS

- Markedly stimulates CNS
- Moderate doses → tremors
- Convulsion (in large doses)
- Stimulation of CNS followed by depression
- Death from respiratory failure

Effect of nicotine on PNS

- Small doses stimulate the ganglion cells & facilitate transmission of impulses.
- Large doses initial stimulation followed very quickly by blockade of transmission.

Biotransformation/Excretion

- Broken down by lung and liver
  - >90% in liver
- Excreted through kidneys (urine)
- Lungs do some excretion

Pharmacokinetics

- Rapidly absorbed from respiratory tract, buccal mucosa & skin.
- Metabolized mainly in liver but also in kidney & lungs.

Table II-2-7. Characteristics of Ganglion Blocking Agents

Effector	System	Effect of Ganglion Blockade
Arterioles	SANS	Vasodilation, hypotension
Veins	SANS	Dilation, ↓ CO, ↓ venous return
Heart	PANS	Tachycardia
Iris	PANS	Mydriasis
Ciliary muscle	PANS	Cycloplegia
GI tract	PANS	↓ Tone and motility—constipation
Bladder	PANS	Urinary retention
Salivary glands	PANS	Xerostomia
Sweat glands	SANS	Anhydrosis

Dependence

- One of the most dependence-producing drugs
  - Pharmacology – Stimulates reward center influences ANS
  - Function – Weight control, coping with negative affect/stress, cognitive enhancement
  - Social Factors – Friends, habit, context

Withdrawal Symptoms of Nicotine

- Lethargy, decreased arousal
- Constipation
- Headaches
- Disrupted sleep cycles
- Irritability/anxiety
- Excessive hunger (blood sugar drop)

PHARMACOLOGY OF THE GANGLION-BLOCKING DRUGS

GANGLION-BLOCKING DRUGS

- Hexamethonium
- Trimetaphan
- Tubocurarine
- Pancuronium
- Atracurium
- Vecuronium

GANGLION-BLOCKING DRUGS

- Hexamethonium
- Mecamylamine
- Prevents reflex changes in H.R
- Most are no longer available clinically because of toxicities.

GANGLION-BLOCKING DRUGS

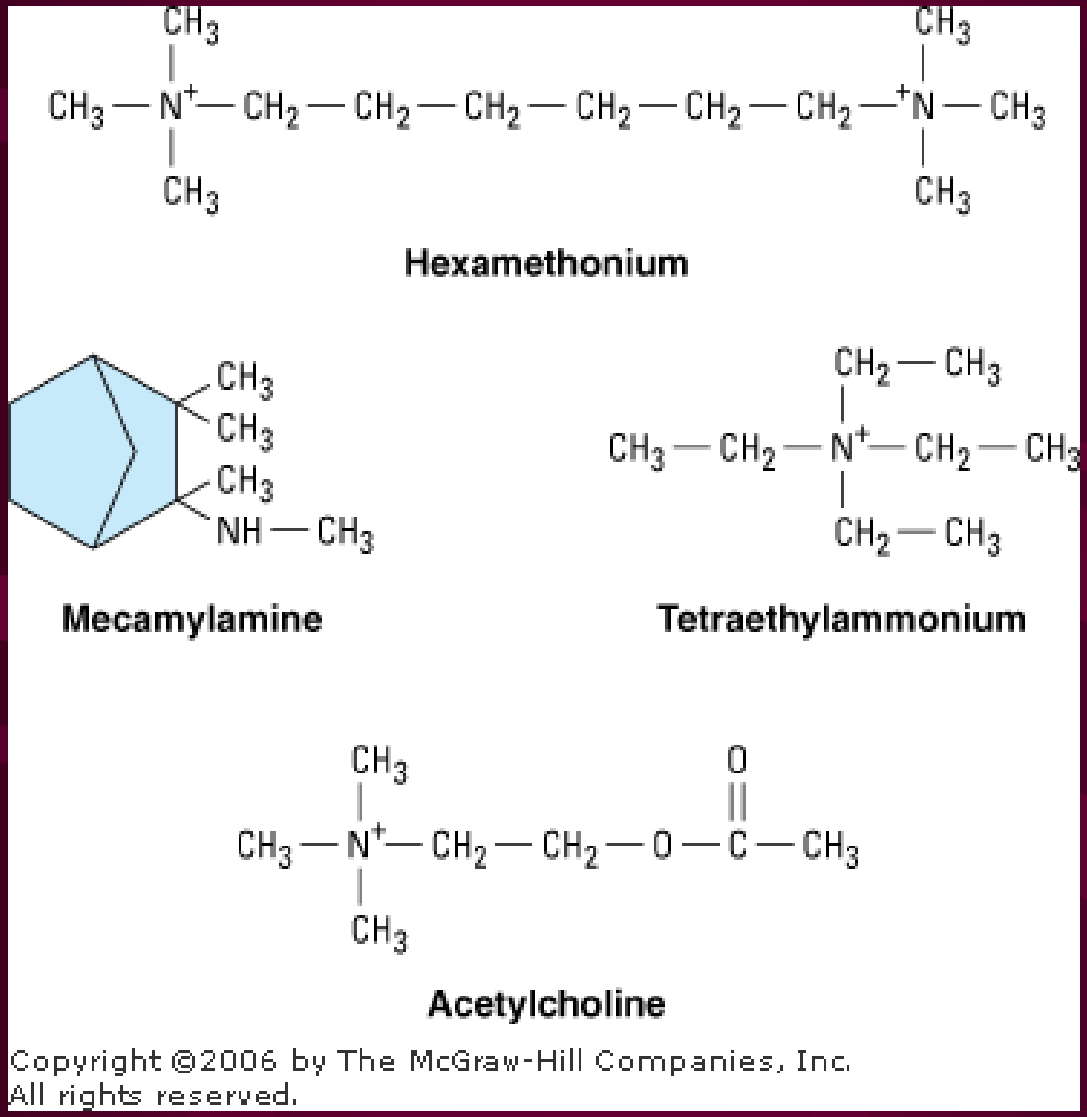
- Prevent reflex changes in H.R elicited by vasoconstriction (α1) & vasodilation (β2).
- Do not prevent changes in H.R elicited directly by the drug (β1 & M2).

Introduction

- Block the action of ACh and similar agonists at nicotinic receptors of both parasympathetic and sympathetic autonomic ganglia.
- Ganglion-blocking drugs are important and used in pharmacologic and physiologic research because they can block all autonomic outflow.
- Lack of selectivity confers such a broad range of undesirable effects that they have limited clinical use.

Chemistry & Pharmacokinetics

- All ganglion-blocking drugs of interest are synthetic amines.
- **Tetraethylammonium (TEA):**
  - The first to be recognized as having this action,
  - Has a very short duration of action.
- **Hexamethonium ("C6")** was developed and was introduced clinically as the first drug effective for management of hypertension.
- **Decamethonium**, the "C10" analog of hexamethonium, is a depolarizing neuromuscular blocking agent.



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- There is an obvious relationship between the structures of the agonist acetylcholine and the nicotinic antagonists tetraethylammonium and hexamethonium.

Chemistry & Pharmacokinetics

- The quaternary amine ganglion-blocking compounds were poorly and erratically absorbed after oral administration.
- **Mecamylamine,** a secondary amine, was developed to improve the degree and extent of absorption from the GIT.
- **Trimethaphan,** a short-acting ganglion blocker, is inactive orally and is given by intravenous infusion.

Pharmacodynamics

MECHANISM OF ACTION

- Ganglionic nicotinic receptors are subject to both depolarizing and nondepolarizing blockade
- Nicotine and even acetylcholine (if amplified with a chE inhibitor) can produce depolarizing ganglion block.
- Drugs now used as ganglion blockers are classified as nondepolarizing competitive antagonists.
  - Hexamethonium actually produces most of its blockade by occupying sites in or on the nicotinic ion channel, not by occupying the cholinoceptor itself.
  - Trimethaphan appears to block the nicotinic receptor, not the channel.

ORGAN SYSTEM EFFECTS

Central Nervous System

- Mecamylamine, unlike the quaternary amine agents and trimethaphan, crosses the BBB and readily enters the CNS.
  - Sedation, tremor, choreiform movements, and mental aberrations

ORGAN SYSTEM EFFECTS

Eye

- Cycloplegia with loss of accommodation because the ciliary muscle receives innervation primarily from the parasympathetic nervous system.
- The effect on the pupil is not so easily predicted,
- Moderate dilation of the pupil because parasympathetic tone usually dominates this tissue.

ORGAN SYSTEM EFFECTS

Cardiovascular System

- Blood vessels receive chiefly vasoconstrictor fibers from the sympathetic nervous system; therefore, ganglionic blockade causes a marked decrease in arteriolar and venomotor tone.
- Hypotension is marked in the upright position
- Cardiac effects include diminished contractility and,
- Sinoatrial node is usually dominated by the parasympathetic nervous system, a moderate tachycardia.

ORGAN SYSTEM EFFECTS

Gastrointestinal Tract

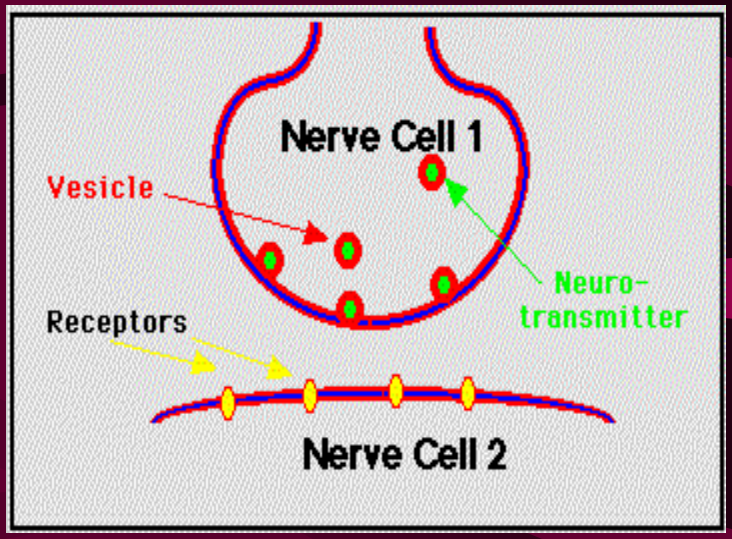
- Secretion is reduced, although not enough to effectively treat peptic disease.
- Motility is profoundly inhibited, and constipation can be marked.

Genitourinary

- Hesitancy in urination
- Erection and ejaculation may be prevented by moderate doses

Clinical Applications & Toxicity

- Use of the ganglion blockers is infrequent
- Mecamylamine is being studied for possible use in reducing nicotine craving in patients
- Trimethaphan is occasionally used:
  - treatment of hypertensive emergencies and dissecting aortic aneurysm;
  - to produce controlled hypotension, which can be of value in neurosurgery to reduce bleeding in the operative field; and in patients undergoing electroconvulsive therapy.
- Toxicity- limited to the autonomic effects already described. For most patients, these effects are intolerable except for acute use.



Adrenergic System and Adrenergic Drugs

CATECHOLAMINES

- **Noradrenaline** (**norepinephrine**1), a transmitter released by sympathetic nerve terminals
- **Adrenaline** (**epinephrine**), a hormone secreted by the adrenal medulla
- **Dopamine**, the metabolic precursor of noradrenaline and adrenaline, also a transmitter/neuromodulator in the central nervous system

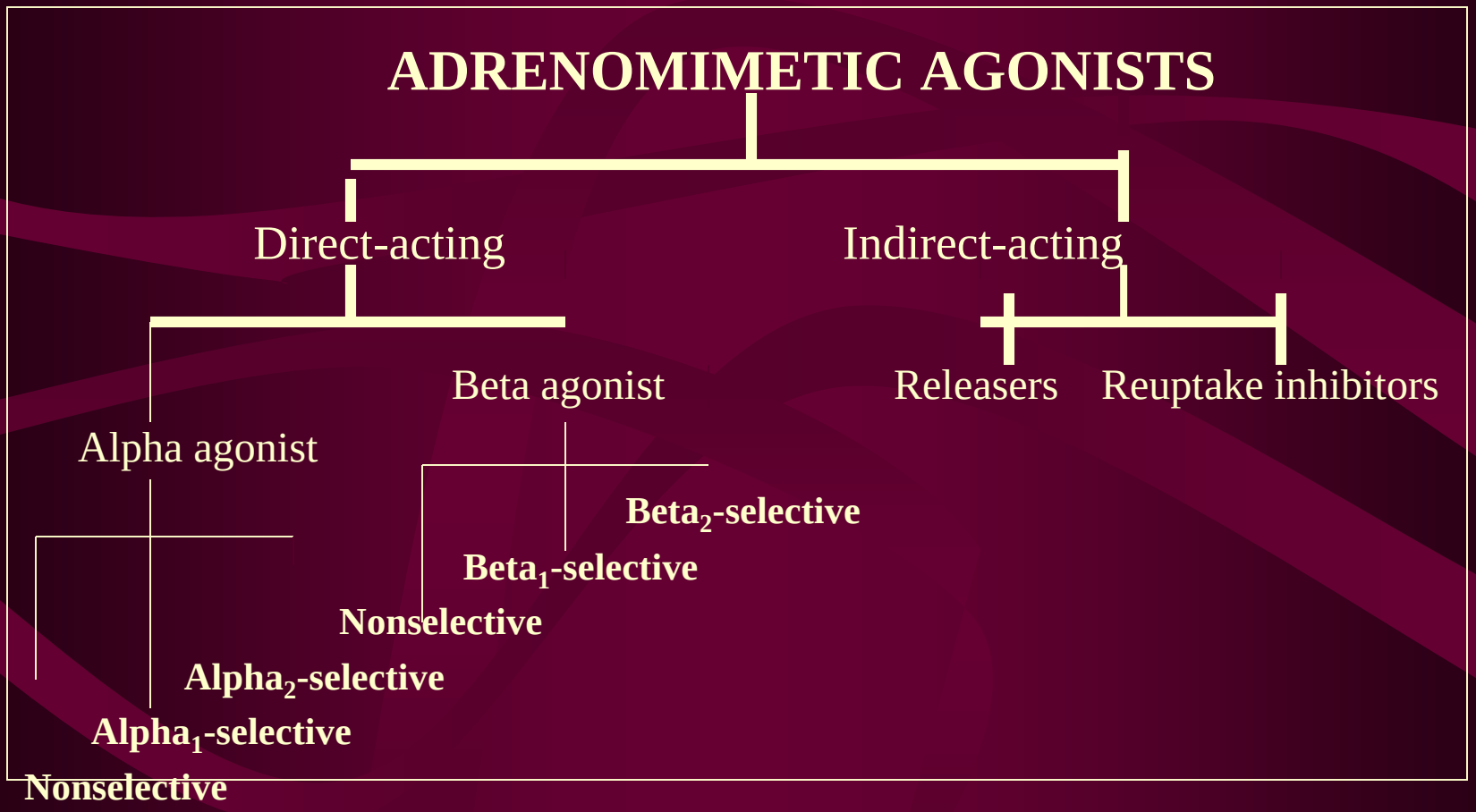
SYMPATHOMIMETICS

- Adrenomimetics
- Constitute a group of drugs used for
  - Cardiovascular
  - Respiratory
  - Other conditions

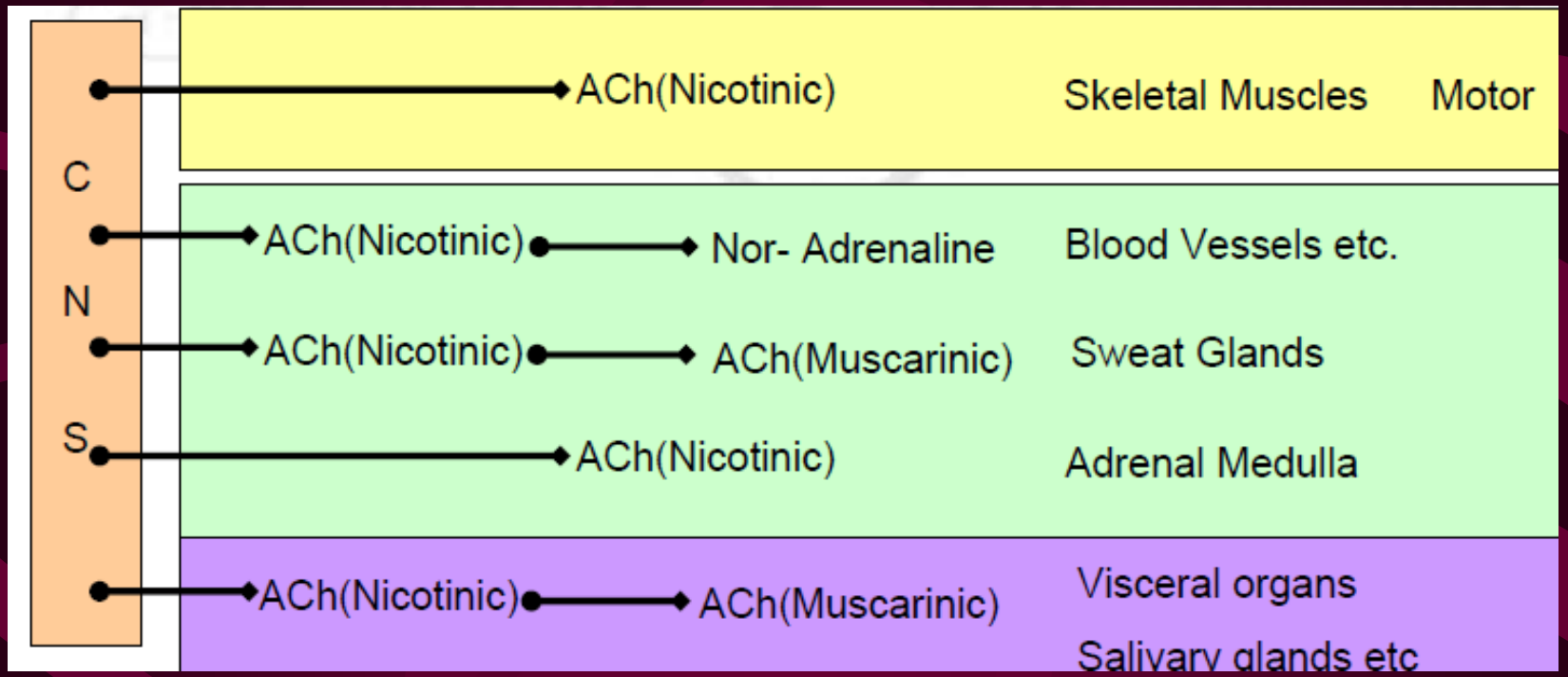
- Subdivided into
  - Mode of action
    - Direct-acting
    - Indirect-acting

- Subdivided into
  - Spectrum of action
    - Alpha
    - Beta
    - Dopamine receptor affinity

SYMPATHOMIMETICS

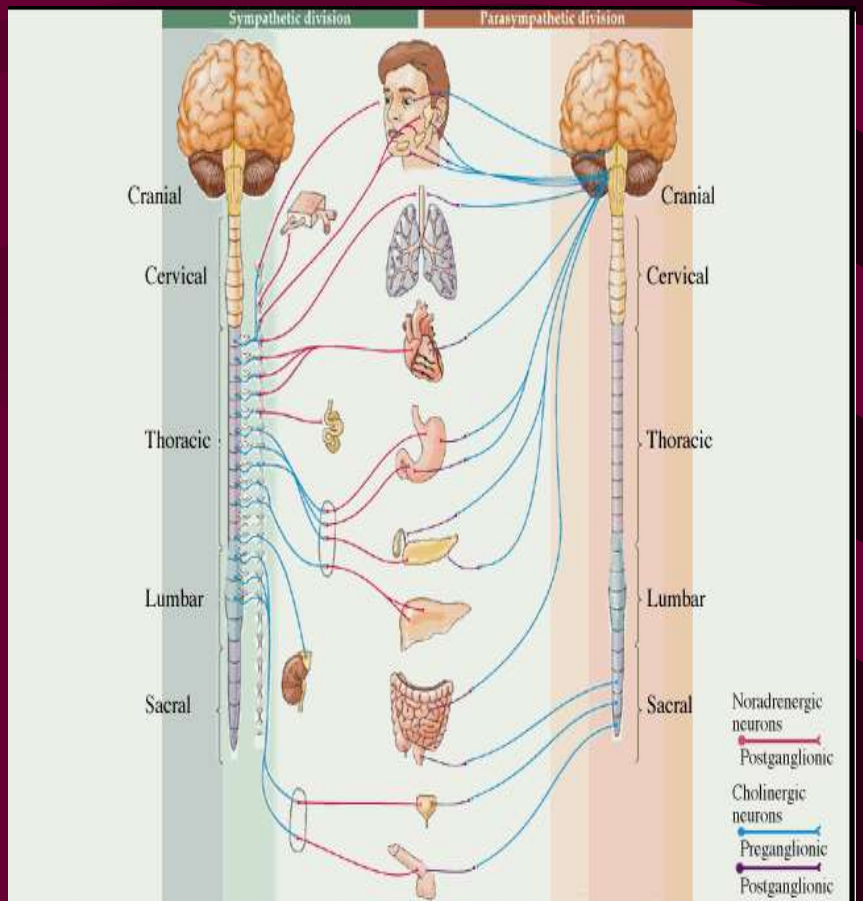


Neurotransmission in ANS



Noradrenergic transmission

- Nor-adrenaline is the major neurotransmitter of the Sympathetic system
- Noradrenergic neurons are postganglionic sympathetic neurons with cell bodies in the sympathetic ganglia
- They have long axons which end in varicosities where NA is synthesized and stored

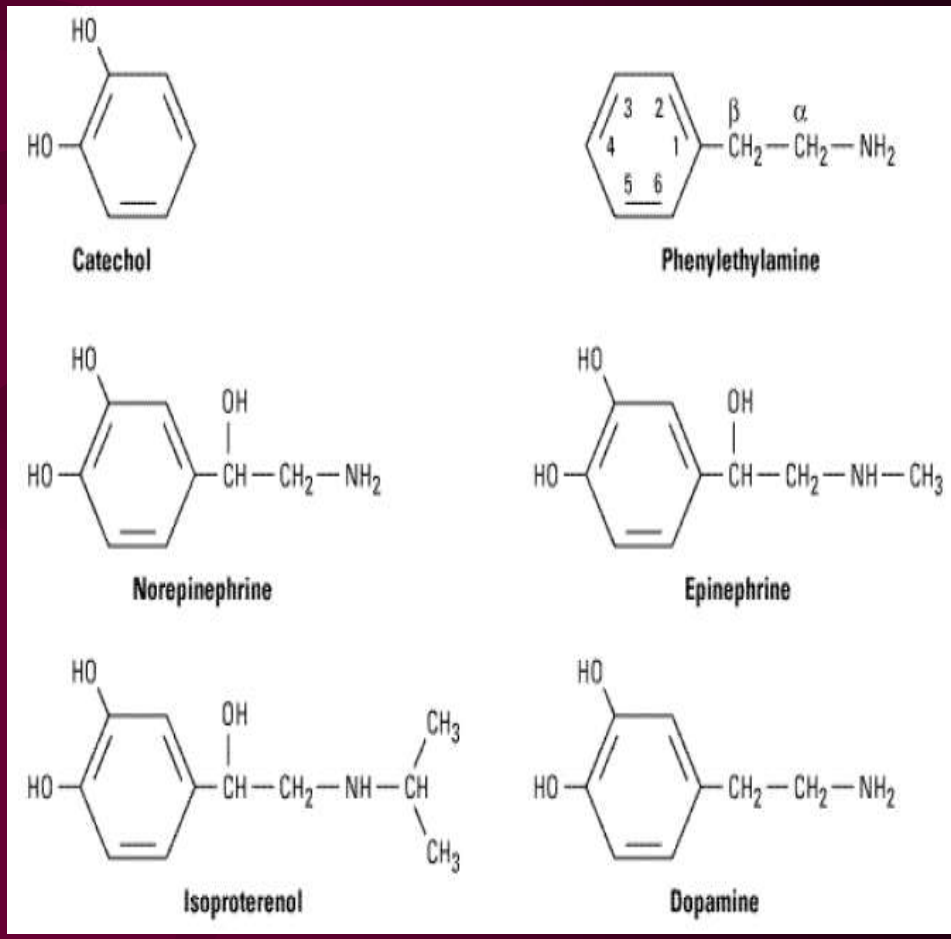


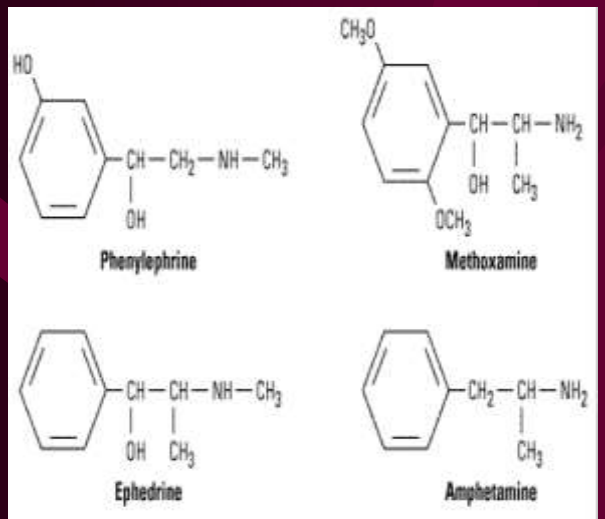
Adrenergic transmission

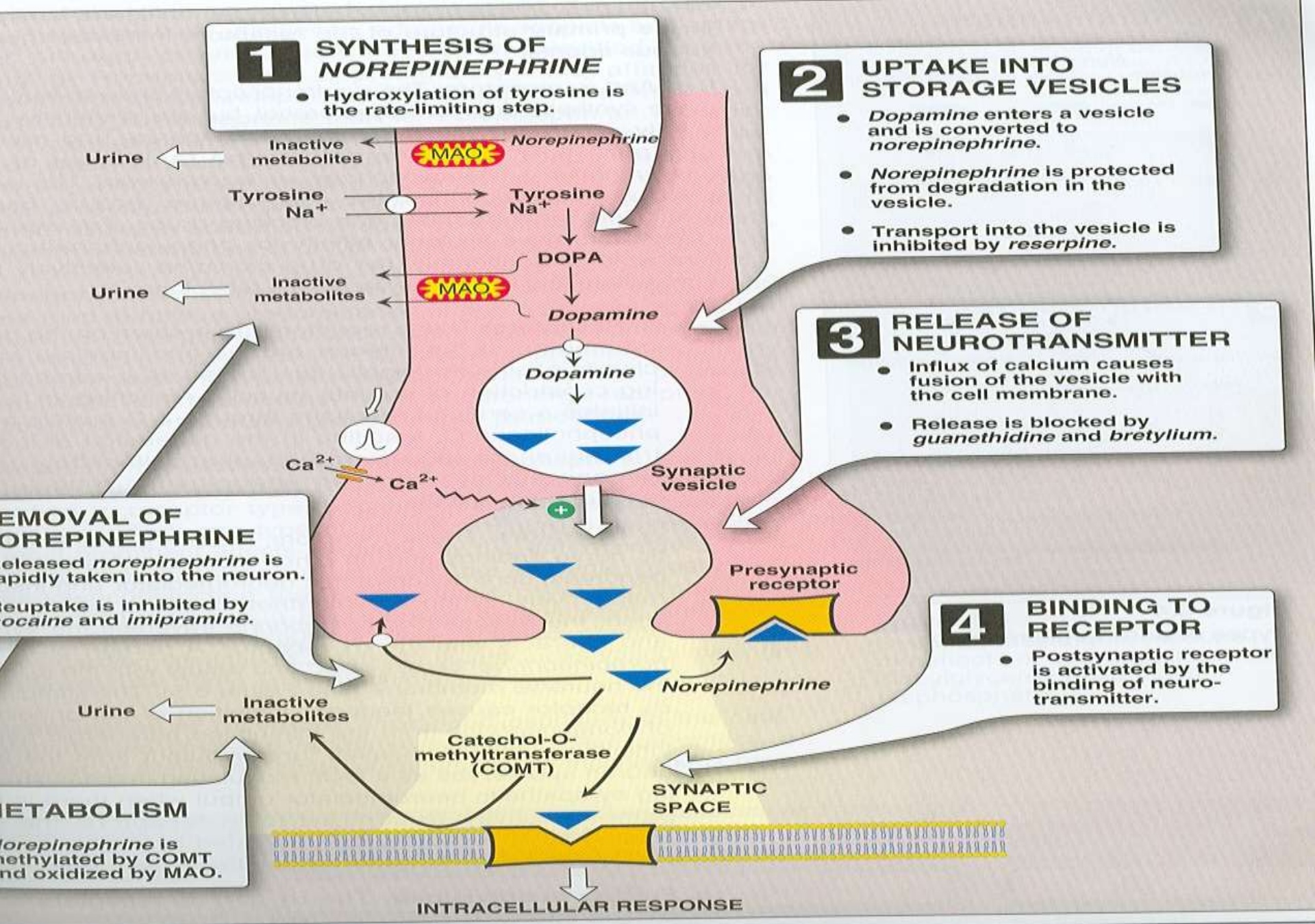
Catecholamines:

- **Natural:** Adrenaline, Noradrenaline, Dopamine
- **Synthetic**: Isoprenaline, Dobutamine
- Non-Catecholamines:
  - Ephedrine, Amphetamines, Phenylepherine, Methoxamine, Mephentermine
- Also called *sympathomimetic amines* as most of them contain an *intact or partially* substituted amino (NH2) group

- **Catecholamines:** Compounds containing a *catechol nucleus* (Benzene ring with 2 adjacent OH groups) and an *amine* containing side chain
- **Non-catecholamines** lack hydroxyl (OH) group





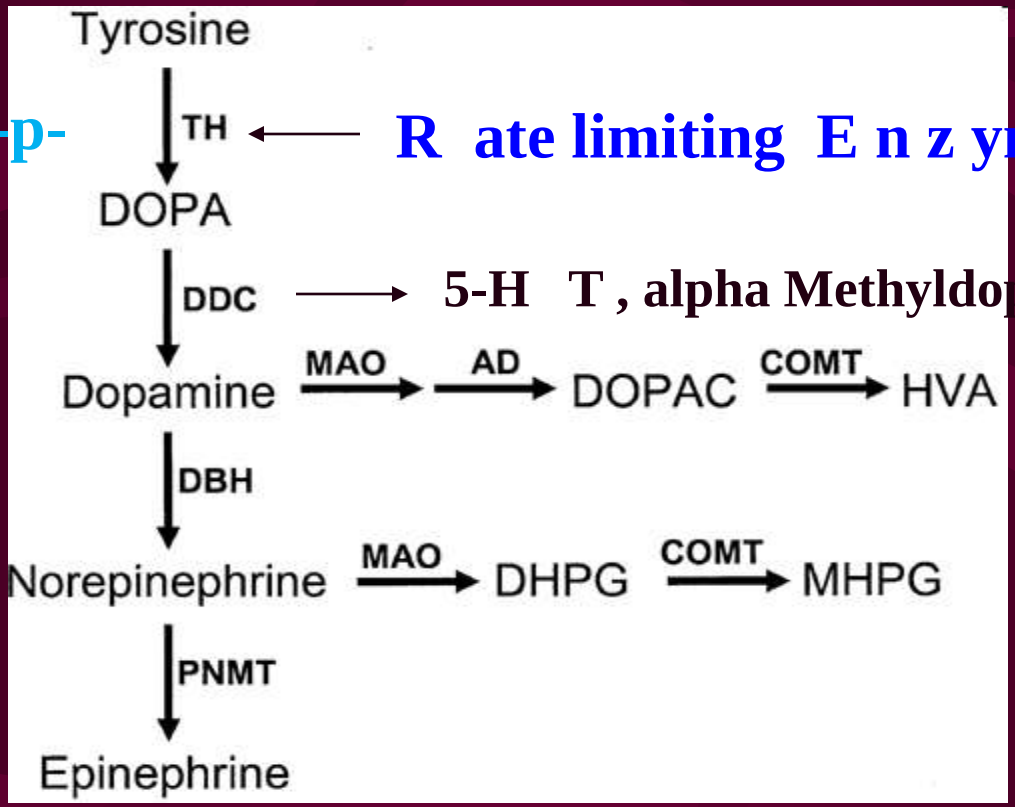


6.3
is and release of *norepinephrine* from the adrenergic neuron. (MAO = monoamine oxidase.)

Biosynthesis of Catecholamines

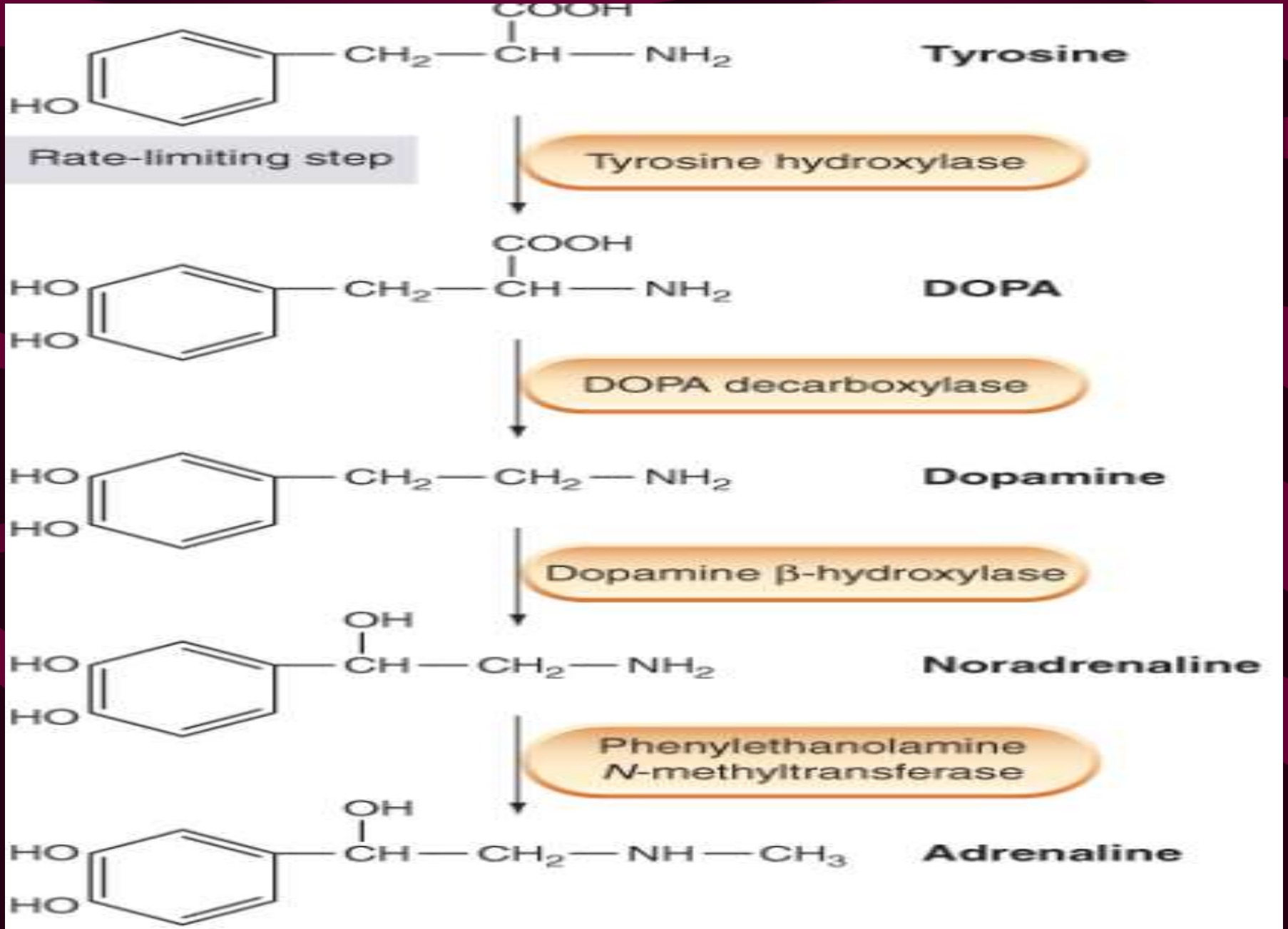
P henylalanine

PH

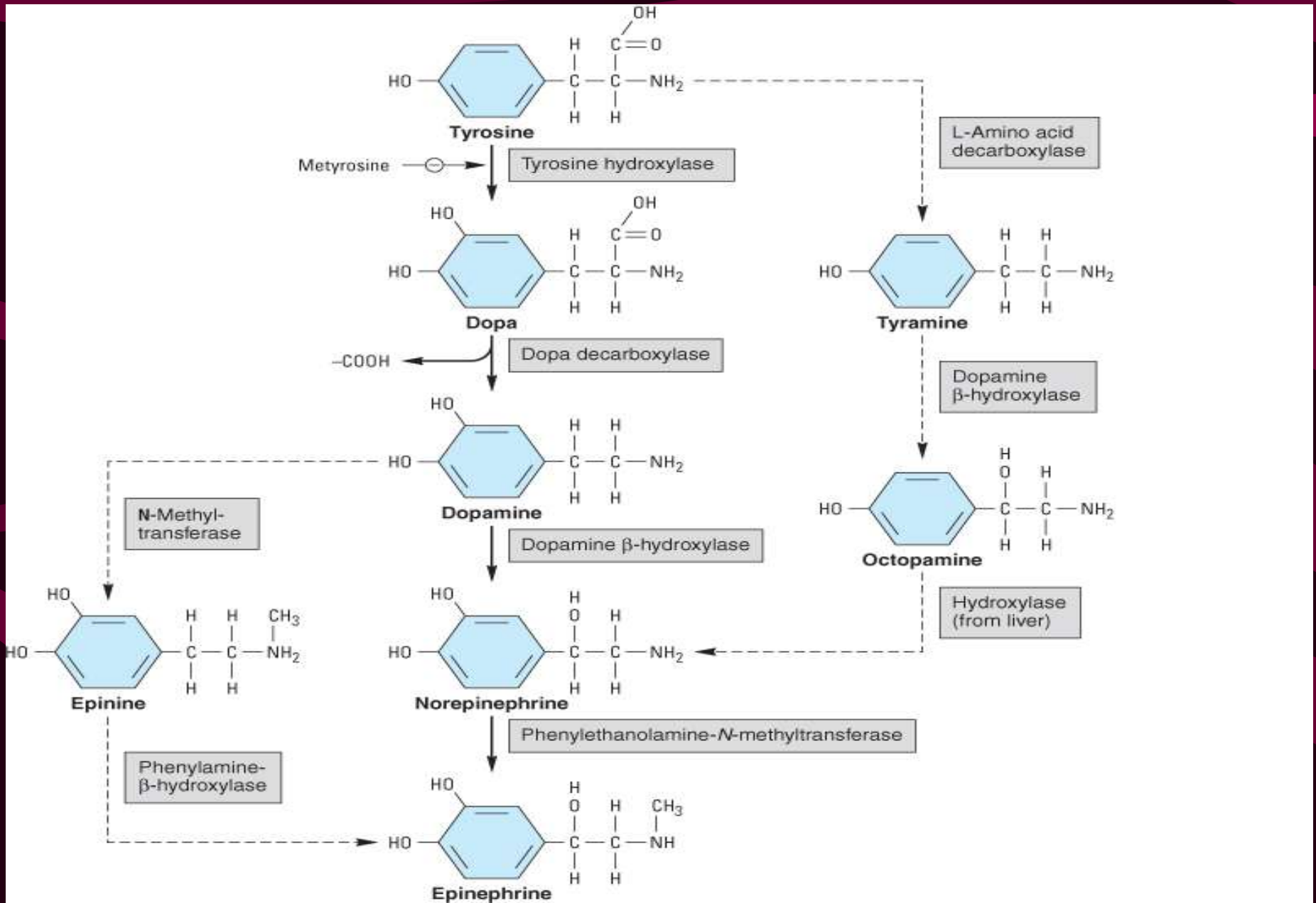


Alpha-methyl-p-tyrosine

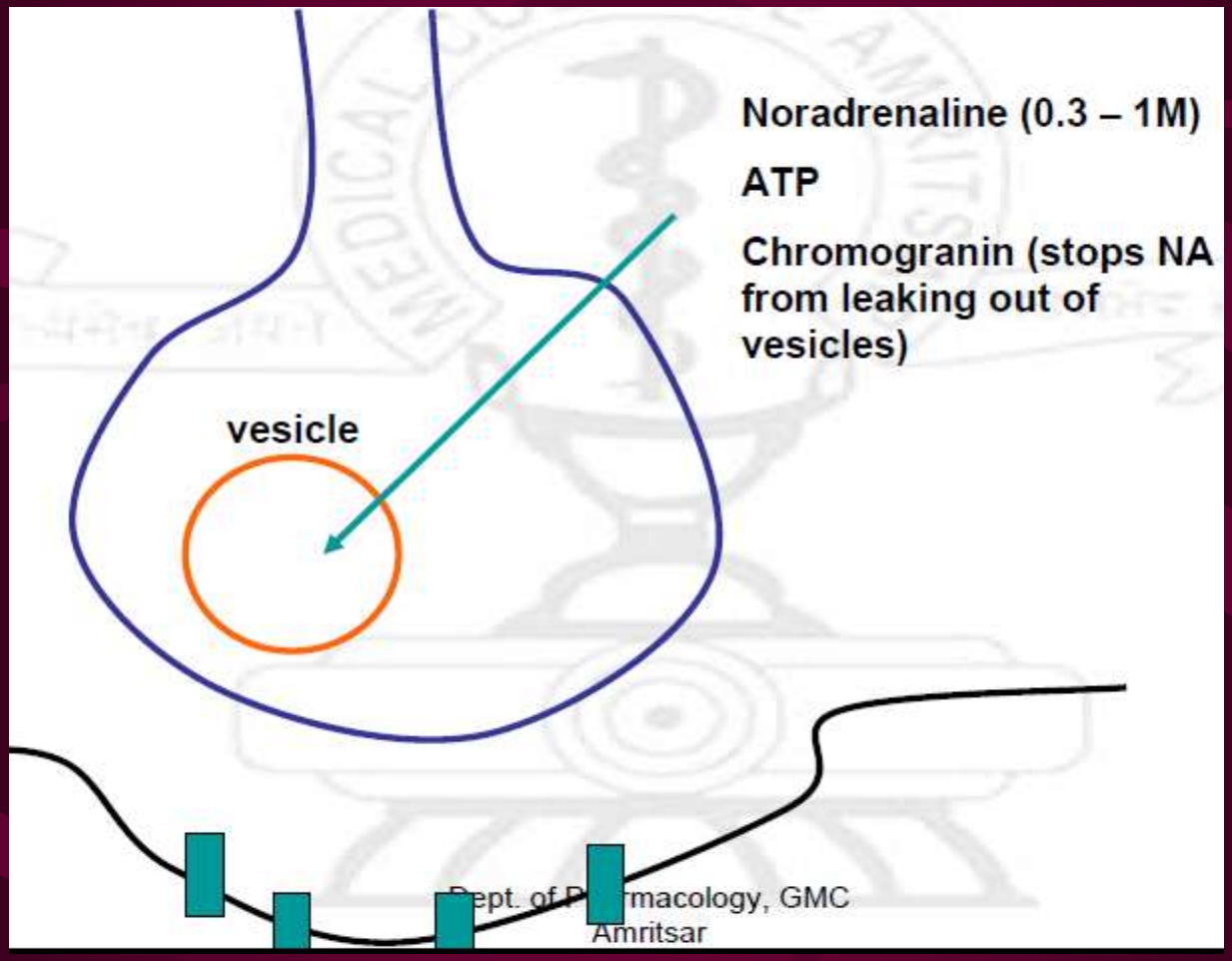
Biosynthesis of catecholamines



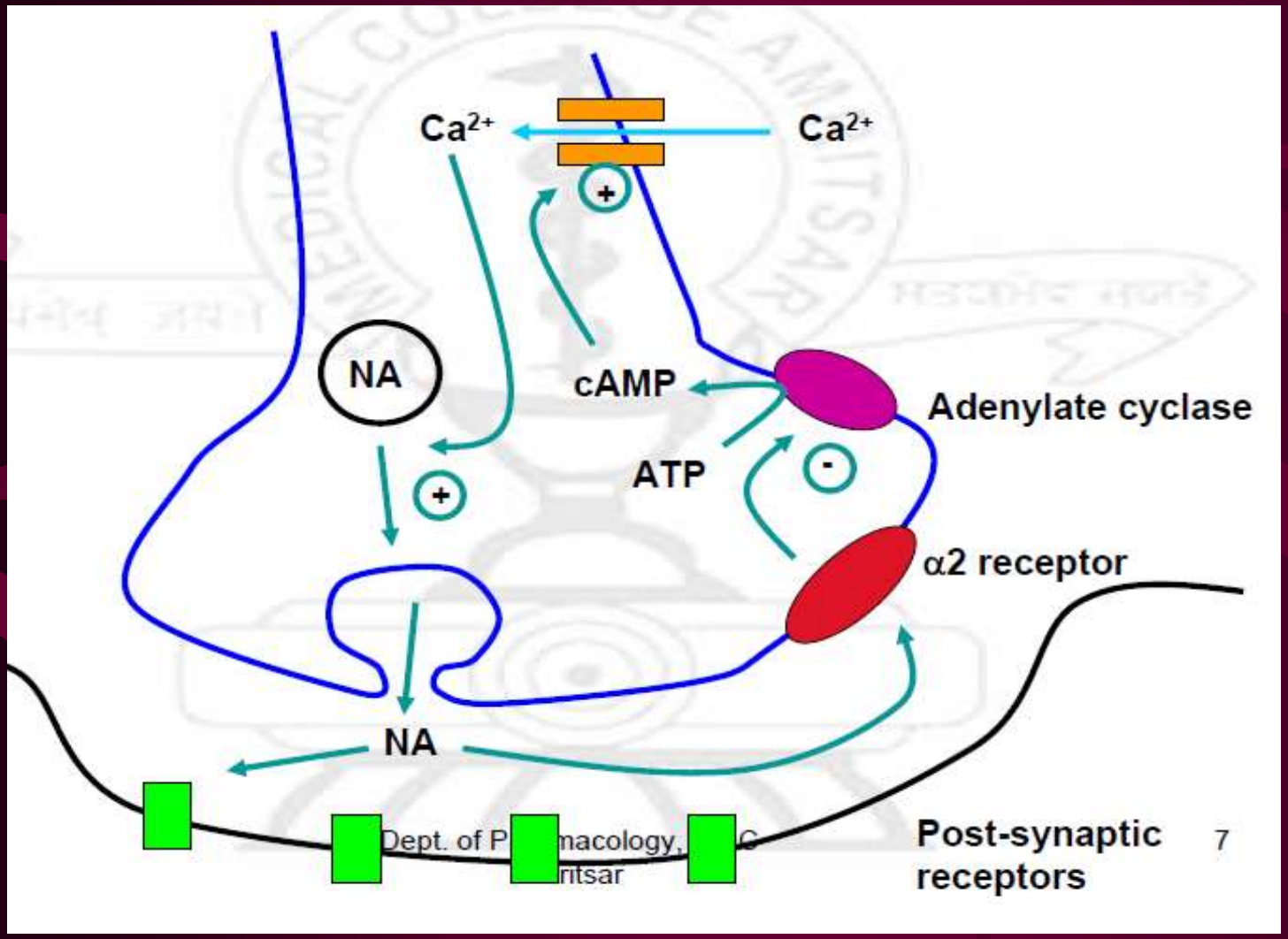
Biosynthesis of catecholamines

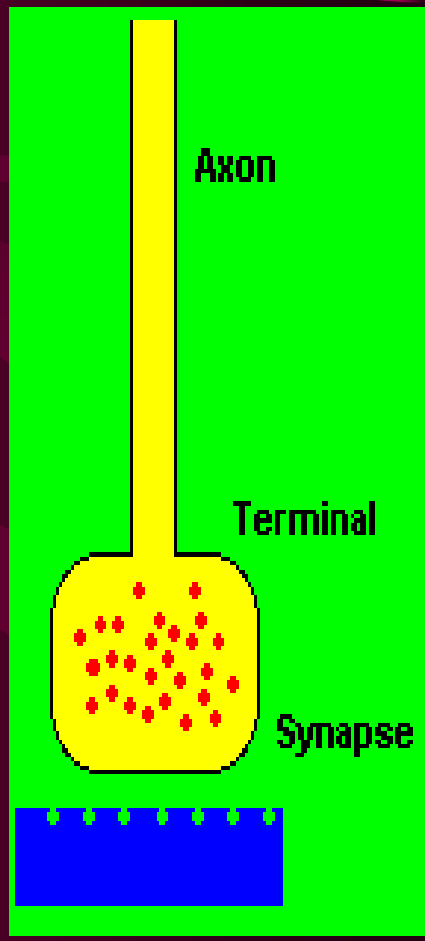


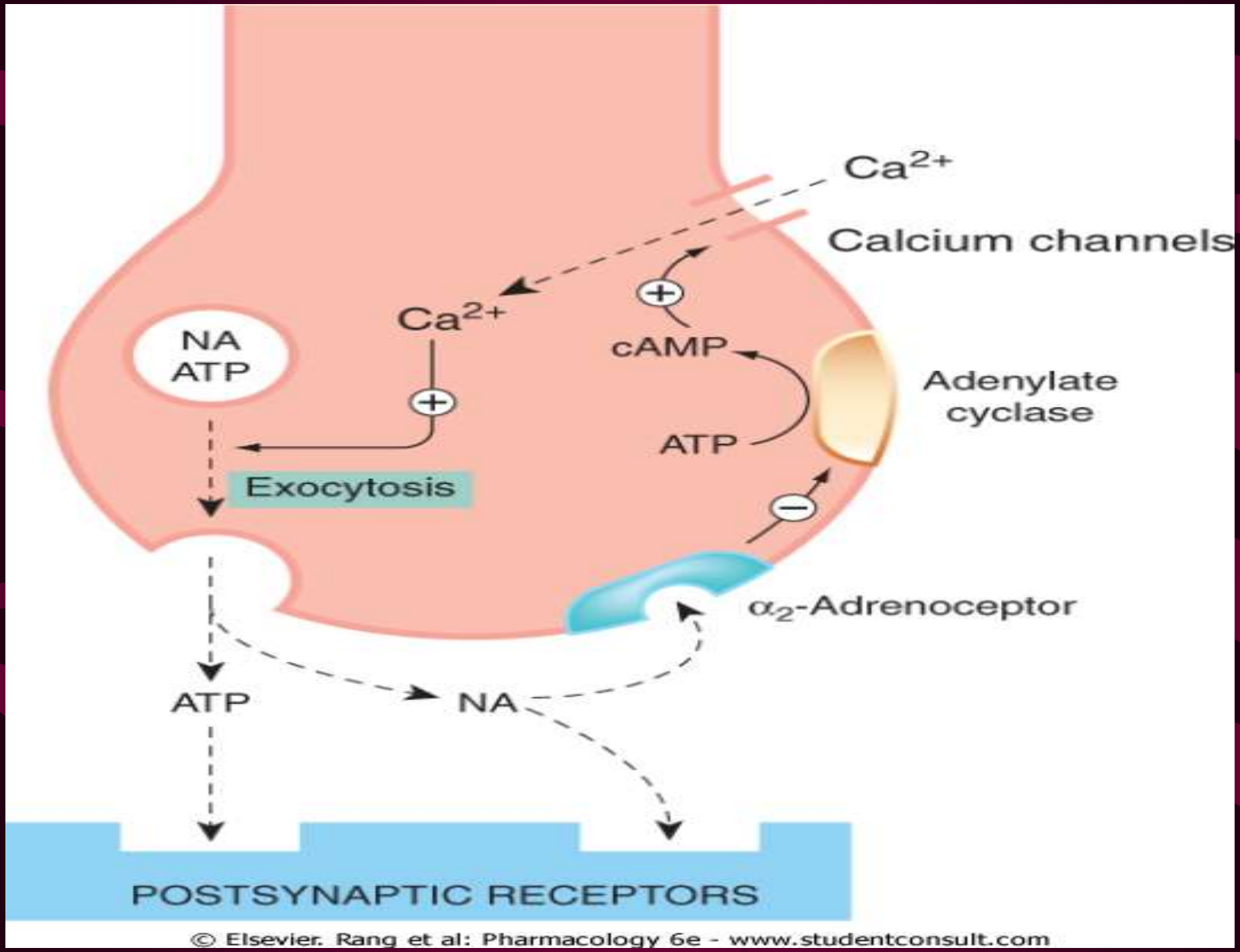
Storage of Noradrenaline



Release of NA – Feedback Control







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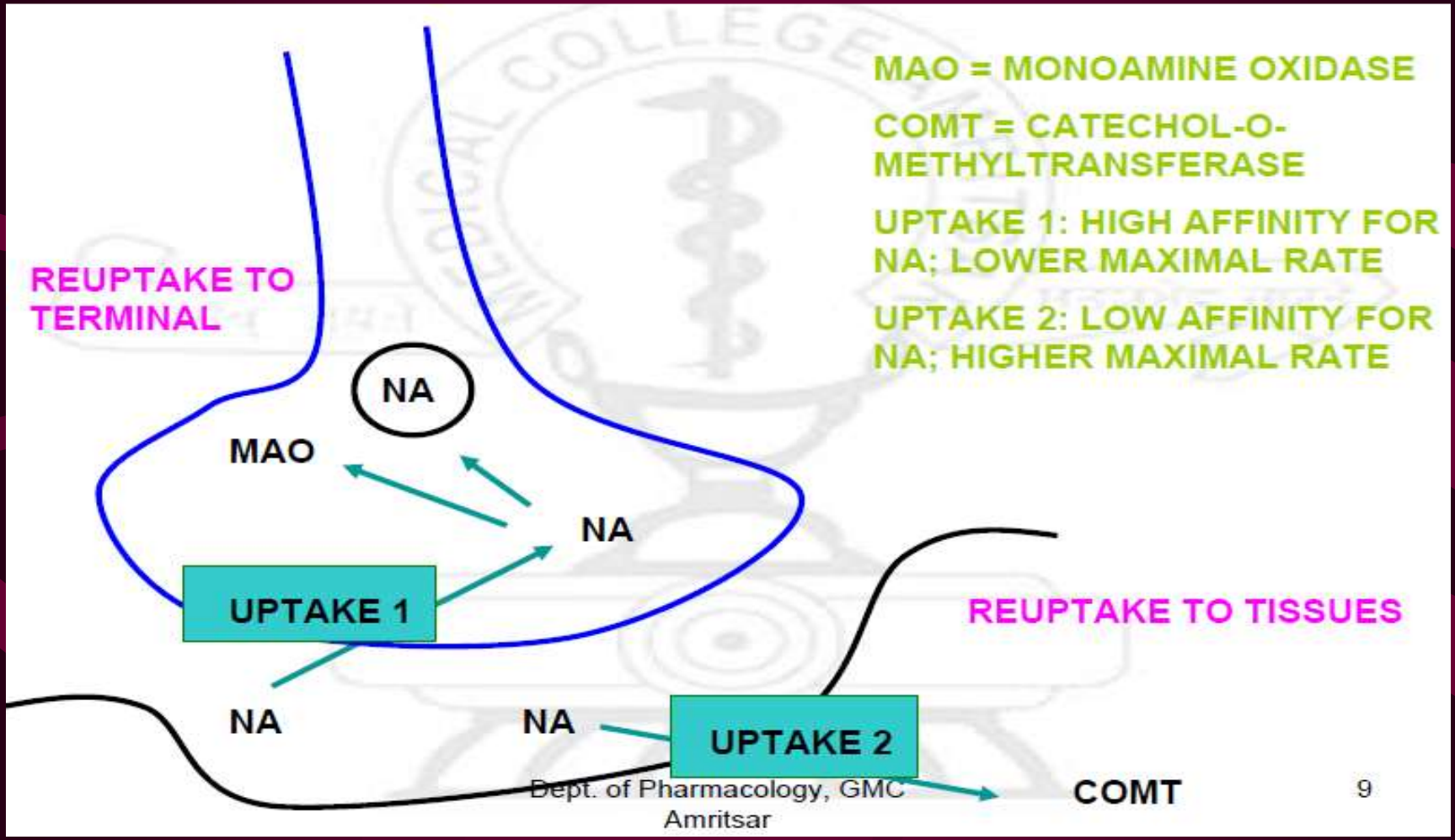
Regulators of NA release

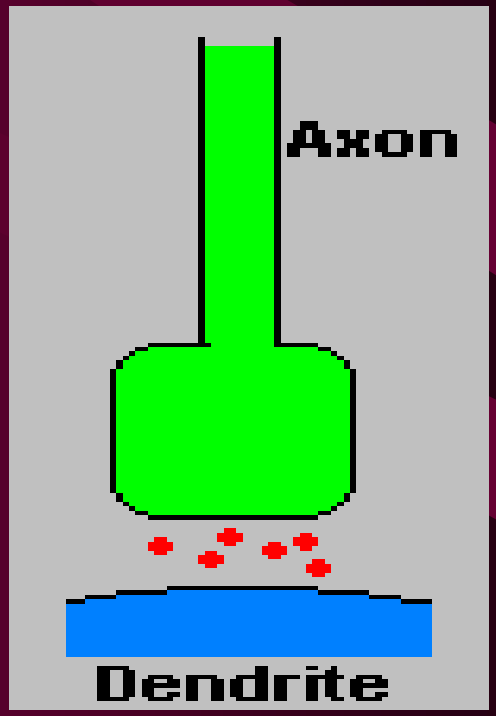
INHIBITORY		EXCITATORY	
ACh	muscarinic	Ad	β2
NA/Ad	α2	AT1	angiotensin II
Seretonin	5-HT1		
Adenosine	P1		
PGE	-----		
Histamine	H2		
Enkephalin	δ		
Dopamine	D2		

Uptake of Catecholamines



Reuptake

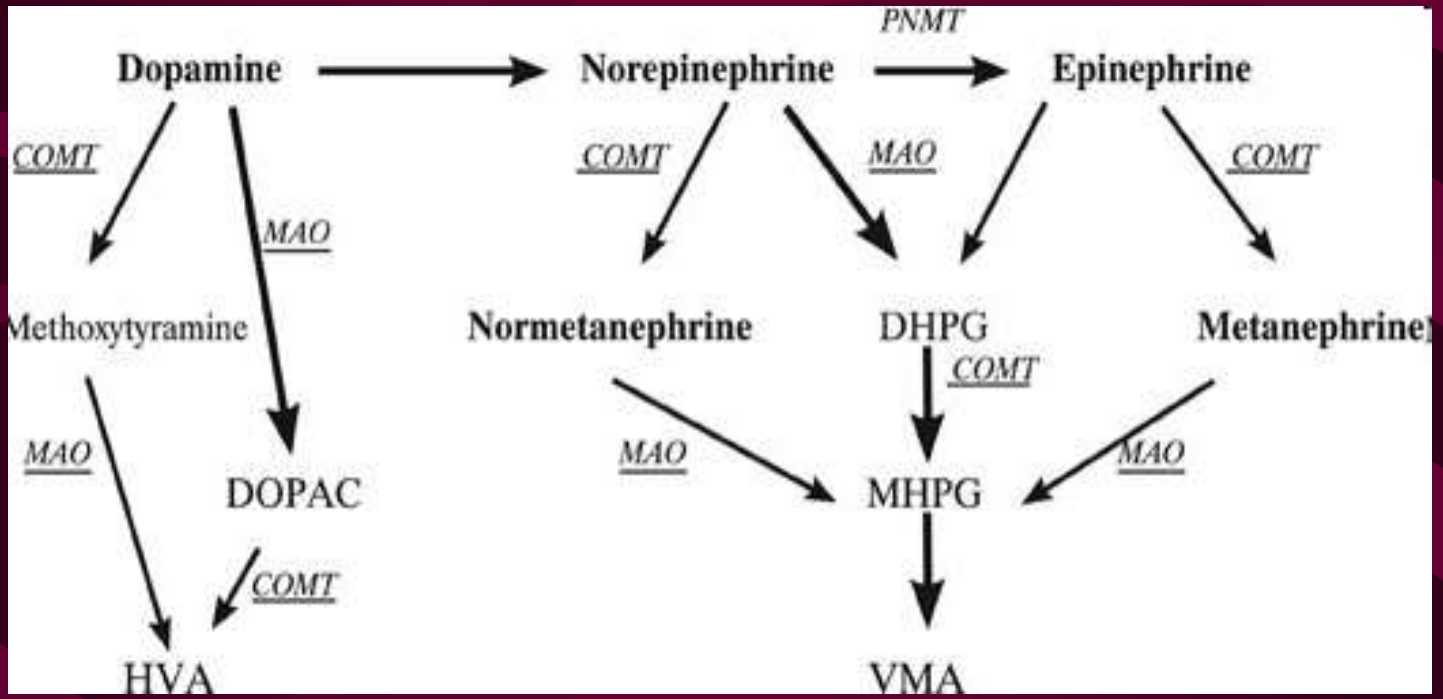
- Sympathetic nerves take up amines and release them as neurotransmitters
- **Uptake I** is a high efficiency system more specific for NA
  - Located in neuronal membrane
  - Inhibited by Cocaine, Amphetamines
- **Uptake 2** is less specific for NA
  - Located in smooth muscle/ cardiac muscle
  - Inhibited by steroids/ phenoxybenzamine
  - No Physiological or Pharmacological importance



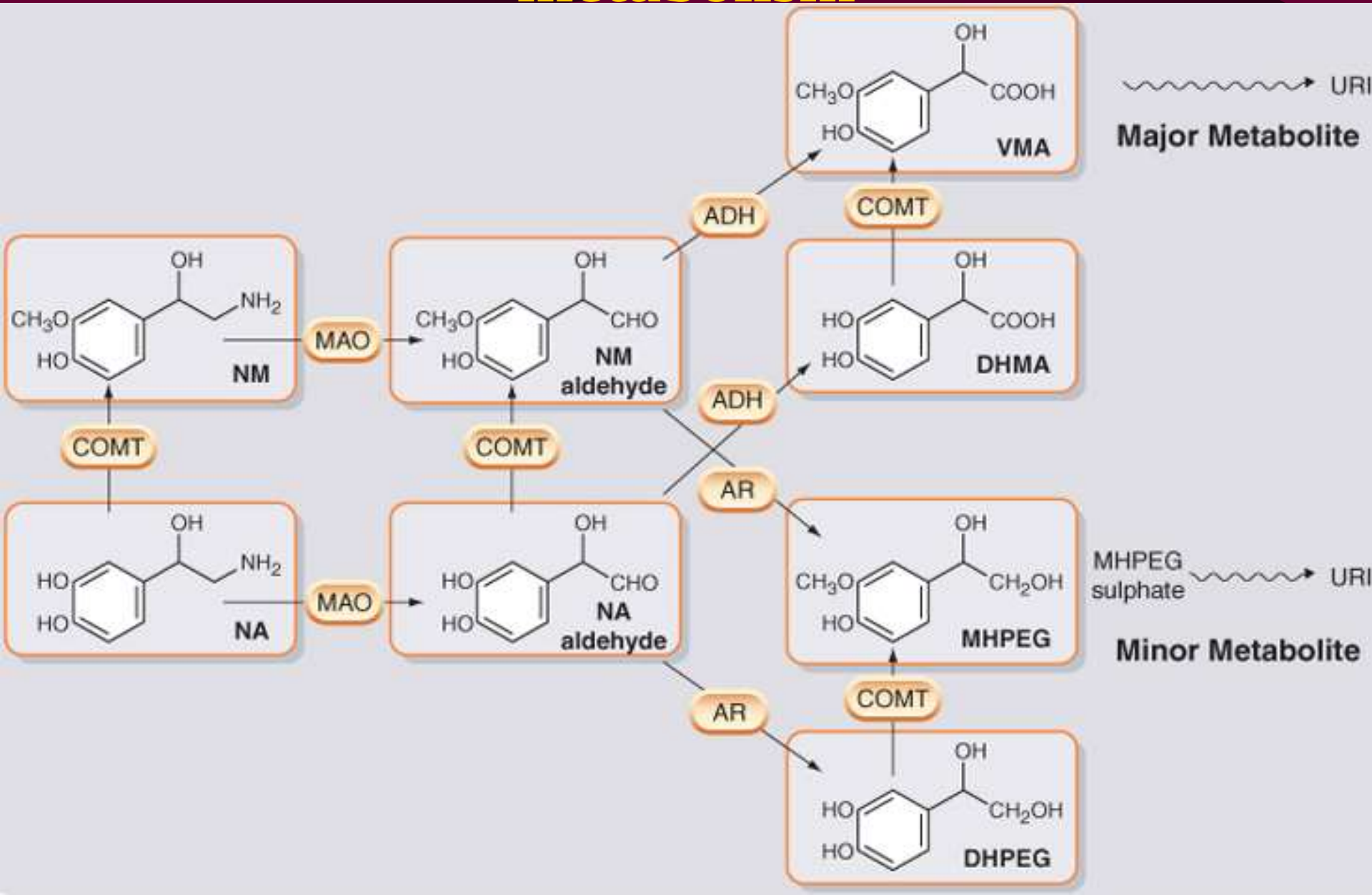
Metabolism of CAs

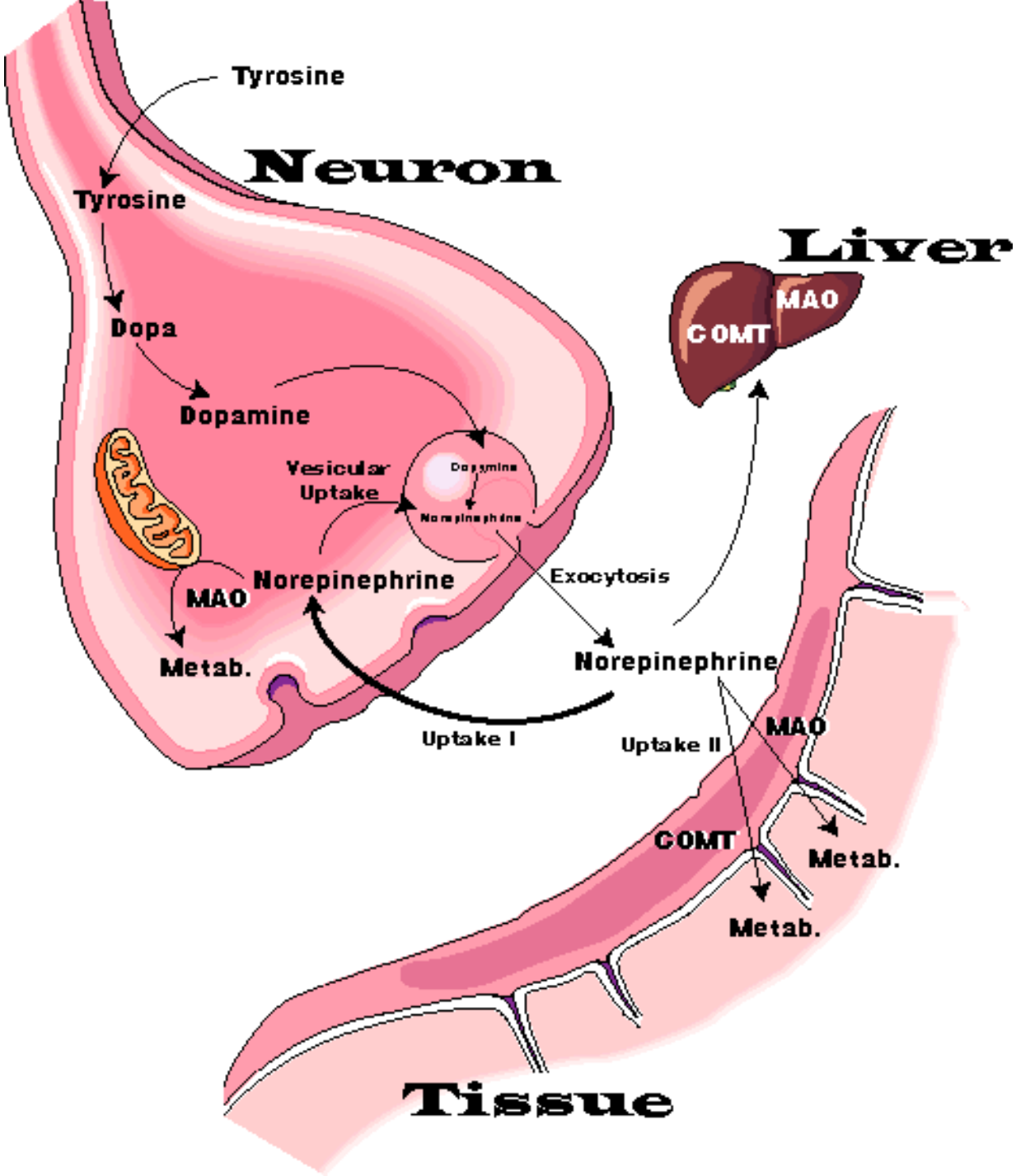
- **MAO**
  - Intracellular bound to mitochondrial membrane
  - Present in NA terminals and liver/ intestine
  - MAO inhibitors are used as antidepressants
- **Catechol-o-methyl-transferase (COMT)**
  - Neuronal and non-neuronal tissue
  - Acts on catecholamines and byproducts
  - VMA levels are diagnostic for tumours

Metabolism of CAs

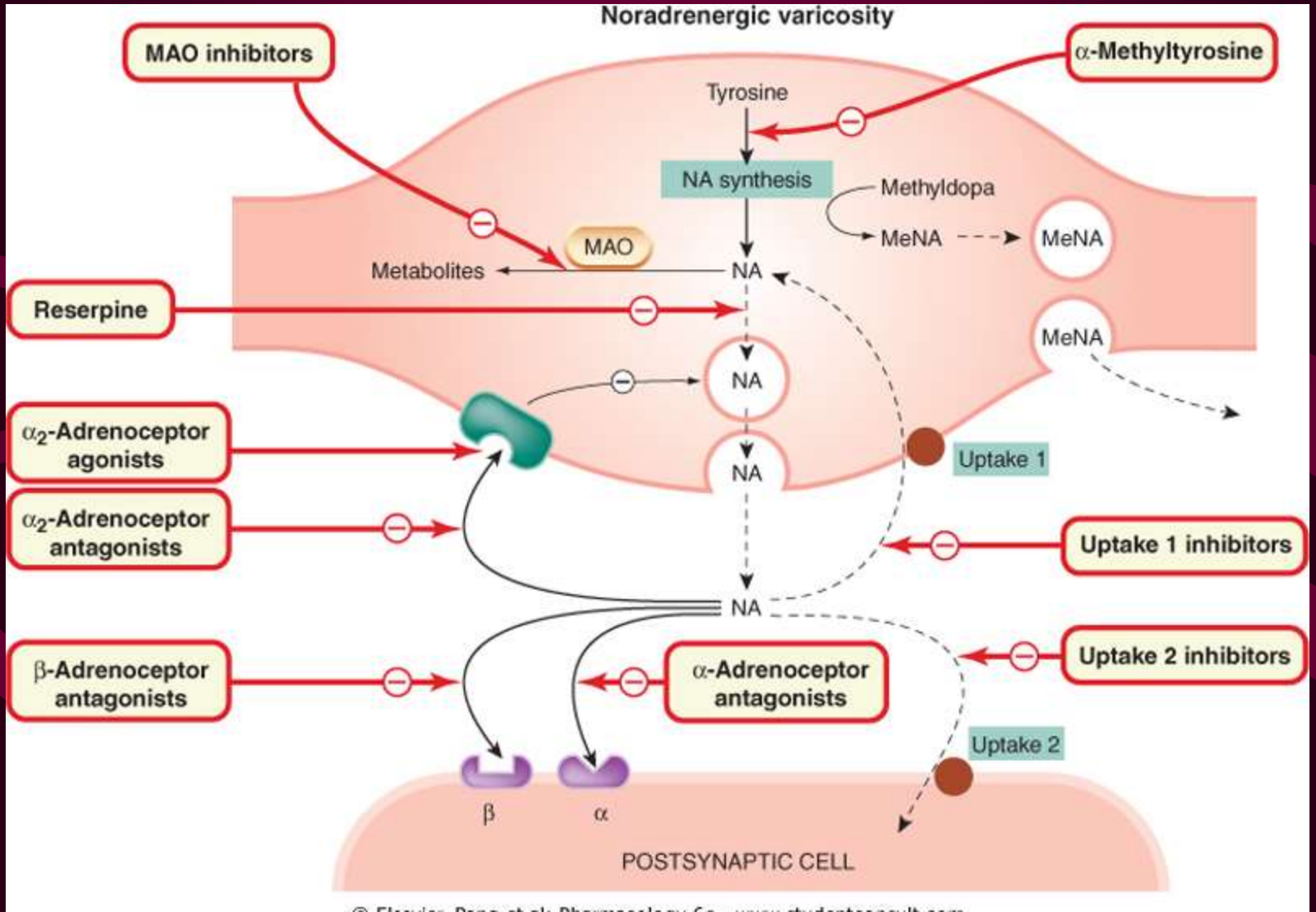


The main pathways of noradrenaline metabolism




Tyrosine
Neuron
Tyrosine
Dopa
Dopamine
Liver
MAO
COMT
Vesicular
Uptake
Dopamine
Norepinephrine
MAO
Norepinephrine
Exocytosis
Metab.
Norepinephrine
Uptake I
Uptake II
MAO
COMT
Metab.
Metab.
Tissue

Schematic representation of adrenergic neurotransmission and its modification by drug



Types of Adrenergic Receptors

- Alpha (α) and Beta (β)
- Agonist affinity of alpha (α):
  - adrenaline > noradrenaline > isoprenaline
  - Phenoxybenzamine
  - IP3 /DAG, cAMP and K+ channel opening
- A and gonist affinity of alpha (β) :
  - isoprenaline > adrenaline > noradrenaline
  - Propranolol
  - cAMP and Ca+ channel opening

Adrenergic receptors - alpha

- **Type α 1**
  - Acts by phospholipase C activation, which forms IP3 and DAG
  - In blood vessels these cause vasoconstriction
  - Blood vessels with alpha-1 receptors are present in the skin and the genitourinary system, and during the fight-or-flight response there is decreased blood flow to these organs
- **Type α 2**
  - Acts by inactivation of adenylate cyclase, cyclic AMP levels within the cell decrease
  - These are found on pre-synaptic nerve terminals

Adrenergic Receptors

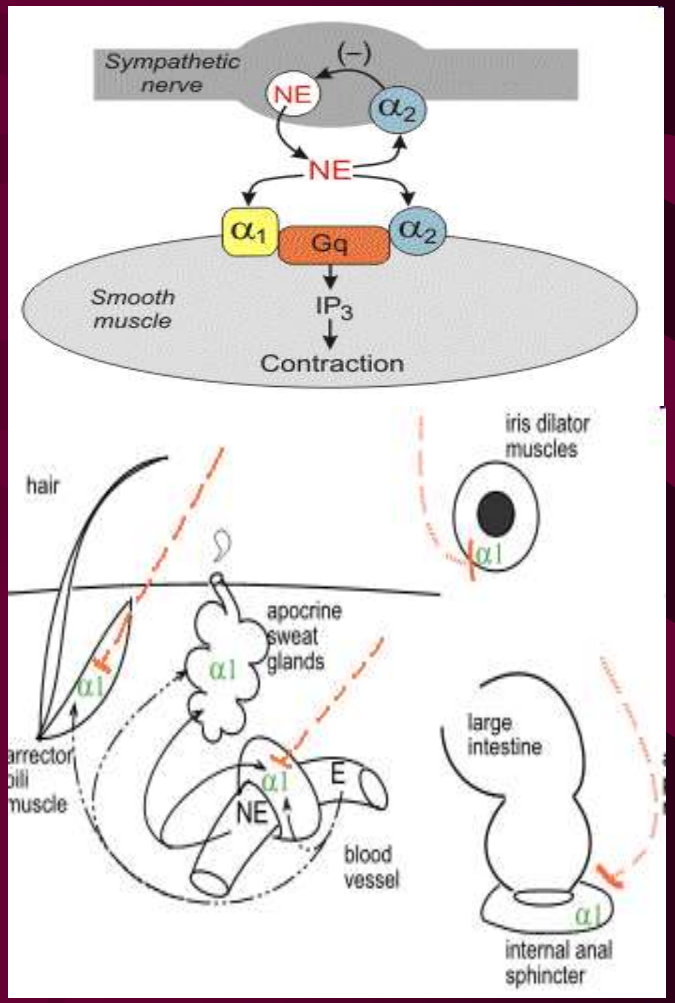
- **Adrenergic receptors** (or **adrenoceptors**) are a class of G-protein coupled receptors that are the target of catecholamines
- Adrenergic receptors specifically bind their endogenous ligands – catecholamines (adrenaline and noradrenline)
  - Increase or decrease of 2nd messengers cAMP or IP3/DAG
- Many cells possess these receptors, and the binding of an agonist will generally cause the cell to respond in a flight-fight manner.
  - For instance, the heart will start beating quicker and the pupils will dilate

Differences between α1 and α2

	Alpha-1	Alpha-2
Location	Post junctional	Prejunctional
Function	Stimulatory – GU, Vasoconstriction, gland secretion, Gut relaxation, Glycogenolysis	Inhibition of transmitter release, vasoconstriction, decreased central symp. Outflow, platelet aggregation
Agonist	Phenylephrine, Methoxamine	Clonidine
Antagonist	Prazosin	Y ohimbine

α_1 Adenoceptor Clinical effects

- Eye -- Mydriasis
- Arterioles – Constriction
- Uterus -- Contraction
- Skin -- Sweat
- Platelet - Aggregation
- Male ejaculation
- Hyperkalaemia
- Bladder Sphincter Contraction
- α2 adrenoceptors on nerve endings mediate negative feedback which inhibits noradrenaline



Beta receptors

- All β receptors activate adenylate cyclase, raising the intracellular cAMP concentration
- **Type β1:**
  - These are present in heart tissue, and cause an increased heart rate by acting on the cardiac pacemaker cells
- **Type β2:**
  - These are in the vessels of skeletal muscle, and cause vasodilatation, which allows more blood to flow to the muscles, and **reduce** total peripheral resistance
  - Stimulated by adrenaline, but not noradrenaline
  - Beta-2 receptors are also present in bronchial smooth muscle, and cause bronchodilatation when activated
  - Bronchodilator salbutamol work by binding to and stimulating the β2 receptors
- **Type β3:**
  - Beta-3 receptors are present in adipose tissue and are thought to have a role in the regulation of lipid metabolism

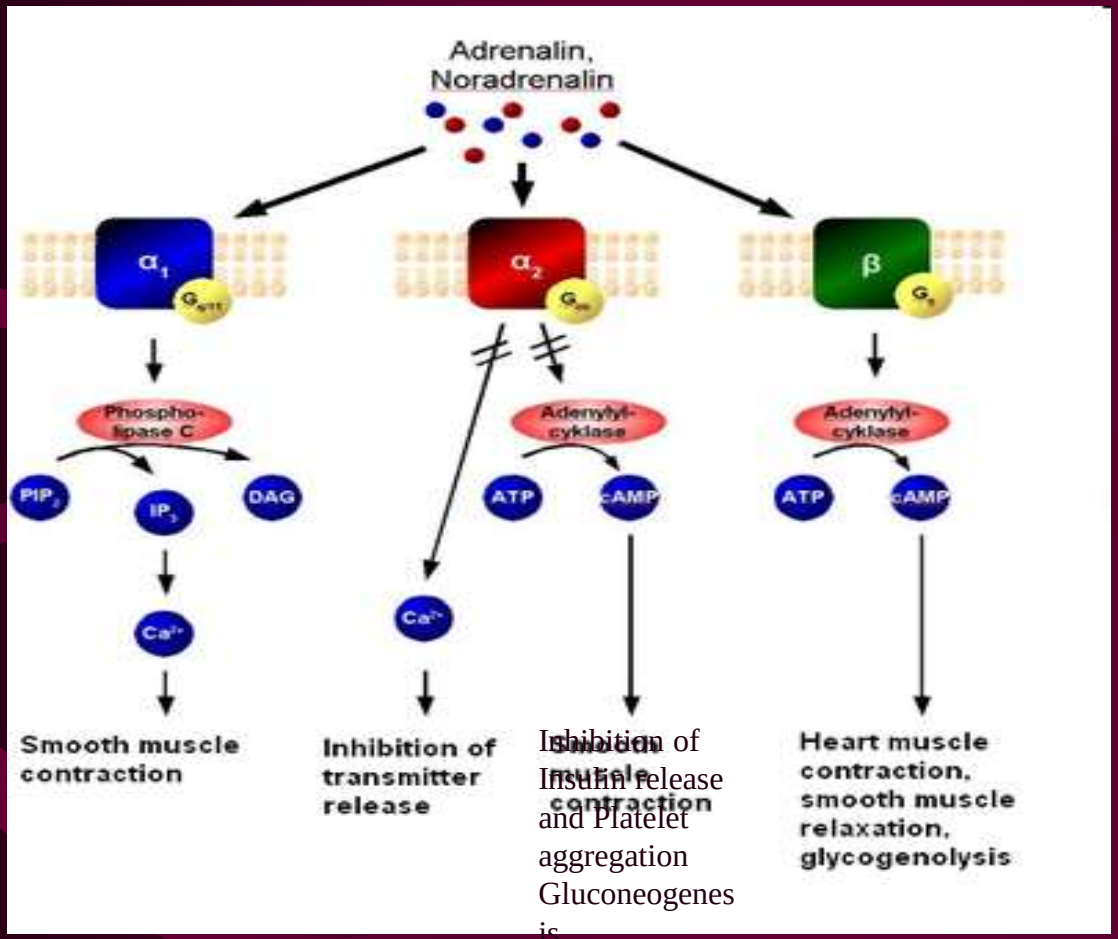
Differences between β1, β2 and β3

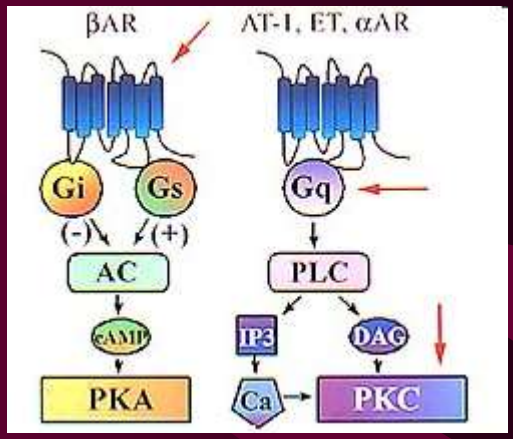
	Beta-1	Beta-2	Beta-3
Location	Heart and JG cells	Bronchi, uterus, Blood vessels, urinary tract, eye	Adipose tissue
Agonist	Dobutamine	Salbutamol	-
Antagonist	Metoprolol, Atenolol	Alpha-methyl propranolol	-
Action on NA	Moderate	Weak	Strong

β2 Adrenoceptor – Clinical Effects

- Bronchi -- Relaxation
- Arterioles -- Dilatation
- Uterus – Relaxation
- Skeletal Muscle - Tremor
- Hypokalaemia
- Hepatic Glycogenolysis

Molecular Basis of Adrenergic Receptors





Dopamine receptors

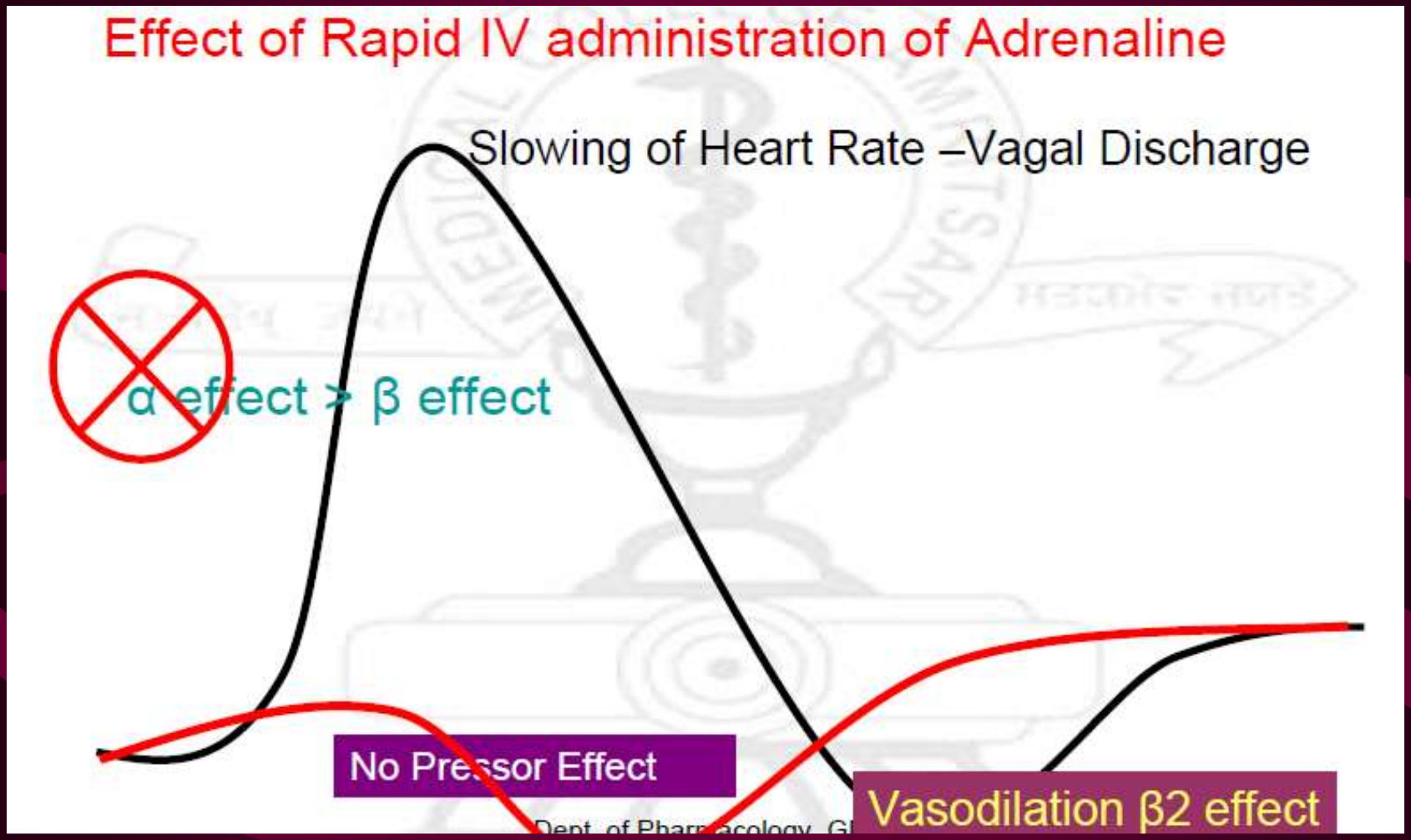
- D1-receptors are post synaptic receptors located in blood vessels and CNS
- D2-receptors are presynaptic present in CNS, ganglia, renal cortex

Pharmacological Actions

Adrenaline as prototype

- Potent stimulant of alpha and beta receptors
- Complex actions on target organs

Dale`s Vasomotor Reversal Phenomenon



Blood Pressure

- Most potent vasopressor known – Both systolic and Diastolic BP rise
- Has a characteristic effect on BP
- Rapid rise to a peak:
  - Direct myocardial stimulation

+ve inotropic

  - Increased heart rate

+ve chronotropic

- Vasoconstriction which leads to increased peripheral resistance
- Reflex Bradycardia- Stimulation of vagus

Heart

- Powerful Cardiac stimulant
- Acts on beta-1 receptors in myocardium, pacemaker cells and conducting tissue
  - Heart rate increases
  - Rhythm is altered
  - Cardiac systole is shorter and more powerful
  - Cardiac output is enhanced
  - Oxygen consumption is increased
  - Cardiac efficiency is markedly decreased

VASCULAR SYSTEM

- Vasoconstriction
- Dopamine causes vasodilatation in the splanchnic and renal vascular beds

Respiratory:

- Powerful bronchodilator β_2
- Relaxes bronchial smooth muscle
  - Beta-$_2$ mediated effect
  - Physiological antagonist to mediators of bronchoconstriction e.g. Histamine

Smooth Muscles:

- Effects on vascular smooth muscle are important
- GIT and Urinary tract smooth muscle are relaxed but are clinically unimportant
- In the pregnant uterus there is inhibition of tone and contractions

Metabolic effects

- Increases concentration of glucose and lactic acid
- Calorigenesis (**β-2 and β-3)**
- Inhibits insulin secretion (**α-2)**
- Decreases uptake of glucose by peripheral tissue
- Simulates glycogenolysis - Beta effect
- Increases free fatty acid concentration in blood

METABOLIC AND HORMONAL EFFECTS

- $Beta_1$ increases renin secretion
- $Beta_2$ increases insulin secretion (gluconeogenesis)
- Increases glycogenolysis in the liver and the resulting hyperglycemia is countered by insulin levels
- Stimulate lipolysis

CNS

- Do not enter the CNS effectively
- Those that enter, like amphetamine, have stimulant effects
- Mild altering or reduction of fatigue, progressing to anorexia, euphoria, and insomnia
- Very high doses
  Marked anxiety or aggressiveness, paranoia and rarely convulsions

EYE

- Pupillary dilator responds with mydriasis
- Accommodation is not significantly affected
- **Facilitate outflow of aqueous humor with subsequent reduction of intraocular pressure**

BRONCHI

- Marked relaxation of the smooth muscles of the bronchi
- Most efficacious and reliable for reversing bronchospasm

GIT

- Well endowed with both alpha and beta receptors
- Located on the smooth muscles and neurons of the enteric nervous system
- Activation of either receptors leads to the relaxation of the smooth muscles

GUT

- Contains alpha receptors in the bladder trigone and sphincter area
- Bladder relaxation and sphincter contraction
- $Beta_2$ agonist causes uterine relaxation in pregnancy

ADME

- Ineffective orally
- Absorbed slowly from subcutaneous tissue
- Faster from IM site
- Inhalation is locally effective
- Not usually given IV
- Rapidly inactivated in Liver by MAO and COMT

Pharmacokinetics of sympathomimetics

Drug	Oral activity	Duration
Catecholamines		
Epinephrine	No	minutes
Norepinephrine	No	minutes
Isoproterenol	Poor	minutes
Dopamine	No	minutes
Dobutamine	No	minutes

Pharmacokinetics of sympathomimetics

Other sympathomimetics

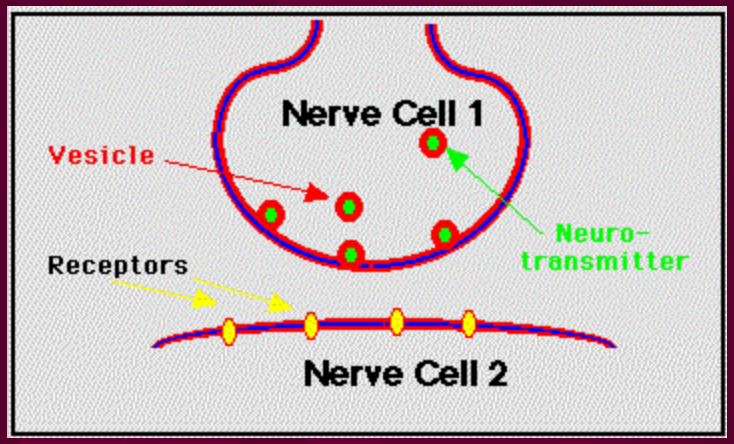
Drug	Oral activity	Duration
Amphetamine,	Yes	Hours
Ephedrine	Yes	Hours
Phenylephrine	Poor	Hours
Albuterol,	Yes	Hours

metaproterenol, terbutaline

Other sympathomimetics

Drug	Oral activity	Duration
Oxymetazoline, xylometazoline	Yes	Hours
Cocaine	No	Minutes to Hours

FIGHT
—or—
FLIGHT
Swerdlow


Nerve Cell 1
Vesicle
Neuro-
transmitter
Receptors
Nerve Cell 2

SYMPATHOMIMETICS

CLINICAL USES

A. ANAPHYLAXIS

EPINEPHRINE

- Drug of choice for the immediate treatment of anaphylactic shock
- Rapid acting
- Pressor agent
- Used for cardiac arrest

SYMPATHOMIMETICS

CLINICAL USES

B. CNS

PHENYLISOPROPYLAMINES (AMPHETAMINES)

- Narcolepsy
- Attention deficit disorder
- With appropriate control, weight reduction

SYMPATHOMIMETICS

CLINICAL USES

C. EYE

PHENYLEPHRINE

- Used topically to produce mydriasis
- Used to reduce conjunctival itching and congestion caused by irritation and allergy

SYMPATHOMIMETICS

CLINICAL USES

D. BRONCHI

TERBUTALINE, ALBUTEROL, METAPROTERENOL

- Drugs of choice in the treatment of acute asthmatic attacks

SYMPATHOMIMETICS

CLINICAL USES

D. BRONCHI

SALMETEROL

- Long-acting
- Recommended for prophylaxis

SYMPATHOMIMETICS

CLINICAL USES

E. CVS

1. Conditions in which an increase in blood flow is desired

- $Beta_1$ agonists maybe useful in situations like
  - Acute cardiac failure
  - Some types of shock

SYMPATHOMIMETICS

CLINICAL USES

E. CVS

2. Conditions in which a decrease in blood flow or increase in BP is desired

- $Alpha_1$ agonists are useful in situations in which vasoconstriction is needed

SYMPATHOMIMETICS

CLINICAL USES

E. CVS

2. Conditions in which a decrease in blood flow or increase in BP is desired

- Local hemostatic
- Decongestant effects
- In spinal shock in which temporary maintenance of BP may help perfuse the brain, heart and the kidneys

SYMPATHOMIMETICS

CLINICAL USES

F. GUT

RITODRINE, TERBUTALINE

- Used to suppress premature labor
- Cardiac stimulant effect may be hazardous to both mother and fetus

SYMPATHOMIMETICS

CLINICAL USES

F. GUT

EPHEDRINE

- Long-acting oral sympathomimetic used to improve urinary incontinence in children and the elderly with enuresis
- Mediated by the alpha receptors in the trigone of the bladder

SYMPATHOMIMETICS

CATHECOLAMINES

EPINEPHRINE

- Pressor agent
- Cardiac arrest
- Anaphylactic shock

SYMPATHOMIMETICS

CATHECOLAMINES

NOREPINEPHRINE

- Pressor agent

ISOPROTERENOL

- Cardiogenic shock

SYMPATHOMIMETICS

CATHECOLAMINES

DOBUTAMINE

- Congestive heart failure
- Inotropic effect

SYMPATHOMIMETICS

CATHECOLAMINES

DOPAMINE

- Inotropic effect
- Lower doses cause renal vasodilation
- Higher doses cause vasoconstriction

SYMPATHOMIMETICS

NON-CATHECOLAMINES

PHENYLEPHRINE

- Pressor agent
- Mydriatic
- Decongestant

SYMPATHOMIMETICS

NON-CATHECOLAMINES

METHOXAMINE

- Pressor agent
- IV form only

SYMPATHOMIMETICS

NON-CATHECOLAMINES

MIDODRINE

- Pressor agent
- Orthostatic hypotension

SYMPATHOMIMETICS

NON-CATHECOLAMINES

DRIXINE

- OXYMETAZOLINE, XYLOMETAZOLINE
- Topical decongestants

SYMPATHOMIMETICS

NON-CATHECOLAMINES

TERBUTALINE

- Bronchial asthma

RITODRINE

- Inhibits premature labor

SYMPATHOMIMETICS

NON-CATHECOLAMINES

COCAINE

- Local vasoconstrictive
- Anesthetic effect

SYMPATHOMIMETICS

NON-CATHECOLAMINES

TYRAMINE

- Derivative of tyrosine
- Increase BP
- Found in cheese and wine

SYMPATHOMIMETICS

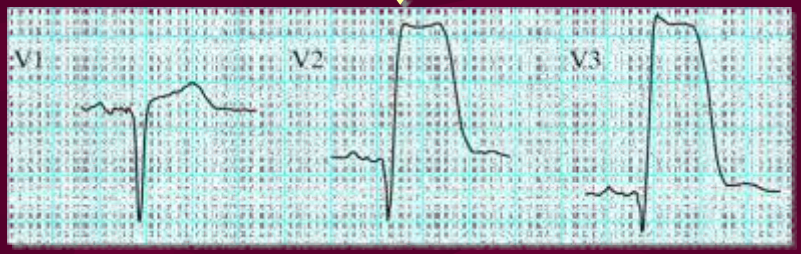
TOXICITY

- Limited penetration to the brain
- Little CNS toxicity when given systemically

β-Blocker

The Heart in Hypertension

Ventricular Ectopy, Ventricular Fibrillation, Sudden Cardiac Death



Coronary Ischemia

Diastolic Heart Failure

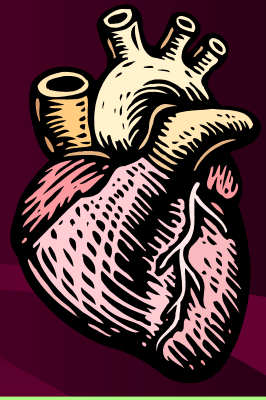
Heart Failure

Systolic Heart Failure

Atrial Fibrillation

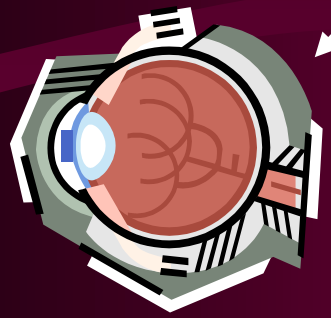
Consequences of Hypertension: Organ Damage

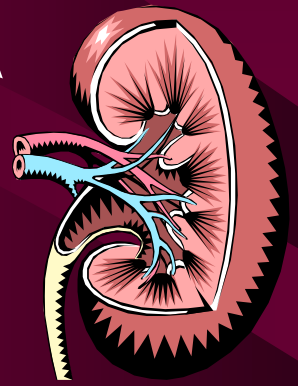
Hypertension



Transient ischemic attack, stroke

LVH, CHD, CHF





Retinopathy

Peripheral arterial disease

Chronic kidney disease

CHF=congestive heart failure; CHD=coronary heart disease; LVH=left ventricular hypertrophy.

Chobanian AV et al. *JAMA.* 2003;289:2560-2572.

β-blocker History

First Generation

Nonselective

Propranolol

Timolol

↓

Second Generation

Selective

Atenolol

Metoprolol

Bisoprolol

↓

Third Generation

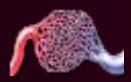
Vasodilatory

Labetalol

Carvedilol

Nebivolol

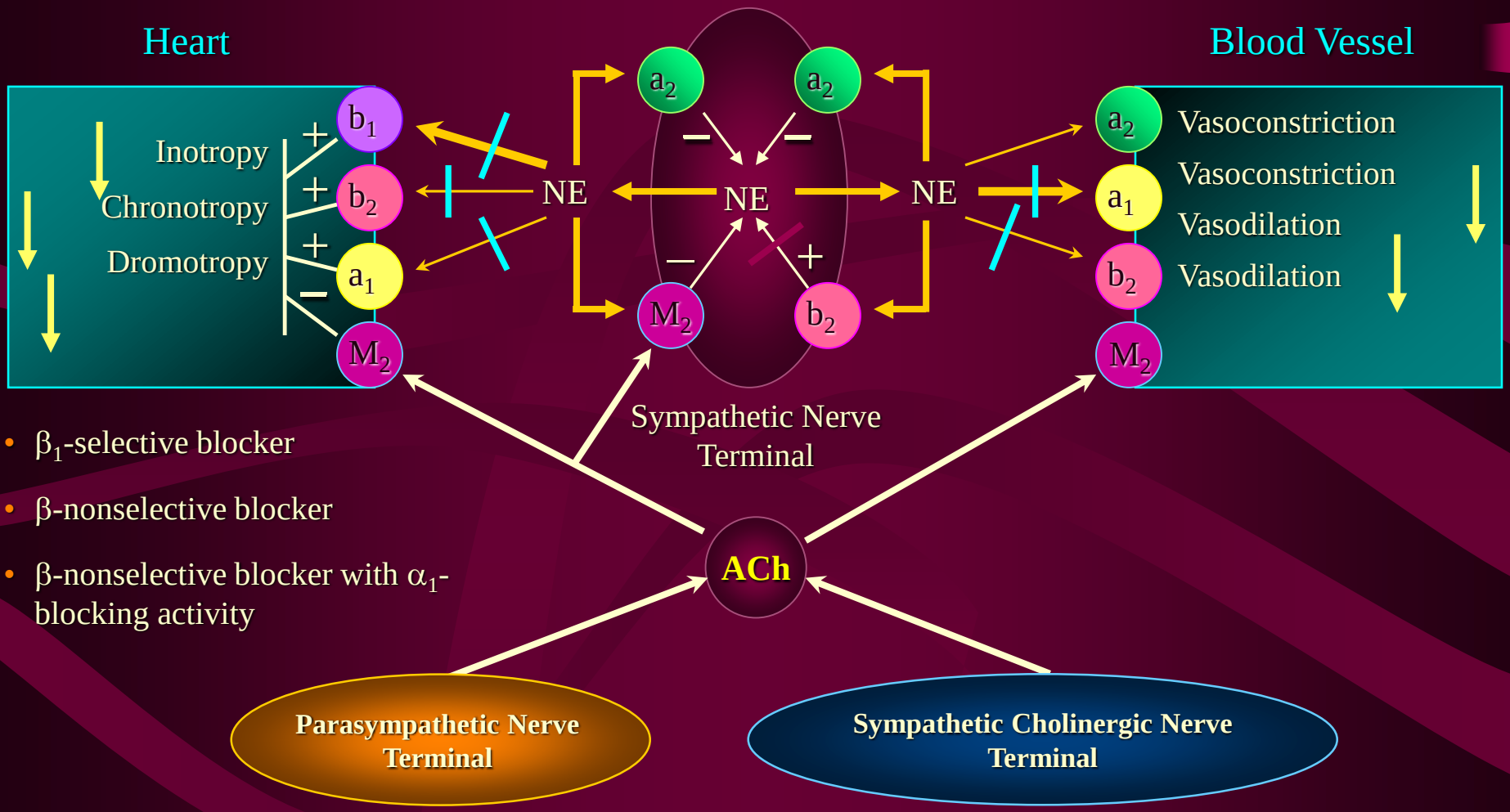
Effects Mediated by β_1- and β_2-adrenoceptors



Tissue	Receptor	Effect
Heart		
SA node	β_1, β_2	↑ heart rate
AV node	β_1, β_2	↑ conduction velocity
Atria	β_1, β_2	↑ contractility
Ventricles	β_1, β_2	↑ contractility, conduction velocity & automaticity of idioventricular pacemakers
Arteries	β_2	Vasodilation
Veins	β_2	Vasodilation
Skeletal muscle	β_2	Vasodilation, ↑ contractility Glycogenolysis, K+ uptake
Liver	β_2	Glycogenolysis and gluconeogenesis
Pancreas (β cells)	β_2	Insulin and glucagon secretion
Parathyroid glands	β_1, β_2	Parathormone secretion
Thyroid gland	β_2	T4→T3 conversion
Fat cells	β_1	Lipolysis
Bronchi	β_2	Bronchodilation
Kidney	β_1	Renin release
Urinary bladder detrusor	β_2	Relaxation
Gallbladder and ducts	β_2	Relaxation
Gastrointestinal	β_2	Relaxation
Uterus	β_2	Relaxation
Nerve terminals	β_2	Promotes noradrenaline release

López-Sendón J, et al. *Eur Heart J* 2004;25:1341-1362.

β-blockers: Targets and Receptor Selectivity

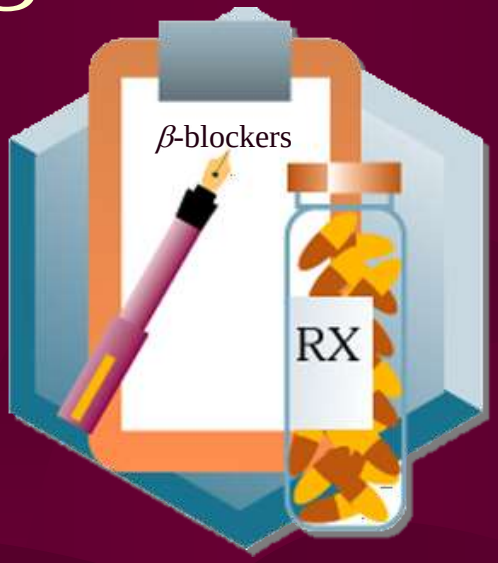


- β_1-selective blocker
- β-nonselective blocker
- β-nonselective blocker with α_1-blocking activity

a=alpha receptor, Ach=acetylcholine, b=beta receptor, M=muscarinic receptor, NE=Norepinephrine

Klabunde RE (ed). *Cardiovascular Physiology Concepts* LWW 2001

ß-blockers: Heterogenous Drug Class



$ß_1/ß_2$ Selectivity

Vasodilatory Properties

Which One?

ß-blockers

Side Effects

Metabolic Profile

Outcomes Trials

β-blocker Characteristics

- Selectivity for the β-receptors:
  - Non-selective: Produce a competitive blockade of both β_1- and β_2-adrenergic receptors:

    1. Carteolol
    2. Nadolol
    3. Penbutolol
    4. Pindolol
    5. Propranolol
    6. Sotalol (not indicated for hypertension)
    7. Timolol

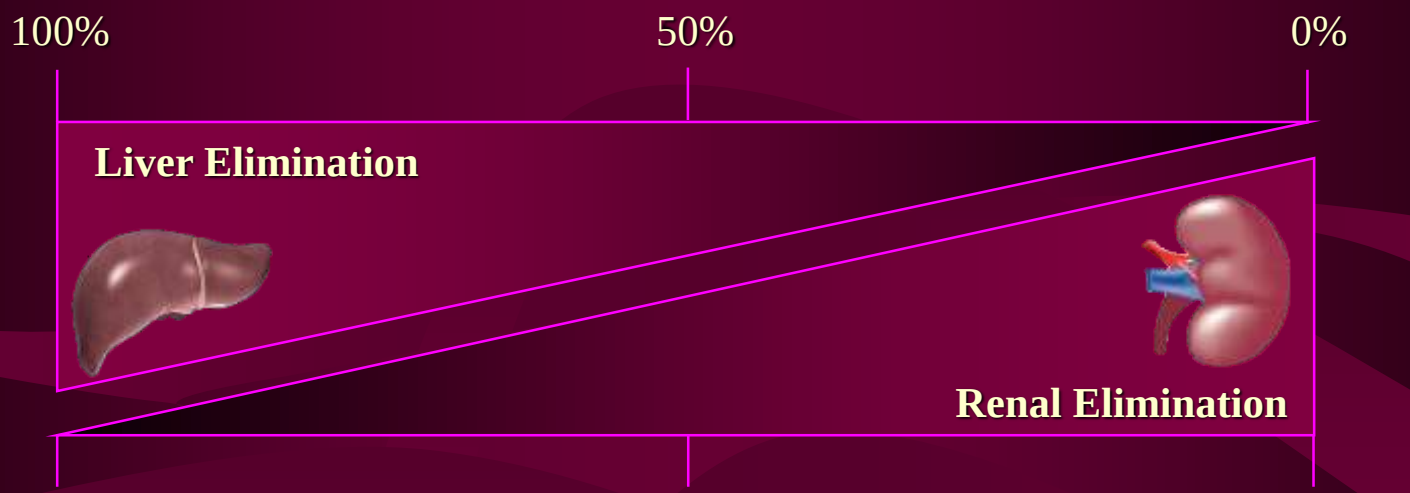
Raynaud's

  - β_1-selective: higher affinity for the β_1- than for the β_2-receptors

β-blocker Characteristics

- Lipophilic (vs hydrophilic) β-blockers
  - Extensively metabolized in the gut wall and liver (first pass effect) → ↓oral bioavailability (10–30%)
  - Drugs may accumulate in patients with reduced hepatic blood flow:
    1. Elderly
    2. Heart failure
    3. Liver cirrhosis
  - Easily enter the central nervous system, which may account for a greater incidence of central side-effects (sedation)
  - Also crosses placenta → fetal bradycardia

Route of Elimination



0%	20%	40%	60%	80%	100%
↑↑↑↑	↑	↑	↑	↑	↑↑↑
Propranolol	Timolol	Pindolol	↑	Diacetolol ‡	Atenolol
Metoprolol	Acebutolol		↑		Nadolol
Labetalol			Bisoprolol		Sotalol *
Betaxolol					Carteolol †
Penbutolol					
Carvedilol					
Nebivolol					

* Antiarrhythmic agent
† No longer available in US for hypertension
‡ Metabolite of acebutolol

Adapted from Meier J. *Cardiology* 1979;64 (Suppl 1):1-13.

β-blocker Characteristics

- Partial Agonist Activity:
  - Also called intrinsic sympathomimetic activity (ISA)
  - Stimulate and block the β-receptor
  - Act as partial β-agonists, but:
    1. Still blocks the *more significant agonist effects* of endogenous catecholamines
    2. Tends to slow the heart rate less
  - Associated with more adverse cardiac events

Characteristics

β-adrenoceptor Blockers

Nonselective

ISA-
Nadolol
Propranolol*
Timolol
Sotalol¶

ISA+
Pindolol
Carteolol
Penbutolol

α_1-blocker

Labetalol 1:4-7*
Carvedilol 1:10*†

Selective

ISA+
Acebutolol*

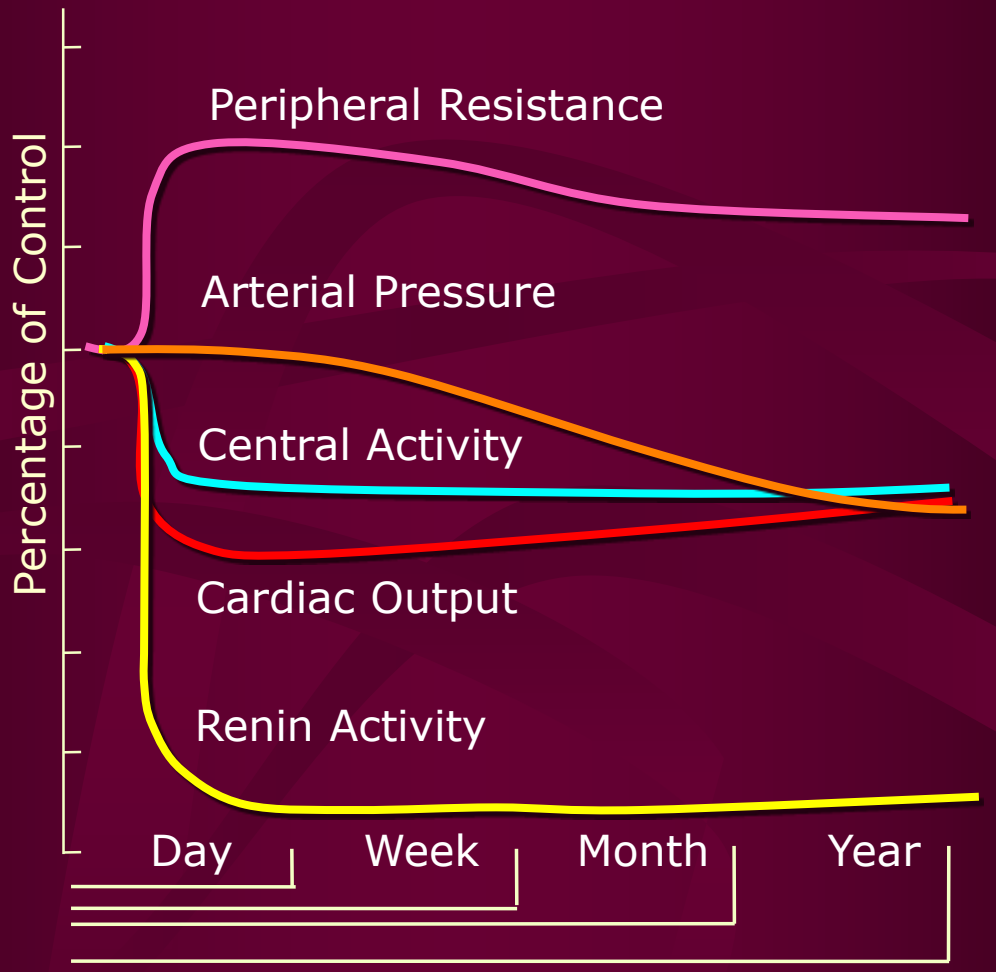
ISA-
Atenolol
Esmolol
Metoprolol
Nebivolol‡
Bisoprolol
Betaxolol*

No β-blocker is cardioselective in large doses
β_2-blockade is needed for tremor and migraines
β_1-blockers are better for bronchospasm & insulin-requiring diabetes

* Membrane Stabilizing Activity
† Antioxidant
‡ Nitric oxide-mediated vasodilation
¶ Class III antiarrhythmic

Modified from Kaplan NM. *Clinical Hypertension* (8ed). 2002;262.

Mechanisms of β-blockers



Man in't Veld AJ, Schalekamp MADH. *Br J Clin Pharmacol* 1982;13(suppl 2):245S-257S.

Summary

- β-blockers are a diverse class of drugs
- They differ in their indications and their effectiveness for various disorders
- Selective, vasodilatory β-blockers are:
  - Indicated for hypertension
  - Proven for heart failure and myocardial infarction
  - Offer advantages in patients with diabetes mellitus
  - Are well tolerated

What is a ß-blocker

A ß-blocker is a substance that blocks (antagonizes) ß receptors throughout the body. Since ß receptors are abundant and widely distributed, they affect many organ systems and are used in many disease states.

Clinical uses of ß-blockers

- Hypertension
- Angina
- Arrhythmias
- Anxiety
- Tremors
- Acute MI
- Pre-op
- Migraines
- Portal HTN
- Glaucoma
- Heart failure
- Hyperthyroidism
- IHSS
- Pheocromocytoma
- EtOH withdrawl

ß-receptors and their function

Organ	Response	ß subtype
Heart	Enhanced inotropy	$ß_1$
	Enhanced chronotropy	$ß_1$
	Enhanced dromotropy	$ß_1$
	Increased automaticity	$ß_1$
Lung	Bronchial smooth muscle relaxation (bronchodilation)	$ß_2$

ß-receptors and their function

Organ	Response	ß subtype
GI tract	Decreased motility/tone	$ß_1$
Liver	Stimulation of glycogenolysis and gluconeogenesis	$ß_2$
Pancreas	Increased glucagon secretion	$ß_2$
Kidney	Increased renin secretion	$ß_1$

ß-receptors and their function

Organ	Response	ß subtype
Skeletal muscle	Increased contractility	$ß_2$
	Increased glycogenolysis	$ß_2$
	Increased triglyceride hydrolysis	$ß_2$
	Intracellular potasium uptake	$ß_2$
	Dilation of blood vessels	$ß_2$
Adipose	Increased adipose lipolysis	$ß_1$
	Increased muscle trig. use	$ß_2$

ß-receptors and their function

Physiology of Beta-Blockers

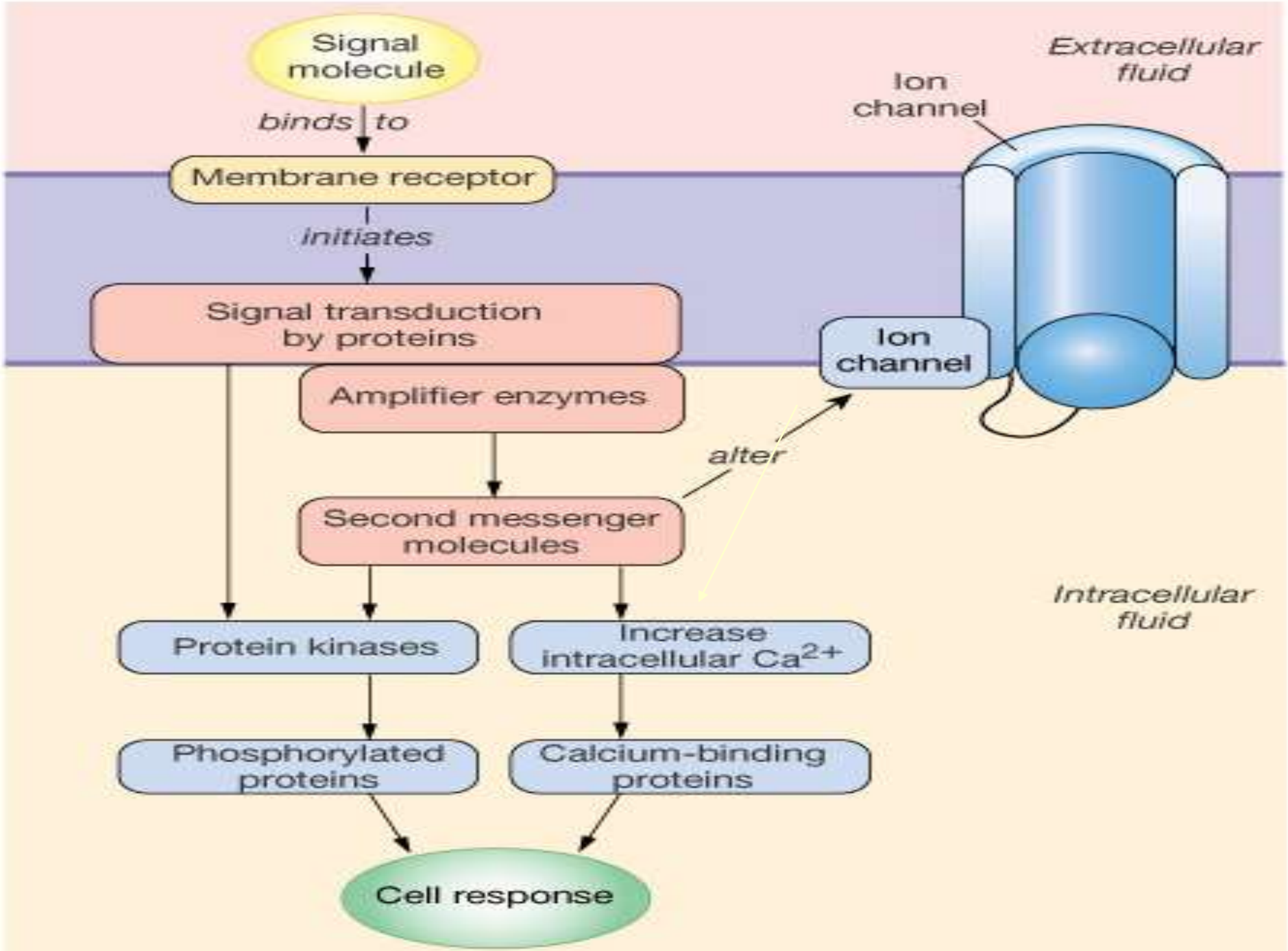
• Beta-blockers would have the exact *opposite* affect that stimulating beta-receptors would have.

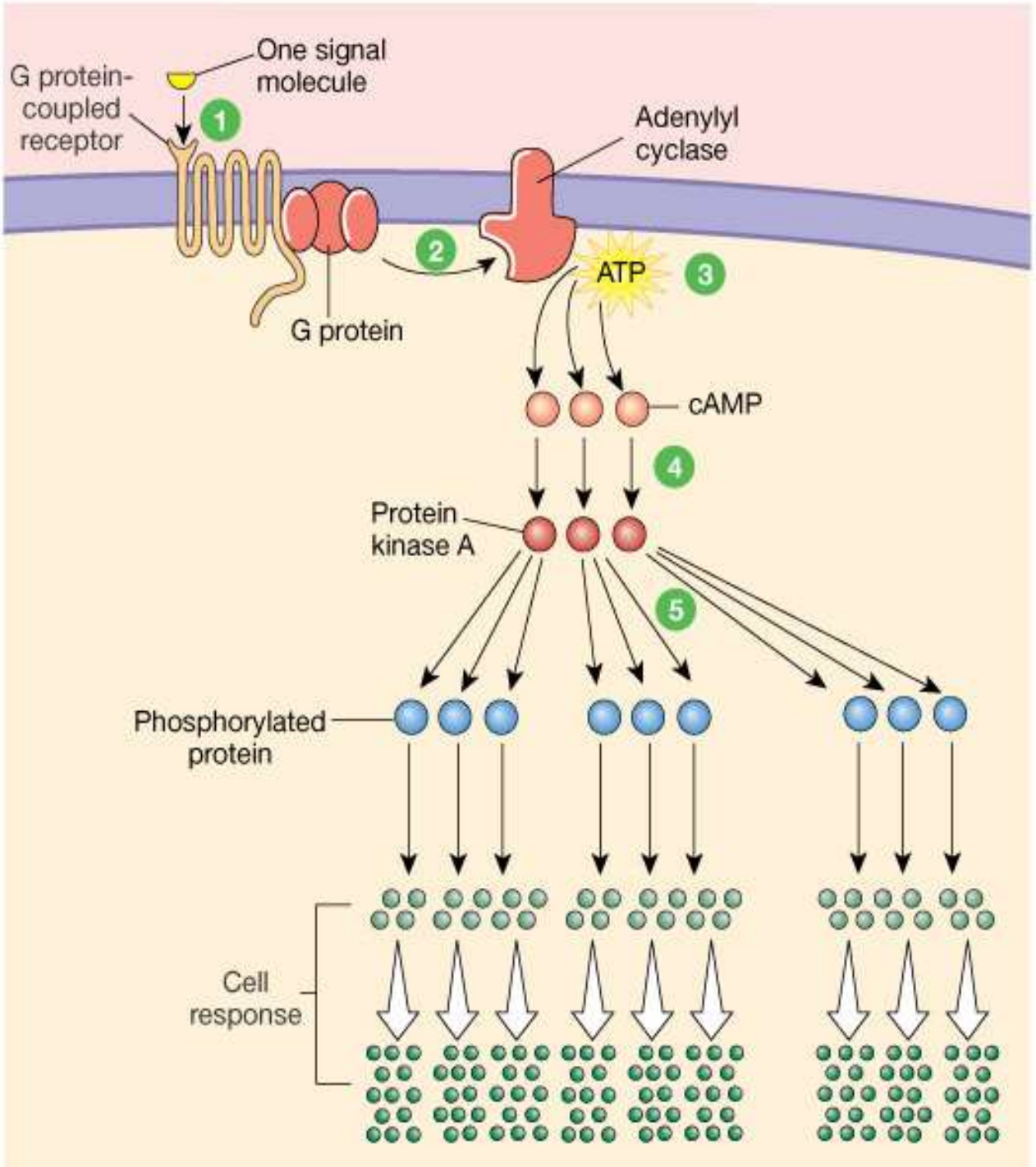
ß-receptor physiology

Summary

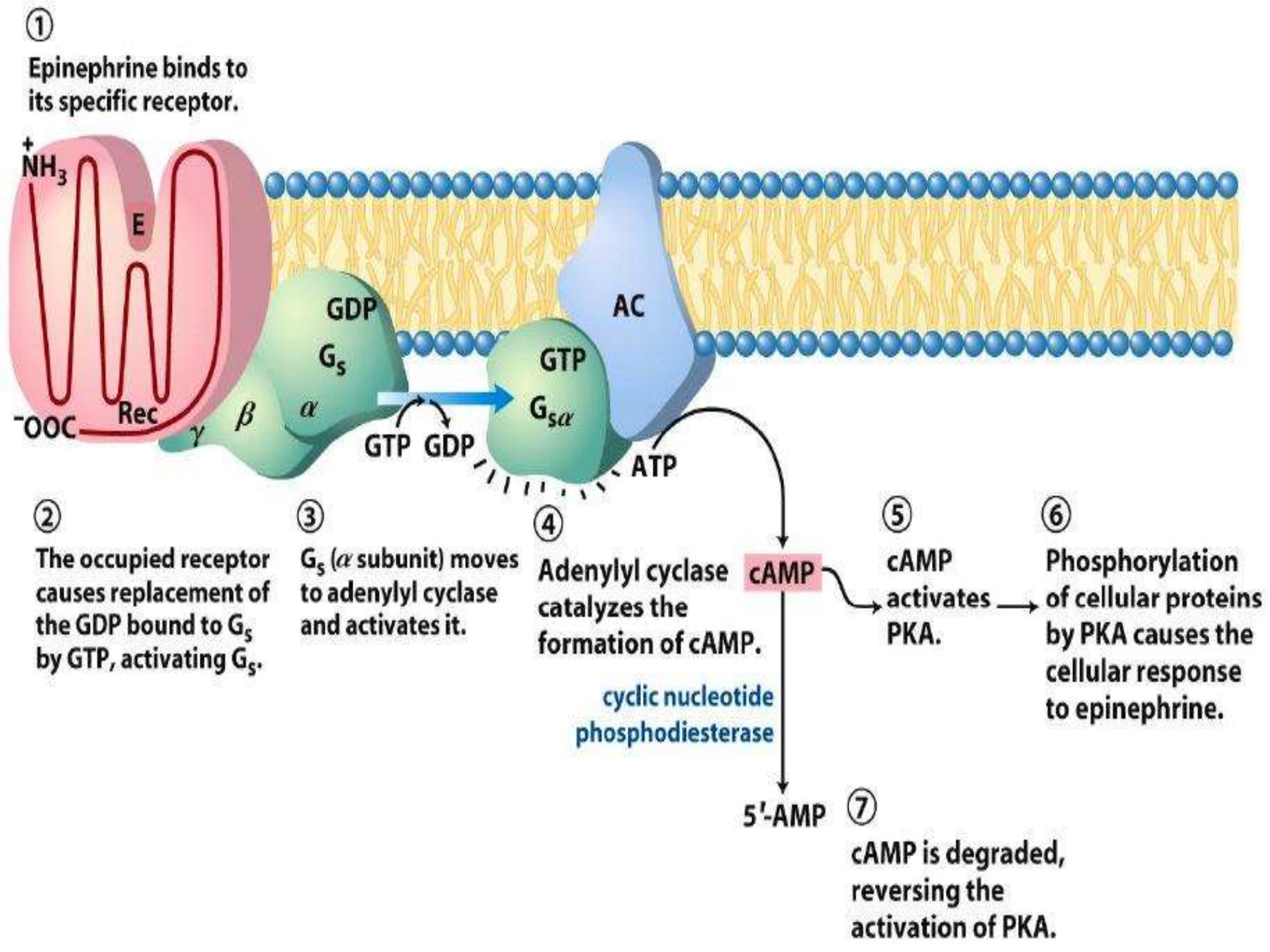
- Beta-receptors are G-protein (G_s) coupled receptors
- Series of events in cellular signaling are as follows:

- Substance (agonist or antagonist) finds the receptor
- Signal transduction is amplified via G_s (by an agonist) by turning *on* adenylyl cyclase or dampened (by an antagonist) simply occupying the receptor site an *not* turning on adenylyl cyclase
- Adenylyl cyclase converts ATP into cAMP
- cAMP activates protein kinase A → phosphorylates proteins → cellular response


Signal molecule
binds to
Membrane receptor
initiates
Signal transduction by proteins
Amplifier enzymes
Second messenger molecules
Protein kinases
Increase intracellular Ca^{2+}
Phosphorylated proteins
Calcium-binding proteins
Cell response
alter
Extracellular fluid
Ion channel
Ion channel
Intracellular fluid



1. Signal molecule binds to G protein-linked receptor, which activates the G protein.
2. G protein turns on adenylyl cyclase, an amplifier enzyme.
3. Adenylyl cyclase converts ATP to cyclic AMP.
4. cAMP activates protein kinase A.
5. Protein kinase A phosphorylates other proteins, leading ultimately to a cellular response.


①
Epinephrine binds to its specific receptor.
+
NH3
E
Rec
-OOC
GDP
Gs
β
α
γ
AC
GTP
GsαGTP GDP
ATP
②
The occupied receptor causes replacement of the GDP bound to Gs by GTP, activating Gs.
③
Gs (α subunit) moves to adenylyl cyclase and activates it.
④
Adenylyl cyclase catalyzes the formation of cAMP.
cAMP
cyclic nucleotide phosphodiesterase
5′-AMP
⑤
cAMP activates PKA.
⑥
Phosphorylation of cellular proteins by PKA causes the cellular response to epinephrine.
⑦
cAMP is degraded, reversing the activation of PKA.

Properties of ß-blockers

Cardioselectivity

- "Cardioselective" ß-blockers block only $ß_1$ receptors
- "Non-cardioselective" ß-blockers block both $ß_1$ and $ß_2$ receptors

Intrinsic sympathomimetic activity

- Some ß-blockers are "partial agonists" at ß receptors

Lipid solubility

- High lipid solubility $\rightarrow$ short $T_{½}$ and high brain penetration
- Low lipid solubility $\rightarrow$ long $T_{½}$ and low brain penetration

Properties of ß-blockers

Cardioselective ß-blockers

• Useful in patients with COPD/Asthma since $ß_2$ blockade inhibits bronchodilation. Inhibiting bronchodilation can result in severe pulmonary symptoms

• Even cardioselective ß-blockers still inhibit $ß_2$ receptors to some extent, so caution must be taken with ANY ß-blocker if the patient has COPD/Asthma

• $ß_2$ receptor stimulation results in muscle triglyceride breakdown. This is important during prolonged low intensity exercise.

Properties of ß-blockers

Lipid solubility

- The higher the lipid solubility, the easier it is for the liver to clear the drug, thus the shorter the half-life
- The higher the lipid solubility, the greater the penetration through the blood brain barrier, thus the greater the central nervous system effects (depression, migraine headache treatment, sleep disorders etc…)

Side effects of ß-blockers

Bradycardia and AV nodal blockade

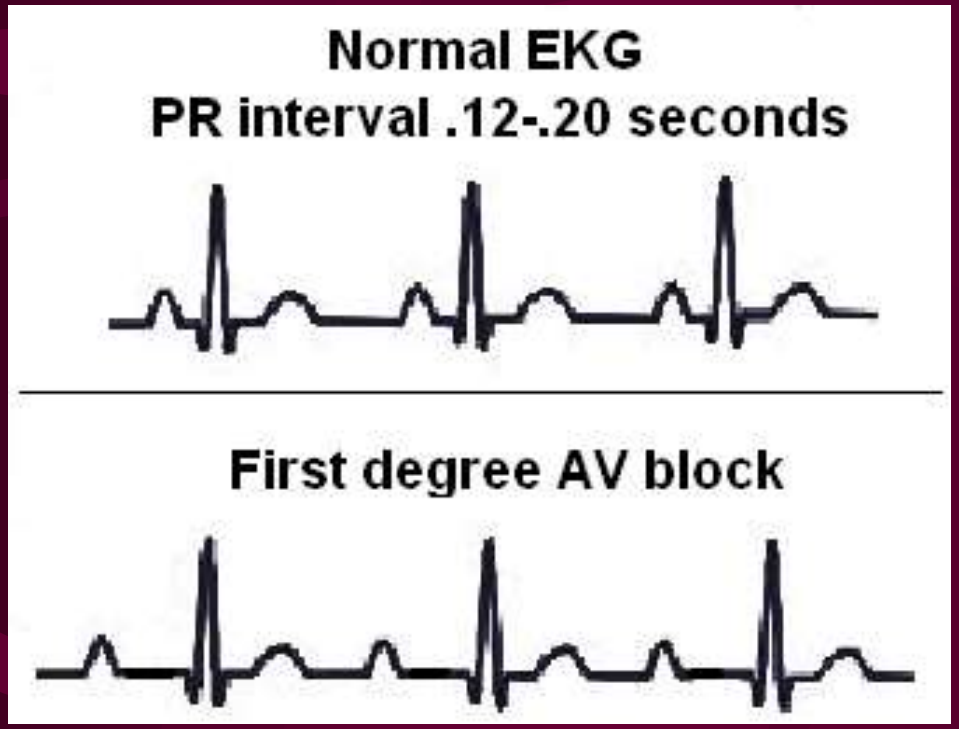
• A HR of less than 40 is usually considered worrisome, however if the patient is symptomatic from the bradycardia regardless of the heart rate, the dose of the ß-blocker should be decreased or it should be discontinued.

• Symptoms of bradycardia include lightheadedness, dizziness, and syncope. These relate to hypotension.

• Chest pain may occur due to diastolic hypotension leading to decreased coronary perfusion, especially in patients with coronary artery disease.

Side effects of ß-blockers

Bradycardia and AV nodal blockade



Side effects of ß-blockers

<u>Congestive heart failure (CHF)</u>

• Beta-blockers can worsen CHF when the patient is in a "decompensated" or "unstable" state

CO = SV X HR

• If the patient has compensated or stable CHF, then beta-blockers are actually the *treatment of choice* via a complex mechanism.

• The American College of Cardiology guidelines state that if a patient's CHF is decompensated enough to require intravenous diuretics, then the patient should not be receiving a beta-blocker due to the potential of worsening their CHF. They should however be discharged on one.

Side effects of ß-blockers

Diabetes Related Side Effects

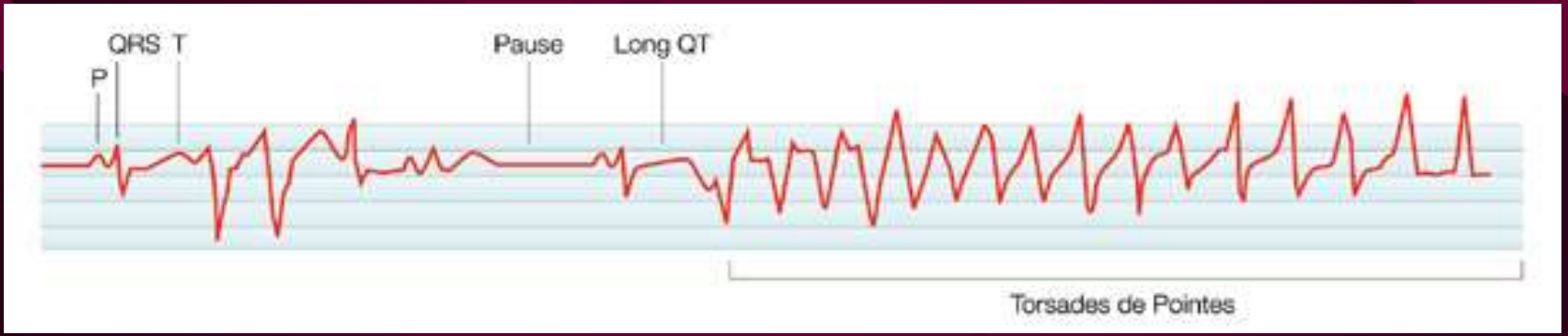
- Masking of symptoms of hypoglycemia such as tachycardia and diaphoresis normally occurs when a patient is hypoglycemic due to an acute increase in SNS activity designed to increase blood sugar.
- Delayed recovery from hypoglycemia since glycogenolysis and gluconeogensis are inhibited. They normally rely on ß-receptor stimulation.
- Insulin resistance state (mechanism unclear)
- Worsening of lipid profile (mechanism unclear)

**Despite the above, ß-blockers are still VERY frequently used in patients with diabetes since the cardiovascular affects are so protective.

Side effects of ß-blockers

Side effects of ß-blockers

- Aggravation of Raynaud's disease and claudication
- Shortness of breath (especially if asthma/COPD)
- Depression, Sleep disturbance
- Dizziness and lightheadedness
- Exercise intolerance
- Sotalol causes QT prolongation and Torsades de Pointes



Side effects of ß-blockers

ß-receptors are highly regulated

1) After prolonged stimulation of ß-receptors (as in frequent intense exercise or CHF), the number of ß-receptors decrease to compensate

**Must start ß-blockers at a low dose or severe bradycardia may develop

2) After prolonged blockade of ß-receptors, the number of receptors increase to compensate

**Must wean a patient off of ß-blockers and not abruptly stop the medication

Side effects of ß-blockers

ß-blocker Overdose

- The main symptoms in overdose of ß-blockers relates to severe hypoglycemia (by *inhibiting* glycogenolysis and gluconeogenesis) and bradycardia.
- Antidote is the combination of atropine (an anti-muscarinic medication used to speed up the heart rate) and glucagon (a hormone with anti-insulin like properties).

Clinical use of ß-blockers

ß-blockers in Hypertension (HTN)

- Indicated as 1st line treatment of HTN according to JNC VII guidelines
- Most commonly used: Toprol XL (metoprolol extended release)
- Thiazide diuretics and ACE inhibitors also indicated for 1st line treatment of HTN

Clinical use of ß-blockers

ß-blockers in Coronary Artery Disease (CAD)

- Used to decrease myocardial oxygen consumption which reduces ischemia
- Reduces chest pain in people with angina pectoris
- Have been shown in multiple large studies to reduce the long-term morality in people with CAD

Clinical use of ß-blockers

ß-blockers in Acute MI (AMI)

- Indicated for all patients with AMI
- Significantly reduces mortality by decreasing oxygen consumption and limiting the extent of the infarction
- Is given in IV form until the HR is reduced to 60 or less

Clinical use of ß-blockers

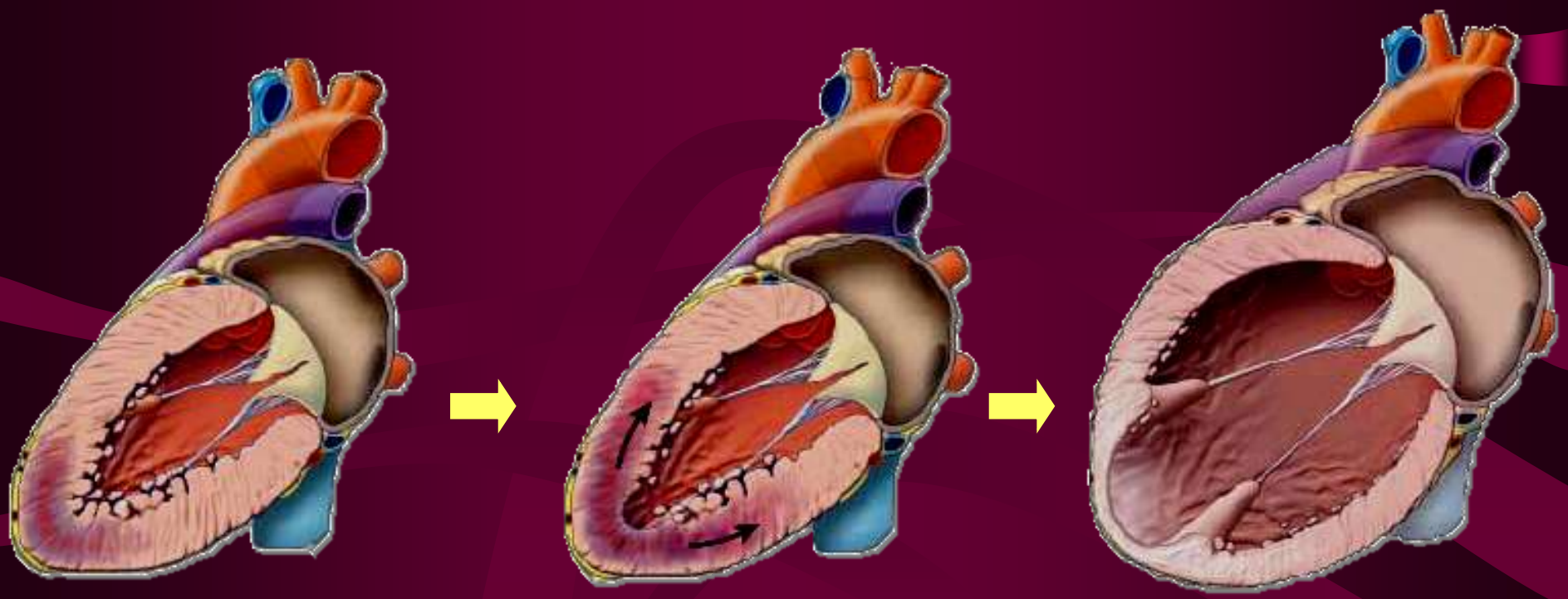
ß-blockers in Congestive Heart Failure (CHF)

- Chronic activation of the SNS that occurs in patients with CHF leads to negative remodeling of the heart and thus further damage. ß-blockers inhibit ↑SNS and thus the negative remodeling This results in an *increase* the CO over time in CHF and significantly reduces mortality in patients with CHF
- Only metoprolol and carvedilol are currently FDA approved for use in CHF

Clinical use of ß-blockers

ß-blockers in other diseases

- Although commonly used in many clinical disorders and disease states, ß-blockers are not absolutely needed in most other situations other than those previously described.
- Exceptions may include atrial fibrillation, IHSS and pheochromocytoma, although the latter two conditions are rare.



Initial Infarct

Expansion of Infarct
Hours to Days

Global Remodeling
Days to Months

GLAUCOMA

What is it?

A disease of progressive optic neuropathy with loss of retinal neurons and the nerve fiber layer, resulting in blindness if left untreated.

GLAUCOMA

"Glaucoma describes a group of diseases that kill retinal ganglion cells."

"High IOP is the strongest known risk factor for glaucoma but it is neither necessary nor sufficient to induce the neuropathy."

Libby, RT, et al: Annu Rev Genomics Hum Genet 6: 15, 2005

GLAUCOMA

What causes it?

There is a dose-response relationship between intraocular pressure and the risk of damage to the visual field.

GLAUCOMA

How do we diagnose it?

- IOP is not helpful diagnostically until it reaches approximately 40 mm Hg at which level the likelihood of damage is significant.
- Visual fields are also not helpful in the early stages of diagnosis because a considerable number of neurons must be lost before VF changes can be detected.
- Optic nerve damage in the early stages is difficult or impossible to recognize.
- 50% of people with glaucoma do not know it!

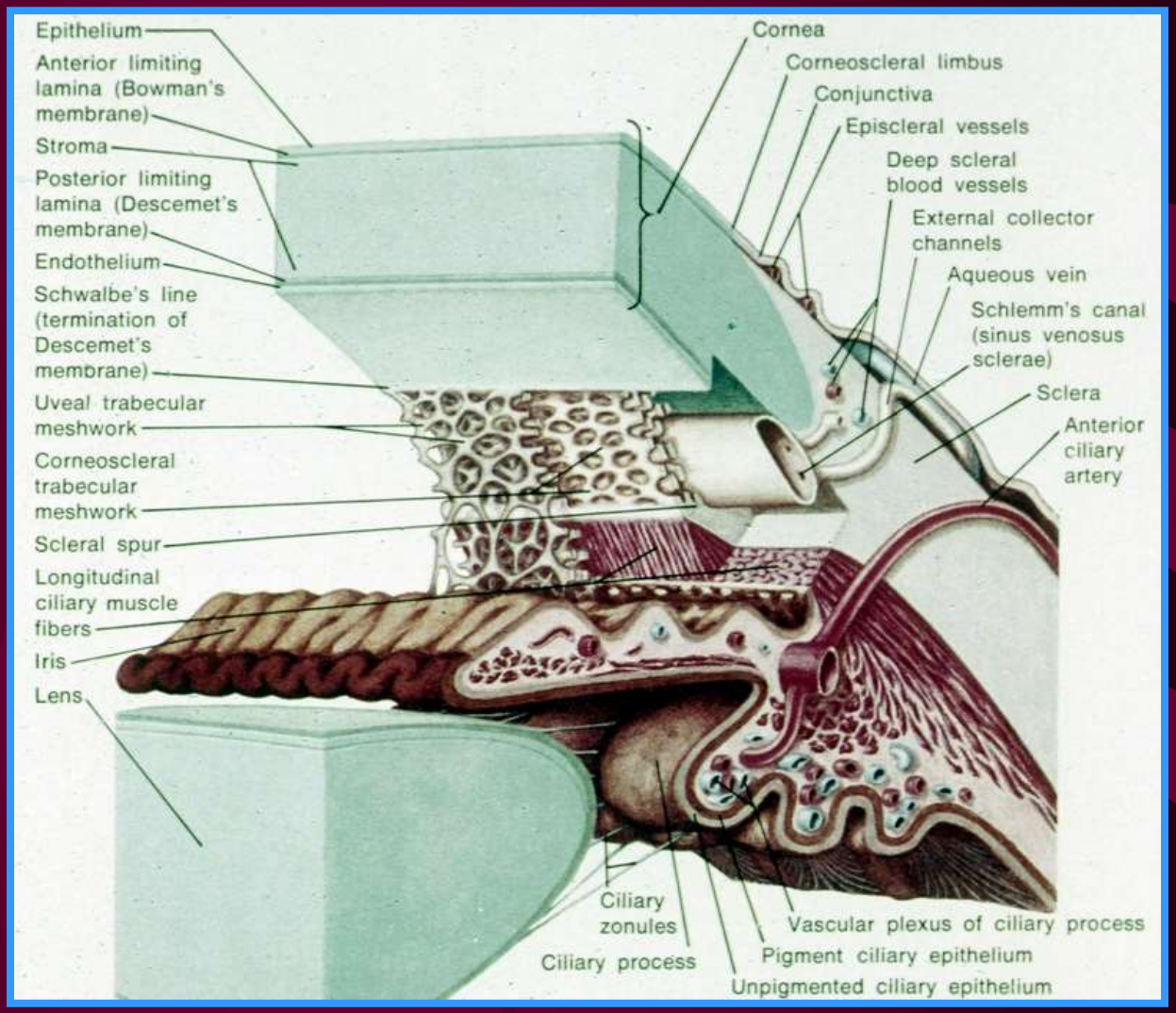
GLAUCOMA

Intraocular pressure is not the only factor responsible for glaucoma!

- **95% of people with elevated IOP will never have the damage associated with glaucoma.**
- **One-third of patients with glaucoma do not have elevated IOP.**
- **Most of the ocular findings that occur in people with glaucoma also occur in people without glaucoma.**

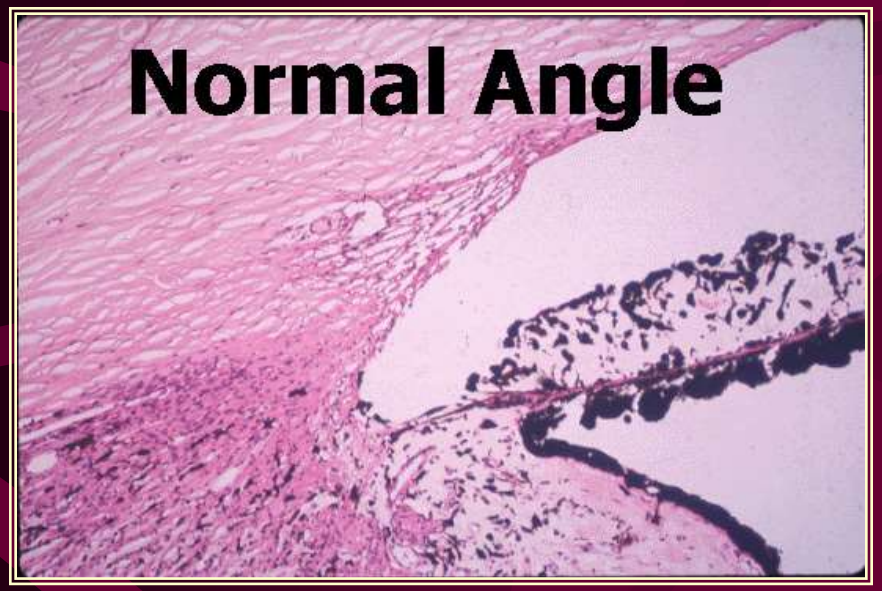
GLAUCOMA

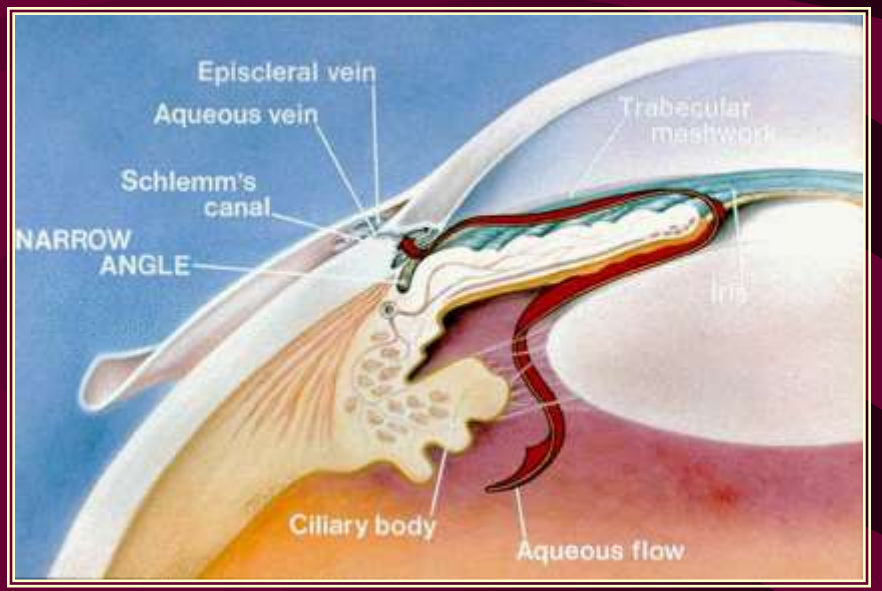
Anatomy of anterior chamber angle



GLAUCOMA

Angle Anatomy

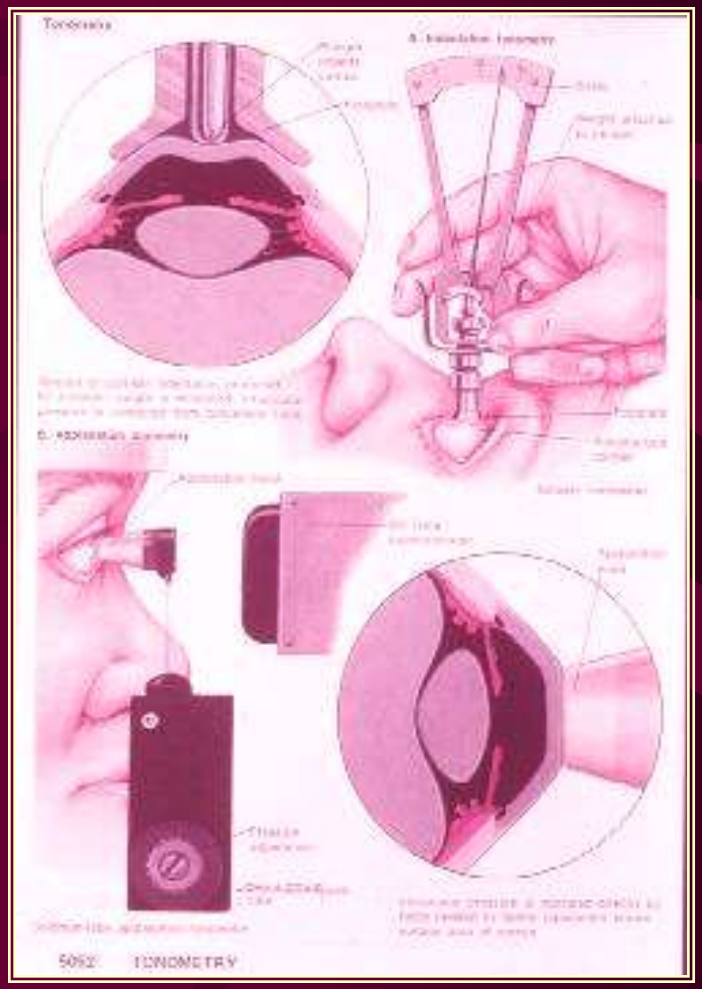




GLAUCOMA

How do we measure IOP?

- Applanation
- Tonopen
- Schiotz
- Air
- Non-contact



GLAUCOMA

Optic nerve signs of glaucoma progression

- Increasing C:D ratio
- Development of disk pallor
- Disc hemorrhage (60% will show progression of visual field damage)
- Vessel displacement
- Increased visibility of lamina cribosa

GLAUCOMA

Ocular hypertension treatment study

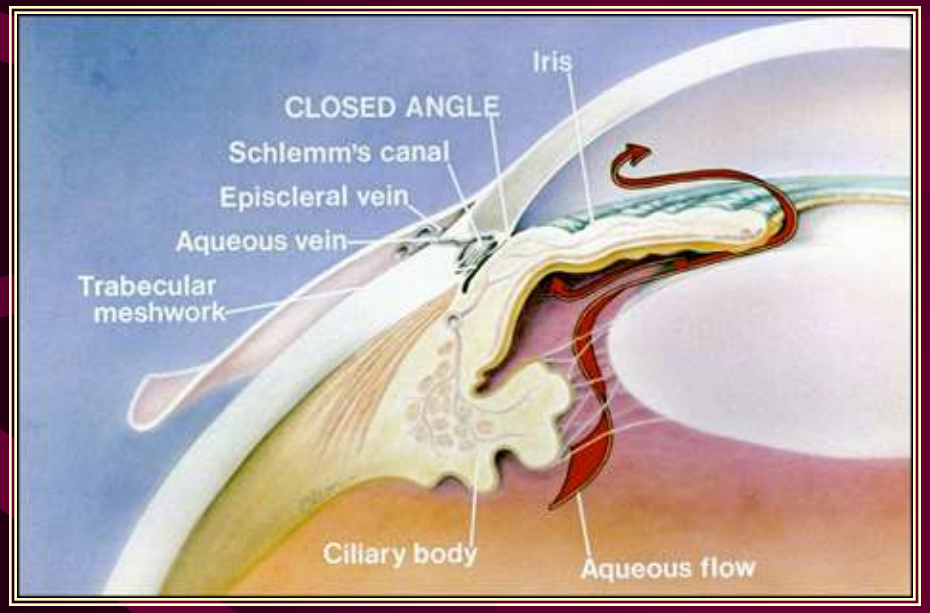
(OHTS study)

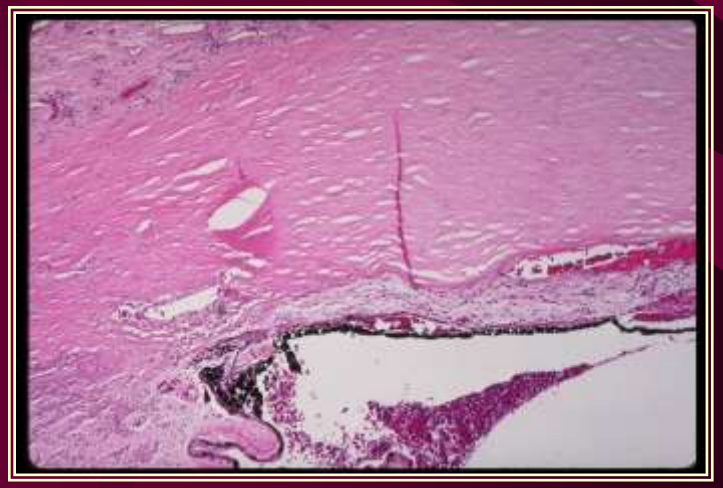
- GOALS: To evaluate the effectiveness of topical ocular hypotensive medications in preventing or delaying visual field loss and/or optic nerve damage in subjects with ocular hypertension at moderate risk for developing open-angle glaucoma (POAG).

- POPULATION: 1636 participants aged 40-80 years with IOP 24-32 mm HG in one eye, and 21-32 in the other, randomly assigned to observation and treatment groups.

GLAUCOMA

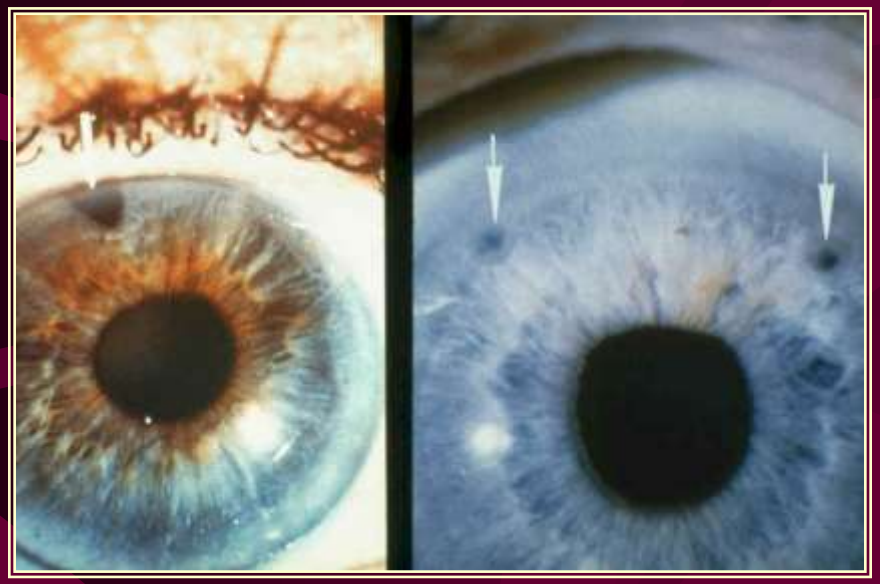
Anatomy of Angle Closure Glaucoma

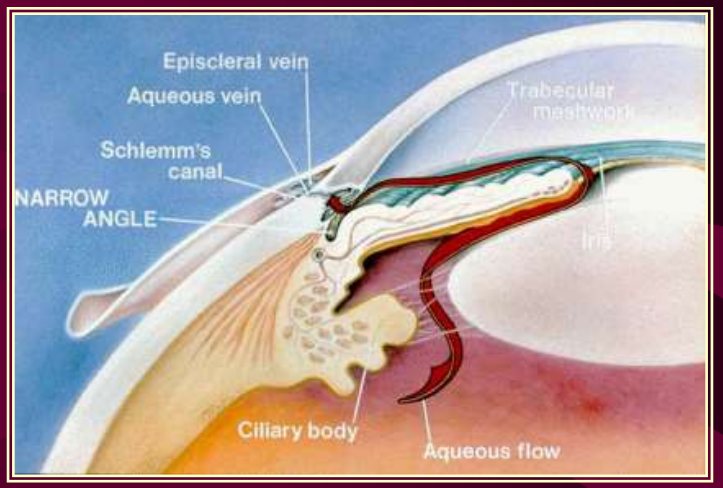


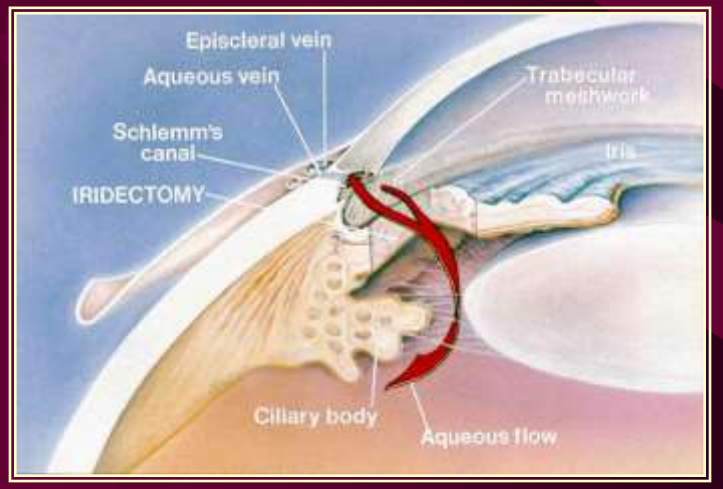


Narrow Angle Glaucoma

Treatment: Peripheral Iridotomy







Open Angle Glaucoma

Aka: chronic simple glaucoma (CSG)

and primary open angle glaucoma (POAG)

Onset: 50+ years of age

Symptoms	Signs
Usually none May have loss of central and peripheral vision late	Elevated IOP Visual field loss Glaucomatous disk changes

Open Angle Glaucoma

Risk factors

HISTORY:

- **Positive family history**
- **African American background**
- **History of trauma**
- **History of steroid use**

EXAMINATION:

- C/D 0.6 or greater
- Vertical elongation of disc
- Inf. rim thinner than sup.
- C/D asymmetry > 0.2

GLAUCOMA

Treatment

Medical

- Miotics
- Beta-blockers
- Carbonic anhydrase inhibitors
- Prostaglandin analogues
- Alpha-2 agonists

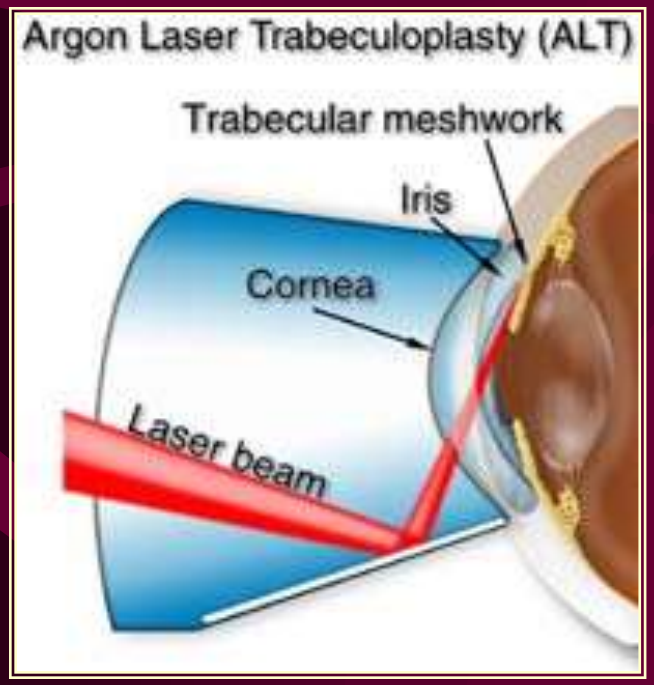
Surgical

- Argon laser trabeculoplasty
- Trabeculectomy
- Filtering procedure
- Cyclocryotherapy
- Cyclolaser ablation
- Iridotomy

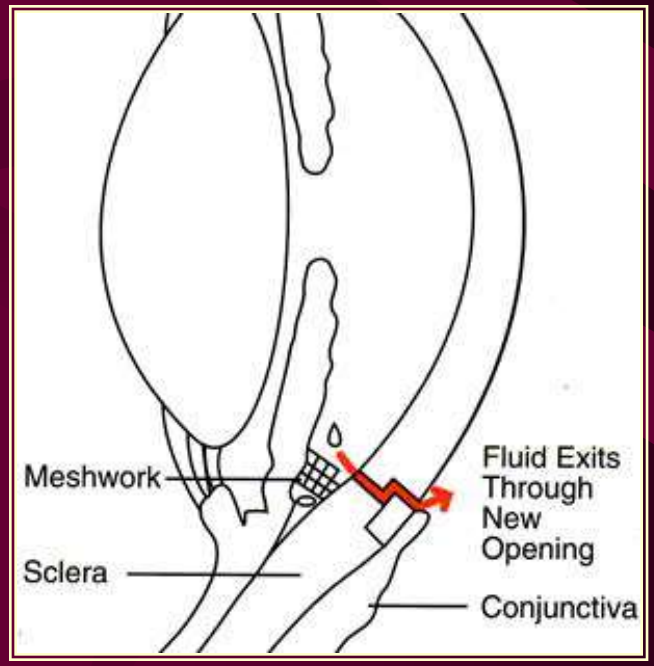
GLAUCOMA

Surgical treatment of glaucoma

Argon laser trabeculoplasty



Filtration procedures



Microencapsulation

Presented by

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Shankarrao Ursal College Of Pharmaceutical Sciences and Research Centre Kharadi,Pune.

CONTENTS:

- Definition
- Advantages
- Disadvantages
- Classifications
- Method of Preparations
- Evaluation Tests
- Applications
- References

Definition:

- **Microencapsulation** is a process by which very tiny droplets or particles of liquid or solid material are surrounded or coated with a continuous film of polymeric materials.

- Particles having diameter between **3-800µm** are known as micro particles.

Advantages:

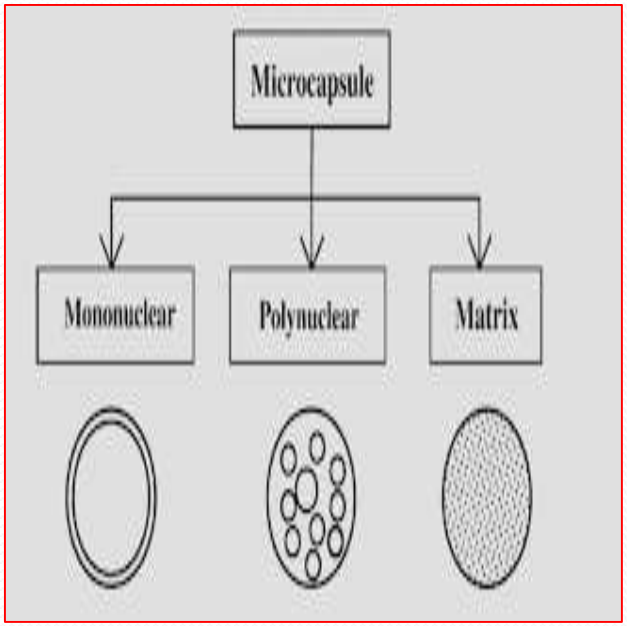
- `To increase of bioavailability.
- To alter the drug release.
- To improve the patient's compliance.
- To decrease evaporation rate of the core material.
- To produce a targeted drug delivery.
- To convert liquid to solid from.

Disadvantages:

- Difficult to achieve continuous and uniform film.
- Shelf life of hygroscopic drugs is reduced.
- Polymer may produce toxic effect.
- Costly.

Classifications:

- Microcapsules can be classified on three type. As follow as-
- **(1)Mono nuclear**-Contain the shell around the core.
- **(2)Poly nuclear**-Having many cores enclosed with in shell.
- **(3)Matrix type**- Distributed homogeneously into the shell material.



Method Of Preparation:

- **(1)Physical Method:**
- (a)Air suspension
- (b)Coacervation phase Separation
- (c)Pan coating
- (d)Spray Drying
- **(2)Chemical Method:**
- (a)Solvent Evaporation
- (b)Polymerization

1.a)Air suspension Method:

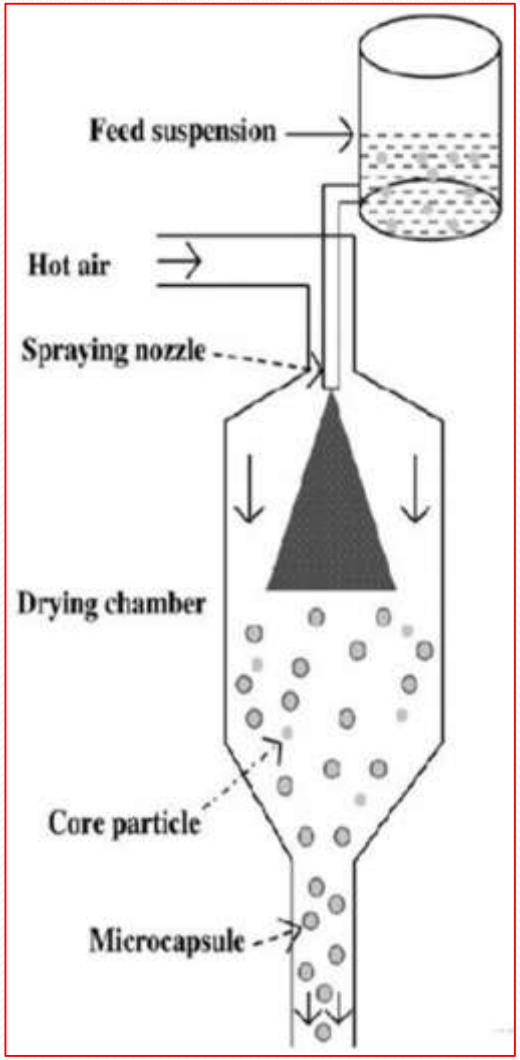
Fine solid core materials are suspended by a vertical current of air

Evaporation of the solvent

The encapsulating material is deposited onto the core material

Achieved the desired film thickness

The encapsulated product is dried by passing the stream air



(b)Coacervation Phase Separation:

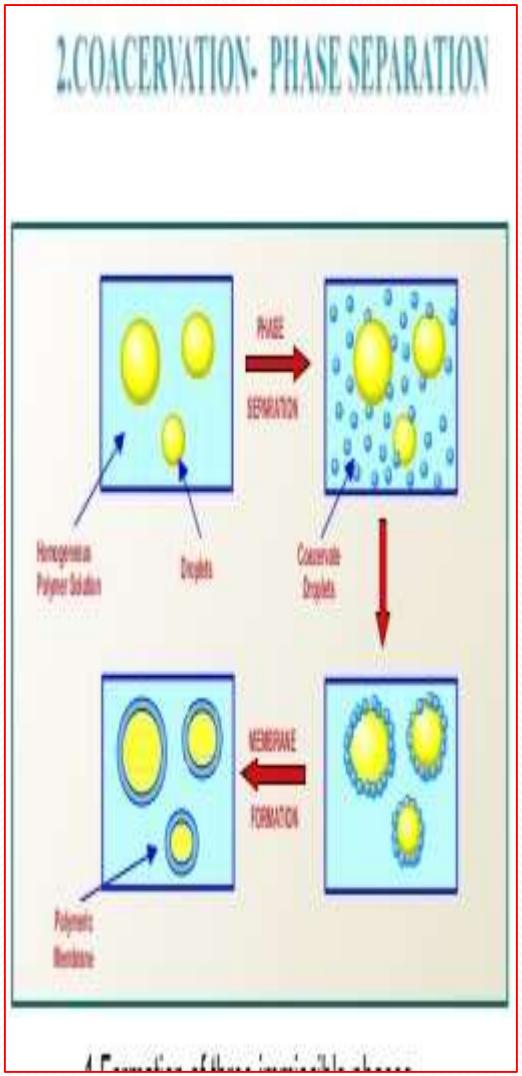
Core material phase is dispersed in the solution of coating polymer

Solvent of polymer is vehicle the phase

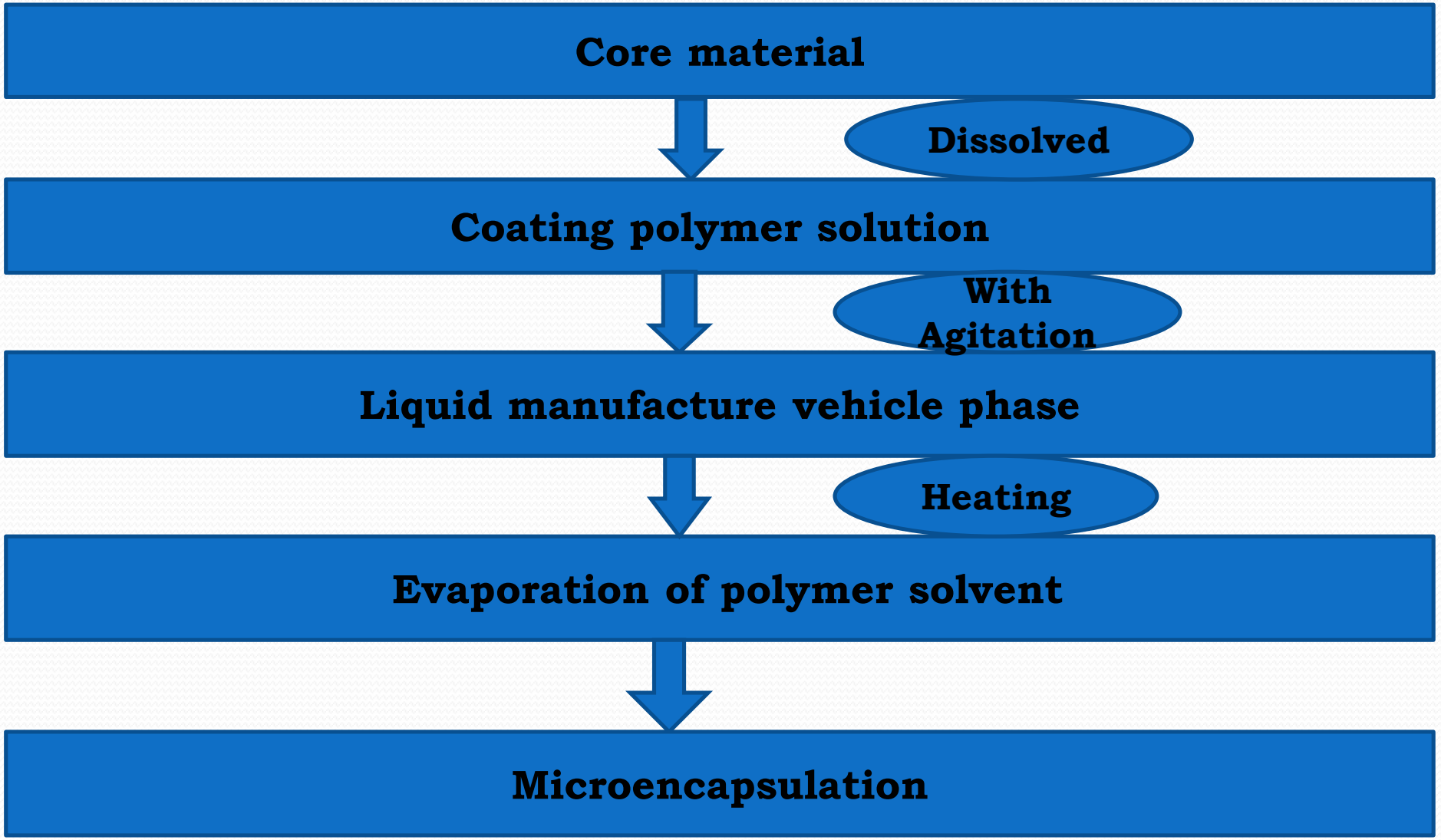
Deposition of polymer solution onto the core material

Deposition of liquid polymer occurs when the polymer is absorbed

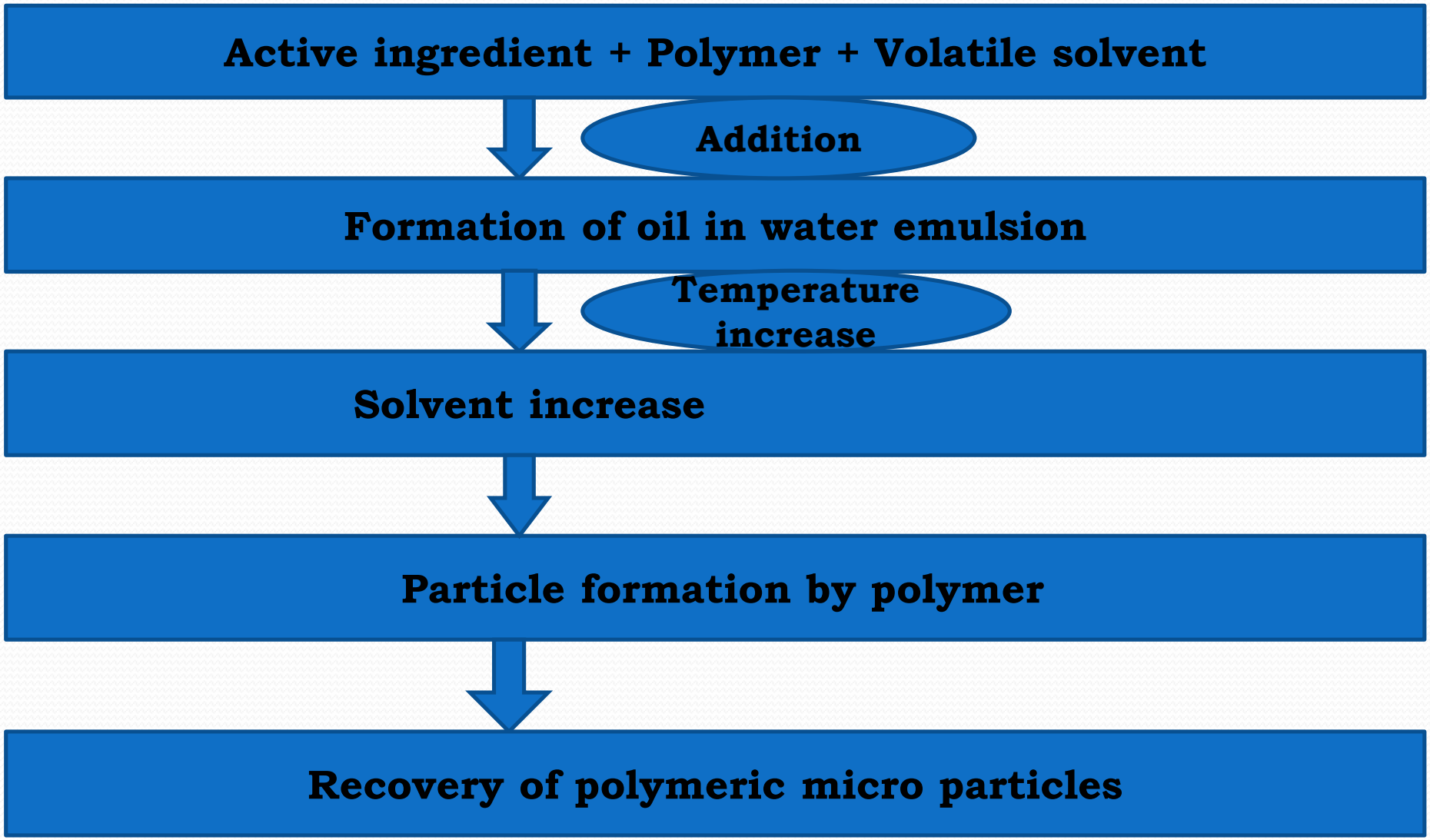
Microcapsule



2.(a)Solvent Evaporation:



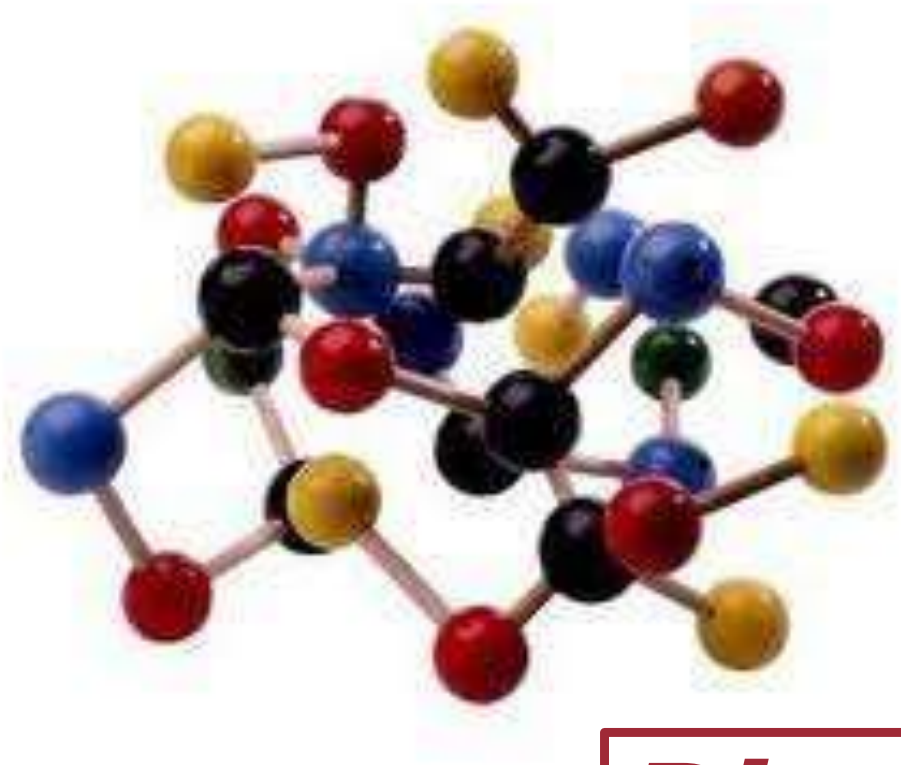
(b)Polymerization:



Application of microencapsulation:

- To improve the flow properties.
  E.g=Thiamine
- To enhances the stability.
  E.g=Vitamins
- To reduced volatility of materials.
  E.g =Methyl salicylate
- To mask the unpleasant taste and odour.
  E.g=castor oil
- To convert liquids into solids.
- **E.g=castor oil**
- To reduce gastric irritation.
- **E.g= endomethacin**

THANK
You!



Biomolecules

BIOMOLECULES

By

Dr. Amit Kasabe
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Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune

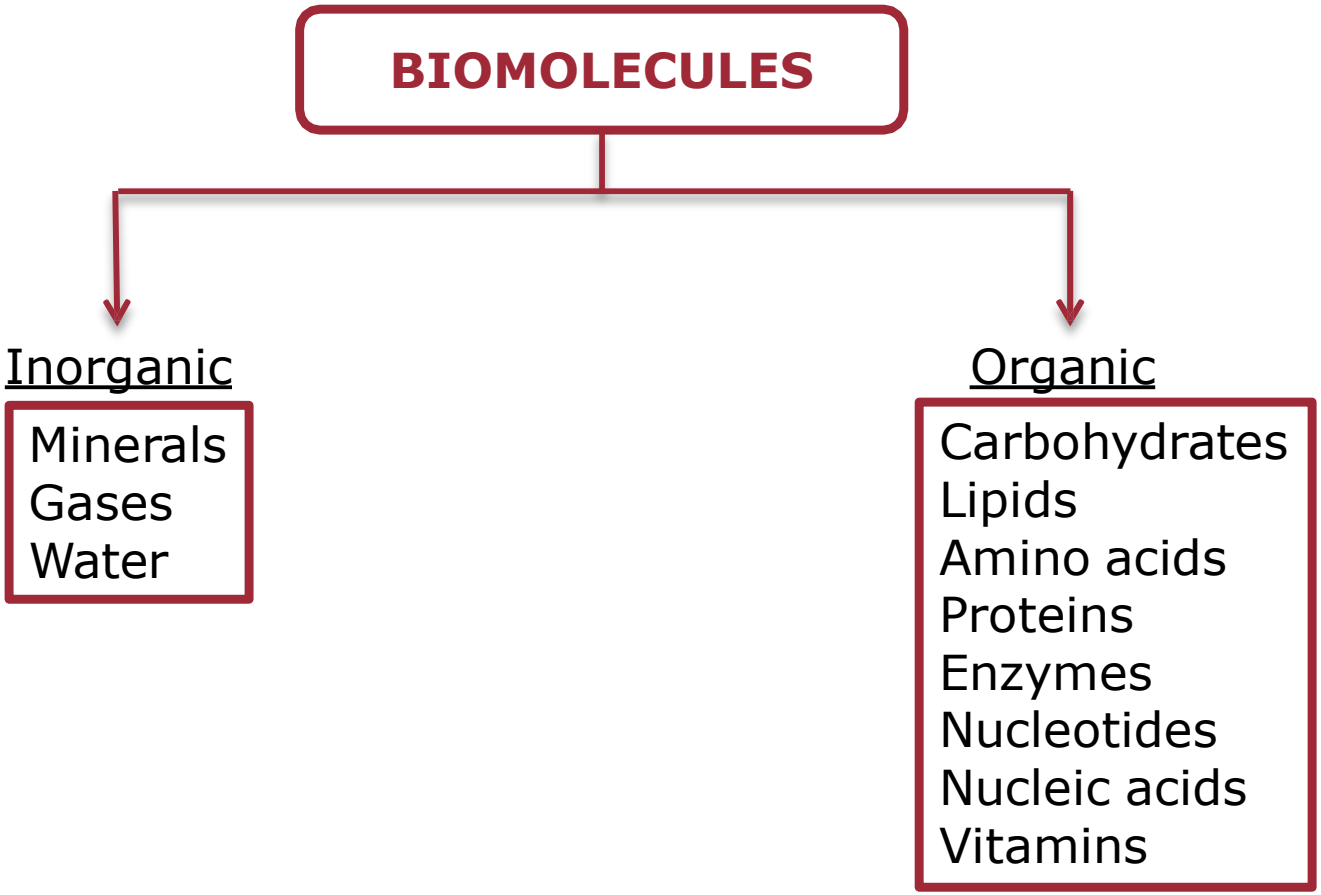
Chemicals or molecules present in the living organisms are known as **Biomolecules**

The sum total of different types of biomolecules, compounds and ions present in a cell is called as **cellular pool**

Biomolecules are compounds of **carbon**.
Hence the chemistry of living organisms is organized around carbon

Carbon is the most versatile and the most predominant element of life.

ELEMENT	Non living (Earth crust)	Living Matter
Hydrogen	0.14	0.5
Carbon	0.03	18.5
Oxygen	46.6	65.0
Nitrogen	Very less	3.3
Sulphur	0.03	0.3
Sodium	2.8	0.2
Calcium	3.6	1.5
Magnesium	2.1	0.1
Silicon	27.7	Very less


BIOMOLECULES
Inorganic
Minerals
Gases
Water
Organic
Carbohydrates
Lipids
Amino acids
Proteins
Enzymes
Nucleotides
Nucleic acids
Vitamins

BIOMOLECULES

Micro molecules

Small sized, low mol wt
Between 18 and 800 daltons
Found in the acid soluble pool

- Minerals
- Gases
- Water
- Sugars
- Amino acids
- nucleotides

Macromolecules

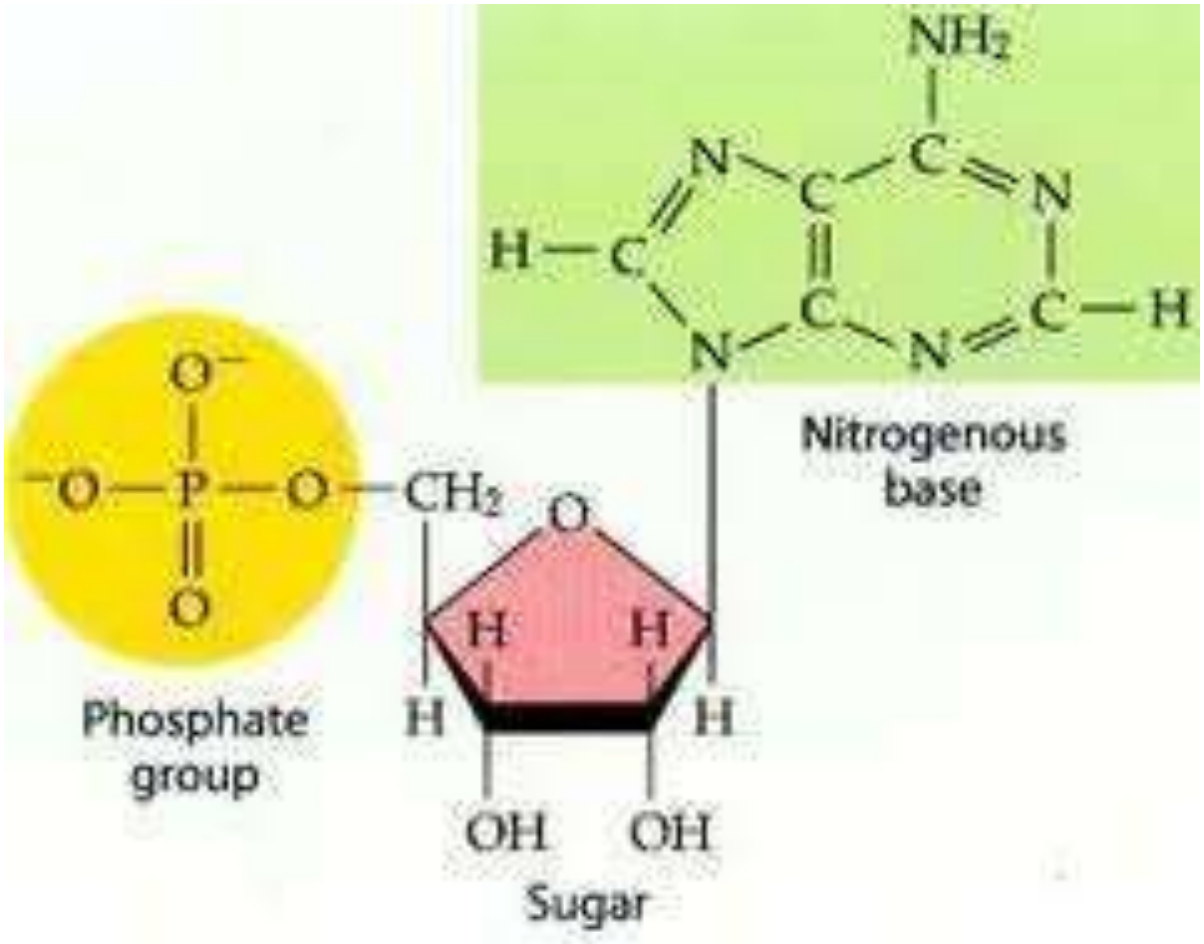
Large sized, high mol wt
Above 10000 daltons
Found in the acid insoluble pool

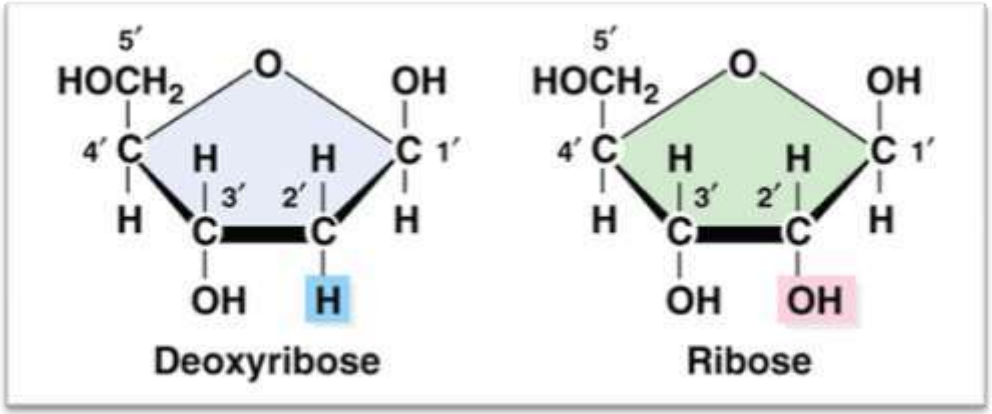
- Carbohydrates
- Lipids
- Proteins
- Nucleic acids

The major complex biomolecules of cells

Biomolecule	Building block	Major functions
Protein	Amino acid	Basic structure and function of cell
DNA	Deoxyribonucleotide	Hereditary information
RNA	Ribonucleotide	Protein synthesis
Polysaccharide	Monosaccharide	Storage form of energy
Lipids	Fatty acids & glycerol	Storage form of energy to meet long term demands

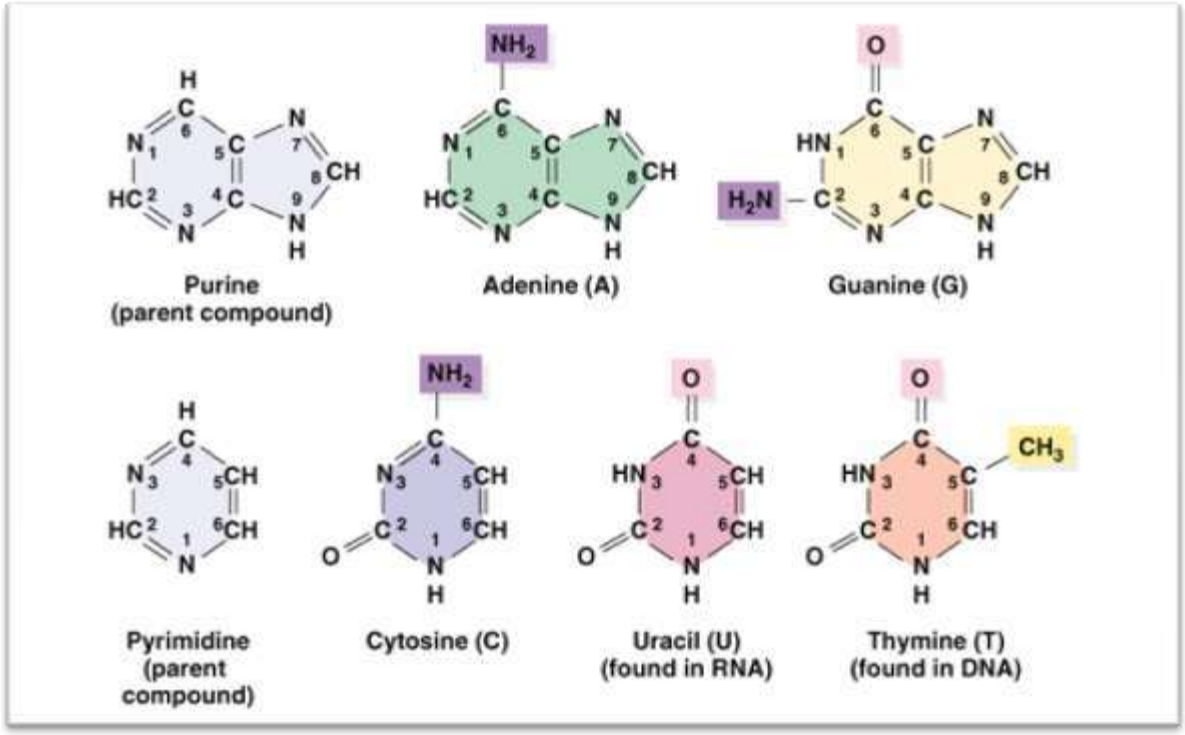
NUCLEOTIDES

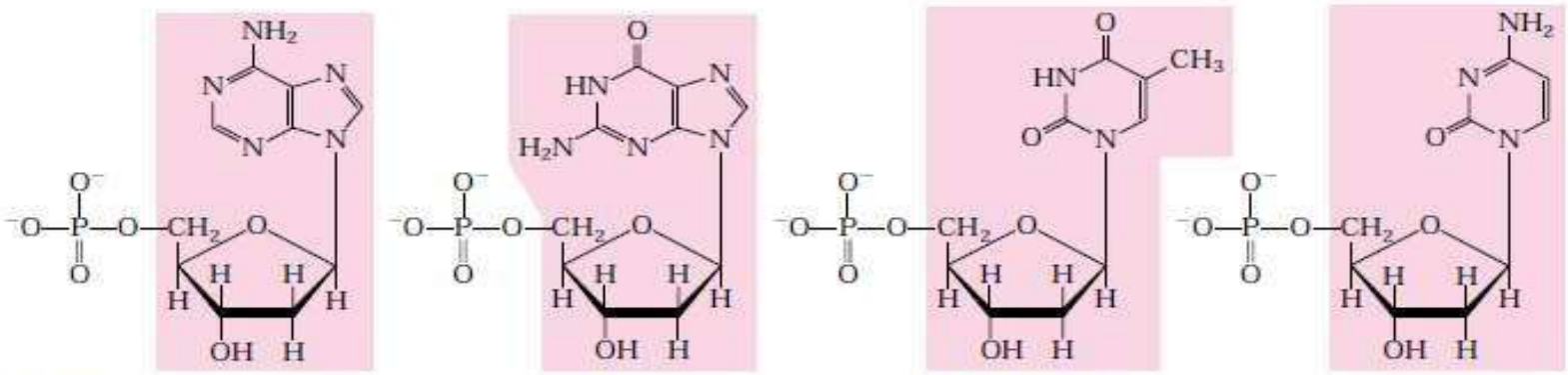




SUGAR

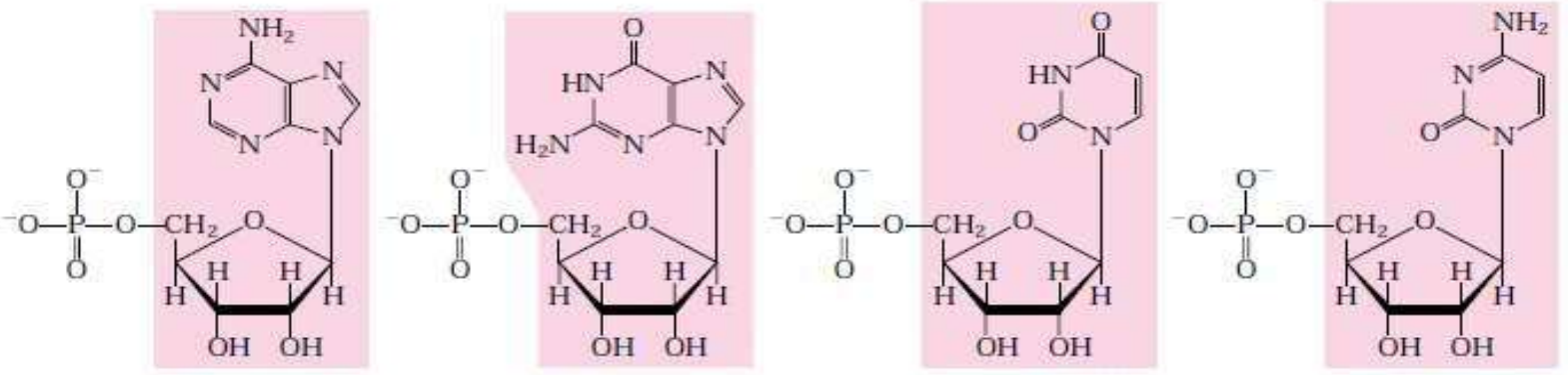
N_2 BASES





	Deoxyadenylate (deoxyadenosine 5′-monophosphate)	Deoxyguanylate (deoxyguanosine 5′-monophosphate)	Deoxythymidylate (deoxythymidine 5′-monophosphate)	Deoxycytidylate (deoxycytidine 5′-monophosphate)
Symbols:	A, dA, dAMP	G, dG, dGMP	T, dT, dTMP	C, dC, dCMP
Nucleoside:	Deoxyadenosine	Deoxyguanosine	Deoxythymidine	Deoxycytidine

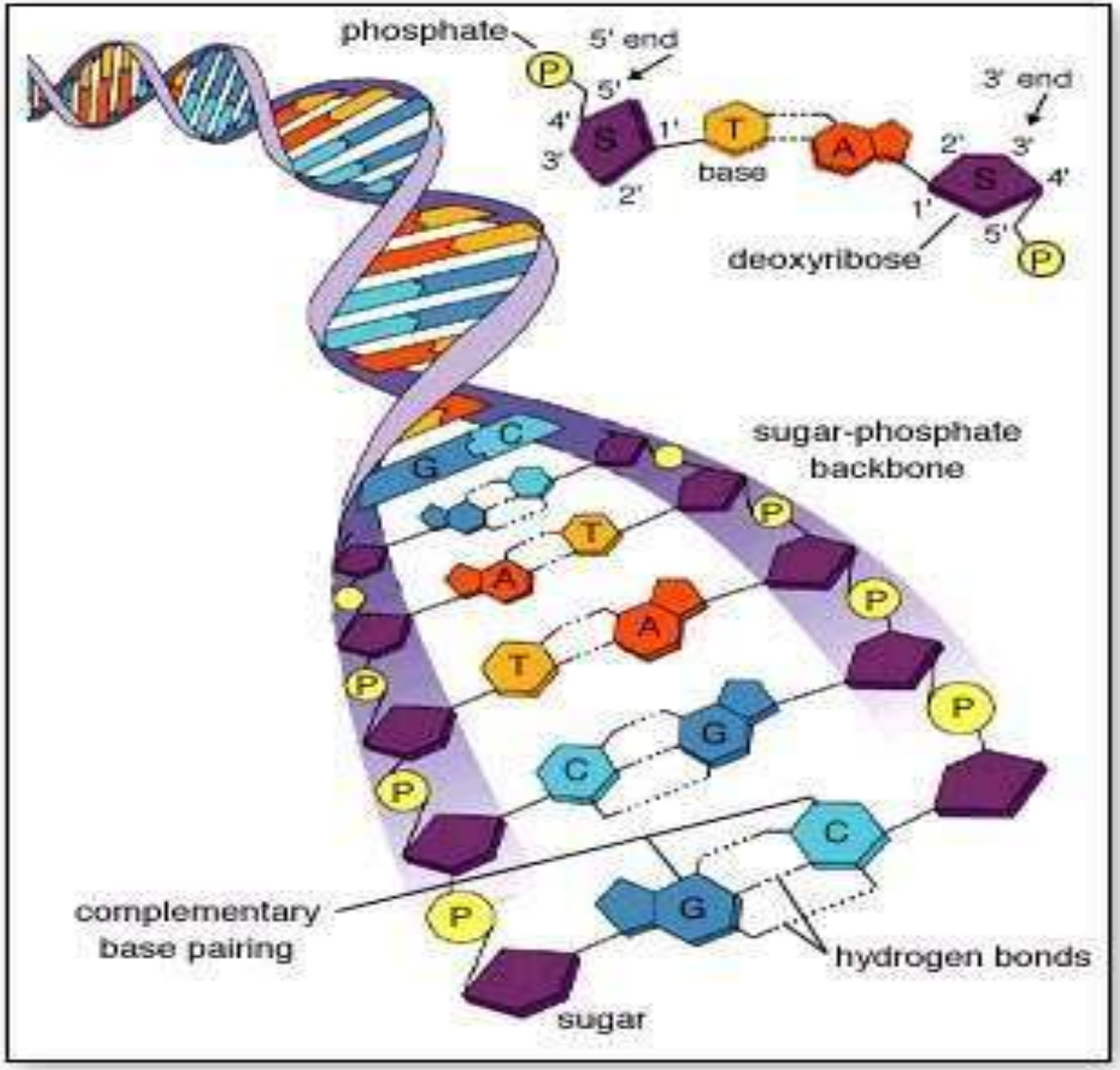
(a) Deoxyribonucleotides

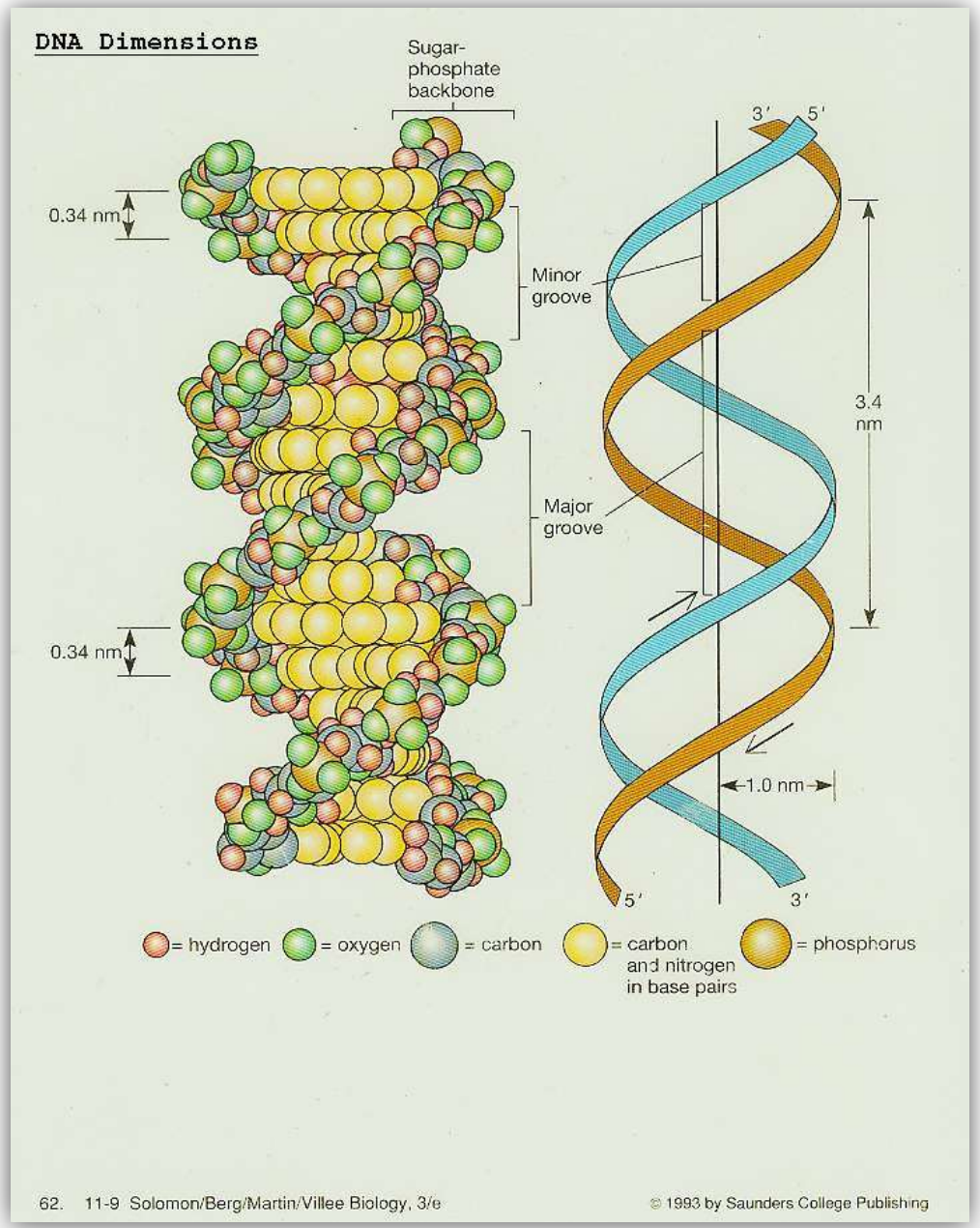


	Adenylate (adenosine 5′-monophosphate)	Guanylate (guanosine 5′-monophosphate)	Uridylate (uridine 5′-monophosphate)	Cytidylate (cytidine 5′-monophosphate)
Symbols:	A, AMP	G, GMP	U, UMP	C, CMP
Nucleoside:	Adenosine	Guanosine	Uridine	Cytidine

(b) Ribonucleotides

DNA


phosphate
5' end
3' end
base
deoxyribose
sugar-phosphate backbone
complementary base pairing
hydrogen bonds
sugar


DNA Dimensions
Sugar-phosphate backbone
3′
5′
0.34 nm
Minor groove
3.4 nm
Major groove
0.34 nm
1.0 nm
5′
3′
= hydrogen
= oxygen
= carbon
= carbon and nitrogen in base pairs
= phosphorus
62. 11-9 Solomon/Berg/Martin/Villee Biology, 3/e
© 1993 by Saunders College Publishing

m- RNA

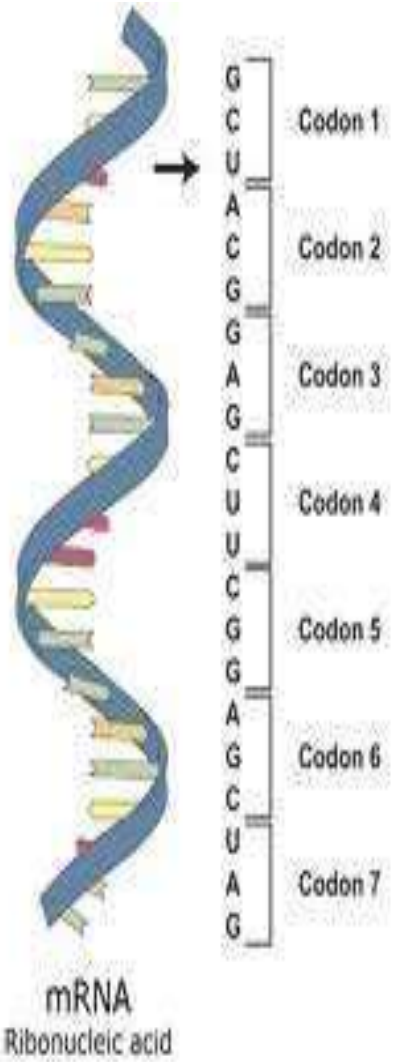
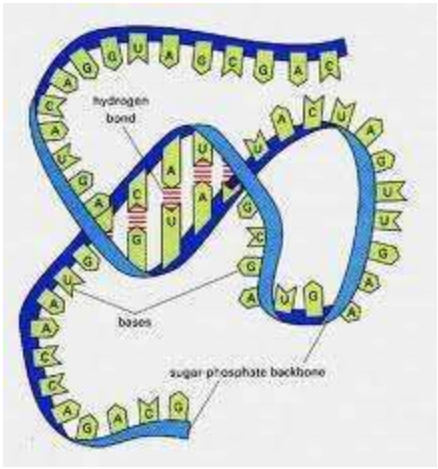
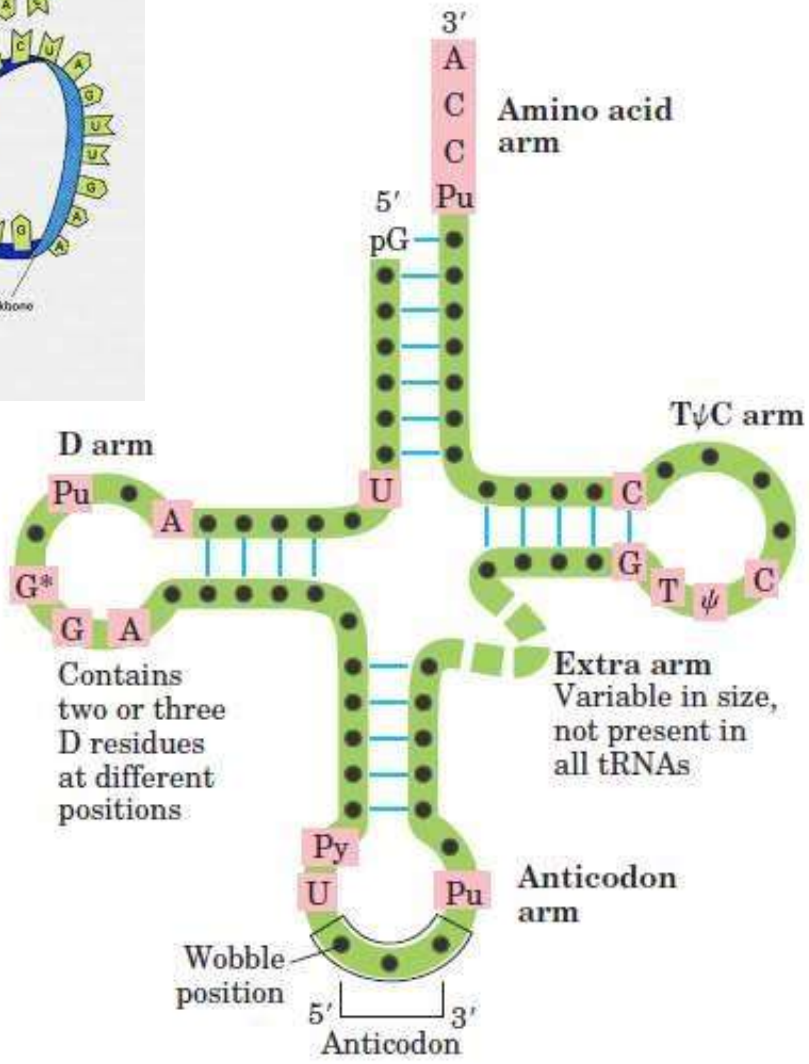


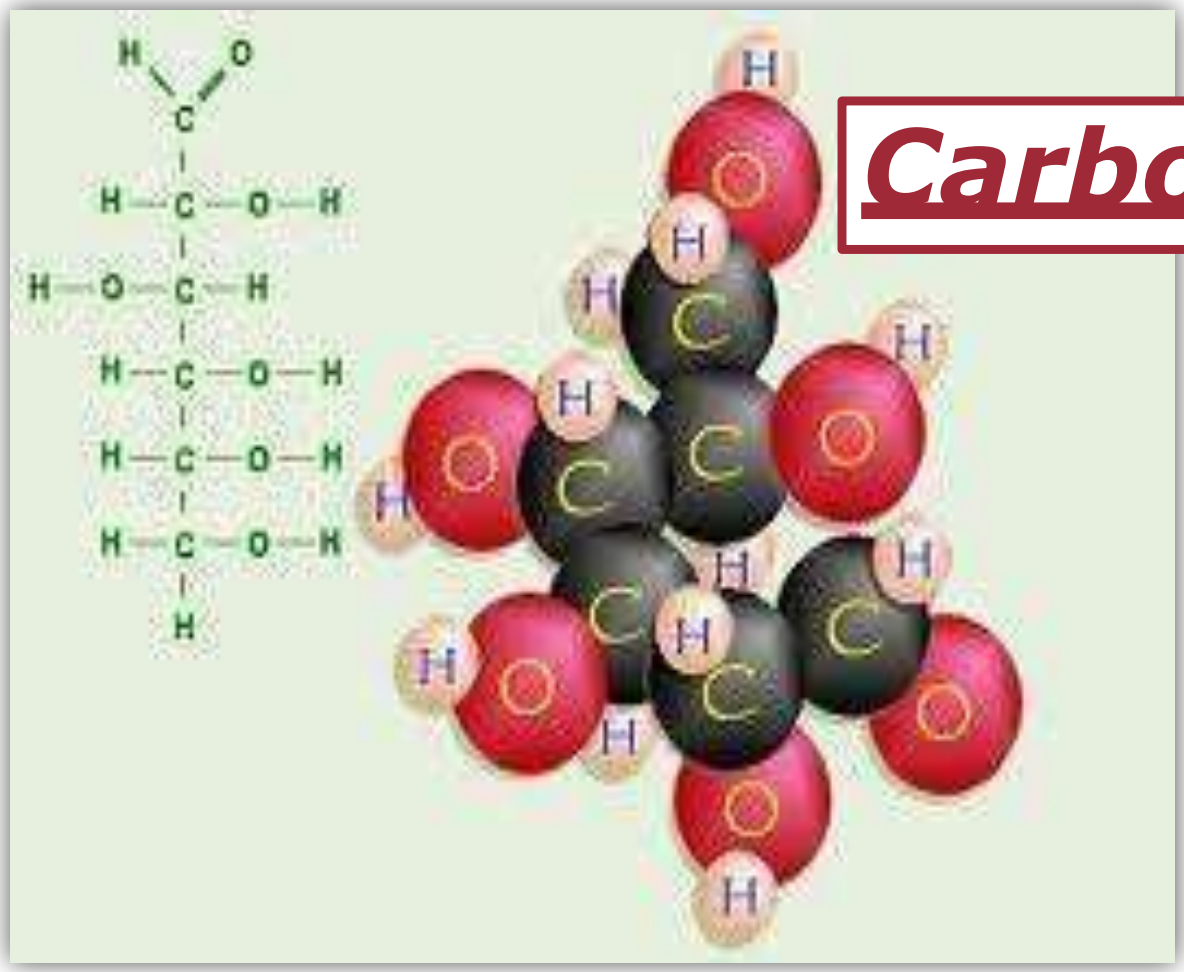
Image adapted from: National Human Genome Research Institute. Talking Glossary of Genetic Terms. Available at: www.genome.gov/Pages/Hyperion/DIR/VIP/Glossary/Illustration/codon.shtml.

r- RNA



t- RNA





Carbohydrates

CARBOHYDRATES

Carbohydrates are the most abundant organic molecules in nature.

The term carbohydrate is derived from the French term *hydrate de carbone* i.e. it is a hydrate of carbon or $C_n(H_2O)_n$

Carbohydrates are defined as organic substances having C, H & O
Wherein H and O are in the ratio 2:1 as found in H_2O

FUNCTIONS OF CARBOHYDRATES

- Most abundant **source of energy** (4 cal/g)
- **Precursors** for many organic compounds **(fats, amino acids)**
- Present **as glycoproteins and glycolipids** in the **cell memebrane** and functions such as cell growth and fertilization
- Present as structural components like **cellulose in plants**, **exoskeleton** of some insects, **cell wall** of microorganisms
- **Storage form of energy** (glycogen) to meet the energy demands of the body.

CARBOHYDRATES

MONOSACCHARIDES

Basic units of carbohydrates
Cannot be hydrolysed into smaller units

a. Based on the no. of C-atoms
a. Based on the type of functional group

OLIGOSACCHARIDES

They can be further hydrolysed

a. Disaccharides
b. Trisachharides
c. Tetrasachharides

POLYSACCHARIDES

Non crystalline, non soluble in water, tasteless, on hydrolysis gives mol of monosaccharides
e.g. starch , cellulose

MONOSACCHARIDES

Based on the no of C-atoms

- **Trioses** ($C_3H_6O_3$)
e.g. Glyceraldehyde,
Dihydroxyacetone

- **Tetroses** ($C_4H_8O_4$)
e.g. Erythrose, Threose

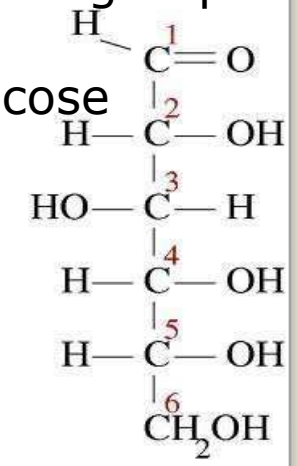
- **Pentoses** ($C_5H_{10}O_5$)
e.g. Ribulose, Xylose
Arabinose
(*deoxyribose – $C_5H_{10}O_4$*)

- **Hexoses** ($C_6H_{12}O_6$)
e.g. glucose, fructose
galactose, mannose

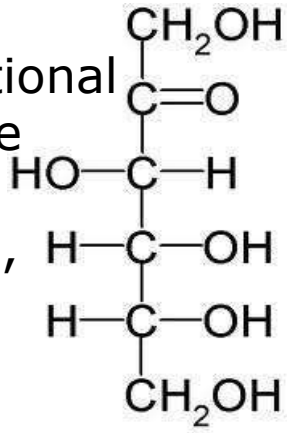
- **Heptoses** ($C_7H_{14}O_7$)
e.g. sedoheptulose
glucoheptose

Based on the functional group

- **Aldoses** : the functional group is Aldehyde –CHO
e.g. Glyceraldehyde, glucose



- **Ketoses** : the functional group is ketone (C = O)
e.g. Dihydroxyacetone, fructose



Derivatives of Monosaccharides

1. **Deoxy Sugars** : Deoxygenation of ribose produces deoxyribose, which is a structural component of DNA
2. **Amino Sugars** : When 1 or more –OH groups of monosaccharides are replaced by $-NH_2$ (amino group) it forms an amino sugar e.g. Glucosamine, which forms chitin, fungal cellulose, hyaluronic acid.
3. **Sugar Acid** : Oxidation of –CHO or –OH group forms sugar acids. Ascorbic acid is a sugar acid
4. **Sugar alcohols** : Reduction of aldoses or ketoses. Glycerol and Mannitol.

OLIGOSACCHARIDES

They are formed by condensation of 2-9 monosaccharides

Depending upon the no. of monosachharide molecules they are :

a. Disaccharides (sucrose, lactose)

b. Trisaccharides (raffinose)

c. Tetrasaccharides (stachyose)

The smallest and the commonest oligosaccharides are Disaccharides

DISACCHARIDES

A disaccharide consists of 2 monosaccharide units (similar or dissimilar) held together by a glycosidic bond

They are crysatalline, water soluble and sweet to taste.

MALTOSE : - is also called as **malt sugar**.
- made up of **2 glucose molecules**

LACTOSE : - is also called **as milk sugar** as it is found naturally in milk
- made up of **glucose and galactose**
- souring of milk is due to conversion of lactose to lactic acid

SUCROSE : -is also called as **cane sugar.** It is the sugar found in sugar cane and sugar beet
- most abundant among naturally occuring sugars.
- Important source of Dietary carbohydrates
- made up of **glucose and fructose**

POLYSACCHARIDES

- Also called as GLYCANS
- Made up of repeating units of monsaccharides held by glycosidic bonds
- During its formation a water molecule is released at each condensation

This helps reduce the bulk making it almost insoluble decreasing its effect on the water potential or osmotic potential of the cell

- Unlike sugars they are not sweet.
- They are ideal as STORAGE AND AS STRUCTURAL COMPONENTS
- They are of 2 types **Homoglycans** and **Heteroglycans**.

HOMOGLYCANS

-Made up of only 1 type of monosaccharide monomers
-For eg **starch, glycogen, cellulose**
-Glucan (made up of glucose)
-Fructan(made up of fructose)
-Galactan (made up of galactose)

HETEROGLYCANS

-Made up condensation of2 or more types of monosaccharides
- For eg **Hyaluronic acid, agar, Chitin, peptidoglycans** etc

STORAGE POLYSACCHARIDES

STARCH

1. **Carbohydrate reserve of plants** and the most important dietary source for animals
2. High content of starch in cereals, roots, tubers, vegetables etc.
3. Homopolymer made up of **GLUCOSE** units. Also called as **GLUCAN**.
4. Starch = Amylose + Amylopectin (polysaccharide components)

GLYCOGEN

1. Carbohydrate reserve in animals. Hence referred as **animal starch**
2. High concentration in Liver, muscles and brain.
3. Also found in plants that do not have chlorophyll (yeast and fungi)
4. **GLUCOSE** is the repeating unit.

INULIN

1. Polymer of fructose i.e. **fructosan**
2. Found in Dahlia, bulbs, garlic, onion etc
3. Easily soluble in water
4. *Inulin is not readily metabolised in the human body and is readily filtered through the kidney. Hence used for testing kidney function (GFR)*

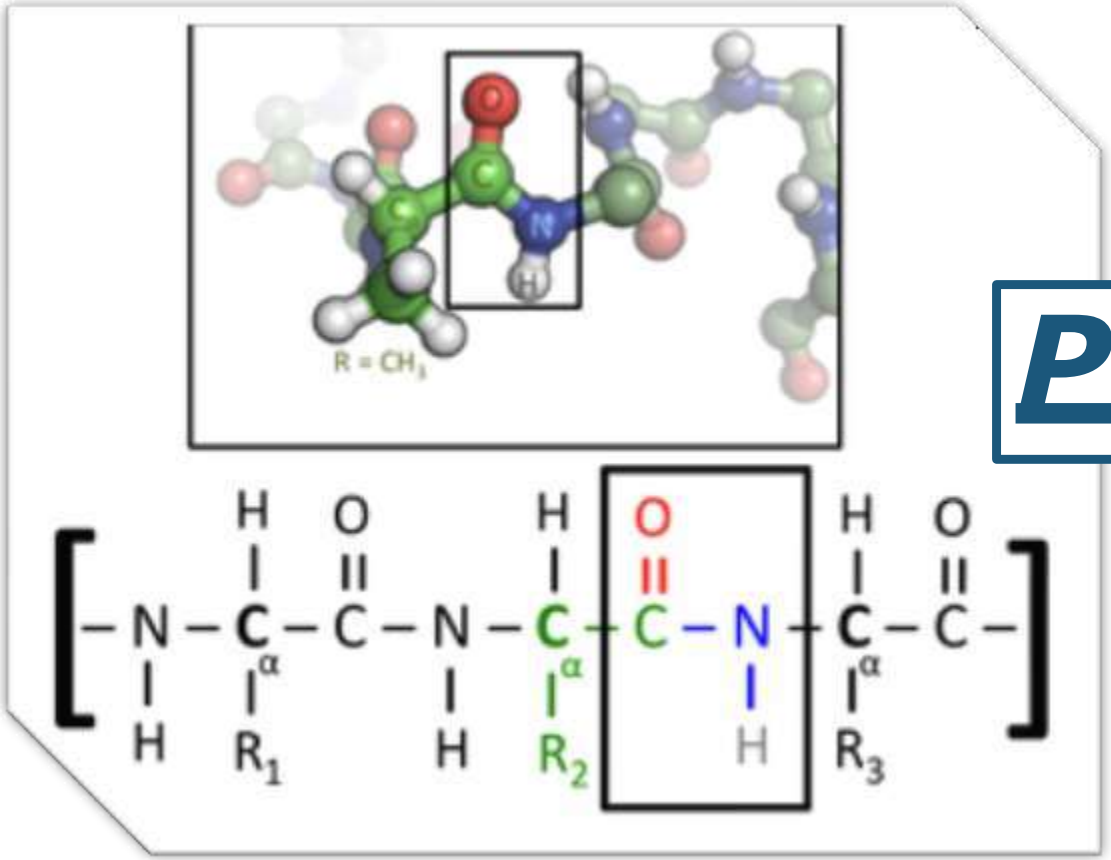
STRUCTURAL POLYSACCHARIDES

CELLULOSE

1. Occurs exclusively in plants and is the **most abundant organic substance** in plant kingdom.
2. Predominant constituent of plant cell wall.
3. **It is totally absent in animals.**

CHITIN

1. **Second most abundant** organic substance.
2. Complex carbohydrate of **Heteropolysaccharide** type.
3. Found in the exoskeletons of some invertebrates like insects and crustaceans. Provides both strength and elasticity.
4. Becomes hard when impregnated with calcium carbonate.



Proteins

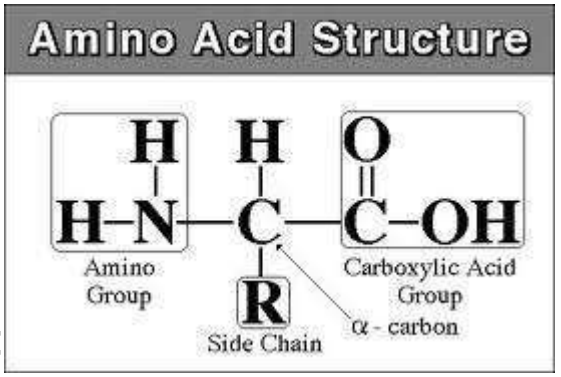
PROTEINS

- Most abundant organic molecules of the living system.
- They form about 50% of the dry weight of the cell.
- They are most important for the architecture and functioning of the cell.

Proteins are polymers of amino acids

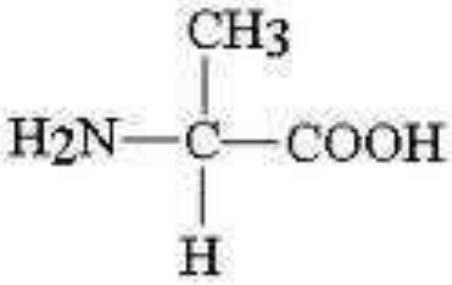
- Proteins on complete hydrolysis yields Amino Acids
- There are 20 standard amino acids which are repeatedly found in the structure of proteins – animal, plant or microbial.
- Collagen is the most abundant animal protein and Rubisco is the most abundant plant protein
- Protein Synthesis is controlled by DNA.

AMINO ACIDS

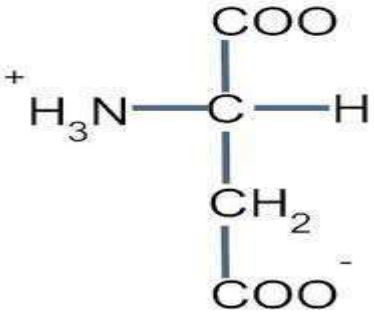


- Amino acids are group of organic compounds having 2 functional groups ($-NH_2$) and (-COOH)
- ($-NH_2$) group is basic whereas (-COOH) is acidic
- R- can be H in *glycine*, CH_3 in *alanin*e, Hydroxymethyl in *serine* in others it can be hydrocarbon chain or a cyclic group
- All amino acids contain C, H, O and N but some of them additionally contain S
- Physical and chemical properties of amino acids are due to amino, carboxyl and R functional groups
- Amino acids are differentiated into 7 groups

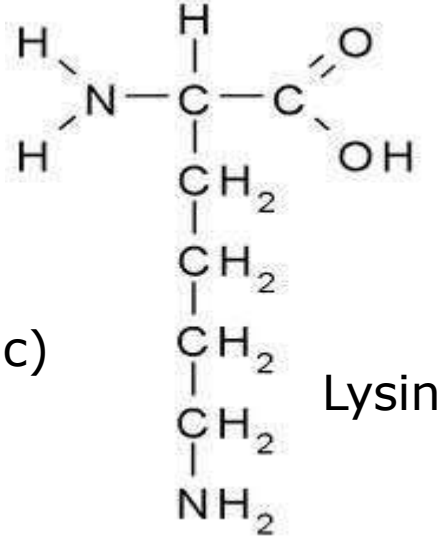
No.	Nature	Amino acids
1.	**NEUTRAL** : Amino acids with 1 amino and 1 carboxyl group	Glycine (Gly), Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (Ile)
2.	**ACIDIC** : 1 extra carboxyl group	Aspartic acid (Asp), Asparagine (Asn), Glutamic acid (Glu), Glutamine (Gln)
3.	**BASIC :** 1 extra amino group	Arginine (Arg), Lysine (Lys)
4.	**S – CONTAINING :** Amino acids have sulphur	Cysteine (Cys), Methionine (Met)
5.	**ALCOHOLIC** : Amino acids having –OH group	Serine (Ser), Threonine (Thr), Tyrosine (Tyr)
6.	**AROMATIC** : Amino acids having cyclic structure	Phenylalanine (Phe), Tryptophan (try)
7.	**HETEROCYCLIC** : amino acids having N in ring structure	Histidine (His), Proline (Pro)



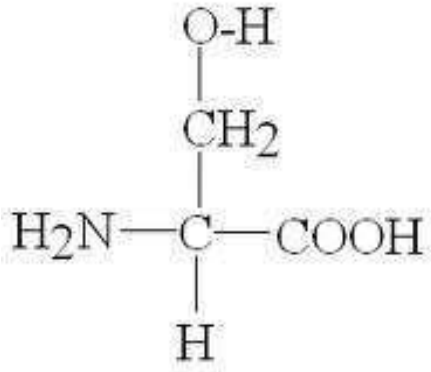
Alanine (neutral)



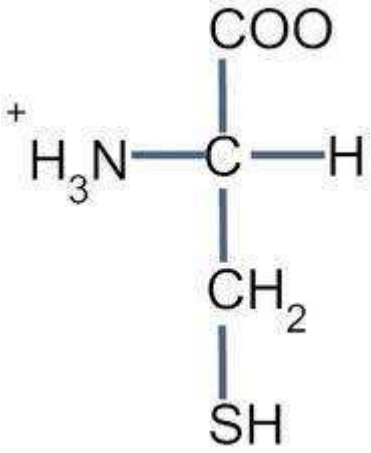
Aspartic acid (acidic)



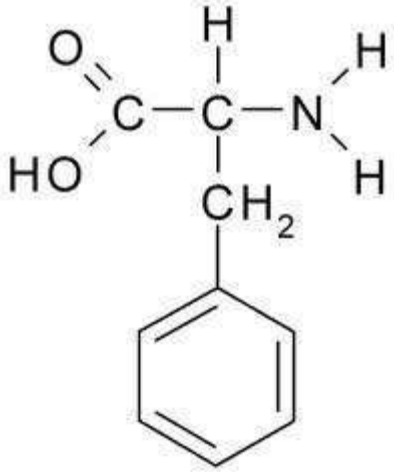
Lysine (basic)



Serine (alcoholic)



Cysteine (suphur)



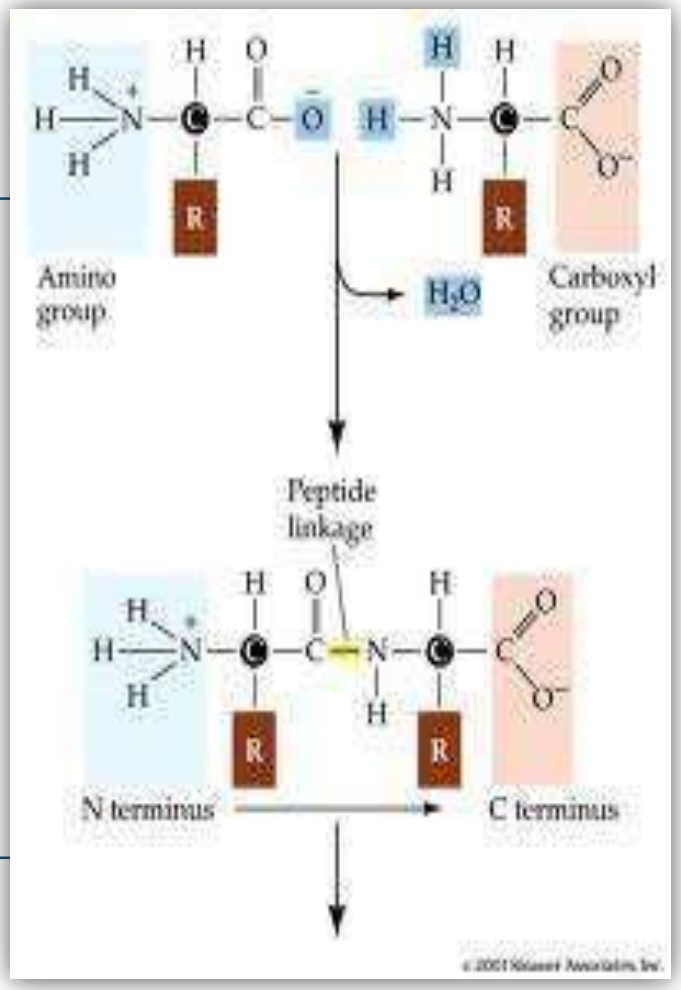
Phenylalanine (aromatic)

PEPTIDE FORMATION

- Amino acids are linked serially by peptide bonds (-CONH-) formed between the
  (-NH2) of one amino acid and the
  (-COOH) of adjacent amino acid

- Chain having 2 amino acids linked by a peptide bond is called as a DIPEPTIDE

- The sequence of amino acids present in a polypeptide is specific for a particular protein.



STRUCTURE OF PROTEIN

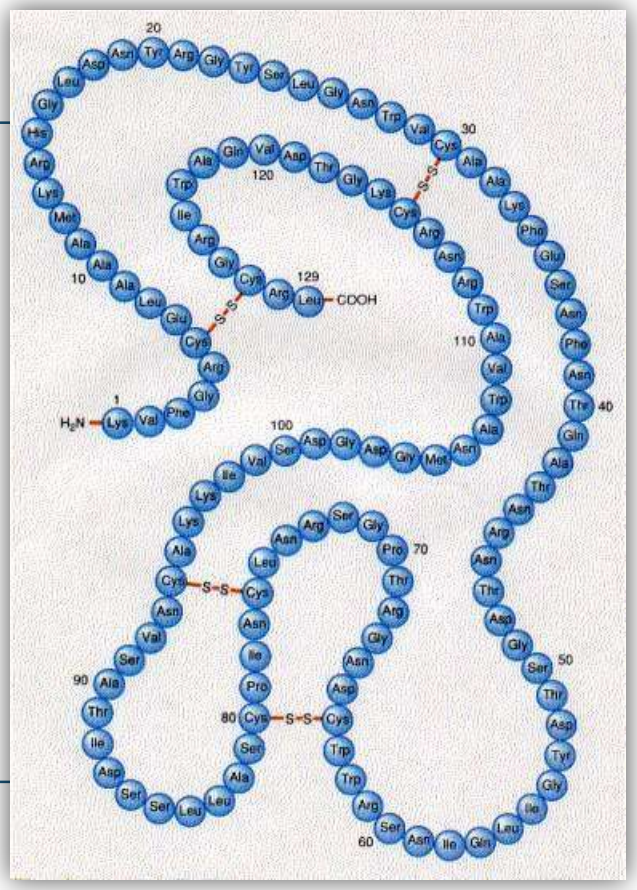
4 basic structural levels are assigned to proteins – primary, secondary, tertiary and quaternary

PRIMARY

The primary structure refers to the number and linear sequence of amino acids in the polypeptide chain and the location of the disulphide bridges

The primary structure is responsible for the function of the protein.

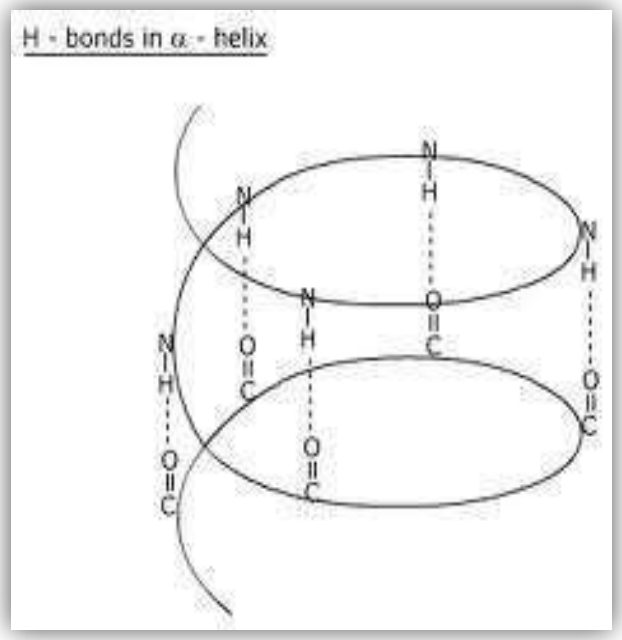
The N-terminal amino acid is written on the left side whereas the C- terminal amino acid is written on the right side



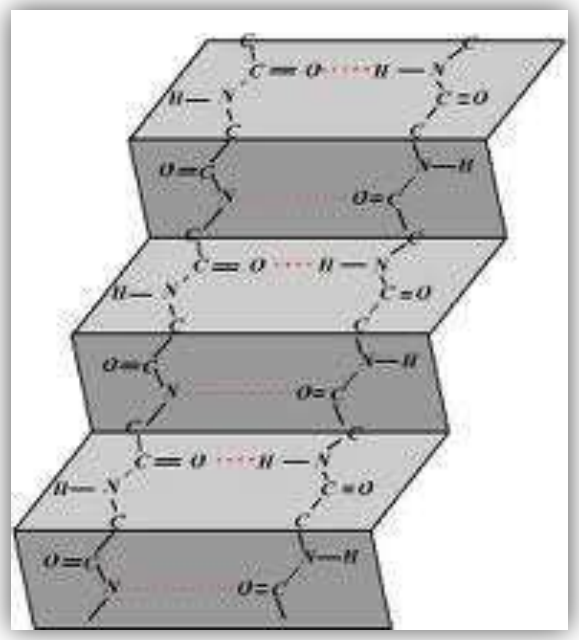
SECONDARY

The folding of the linear chain into a specific coiled structure is called as secondary structure.

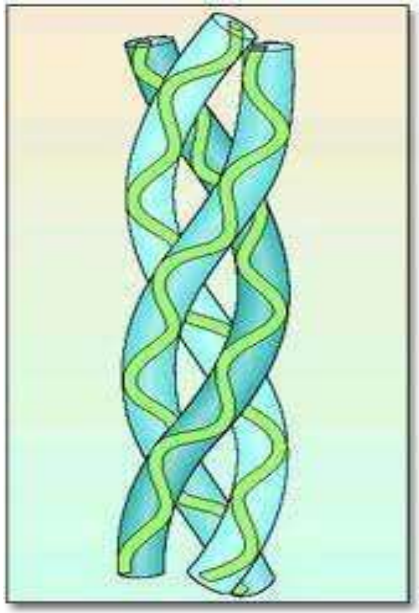
3 types : α- helix, β- pleated sheet and collagen helix



α- helix

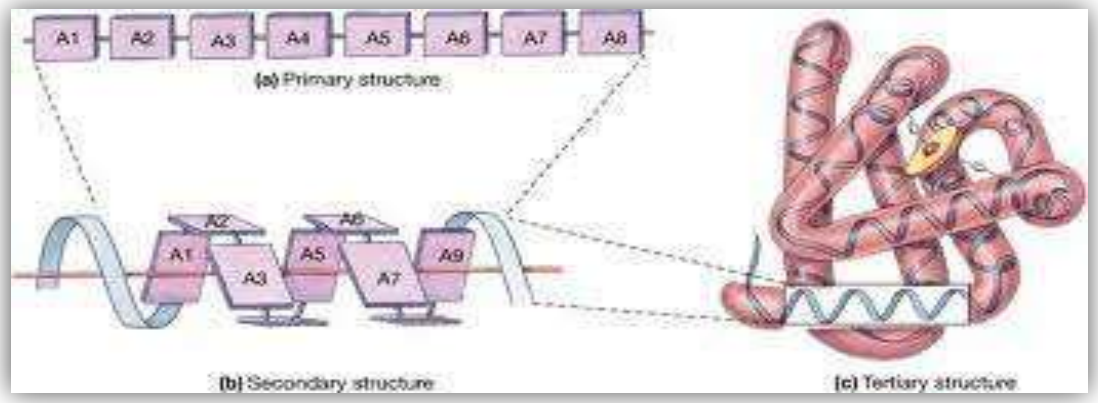


β- pleated sheet



Collagen helix

TERTIARY



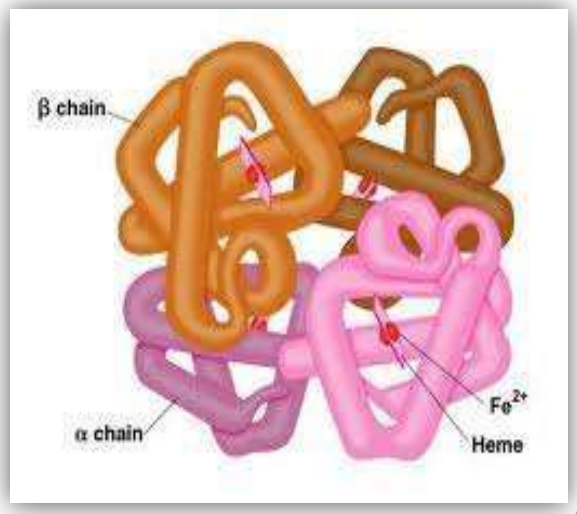
The helical polypeptide may fold upon itself and assume a complex but specific form – spherical, rod like or something in between.

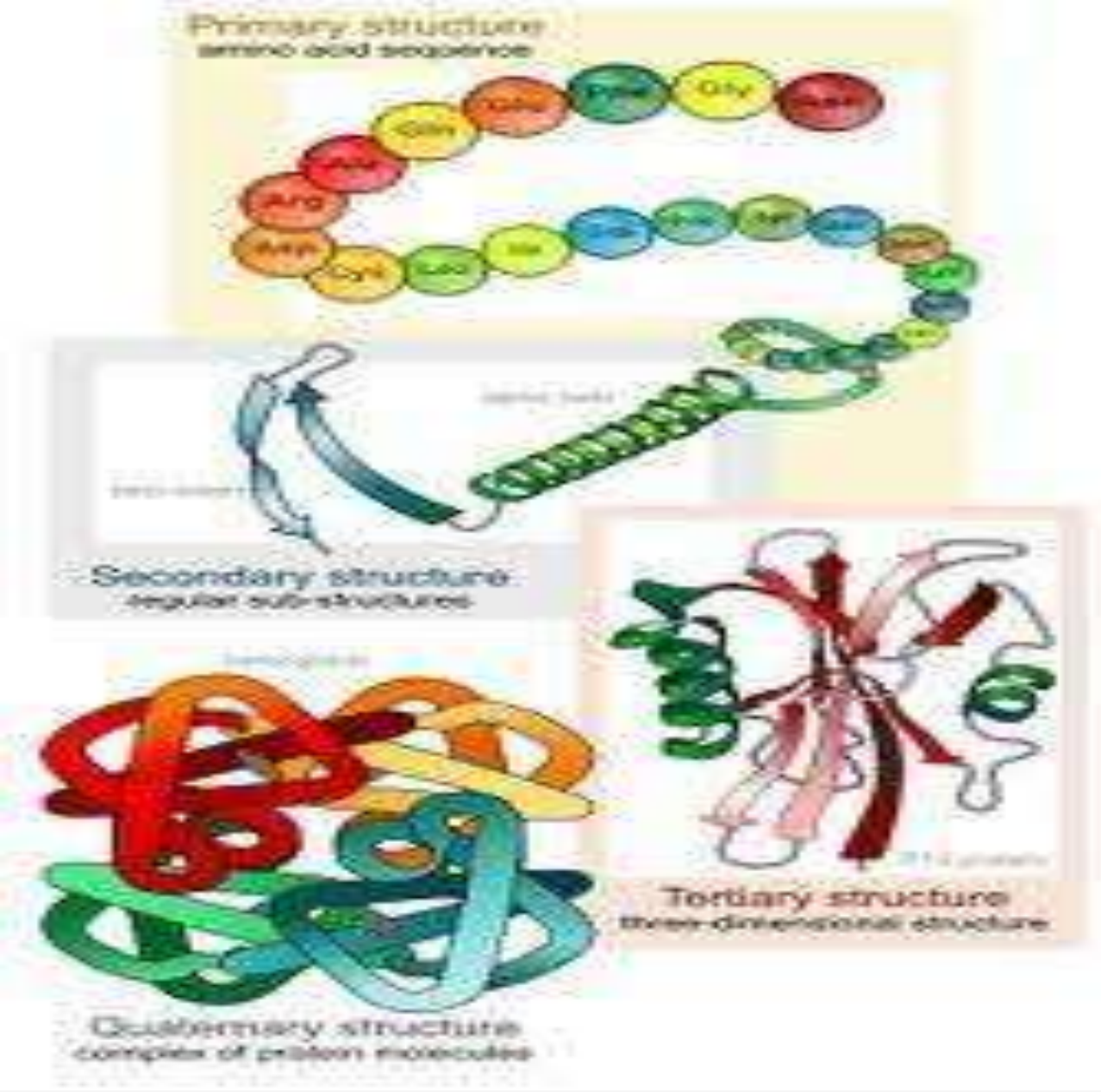
These geometrical shapes are known as tertiary (3^0) structure

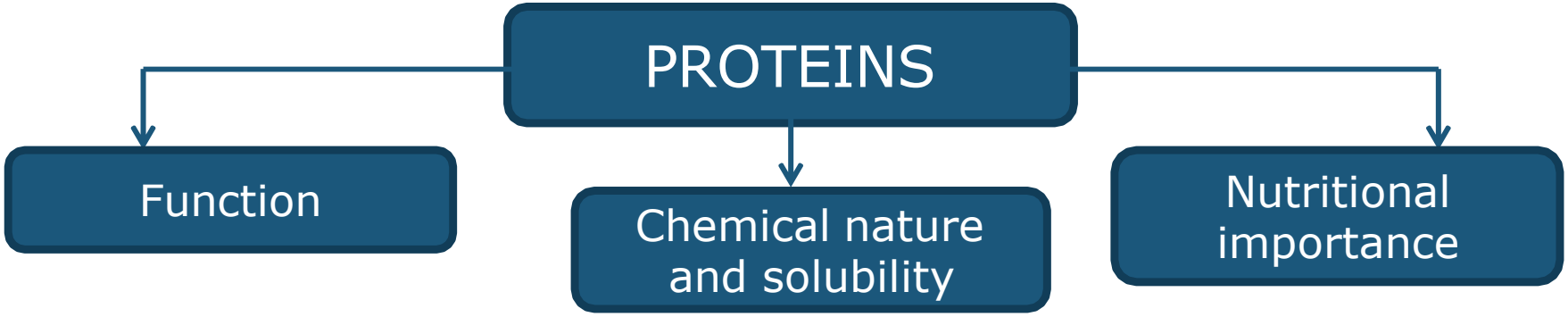
QUATERNARY

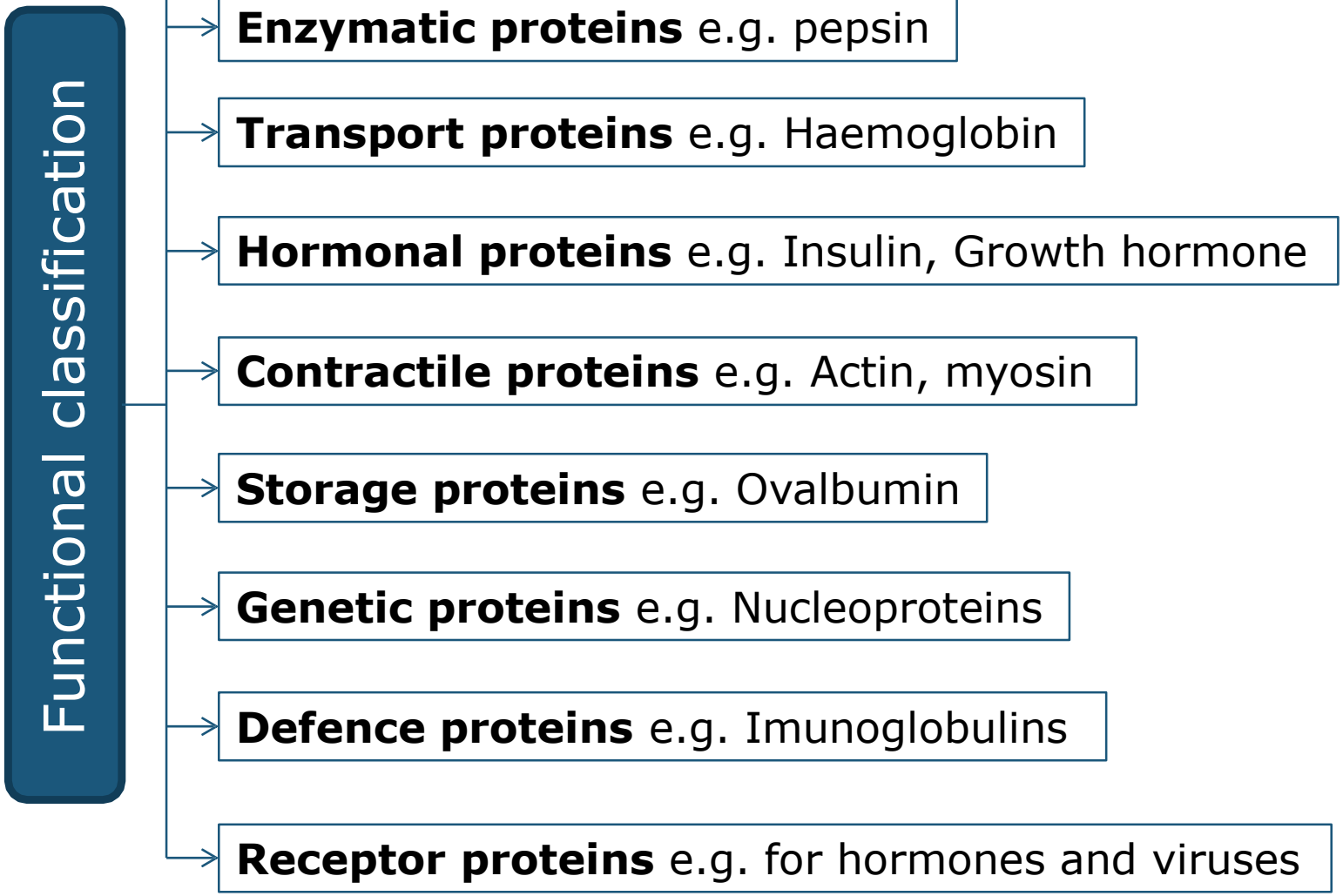
Proteins are said to be quaternary in structure If they have 2 or more polypeptide chains

Haemoglobin is an excellent example




Primary structure
amino acid sequence
Secondary structure
regular sub-structures
Tertiary structure
three-dimensional structure
Quaternary structure
complex of protein molecules


PROTEINS
Function
Chemical nature and solubility
Nutritional importance

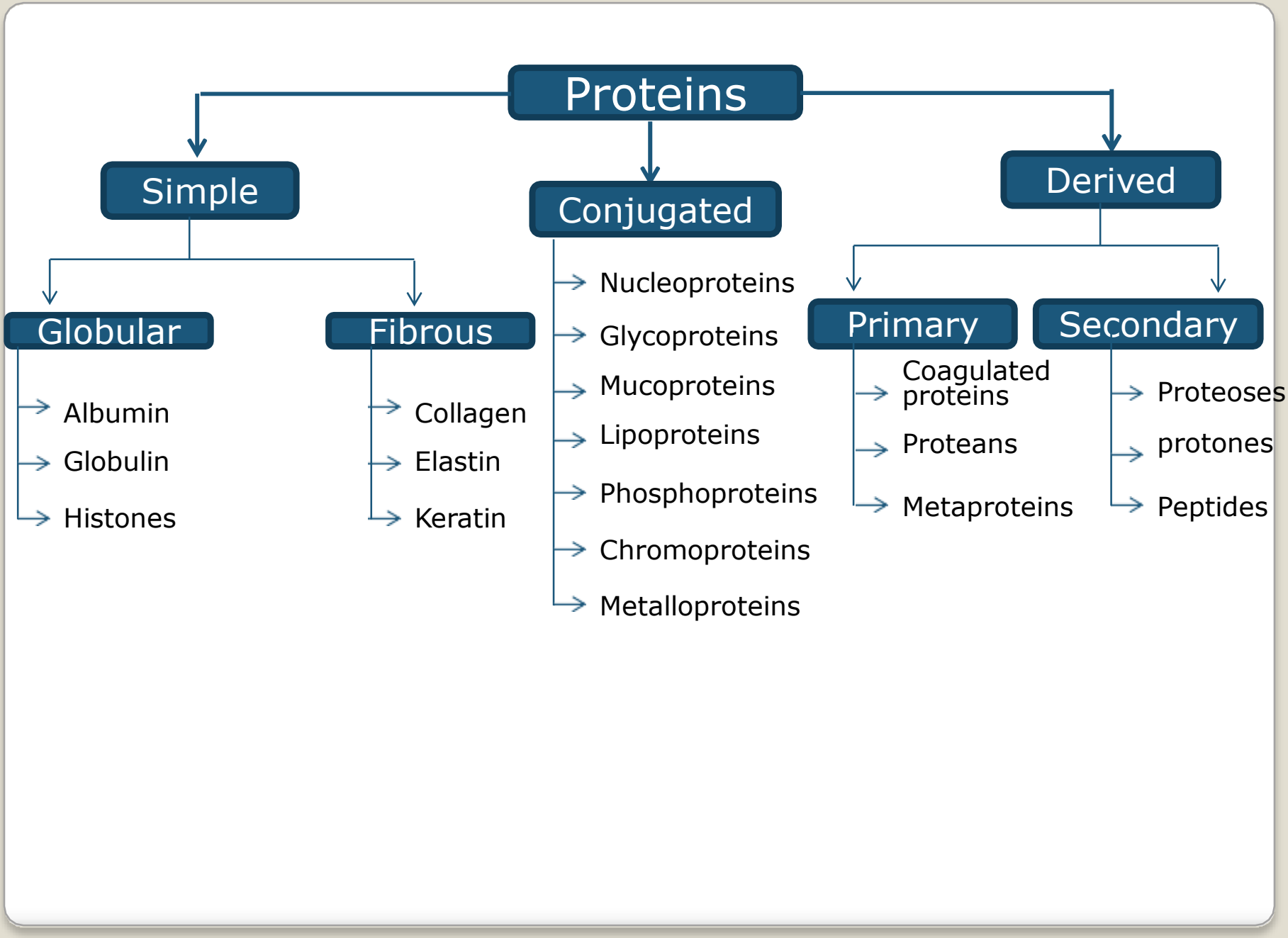

Functional classification
Structural proteins e.g. keratin, collagen
Enzymatic proteins e.g. pepsin
Transport proteins e.g. Haemoglobin
Hormonal proteins e.g. Insulin, Growth hormone
Contractile proteins e.g. Actin, myosin
Storage proteins e.g. Ovalbumin
Genetic proteins e.g. Nucleoproteins
Defence proteins e.g. Imunoglobulins
Receptor proteins e.g. for hormones and viruses

Classification based on chemical nature and solubility

1. **Simple proteins** : They are composed only of amino acid residues

2. **Conjugated proteins** : Along with amino acids , there is a non-protein prosthetic group.

3. **Derived proteins :** They are denatured or degraded products of the above two


Proteins
Simple
Conjugated
Derived
Globular
Fibrous
Albumin
Globulin
Histones
Collagen
Elastin
Keratin
Nucleoproteins
Glycoproteins
Mucoproteins
Lipoproteins
Phosphoproteins
Chromoproteins
Metalloproteins
Primary
Secondary
Coagulated proteins
Proteans
Metaproteins
Proteoses
protones
Peptides

LIPIDS

With the water, I say, Touch me not,

To the tongue, I am tasteful,

Within limits, I am dutiful,

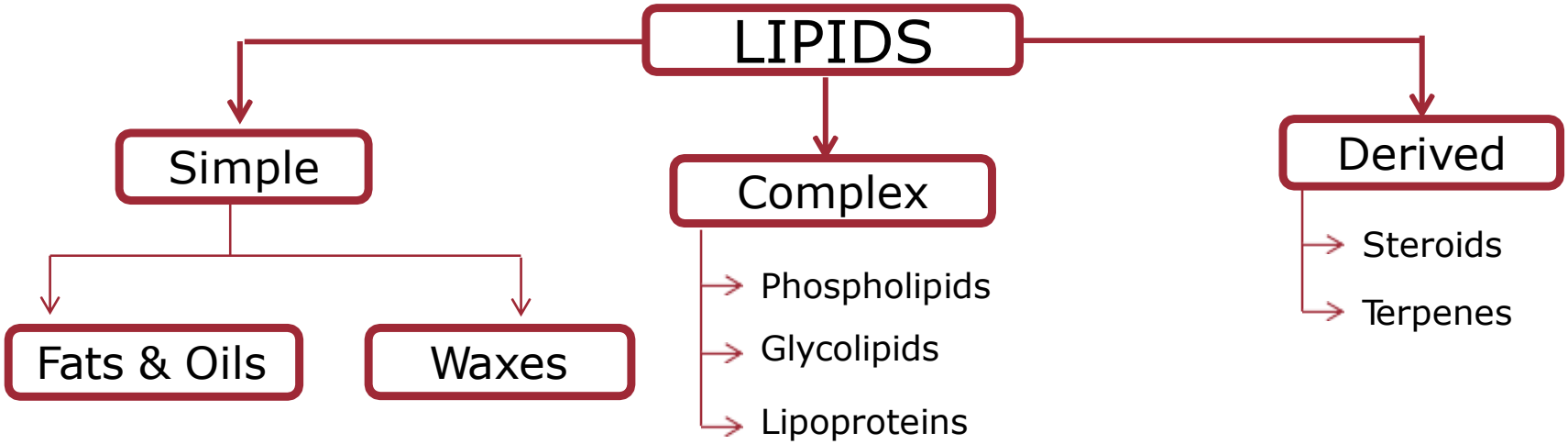
In excess, I am dangerous

Lipids are the chief concentrated storage form of energy forming about 3.5% of the cell content.

Lipids are organic substances relatively insoluble in water but soluble in organic solvents (alcohol, ether)

Functions :

1. They are the concentrated **fuel reserve** of the body.
2. Lipids are **constituents of membrane structure** and regulate the **membrane permeability**.
3. They serve as source **of fat soluble vitamins**
4. Lipids are important cellular **metabolic regulators**
5. Lipds protect the internal organs and serve as **insulating materials**


LIPIDS
Simple
Fats & Oils
Waxes
Complex
Phospholipids
Glycolipids
Lipoproteins
Derived
Steroids
Terpenes

SIMPLE LIPIDS

They are esters of fatty acids with alcohol. They are of 2 types :

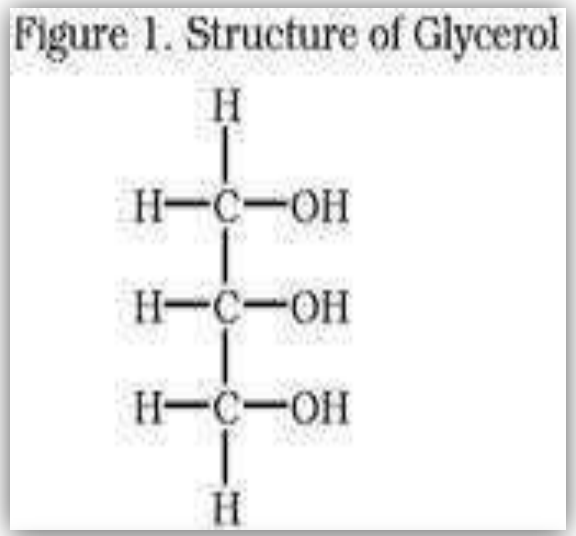
1. Neutral or true fats : Esters of fatty acids with glycerol
2. Waxes : Esters of fatty acids with alcohol other than glycerol.

Neutral / True fats

True fats are made up of C, H, & O but O is less
A fat molecule is made up of 2 components :
a) GLYCEROL
b) FATTY ACIDS (1-3 mol, of same or diff long chained)

Glycerol

A glycerol mol has 3 carbons each bearing a –OH group

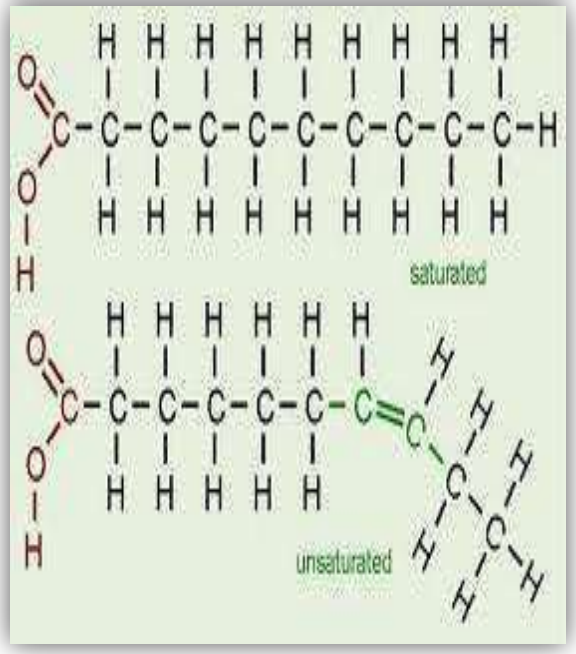


Fatty acid

A fatty acid mol is an unbranched chain of C-atoms.

It has a –COOH group at one end and a H bonded to almost all the C-atoms

Fatty acids may be saturated or unsaturated



WAXES

Lipids which are long chain saturated fatty acids and a long chain Saturated alcohol of high mol wt other than glycerol

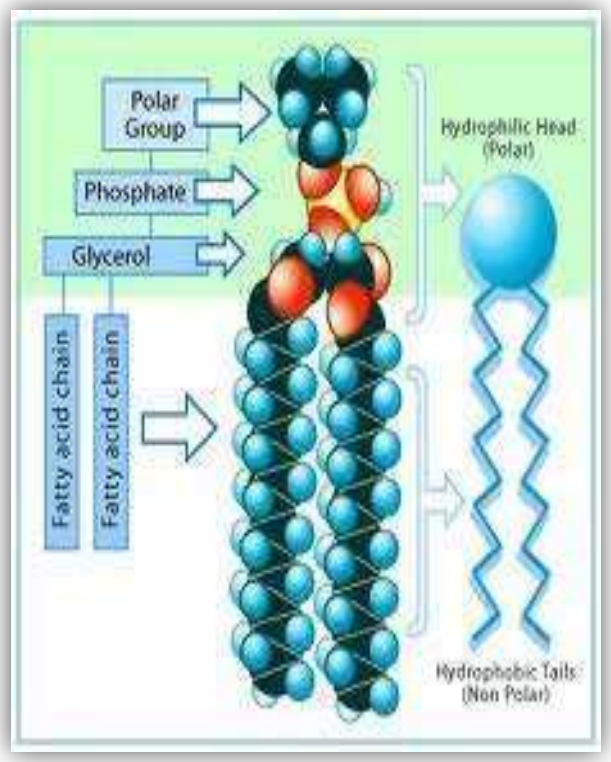
Example :

1. **Bees wax :** secretion of abdominal glands of worker honey bees
2. **Lanolin or wool fat :** Secretion of cutaneous glands and obtained from the wool of sheep
3. **Sebum :** secretion of sebaceous glands of skin
4. **Cerumen :** soft and brownish waxy secretion of the glands in the external auditory canal. Also called as Earwax
5. **Plant wax :** Coating formed on the plant organs to prevent transpiration
6. **Paraffin wax :** A translucent waxy substance obtained from petroleum

COMPLEX LIPIDS

They are derivatives of simple lipids having additional group like phosphate, N_2-base, Protein etc. They are further divided into **Phospholipids, Glycolipids, Lipoproteins**.

Phospholipid



They are made up of a molecule of glycerol Or other alcohol having

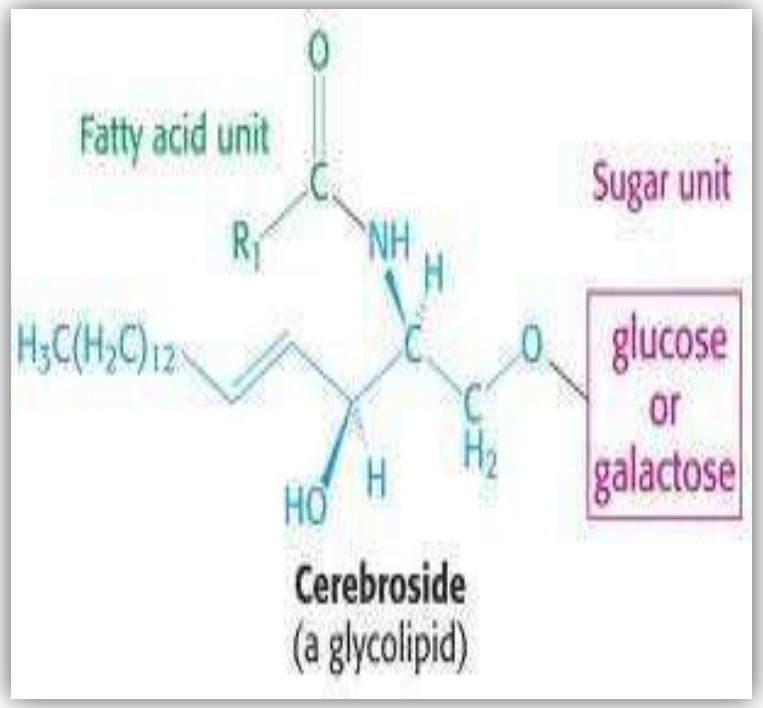
1. *A phos group at 1 of its –OH groups*
2. *2 fatty acid molecules at other 2 –OH groups*
3. *A nitrogen containing base attatched to phos group*

A phospholipid molecule has a **hydrophobic** tail (fatty acids) and a **hydrophilic** head(phos group)

Glycolipid

They are components of **cell membranes,** particularly myelin sheath and chloroplast membranes

CEREBROSIDE are the most simplest form of glycolipids



Lipoprotein

They contain lipids and proteins in their molecules.
They are main constituent of membranes.
They are found in milk and Egg yolk.

Lipids are transported in blood and lymph as lipoproteins.

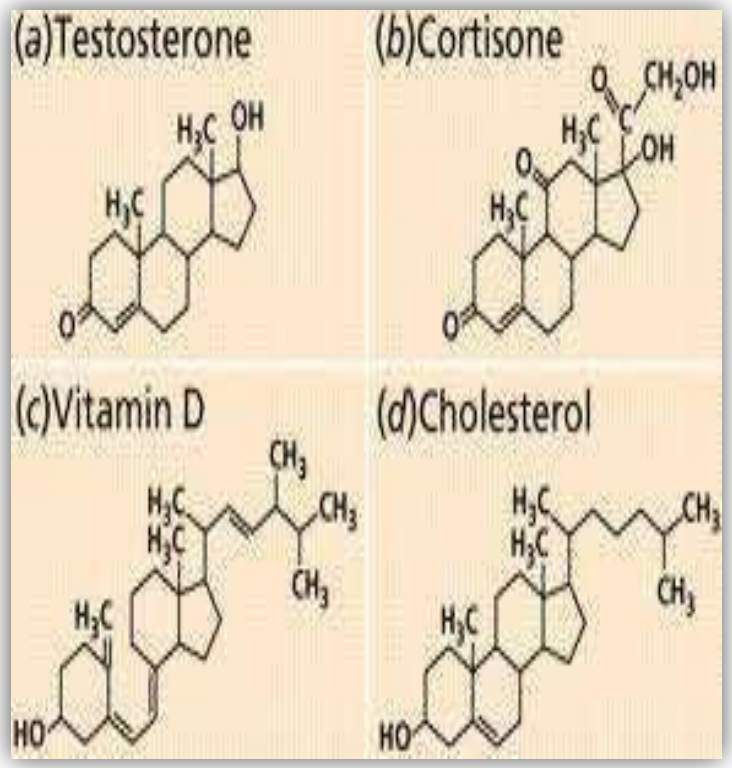
5 types of lipoproteins :

1. chylomicrons
2. VLDL
3. LDL
4. HDL
5. Free fatty acid albumin complex

DERIVED LIPIDS

They are derivatives obtained on the hydrolysis of the simple and complex lipids.
e.g. **steroids, terpenes and prostaglandins**

Steroid



The steroids do not contain fatty acids but are included in lipids as they have fat-like properties.

They are made up of 4 fused carbon rings

Cholesterol, Vit D, testosterone, adrenocortical hormones.

The most common steroids are **STEROLS.**

Common sterols are **Cholesterol and ergosterol**

Terpenes

Terpenes are a major component of essential oils produced by plants. They give fragrance to the plant parts.

Vitamins A, E and K contain a terpenoid called **phytol**

Carotenoid pigment is precursor for Vitamin A

Lycopene, a pigment present in tomatoes is a terpenoid

Gibberellins, the plant hormone is also a terpene

ENZYMES

Enzymes are a group of catalysts functioning in a biological system

They are usually proteinaceous substances produced by the living cell

Without themselves getting affected.

Enzymes enhance the rate of reaction and are formed in the cell under the instructions of genes

ENZYMOLOGY is the branch of science that deals with the study of Enzymes in all the aspects like nomenclature, reactions and functions

Enzymes occur in colloidal state and are often produced in inactive form called **proenzymes (zymogen),** which are converted to their active forms by specific factors like pH, substrate etc.

The enzymes that are produced **within** a cell for metabolic activities are known as **endoenzymes** and those which act **away** from the site of synthesis are called **exo-enzymes**

GENERAL PROPERTIES OF ENZYME AND FACTORS AFFECTING THEIR ACTIVITY

1) Enzymes **accelerate** the reaction but do not initiate it.

2)Enzymes themselves do not participate in the reaction and remain **unchanged** at the end of the reaction. Enzymes, are therefore, needed in **small amounts**.

3) The molecule of an enzyme is **larger** than that of substrate molecule and hence during reaction a specific **part** of enzyme molecule comes in contact with the substrate molecule. That part is called **active site** of enzyme.

4)Amphoteric nature: Chemically most of the enzymes are proteins and, therefore, show amphoteric nature. The enzymes can react with **acidic** substances as well as **alkaline** substances.

5) **Specificity:** Most of the enzymes are specific in their action. A single enzyme acts upon a **single** substrate or a group of closely related substrates.

For example, the enzyme urease can act only upon urea invertase can act upon sucrose only

A slight change in the configuration of the substrate molecule requires action by a different enzyme.

6)Colloidal nature: All enzymes are colloidal in nature and thus provide **large surface area** for reaction to take place. Colloids (colloids- gel like) are mixtures of two components i.e. dispersed particles and dispersion medium. The size of the dispersed particles is larger than dispersion medium.

7) **<u>Enzyme optima</u> :** Enzymes generally work best under certain **narrowly defined conditions** referred to as optima. These include appropriate temperature and PH.

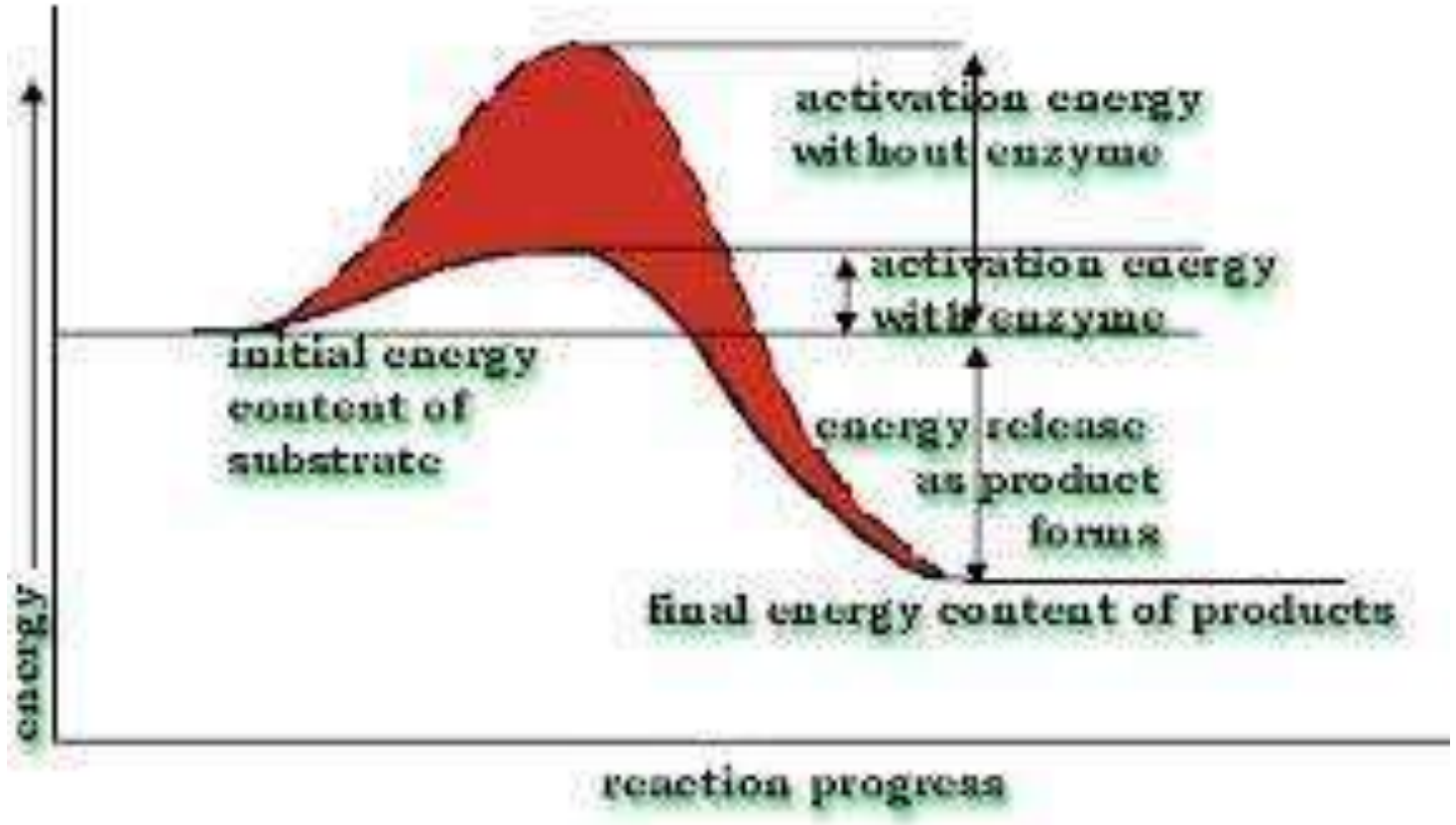
a) **Temperature sensitivity :** Since the enzymes are proteins, they are affected by change in temperature. With increase in temperature, **increase** in enzyme activity takes place (up to 40 C). However, when temperature increases **above 60 C** the proteins undergo **denaturation** or even complete **breakdown**. When the temperature is reduced to **freezing point** or below freezing point the enzymes become **inactivated** but they are not destroyed. The rate of reaction is more at optimum temperature.

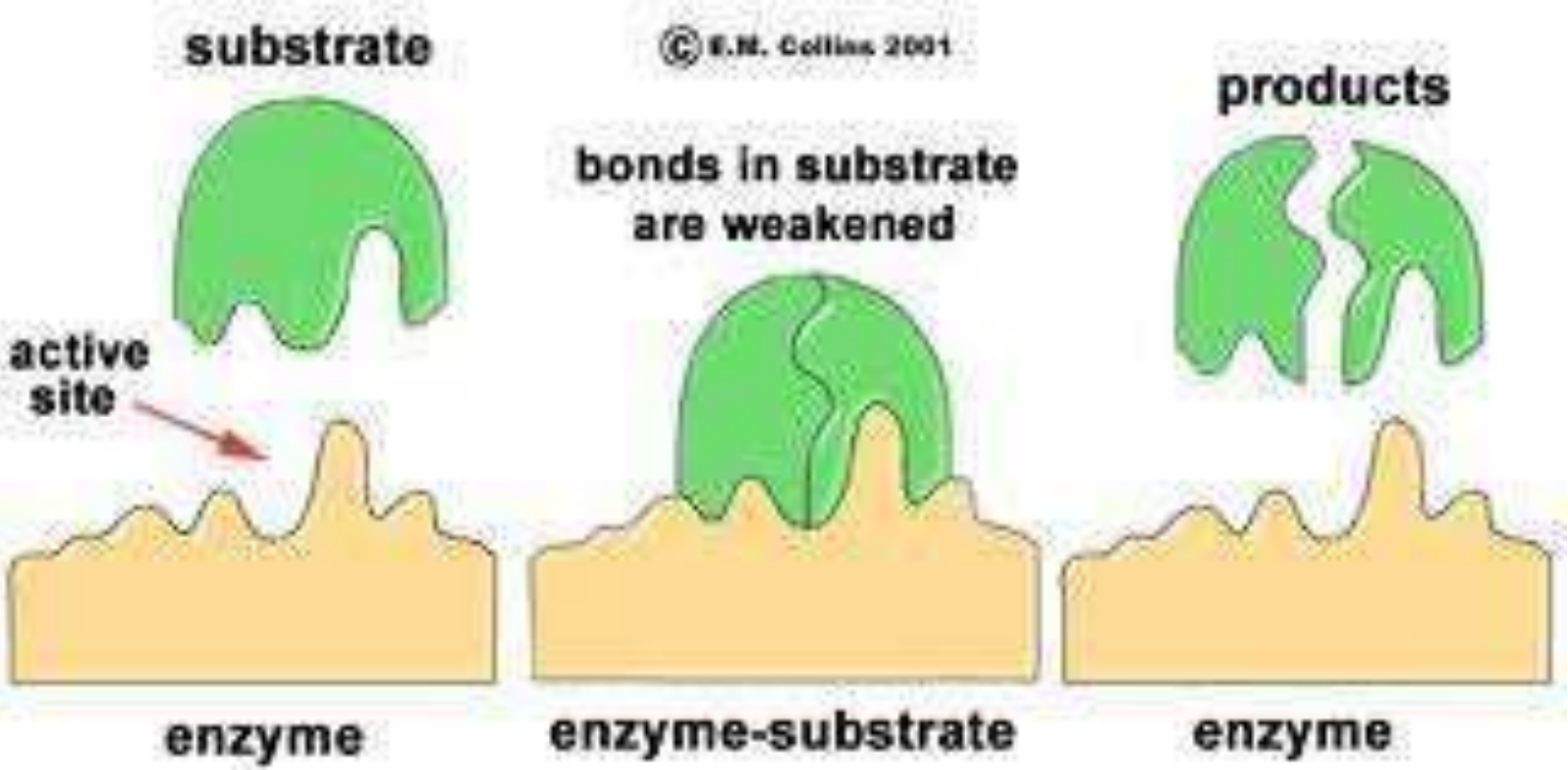
b) pH sensitivity : Most of the enzymes are **specific** to **pH** and remain active within particular range of pH. The strong acid or strong base denatures enzymes. Most of the intracellular enzymes function best around **neutral pH**

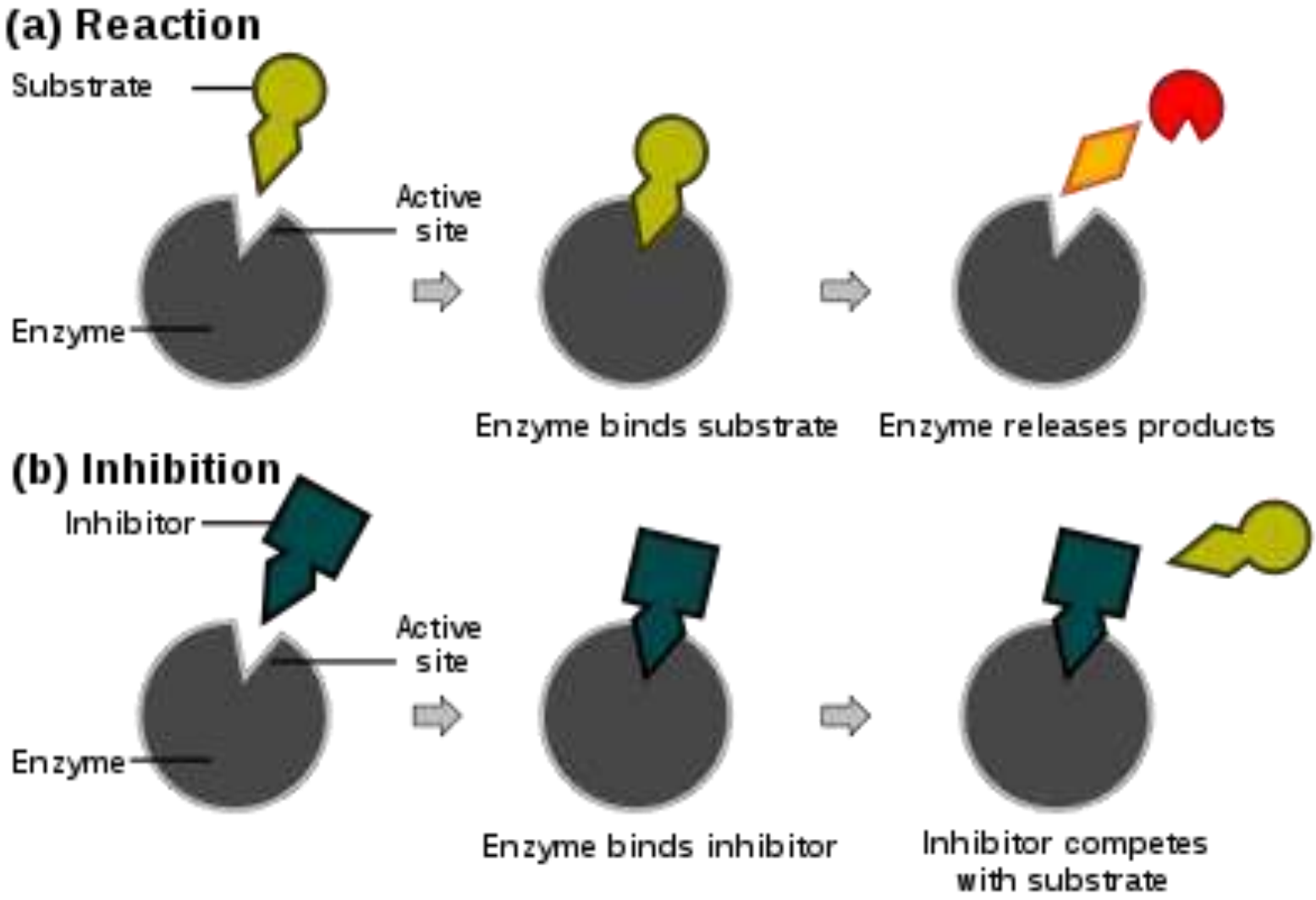
8)Concentration of enzyme and substrate : The rate of reaction is proportionate to the concentration of the reacting molecules. If the **substrate concentration** is increased the rate of **enzyme action** also increases up to certain limit. Beyond a certain concentration, the enzyme molecules remain saturated with substrate molecules and the activity becomes steady.

9)Enzyme inhibitors : Enzyme inhibitors are certain products which inhibit enzyme activity. During the reaction, if the **active site** of enzyme is occupied by these **inhibitors** instead of substrate molecules and the **activity** of enzyme is **lost**. These substances are like substrate molecules in their **structure** and are called **competitive inhibitors**.

Group of Enzymes	Reactions catalysed	Examples
1. Oxidoreductases	Transfer of O_2 or H_2 atoms or electrons from one substrate to another	Dehydrogenase Oxidases
2.Transferases	Transfer of a specific group from one substrate to another	Transaminase
3.Hydrolases	Hydrolysis of a substrate	Digestive enzymes
4. Isomerases	Change of the molecular form of the substrate	Phospho Hexo isomerase
5.Lyases	Non hydrolytic removal or addition of a group to a substrate	Decarboxylase Aldolase
6. Ligases	Joining of 2 molecules by formation of new bonds	Citric acid synthetase


activation energy without enzyme
activation energy with enzyme
initial energy content of substrate
energy release as product forms
final energy content of products
energy
reaction progress


substrate
© E.N. Collins 2001
products
bonds in substrate
are weakened
active
site
enzyme
enzyme-substrate
enzyme


(a) Reaction
Substrate
Active site
Enzyme
Enzyme binds substrate
Enzyme releases products
(b) Inhibition
Inhibitor
Active site
Enzyme
Enzyme binds inhibitor
Inhibitor competes with substrate

Pune District Education Associations
Shankarrao Ursal College of Pharmaceutical
Sciences & Research Center kharadi pune

Genetic Engineering

tin Neharkar
ant professor,
t of pharmacutices
o ursal college of
al science & research
kharadi pune.

CONTENTS

- Introduction
- History of Genetic Engineering
- Tools of Genetic Engineering
- Principles of rDNA technology
- Applications of Genetic Engineering
- Disadvantage
- Conclusion

GENETIC ENGINEERING

Genetic recombination is the exchange of information between two DNA segments.

- This is a common occurrence within the same species.
- But by artificial means, when a gene of one species in transferred to another living organism, it is **called recombinant DNA technology.**

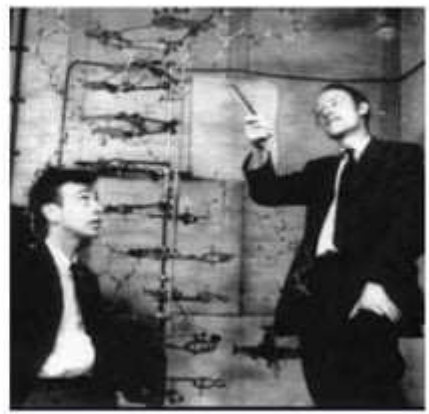
- This refers to the processes that allow introduction of new DNA into a cell.
- This allows the taking of genes from one organism and placing them in the chromosomes of another producing a transgenic organism.

The genes can come from individuals of the same species (eg transferring a healthy gene from one person into a person with a genetic disease = gene therapy) OR

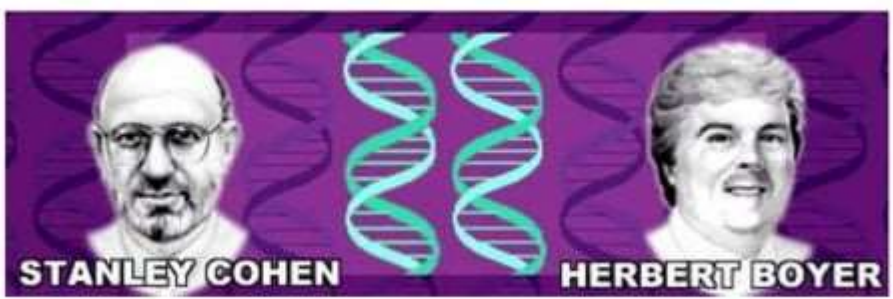
From a different species (eg placing insulin producing gene into a bacteria)

History of Genetic Engineering

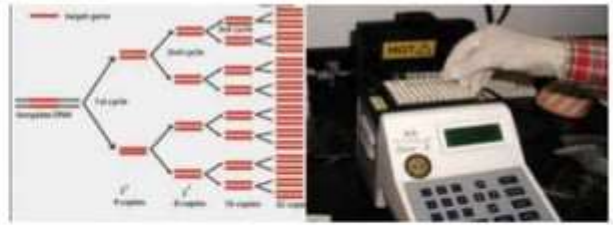
- In 1951-The term **"genetic engineering"** was first coined by Jack Williamson in his science fiction novel (Dragon's Island).
- In 1953, James Watson and Francis Crick proposed the **double helix structure of DNA**.

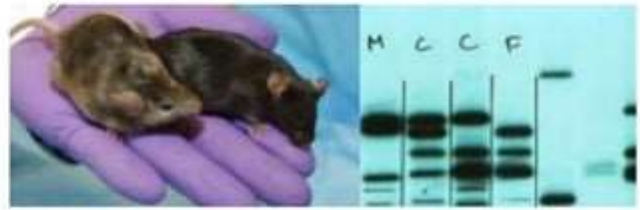


- **1972**- Paul berg created the first recombinant DNA molecules by combining DNA from the monkey virus SV40 (Simian virus 40) with that of the lambda virus (bacteriophage)
- **1973**- The first GMO was a bacterium generated by Herbert Boyer and Stanley Cohen



- In 1980s invention of polymerase chain reaction (PCR).
- Development of genetic fingerprinting.
- Transgenic mice produced carrying human genes.
- Transgenic plants produced resistant to a herbicide.
- In1996 The birth of the first cloned animal, Dolly the sheep, was announced.





Tools of Genetic Engineering

Restriction Endonucleases

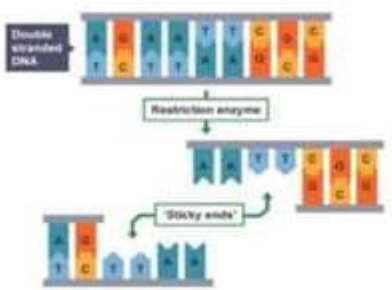
- Bacterial enzymes that can cut/split DNA at specific sites. These were first discovered in E.coli restricting the replication of bacteriophages ,by cutting the viral DNA.

Recognition sequences:

- Recognition sequence is the site where the DNA is cut by a restriction endonuclease.
- Restriction endonucleases can specifically recognise DNA with a particular sequence of 4-8 nucleotides & cleave

Cleavage patterns:

- The cut DNA fragments by restriction endonucleases may have mostly sticky ends or blunt ends.
- DNA fragments with sticky ends are particularly useful for rDNA experiments,since single stranded sticky DNA ends can easily pair with any other DNA fragment having complementary .

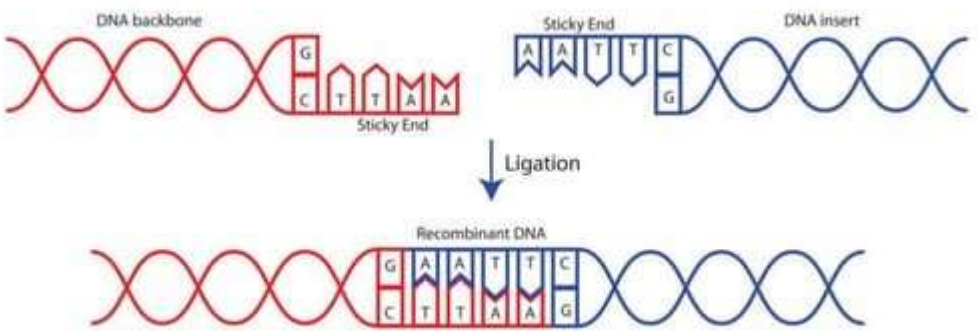


First cut the DNA using **restriction enzymes,** these cut the DNA between specific base pairs.. such as GAATTC

Many restriction enzymes cut DNA in a **staggered pattern** and leave **overhanging** ends with unpaired bases exposed. These are known as **"sticky ends"**

DNA ligases

- These were originally isolated from viruses, also occur in E.coli & eukaryotic cells.
- The cut DNA fragments are covalently joined together by DNA ligases



Host cells

- The hosts are the living systems or cells in which the carrier of rDNA molecule or vector can be propagated.
- Microorganisms are preferred as host cells, since they multiply faster compared to cells of higher organisms.

Group	*Examples*
Prokaryotic	
Bacteria	*Escherichia coli*
	Bacillus subtilis
	Streptomyces sp
Eukaryotic	
Fungi	*Saccharomyces cerevisiae*
	Aspergillus nidulans
Animals	Insect cells
	Oocytes
	Mammalian cells
	Whole organisms
Plants	Protoplasts
	Intact cells
	Whole plants

E.coli

- This was the first organism used in the DNA technology experiments.
- The major drawback is that it cannot perform post translational modifications.

Eukaryotic Hosts

- These are preferred to produce human proteins, since these have complex structure suitable to synthezise complex proteins.
- Mammalian cells possess the machinery to modify the protein to the active form.(post translational modifications)

  E.g., Tissue plasminogen activator

Vectors

- DNA molecules, which can carry a foreign DNA fragment to be cloned.
- These are self replicating in an appropriate host cell.
    - Plasmids - E.coli
    - Bacteriophages - phage λ, phageM13
    - Cosmids
    - Artificial chromosome vectors.

Principles of rDNA technology

GENERATION OF DNA FRAGMENTS

(Restriction endonuclease digestion, cDNA synthesis, PCR, chemical synthesis)

INSERTION INTO A CLONING VECTOR

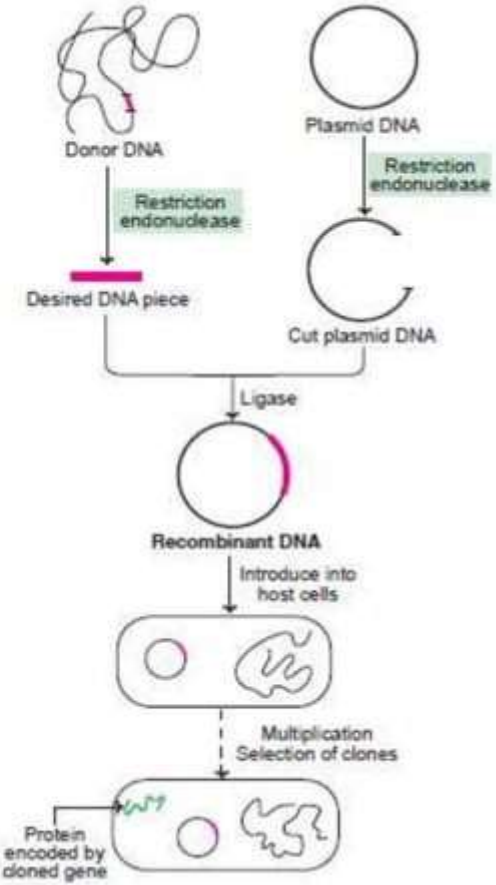
(Ligation of blunt ends or cohesive ends, homopolymer tailing, linker molecules)

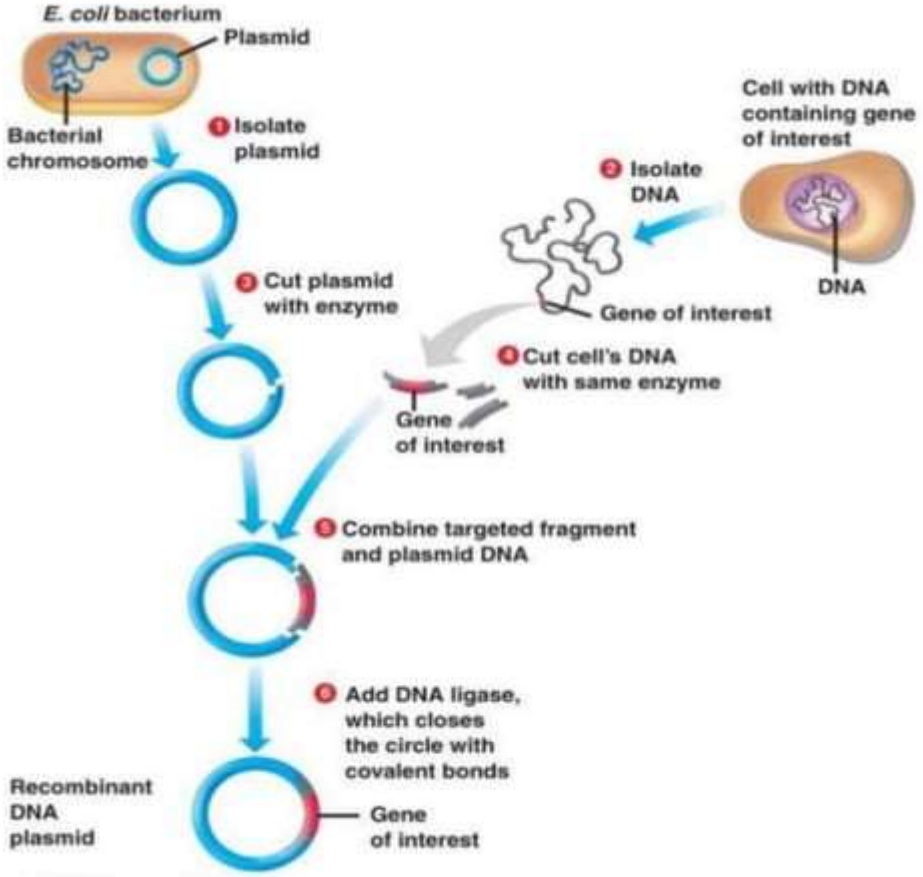
INTRODUCTION INTO HOST CELLS

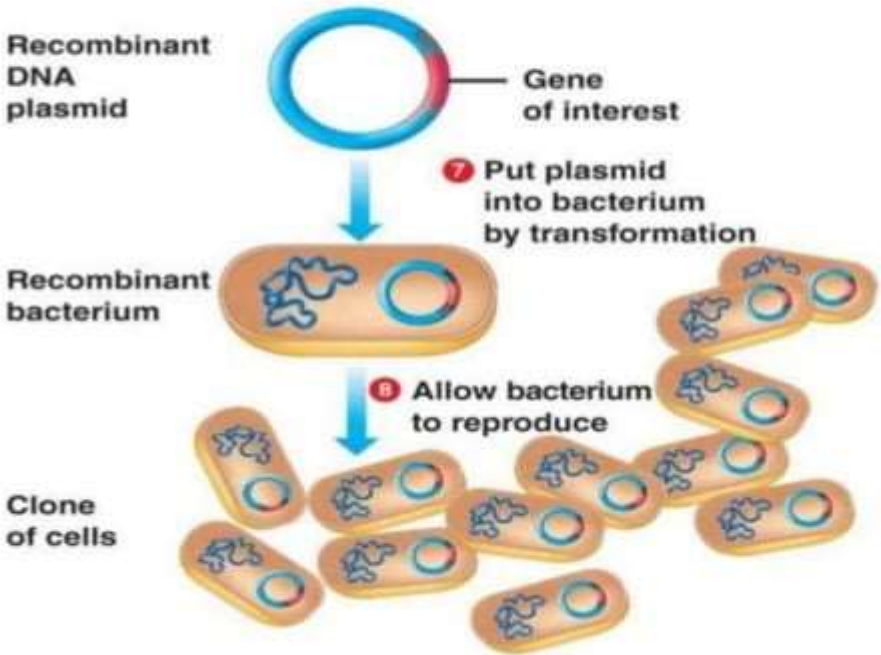
(Transformation, transfection, transduction)

SELECTION OR SCREENING

(Hybridization, PCR, immunochemical methods, protein–protein interactions, functional complementation)




E. coli bacterium
Plasmid
Bacterial chromosome
1 Isolate plasmid
Cell with DNA containing gene of interest
2 Isolate DNA
DNA
Gene of interest
3 Cut plasmid with enzyme
4 Cut cell's DNA with same enzyme
Gene of interest
5 Combine targeted fragment and plasmid DNA
6 Add DNA ligase, which closes the circle with covalent bonds
Recombinant DNA plasmid
Gene of interest


Recombinant DNA plasmid
Gene of interest
7 Put plasmid into bacterium by transformation
Recombinant bacterium
8 Allow bacterium to reproduce
Clone of cells

GENE THERAPY - TYPES

SOMATIC:

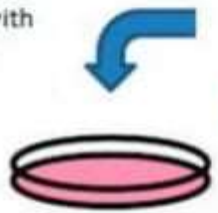
The somatic gene therapy involves changes in the target cells, however, that are **not transferred to the next generation**.

GERM LINE THERAPY:

Germ line gene therapy, germ cells i.e. sperm or eggs are modified by the introduction of functional genes, which are ordinarily integrated into their genomes. Therefore, the change due to therapy would be heritable and would be **passed on to later generations.**

EX VIVO GENE TRANSFER

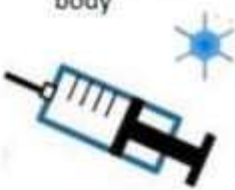
Tissue cells from patient are cultured and transduced with modified vector

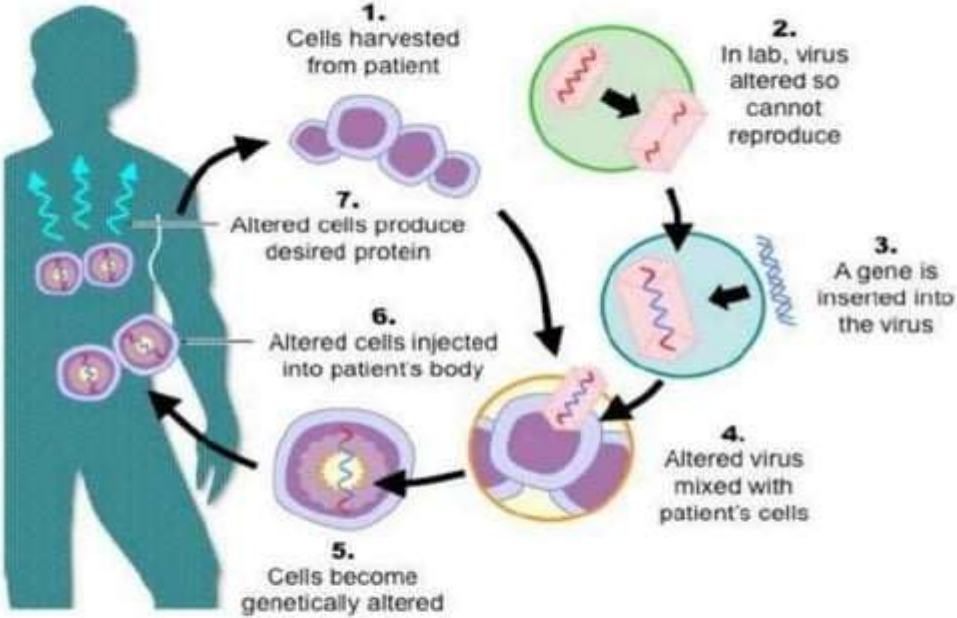


Transduced cells that produce therapeutic protein are infused back into the body

IN VIVO GENE TRANSFER

The modified vector is directly injected into the body




1.
Cells harvested from patient
2.
In lab, virus altered so cannot reproduce
3.
A gene is inserted into the virus
4.
Altered virus mixed with patient's cells
5.
Cells become genetically altered
6.
Altered cells injected into patient's body
7.
Altered cells produce desired protein

APPLICATION OF GENETIC ENGINEERING

AGRICULTURE

Genetically modified organism

Applications of Genetic Engineering in Medicine

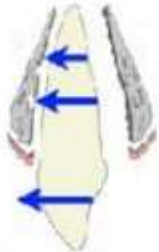
- Human insulin gene inserted into the bacterium E.coli to produce synthetic "human" insulin, for the treatment of insulin-dependent diabetes .
- Today, the microorganism (such as: yeast) is used to produce virus antigen used as a vaccine, that stimulate human immune system against this virus.

- Producing human growth hormones: to treat growth retardation (dwarfism).
- Producing Follistim injection: (contains the FSH hormone) for treating infertility.
- Other biopharmaceuticals under development through genetic engineering, include: anti-cancer drug and a possible vaccine for AIDS, malaria, etc

Genetically engineered bacteria can produce growth hormone for human use.

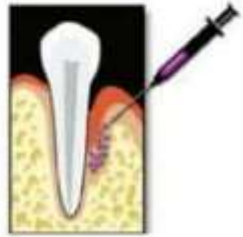
Orthodontic Tooth Movement

New Teeth



DNA Vaccine

Bone Repair



GENE THERAPY
APPLICATIONS IN DENTISTRY

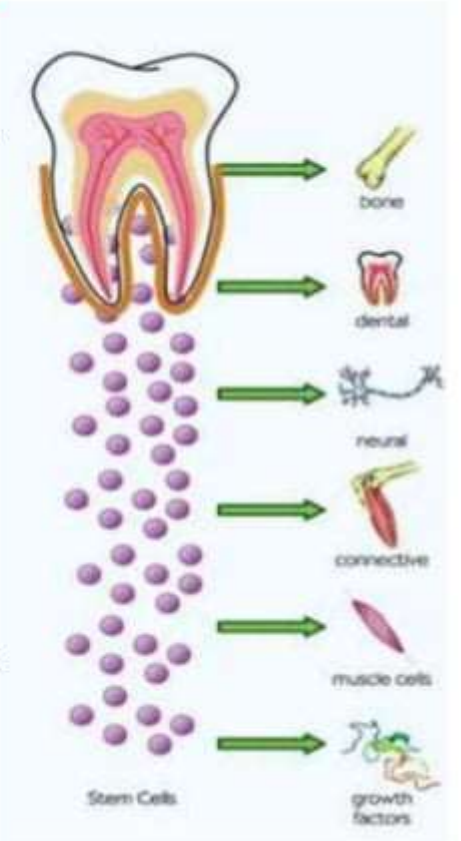
Salivary Glands

Chronic Pain

Squamous Cell Carcinoma

Stem cells

- Stem cells in the pulp of primary teeth, characterized as multipotent cells
- Helps in treatment of periodontal disease, diabetes, spinal cord injury, stroke, heart attack, burn, rheumatoid arthritis and Parkinson's and Alzheimer's, and regenerate many types of tissue in the body.

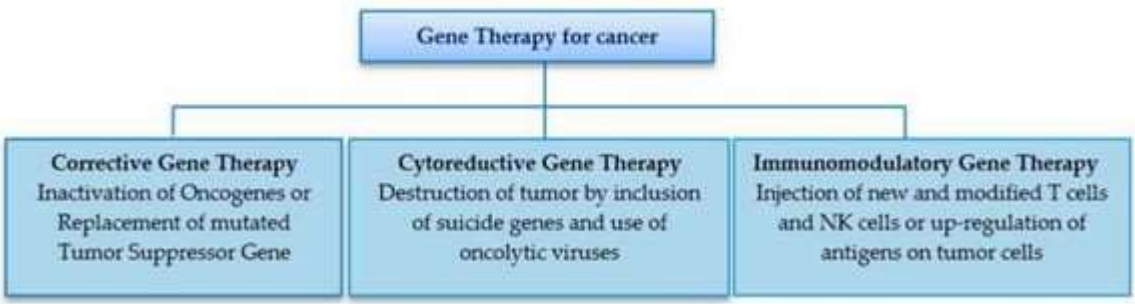


TO REDUCE OROFACIAL PAIN

- Firstly, the modified adenovirus, adeno associated virus (AAV) or lipid encapsulated plasmids coding for Interleukin-10, a therapeutic protein, may be injected into the sub arachnoid space to transduce the pia mater cells
- Secondly, a modified herpes virus may be introduced into the nerves of Dorsal Root Ganglia (DRG) via an intradermal injection to the skin.
- In the DRG, it codes for an inhibitory neurotransmitter, an anti-inflammatory peptide or decreases the synthesis of an endogenous nociceptive molecule that results in alleviation of pain

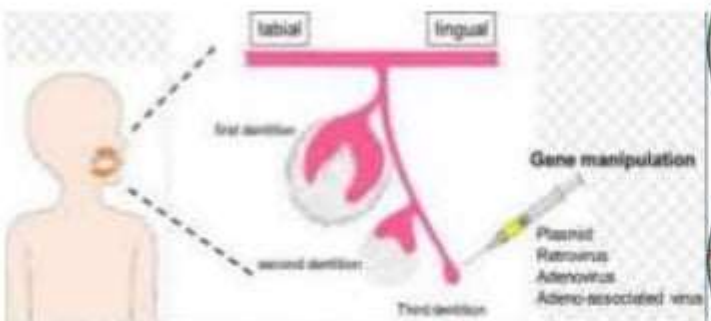
IN CARCINOGENESIS..

- In 2003, the first gene therapy drug Gendicine, a recombinant human adenovirus p53, was formulated and approved by the Chinese authorities for the treatment of SCCHN and other cancerous lesions.
- In 2005, the first genetically engineered oncolytic virus, H101 Adenovirus, was approved for treatment of SCCHN in China



MISSING TOOTH..

- A tooth germ can be created in vitro or ex vivo with a culture of epithelial and mesenchymal stem cells. The tooth germ is implanted into the alveolar bone to develop into a fully functional, non-metal tooth implant.



CONCLUSION

- Genetic Engineering is a vast field and accounts for tremendous depth of knowledge yet to be understood.
- Many application of the Genetic Engineering applied in various fields such as in Agriculture, Horticulture, Various medications, vaccines productions etc has proved to be beneficial
- There's no doubt that this field will only improve the quality of Life of Humans, But there still remains the question reliability in the future.

REFERENCE

1. www.ieeeexplore.ieee.org
2. www.wikipedia.com/genetic engineering

thank you

CARBOHYDRATES

By

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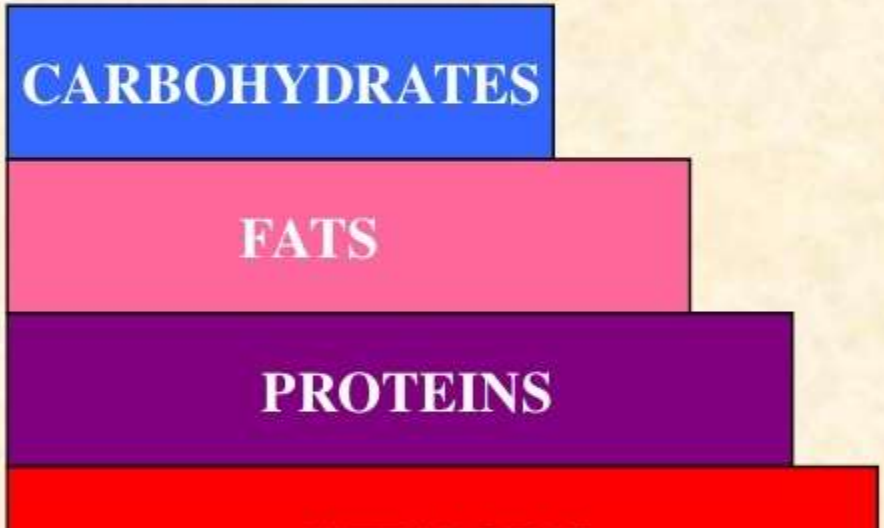
INTRODUCTION

NUTRITION

- Nutrition is defined as " the science of how the body utilizes food to meet requirements for development growth, repair and maintenance."



Daily Intake

- **Nutrient Quantity Per Day**

✓ Energy = 8,700 kilojoules

✓ Protein = 50 grams

✓ Fat = 70 grams

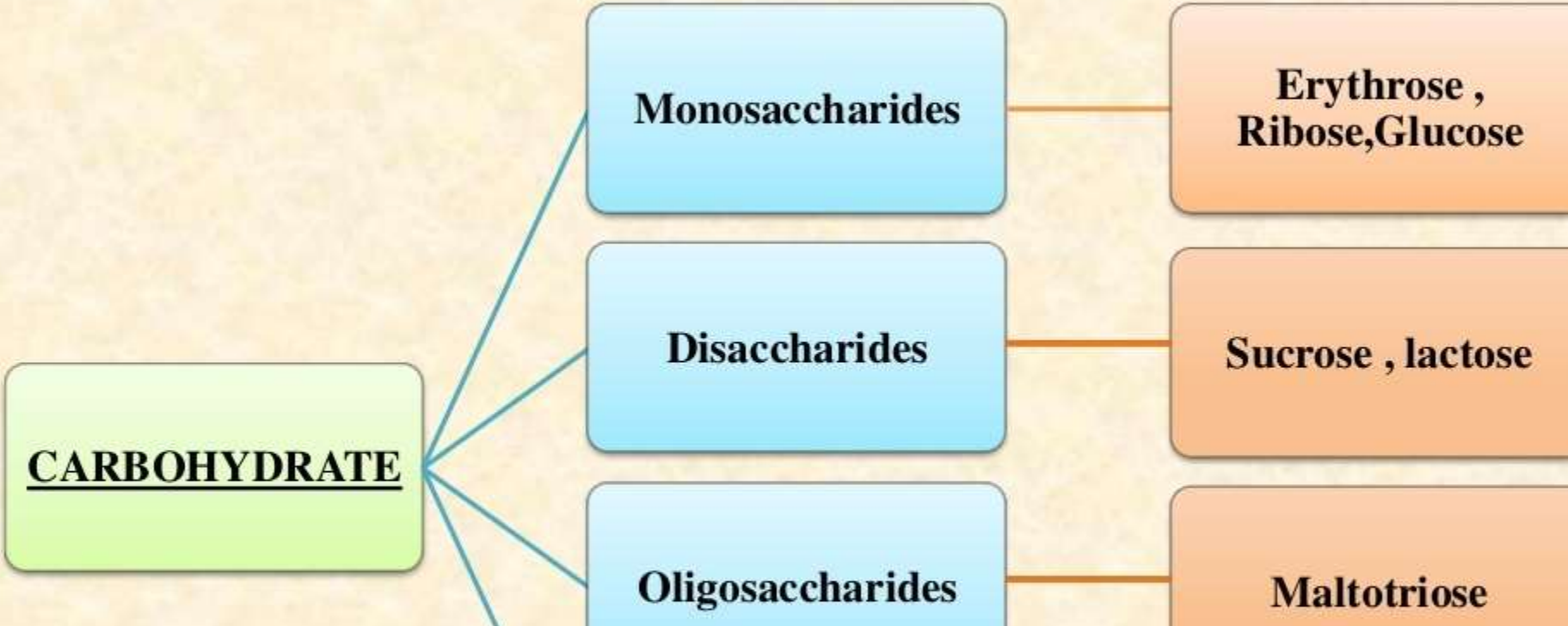
✓ Carbohydrates = 310 grams

✓ Sugars = 90 grams

CARBOHYDRATE:

- Most abundant organic molecule on earth.
- *Carbohydrates are defined as aldehyde or keto derivatives of polyhydric alcohols.*
- For example: Glycerol on oxidation is converted to D-glyceraldehyde, which is a carbohydrate derived from the trihydric alcohol (glycerol).
- All carbohydrates have the general formula $C_x H_{2y} O_y$ [or it

CLASSIFICATION OF CARBOHYDRATE



FUNCTIONS OF CARBOHYDRATES

- Main source of energy in the body. Energy production from carbohydrates will be 4 k calories/g (16 k Joules/g).
- Storage form of energy (starch and glycogen).
- Excess carbohydrate is converted to fat.
- Glycoproteins and glycolipids are components of cell membranes and receptors

Biomedical Importance Of Glucose

- Glucose is a major carbohydrate
- It is a major fuel of tissues
- It is converted into other carbohydrates

✓ Glycogen for storage.

✓ Ribose in nucleic acids.

✓ Galactose in lactose of milk.

✓ They form glycoproteins & proteoglycans

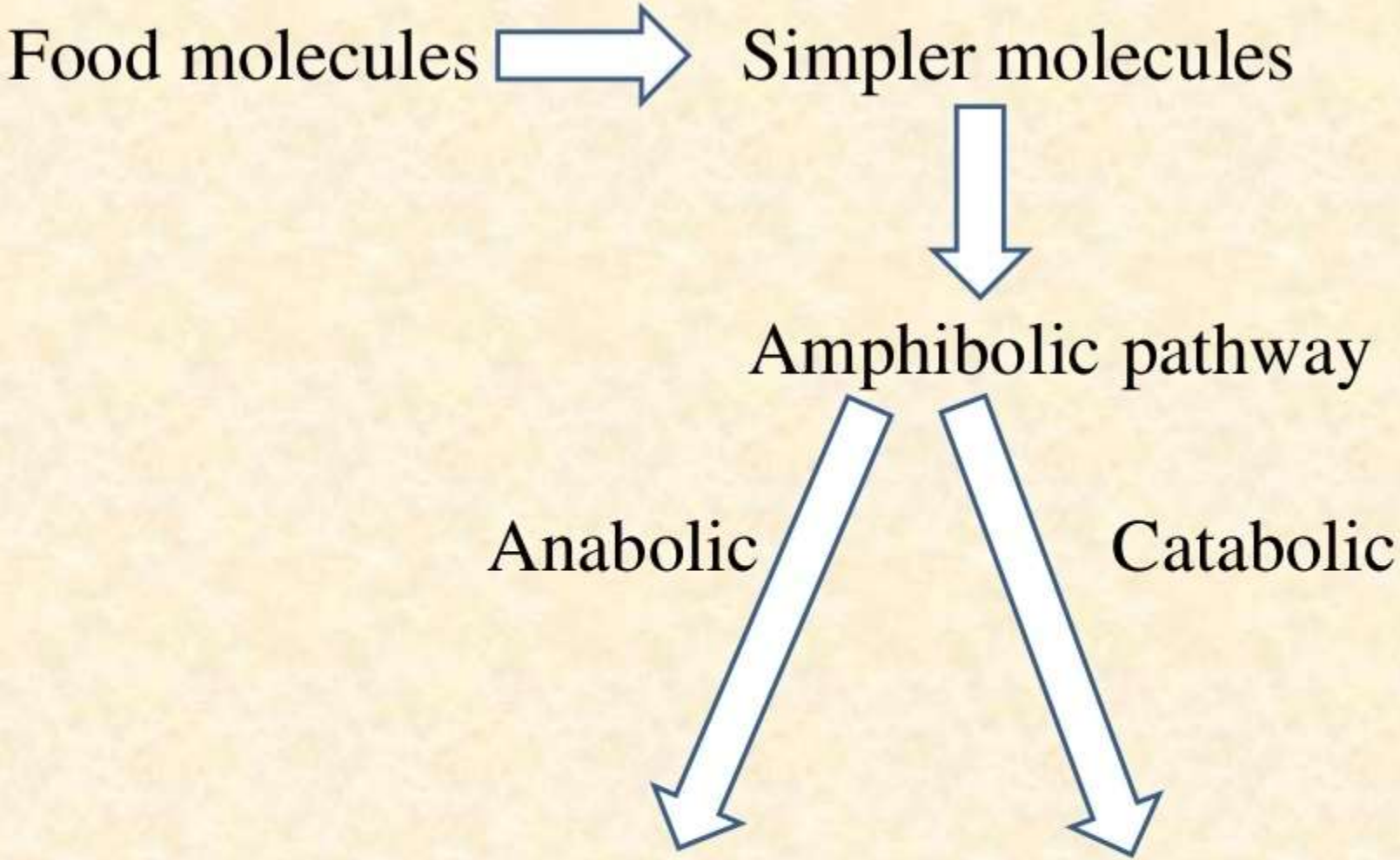
✓ They are present in some lipoproteins (LDL) .

METABOLISM

Thousands of chemical reactions are taking place inside a cell in an organized, well co-ordinated and purposeful manner; all these reactions are called as METABOLISM.

TYPES OF METABOLIC PATHWAY:

- ✓Catabolic Pathway
- ✓Anabolic Pathway
- ✓Amphibolic Pathway


Food molecules
Simpler molecules
Amphibolic pathway
Anabolic
Catabolic

MAJOR PATHWAYS OF CARBOHYDRATE METABOLISM

1) Glycolysis

2) Citric Acid Cycle

3) Gluconeogenesis

4) Glycogenesis

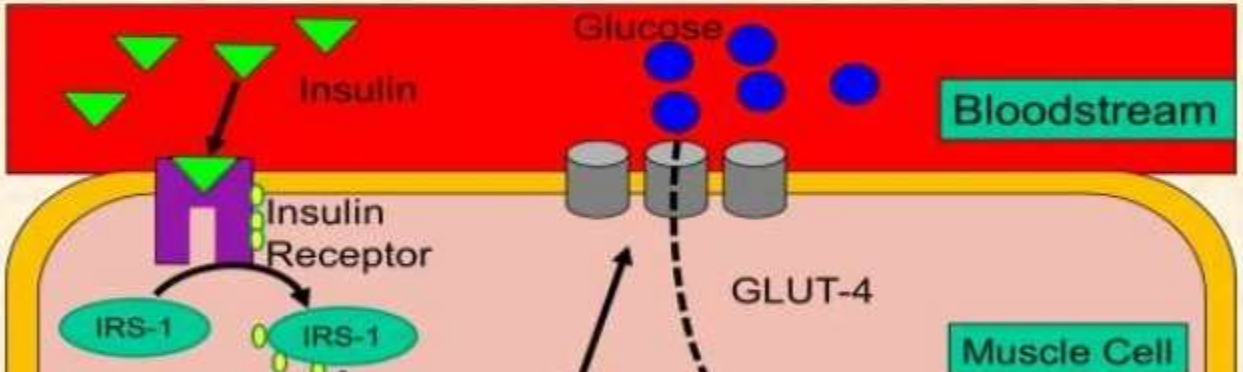
5) Glycogenolysis

6) Hexose monophosphate shunt

7) Uronic Acid Pathway

Entry of Glucose into cells

1) Insulin-independent transport system of glucose: Not dependent on hormone insulin. This is operative in – hepatocytes, erythrocytes (GLUT-1) and brain.
2) Insulin-dependent transport system: Muscles and adipose tissue (GLUT-4).



Type 2 diabetes melitus:
-Due to reduction in the quantity of

GLYCOLYSIS

EMBDEN-MEYERHOF PATHWAY

(OR)

E.M.PATHWAY

Definition:

Salient features:

1) Takes place in all cells of the body.
2) Enzymes present in "cytosomal fraction" of the cell.
3) Lactate – end product – anaerobic condition.
4) Pyruvate(finally oxidized to CO_2 & H_2O) – end product of aerobic condition.
5) Tissues lacking mitochondria – major pathway – ATP synthesis.
6) Very essential for brain – dependent on glucose for energy

Reactions of Glycolysis

1) Energy Investment phase (or) priming phase

2) Splitting phase

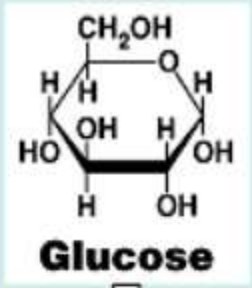
Energy Investment Phase

- **Glucose** is phosphorylated to **glucose-6-phosphate** by *hexokinase (or) glucokinase*.
- Glucose-6-phosphate undergoes isomerization to give **fructose -6- phosphate** in the presense o *phospho-hexose isomerase* and Mg^{2+}
- Fructose-6-phosphate is phoshorylated to **fructose 1,6-bisphosphate** by *phosphofructokinase*.

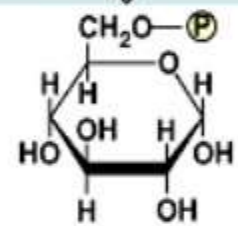
Splitting Phase

- Fructose 1,6-bisphosphate → **glyceraldehyde 3-phosphate + dihydroxyacetone phosphate.**(*aldolase enzyme*)
- **2 molecules** of glyceraldehyde 3-phosphate are obtained from 1 molecule of glucos

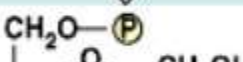
- Glyceraldehyde 3-phosphate → **1,3-bisphosphoglycerate**(*glyceraldehyde 3-phosphate hydroge*

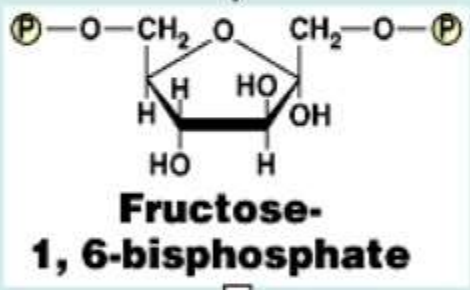


Hexokinase

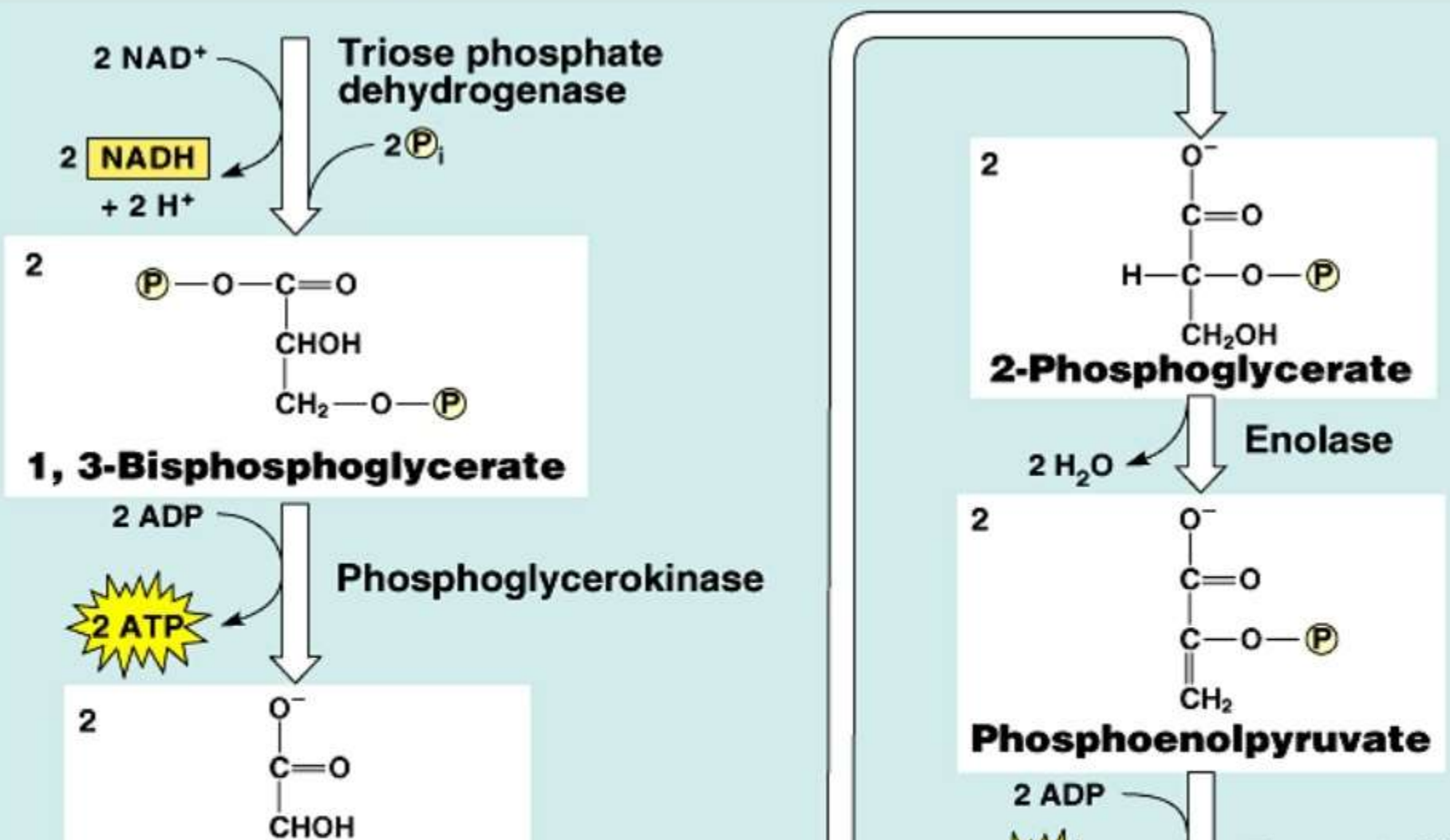


Phosphoglucoisomerase





Aldolase


2 NAD+
Triose phosphate dehydrogenase
2 NADH
+ 2 H+
2 Pi
2
1, 3-Bisphosphoglycerate
2 ADP
2 ATP
Phosphoglycerokinase
2
2-Phosphoglycerate
2 H2O
Enolase
2
Phosphoenolpyruvate
2 ADP

Energy production of glycolysis:

ATP production = ATP produced - ATP utilized

	ATP produced	ATP utilized	Net energy
In absence of oxygen (anaerobic glycolysis)	4 ATP (Substrate level phosphorylation) 2ATP from 1,3 DPG. 2ATP from phosphoenol pyruvate	2ATP From glucose to glucose - 6-p. From fructose -6-p to fructose 1,6 p.	2 ATP
In presence of oxygen (aerobic glycolysis)	4 ATP (substrate level phosphorylation) 2ATP from 1,3 BPG.	2ATP -From glucose to glucose - 6-p. From fructose -6-p to	8 ATP / 6 ATP (Pyruvate dehydrogenase 2NADH,ETC, Oxidative

CLINICAL ASPECT

1) Lactic acidosis

- Normal value – 4 to 15 mg/dl.
- Mild forms – strenous exercise, shock, respiratory diseases, cancers
- Severe forms – Impairment/collapse of circulatory system – myocardial infarction,

2) Cancer and glycolysis :

- Cancer cells – increased uptake of glucose and glycolysis.

- Blood vessels unable to supply adequate oxygen – HYPOXIC condition – Anaerobic glycolysis / hypoxic glycolysis – Involvement of Hypoxic inducible transcription factor (HIF).

- ✓ Pasteur effect : Inhibition of glycolysis by oxygen (Phosphofructokinase) .

- ✓ Crabtree effect : The phenomenon of inhibition of oxygen consumption by the addition of glucose to tissues having high aerobic glycolysis.

RAPARPORT – LEUBERING CYCLE

- Supplementary pathway/ Shunt pathway to glycolysis .
- Erythrocytes
- Synthesis of 2,3-bisphosphoglycerate (2,3-BPG).
- Without the synthesis of ATP.

CITRIC ACID CYCLE

KREBS CYCLE / TRICARBOXYLIC ACID/ TCA CYCLE

Essentially involves the oxidation of acetyl CoA

Brief History:

- Hans Adolf Krebs
- 1937
- Studies of oxygen consumptiom

Location of TCA

- Mitochondrial matrix
- In close proximity to the electronic transport chain.

Overview

- 65-70% of the ATP is synthesized
- Name : TCA used because at the ouset of the cycle

Reactions of citric acid cycle

1) **Formation of citrate** : Condensation of acetyl CoA an
oxaloacetate → catalysed by citrate synthase.

2) & 3) **Citrate is isomerized to isocitrate** → aconitase (t
steps).

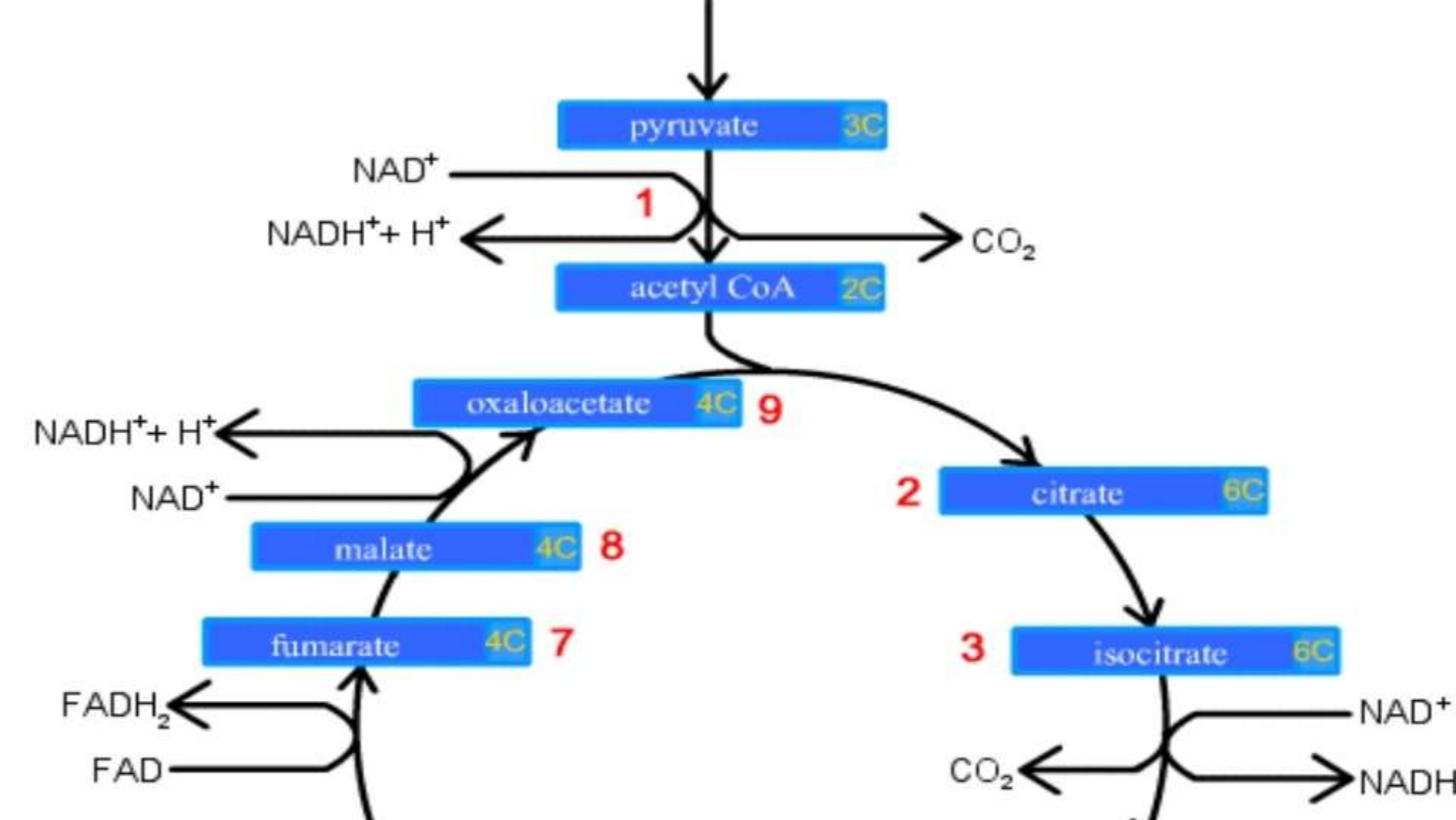
4) & 5) **Formation of ḍ-ketoglutarate** : enzyme isocitrate
dehydrogenase.

7)**Formation of succinate** : enzyme succinate thiokinase

GTP + ADP ←→ ATP + GDP (nucleoside diphosphate kinase)

8)**Conversion of succinate to fumarase** : enzyme succinate dehydrogenase

9)**Formation of malate** : enzyme fumarase


pyruvate 3C
NAD+
1
NADH+ + H+
CO2
acetyl CoA 2C
oxaloacetate 4C
9
NADH+ + H+
NAD+
2
citrate 6C
malate 4C
8
fumarate 4C
7
3
isocitrate 6C
FADH2
FAD
NAD+
CO2
NADH

- TCA cycle is strictly **aerobic** in contrast to glycolysis.

- Total of **12 ATP** are produced from one acetyl CoA :-

✓ During the process of oxidation of acetyl CoA via citric acid cycle → 3 NADH & 1 FADH2.

✓ Oxidation of 3 NADH by electron transport chain coupled with oxidative phosphorylation results in 9 ATP. FADH2 → 2 ATP.

APPLIED ASPECTS OF TCA CYCLE

Mitochondrial encephalopathy occurs due to fumarase deficiency .

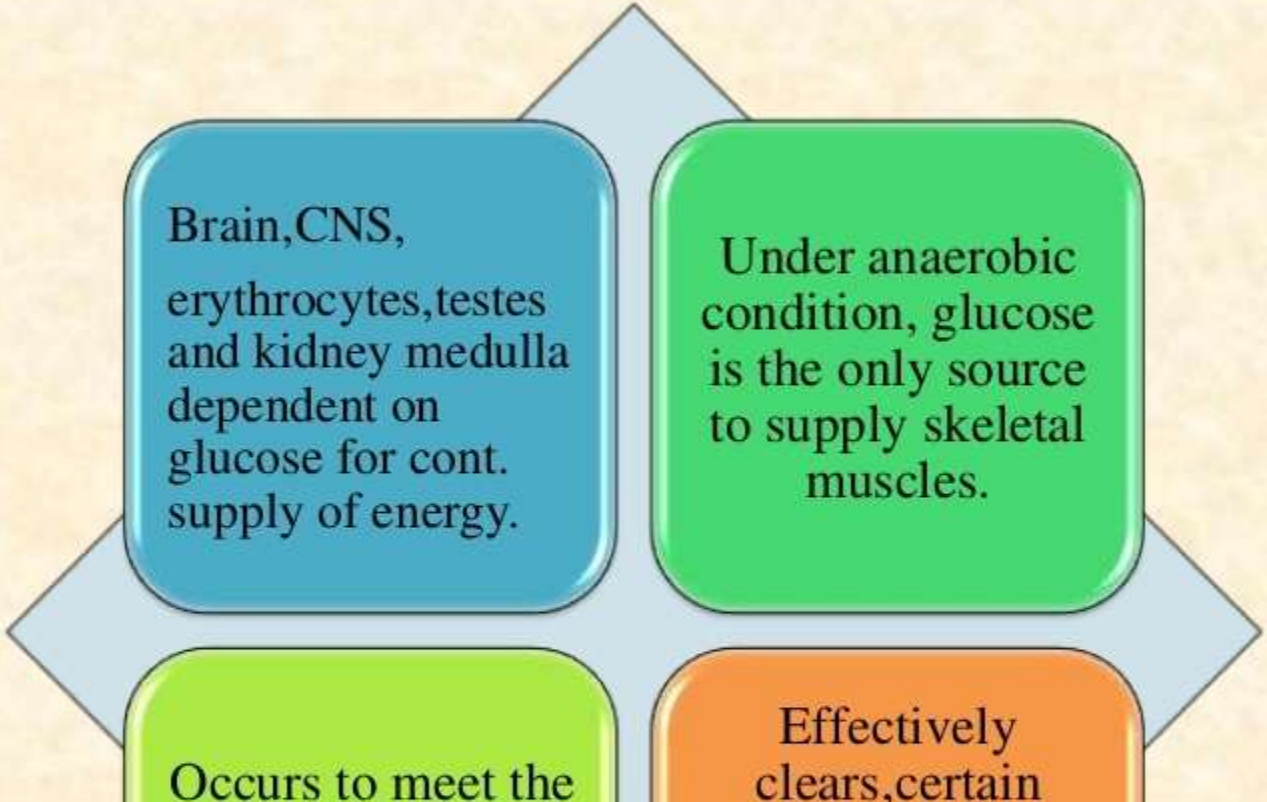
It is a

GLUCONEOGENESIS

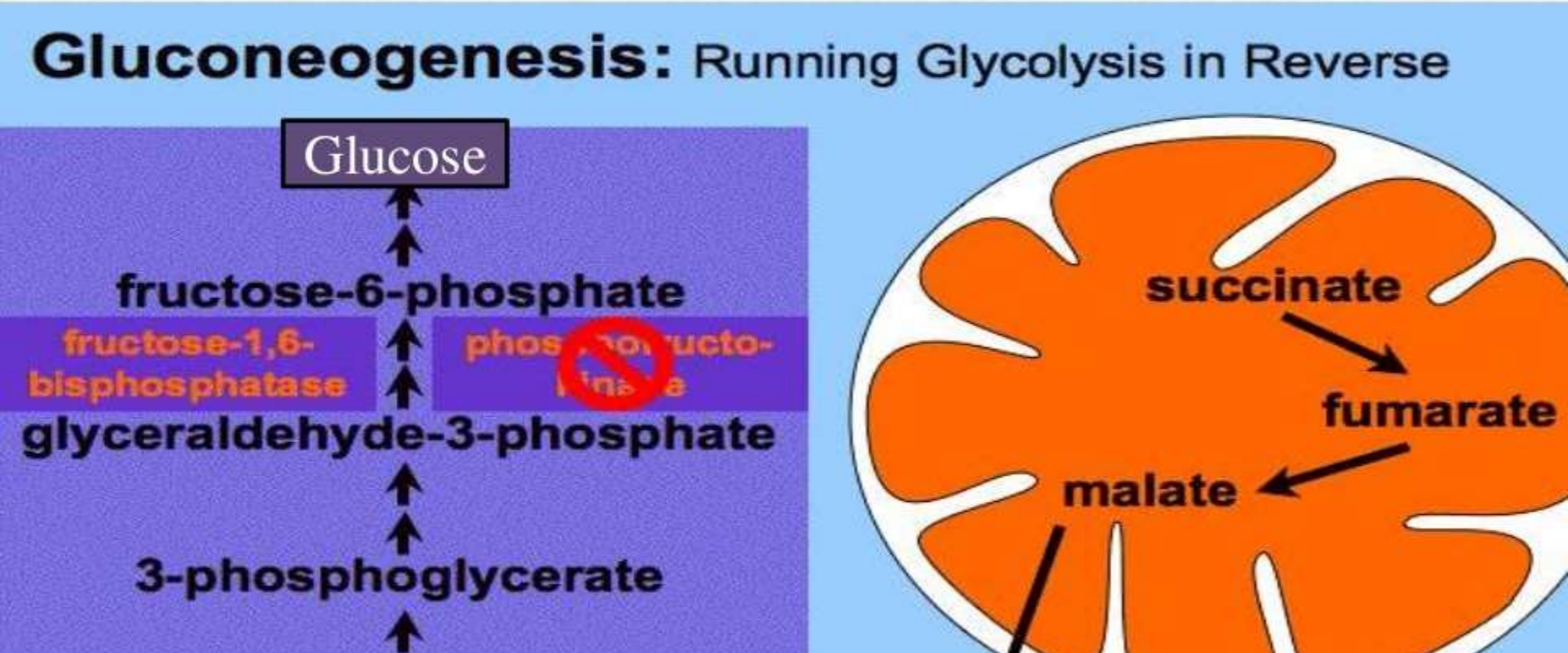
The synthesis of glucose from non-carbohydrate compounds is known as gluconeogenesis.

Major substrate/precursors : lactate, pyruvate, glycogenic amino acids, propionate & glycerol.

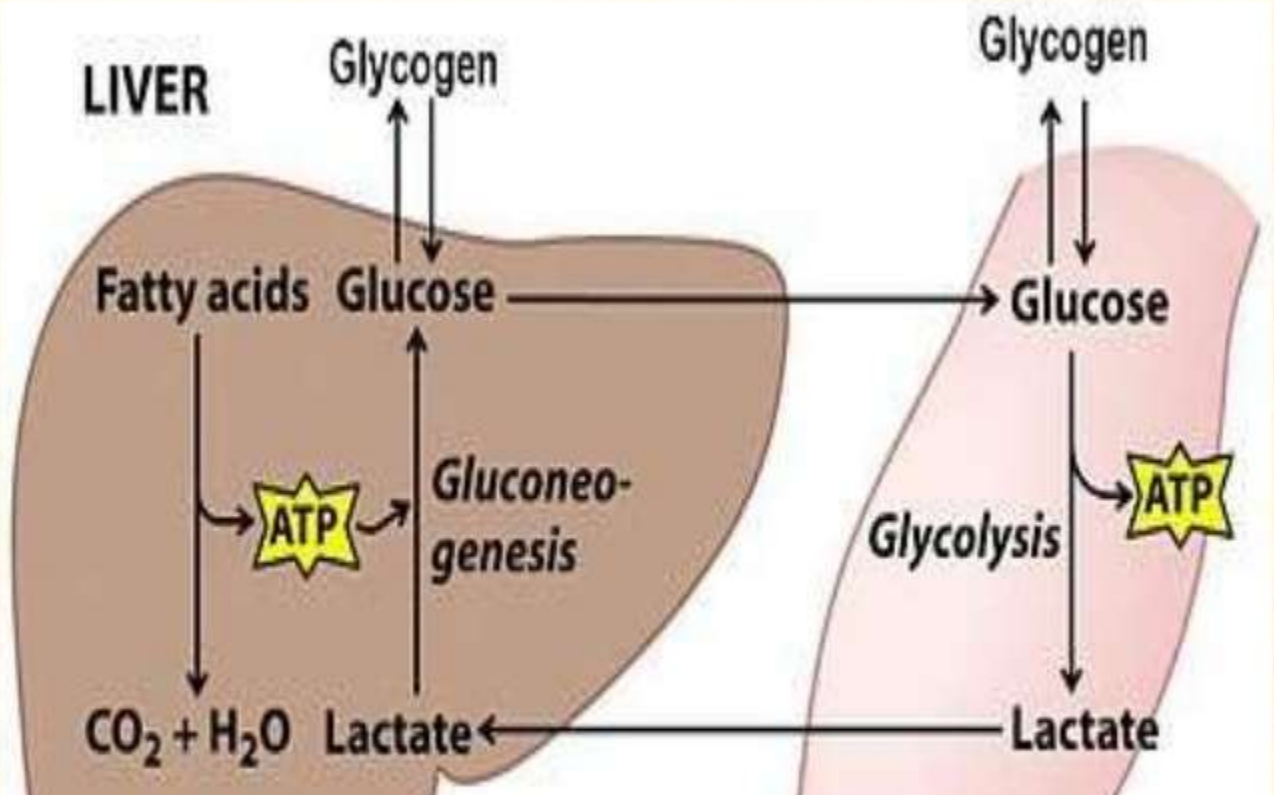
Importance of Gluconeogenesis



Reaction of Gluconeogenesis

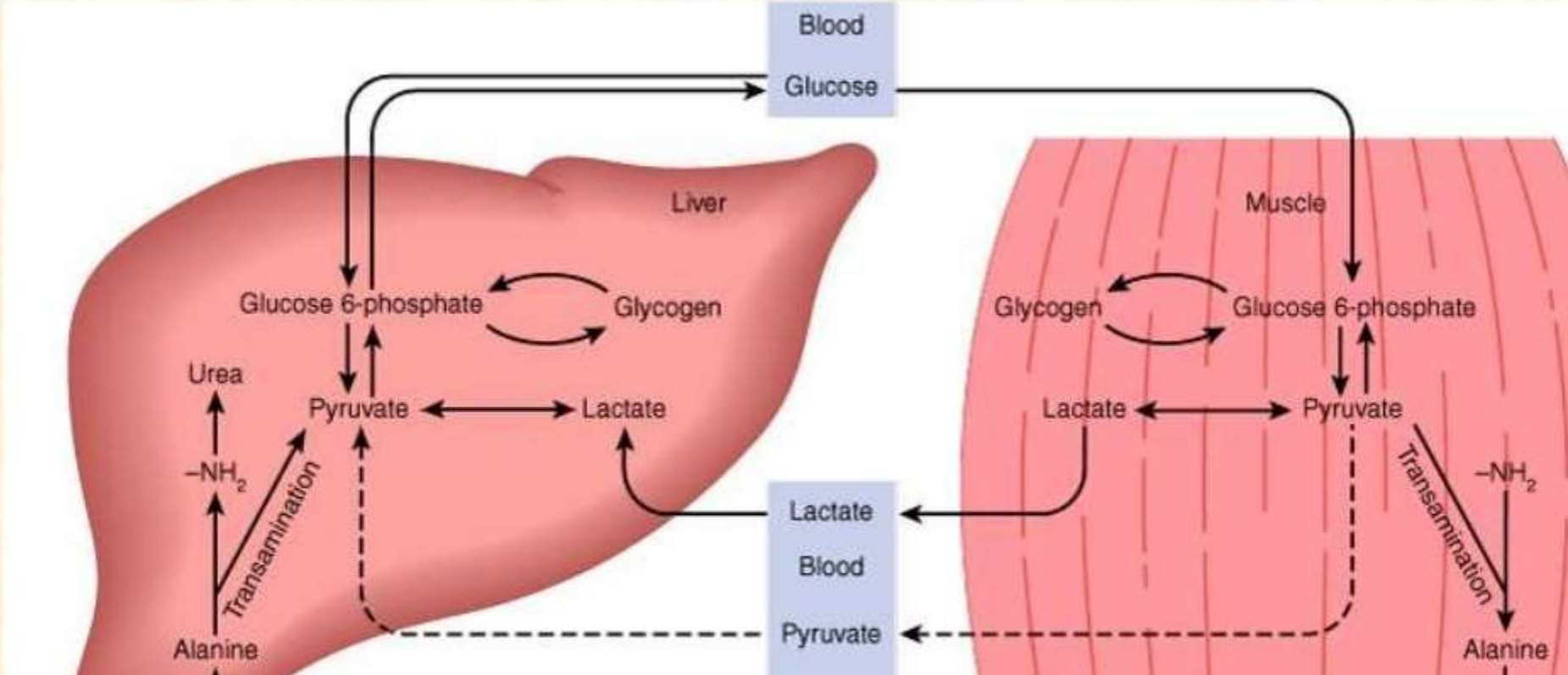


Cori Cycle



The cycle involveing the **synthesis of glucose in liver from the skeletal muscle lactate** and the reuse of glucose thus synthesized by the

Glucose-Alanine Cycle



Clinical Aspects

* **Glucagon** stimulates gluconeogenesis:

1)Active pyruvate kinase converted to inactive form

2)Reduces the concentration of fructose 2,6-bisphosphate.

* **Glycogenic amino acids** have stimulating influence on gluconeogenesis.

* **Diabetes mellitus** where amino acids are mobilized from muscle protein for the purpose of gluconeogenesis.

Acetyl CoA promotes gluconeogenesis:

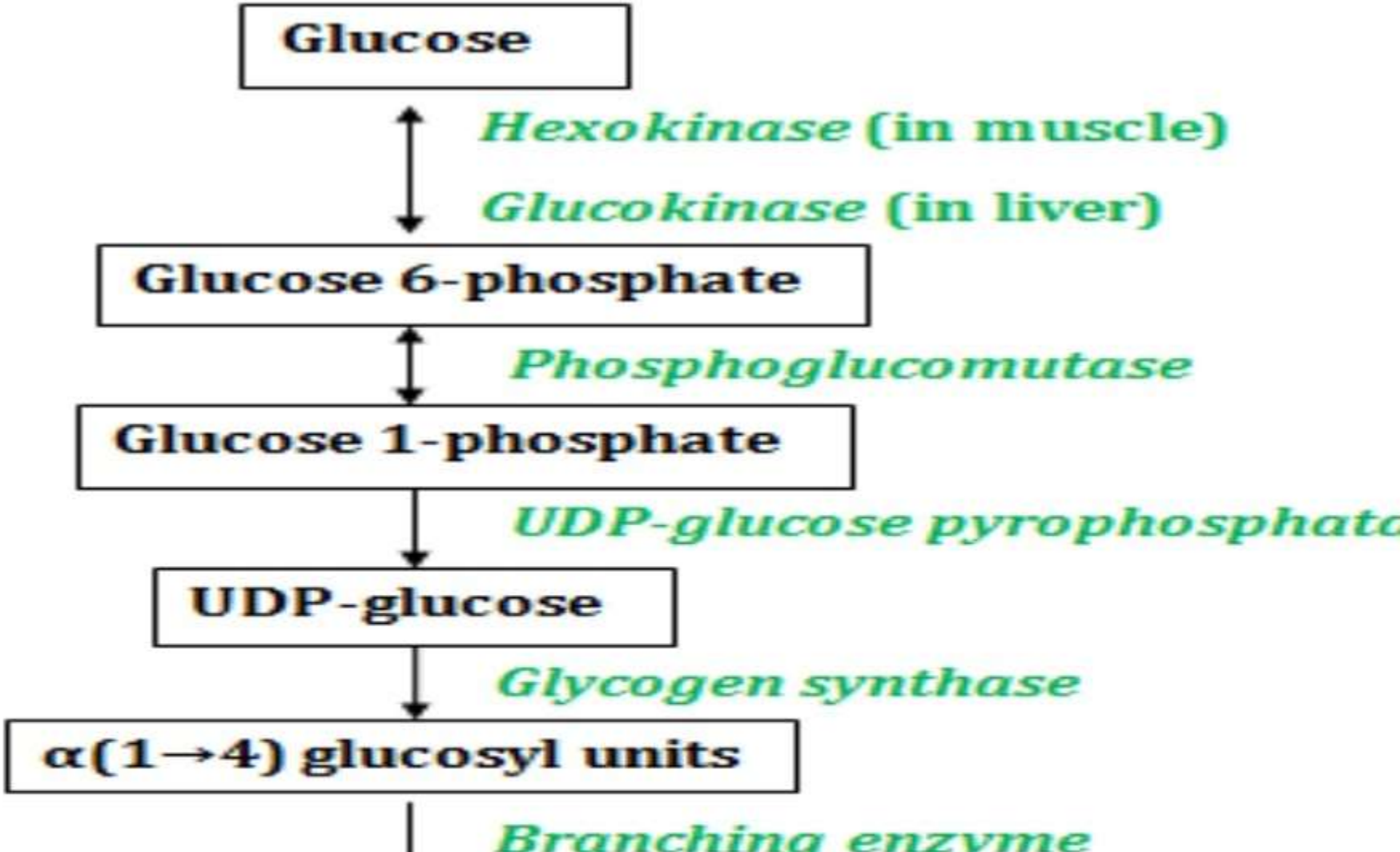
* **During starvation** – due to excessive lipolysis in adipose tissue –acetyl CoA accumulates in the liver.

GLYCOGEN METABOLISM

- ✓Glycogen is a storage form of glucose in animals.
- ✓Stored mostly in liver (6-8%) and muscle (1-2%)
- ✓Due to muscle mass the quantity of glycogen in muscle = 250g and liver =75g

GLYCOGENESIS

- Synthesis of glycogen from glucose.
- Takes place in cytosol.
- Requires UTP and ATP besides glucose.
- Steps in synthesis :


Glucose
Hexokinase (in muscle)
Glucokinase (in liver)
Glucose 6-phosphate
Phosphoglucomutase
Glucose 1-phosphate
UDP-glucose pyrophosphato
UDP-glucose
Glycogen synthase
α(1→4) glucosyl units
Branching enzyme

GLYCOGENOLYSIS

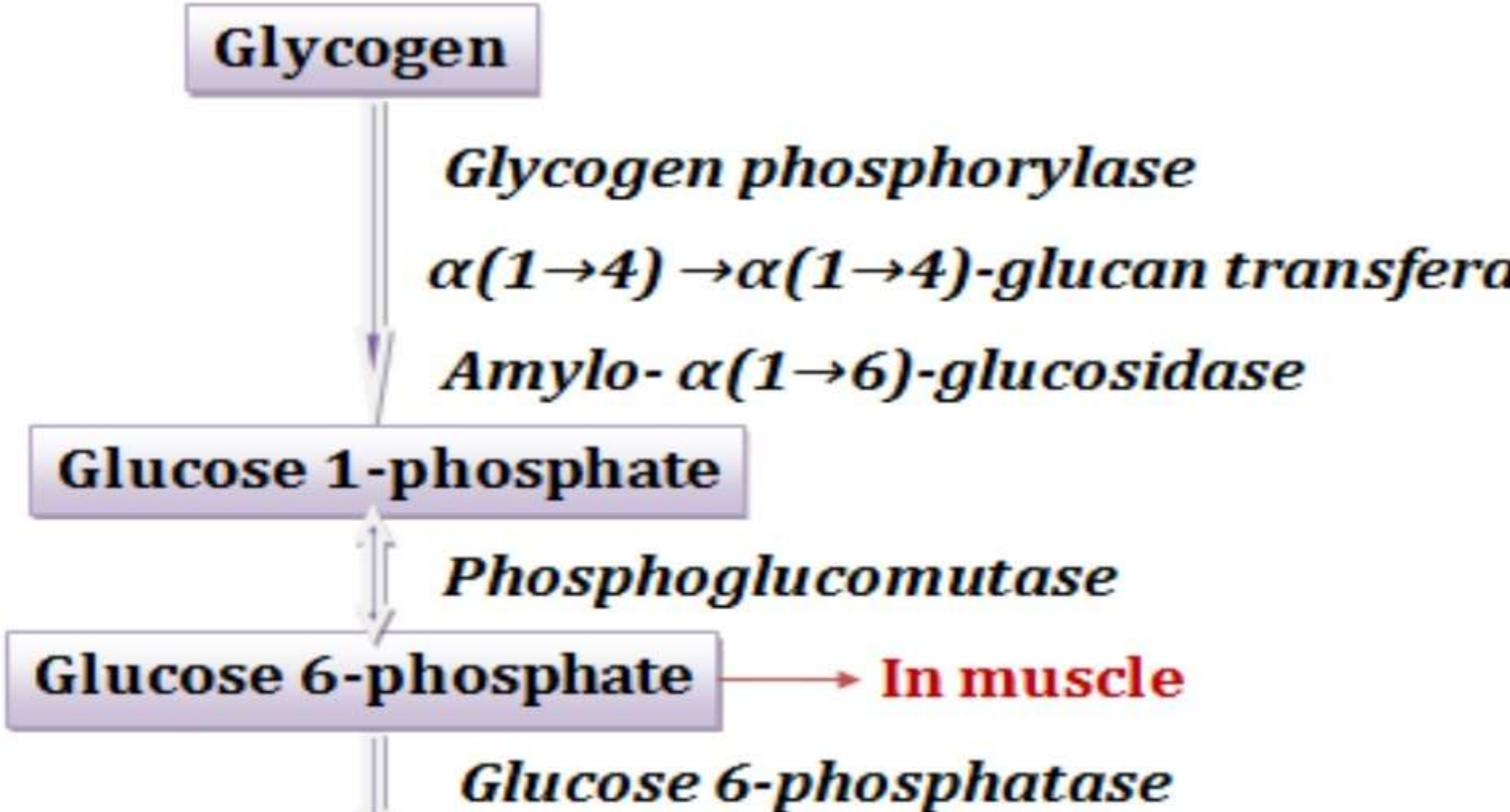
- Degradation of stored glycogen in liver and muscle constitutes glycogenolysis.

- Irreversible pathway takes place in cytosol.

- Hormonal effect on glycogen metabolism :

1) Elevated glucagon – increases glycogen degradation
2) Elevated insulin – increases glycogen synthesis

- Degraded by breaking majorly α-1,4- and α-1,6-glycosidic bonds.


Glycogen
Glycogen phosphorylase
α(1→4) →α(1→4)-glucan transfera
Amylo- α(1→6)-glucosidase
Glucose 1-phosphate
Phosphoglucomutase
Glucose 6-phosphate
In muscle
Glucose 6-phosphatase

GLYCOGEN STORAGE DISEASES

TYPE	ENZYME DEFECT	CLINICAL FEATURES
Type I (Von Gierke's disease)	Glucose-6-phosphatase deficiency.	Hypoglycemia, enlarged liver and kidneys, gastro-intestinal symptoms, Nose bleed, sh stature, gout
Type II (Pompe's disease)	Acid maltase deficiency	Diminished muscle tone, heart failure, enla tongue
Type III (Cori's disease,Forbe disease)	Debranching enzyme deficiency	Hypoglycemia, enlarged liver, cirrhosis, m weakness, cardiac involvement
Type IV (Andersen's	Branching enzyme	Enlarged liver & spleen, cirrhosis, diminish

TYPE	ENZYME DEFECT	CLINICAL FEATURES
Type VI (Her's disease)	Liver phosphorylase deficiency	Mild hypoglycemia, enlarged liver, short stature in childhood
Type VII (Tarui's disease)	Phosphofructokinase deficiency	Muscle pain, weakness and decreased endurance
Type VIII	Liver phosphorylase kinase	Mild hypoglycemia, enlarged liver, short stature in childhood, possible muscle weak and cramps

Von Gierke's disease)

Cori's disease, Forbe disease

HEXOSE MONOPHOSPHATE SHUNT

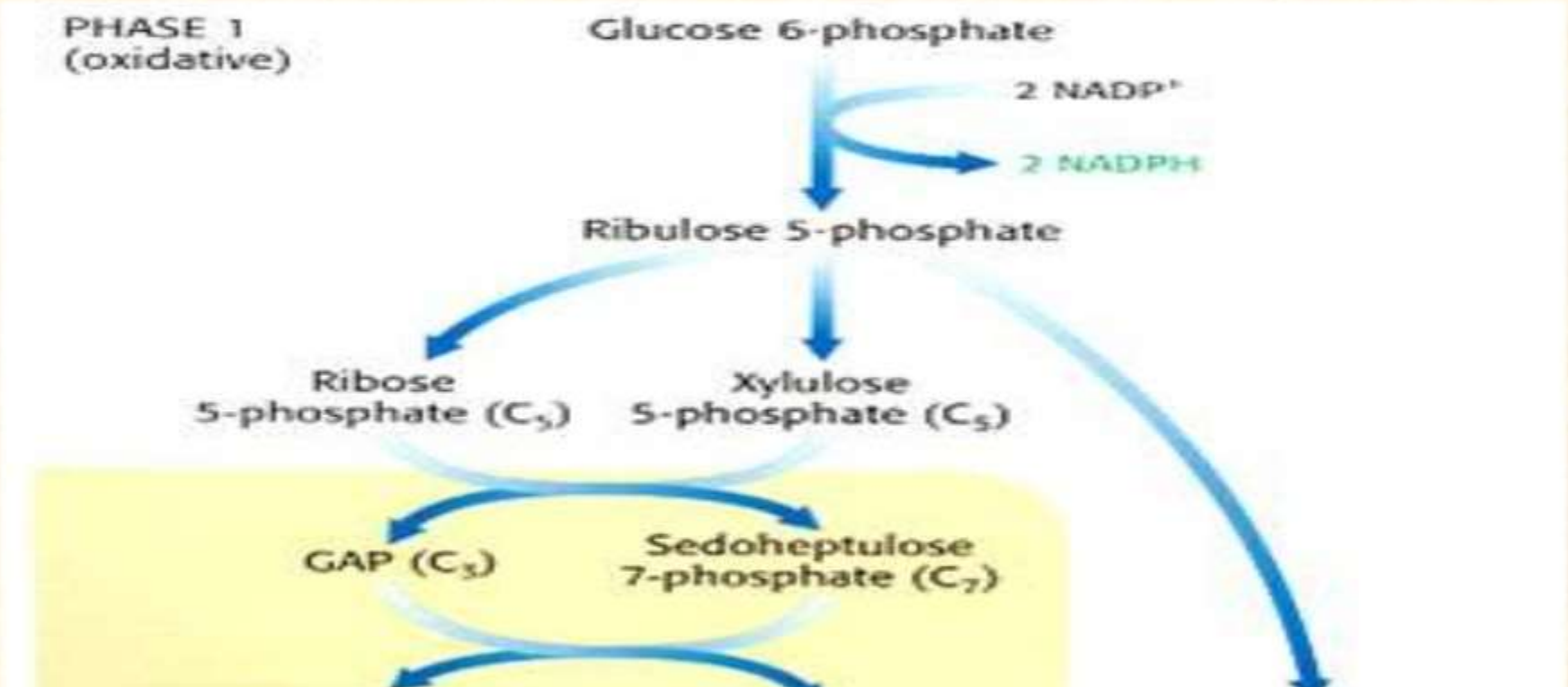
HMP Shunt/ Pentose Phosphate Pathway/ Phosphogluconate Pathway

* This is an alternative pathway to glycolysis and TCA cyc for the oxidation of glucose.

* Anabolic in nature, since it is concerned with the biosynthesis of NADPH and pentoses.

* Unique multifunctional pathway

Reactions of the HMP Shunt Pathway



Significance of HMP Shunt

- Pentose or its derivatives are useful for the synthesis of nucleic acids and nucleotides.

- NADPH is required :

  - For reductive biosynthesis of fatty acids and steroids.

  - For the synthesis of certain amino acids.

Clinical Aspects

- Glucose-6-Phosphate dehydrogenase deficiency :
  - Inherited sex-linked trait
  - Red blood cells
  - Impaired synthesis of NADPH
  - hemolysis , developing hemolytic anemia

Clinical Aspects

- Wernicke-Korsakoff syndrome :
  - Genetic disorder
  - Alteration in transketolase activity
  - Symptoms : mental disorder, loss of memory, partial paralysis

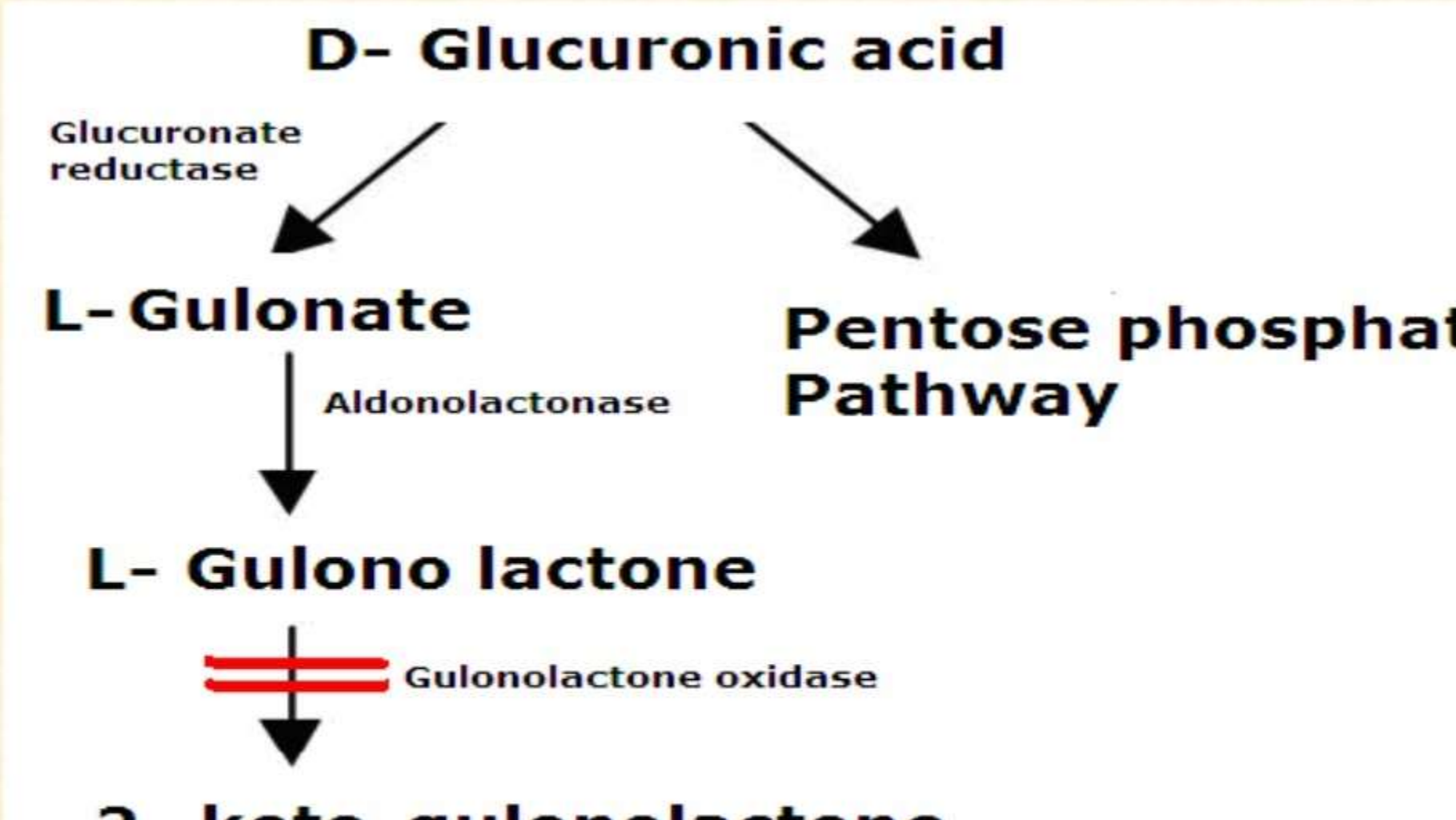
URONIC ACID PATHWAY

(OR)

GLUCORONIC ACIS PATHWAY

- ✓ Alternative oxidative pathway for glucose.
- ✓ synthesis of glucorinc acid,pentoses and vitamin (ascorbic acid).
- ✓ Normal carbohydrate metabolism ,phosphate esters are involved – but in uronic acid pathway free sugars and sugar acids are involved.
- ✓ Steps of reactions :

1) Formation of UDP-glucoronate


D- Glucuronic acid
Glucuronate reductase
L- Gulonate
Pentose phosphat
Pathway
Aldonolactonase
L- Gulono lactone
Gulonolactone oxidase

Clinical Aspects

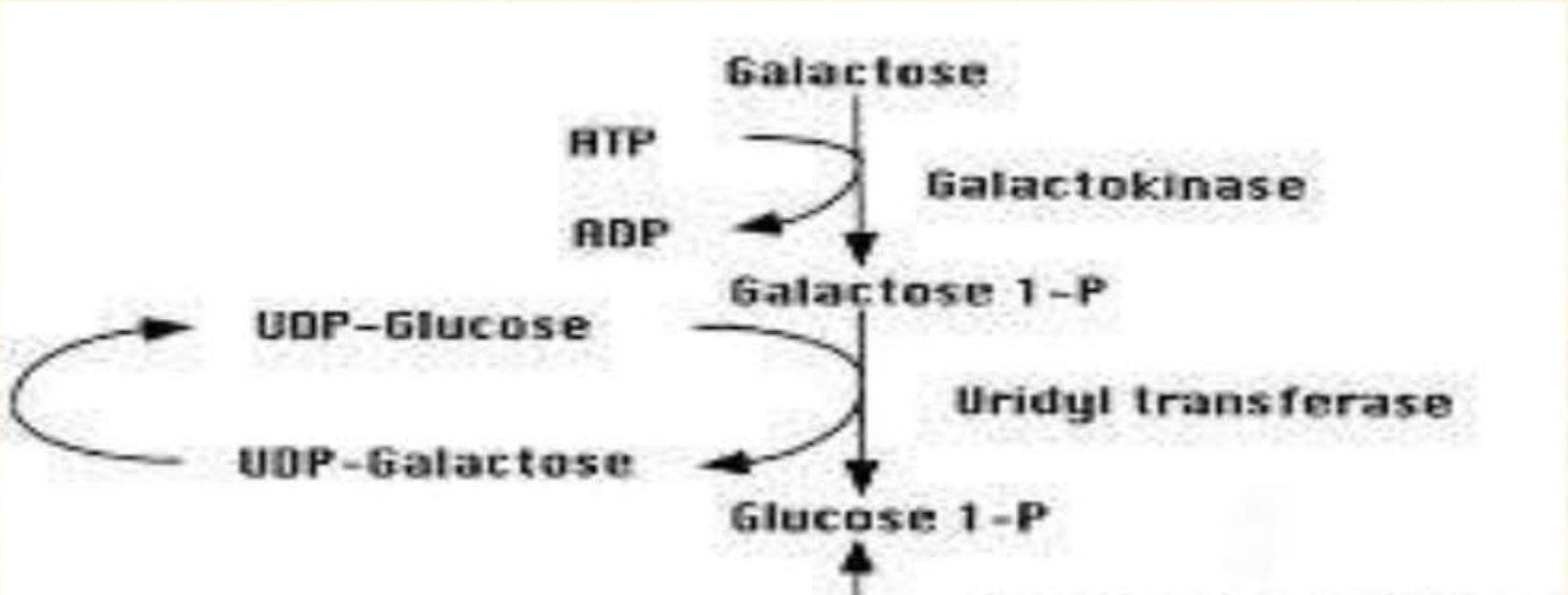
- Effects of drugs : increases the pathway to achieve more synthesis of glucaronate from glucose .

  - barbital,chloro-butanol etc.

- Essential pentosuria : deficiency of xylitol-dehydrogenase

- Rare genetic disorder

METABOLISM OF GALACTOSE



✓ Disaccharide lactose present in milk – principle source of of galactose.

✓ Lactase of intestinal mucosal cells hydrolyses lactose to galactose and glucose

✓ Within cell galactose is produced by lysosomal degradation of glycoproteins and glycolipids.

✓ CLINICAL ASPECTS :

- **Classical galactosemia** : deficiency of galactose-1-phosphate uridyltransferase. Increase in galactose level.

- **Galactokinase deficiency** : Responsible for galactosemia and galactosuria.

- **Clinical symptoms** : loss of weight in infants, hepatosplenomegaly, jaundice

METABOLISM OF FRUCTOSE

Sorbitol/Polyol Pathway:

- ✓ Conversion of glucose to fructose via sorbitol.
- ✓ Glucose to Sorbitol reduction by enzyme aldolase (NADPH).
- ✓ Sorbitol is then oxidized to fructose by sorbitol dehydrogenase and NAD^+.
- ✓ Fructose is preferred carbohydrate for energy needs of sperm cells

METABOLISM OF AMINO SUGARS

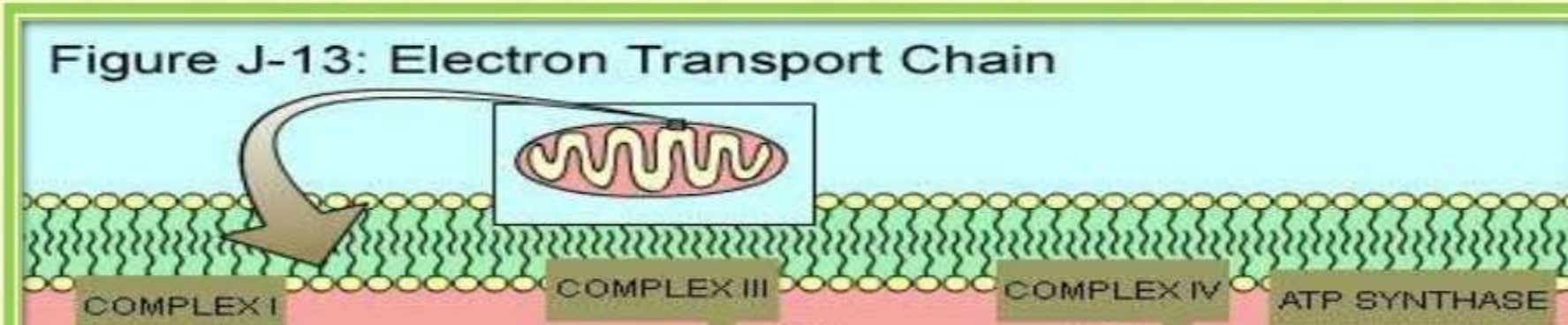
- When the hydroxyl group of the sugar is replaced by the amino group, the resultant compound is an amino sugar.

- Eg. Glucosamine,galactosamine,mannosamine,sialic acid etc.

- Essential components of glycoproteins, glycosaminoglycans, glycolipids.

ELECTRON TRANSPORT CHAIN REACTIONS

- Electron transport chain is a series of protein complexes located in the inner membrane of mitochondria .



POLYSACCHARIDES

&

CLINICAL ASPECTS

Proteoglycans & Glycosaminoglycans

✓ Seven glycosaminoglycans :

1) Hyaluronic acid
2) Chondriotin sulfate
3) Keratan sulfate I
4) Keratan sulfate II
5) Heparin

Functions of glycoaminoglycans

- Structural components of extracellular matrix.
- Act as sieves in extracellular matrix.
- Facilitate cell migration.
- Corneal transparency.

Mucopolysaccharidoses

MPS	Defect	Symptoms
MPS I (Hurler syndrome)	Alpha-L-Iduronidase	Mental retardation, micrognathia, coarse f features, macroglossia, retinal degeneratio corneal clouding, cardiomyopathy, hepatosplenomegaly
MPS II (Hunter syndrome)	Iduronate sulfatase	Mental retardation (similar, but milder, symptoms to MPS I). This type exceptiona has X-linked recessive inheritance
MPS IIIA (Sanfilippo A)	Heparan sulfate N sulfatase	

MPS	Defect	Symptoms
MPS IVA (Morquio A)	Galactose-6-sulfatase	Severe skeletal dysplasia, short stature, m dysfunction
MPS IVB (Morquio B)	Beta galactosidase	
MPS VI (Maroteaux Lamy syndrome)	N acetylgalactosamine 4 sulfatase	Severe skeletal dysplasia, short stature, m dysfunction, kyphosis, heart defects
MPS VII (Sly)	Beta glucoronidase	Hepatomegaly, skeletal dysplasia, short stature, corneal clouding, developmental d

Hunter's syndrome

- Short and broad mandible
- Localized radiolucent lesions of the jaw
- Flattened temporomandibular joints
- Macroglossia
- Conical peg-shaped teeth with generalized wide spacing

ROLE OF HORMONES IN CARBOHYDRATE METABOLISM

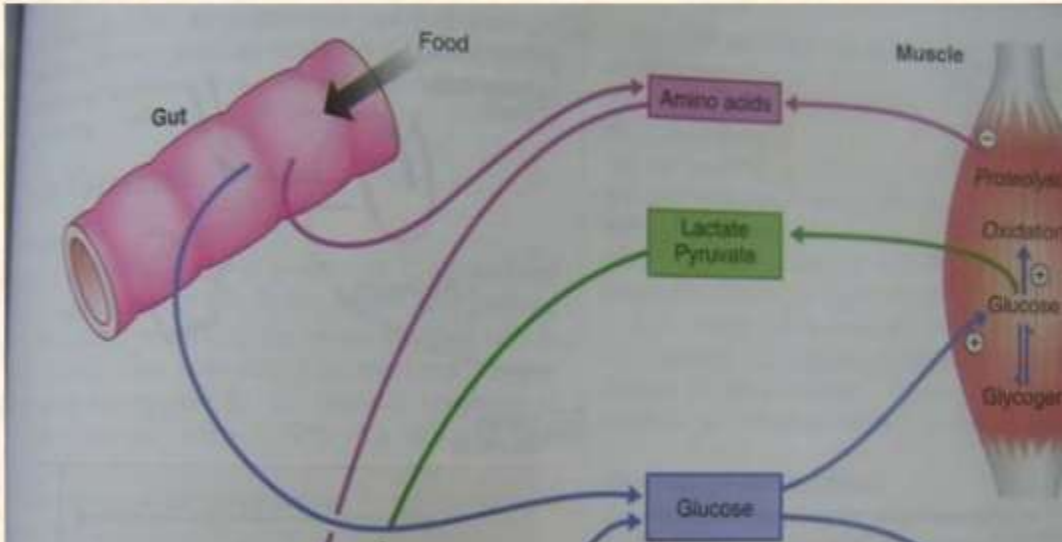
Regulation of Blood glucose

- Postabsorptive state: Blood glucose is 4.5-5.5mmol/L.
- After carbohydrate meal: 6.5-7.2mmol/L
- During fasting : 3.3-3.9mmol/L

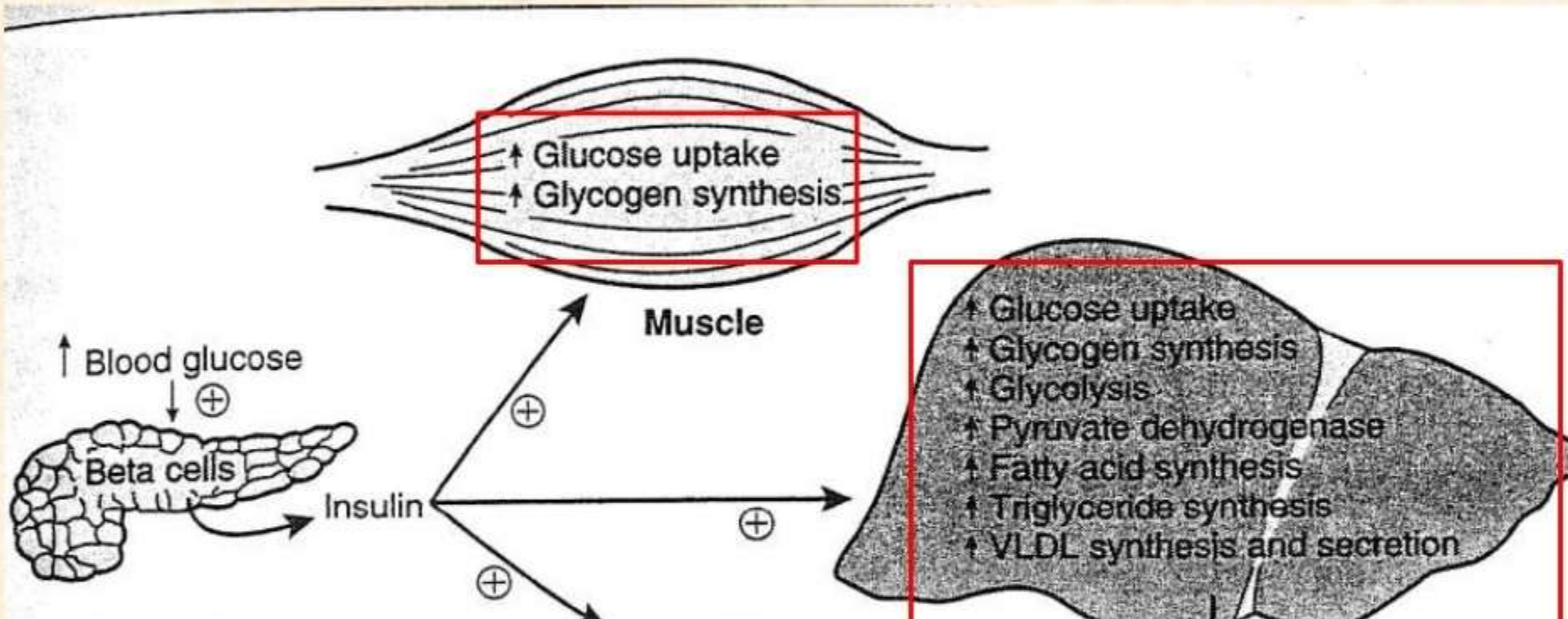
Metabolic & hormonal mechanisms regulate blood glucose level

Maintenance of stable levels of glucose in blood is by

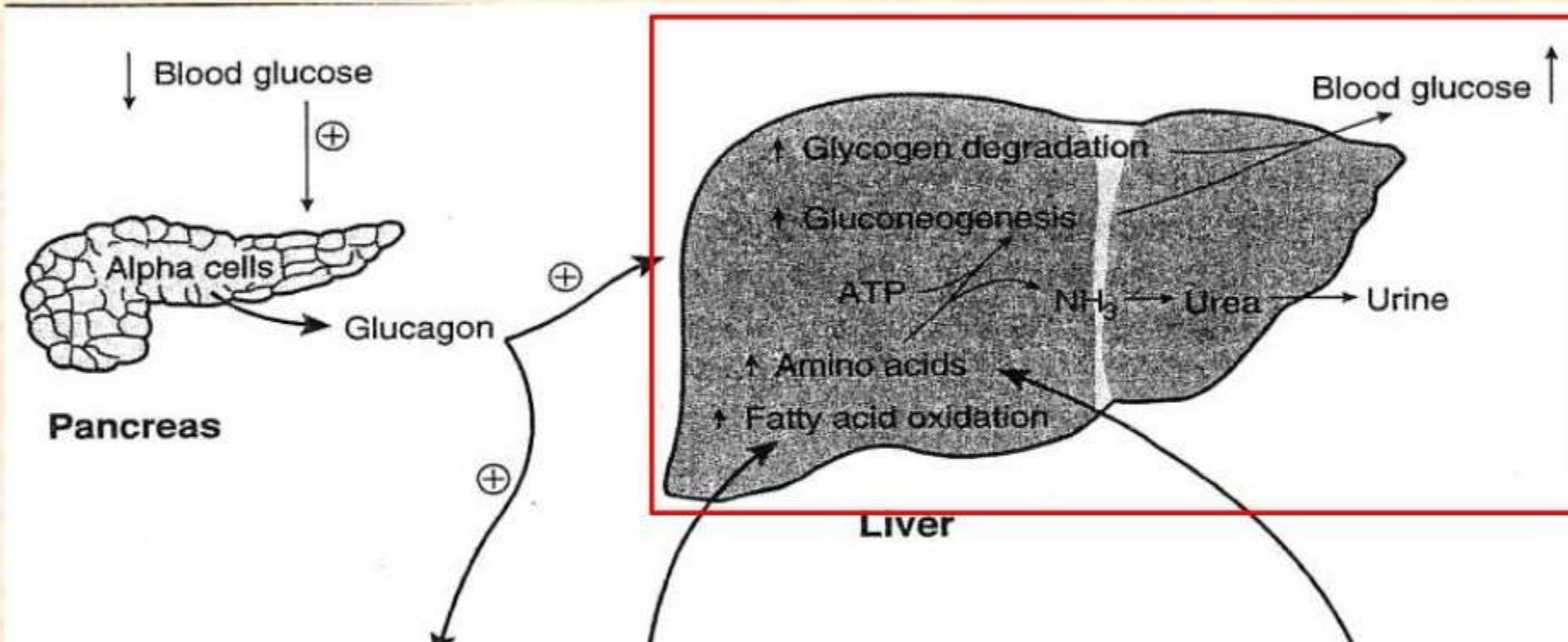
- ✓ Liver.
- ✓ Extrahepatic tissues.
- ✓ Hormones.



Regulation of blood glucose levels
Insulin



Role of glucagon



Role of thyroid hormone

✓ It stimulates glycogenolysis & gluconeogenesis.

Hypothyroid

- Fasting blood glucose is lowered.
- Patients have decreased ability to utilise glucose.

Hyperthyroid

- Fasting blood glucose is elevated
- Patients utilise glucose at normal or

Glucocorticoids

- ✓ Glucocorticoids are antagonistic to insulin.
- ✓ Inhibit the utilisation of glucose in extrahepatic tissues.

Epinephrine

- ✓ Secreted by adrenal medulla.
- ✓ It stimulates glycogenolysis in liver & muscle.

Other Hormones

- Anterior pituitary hormones

Growth hormone:

- ✓ Elevates blood glucose level & antagonizes action of insulin.
- ✓ Growth hormone is stimulated by hypoglycemia

SEX HORMONES

- ✓Estrogens cause increased liberation of insulin.

- ✓Testosterone decrease blood sugar level

Hyperglycemia

- Thirst, dry mouth
- Polyuria
- Tiredness, fatigue
- Blurring of vision.
- Nausea, headache,
- Hyperphagia
- Mood change

Hypoglycemia

- Sweating
- Trembling,pounding heart
- Anxiety, hunger
- Confusion, drowsiness
- Speech difficulty
- Incoordination.

Clinical aspects

- ✓ Glycosuria: occurs when venous blood glucose concentration exceeds 9.5-10.0mmol/L

Diabetes Mellitus

A multi-organ catabolic response caused by insulin insufficiency

Muscle

- Protein catabolism for gluconeogenesis

Adipose tissue

- Lipolysis for fatty acid release

Liver

- Ketogenesis from fatty acid oxidation

DENTAL ASPECTS OF CARBOHYDRATES METABOLISM

Role of carbohydrates in dental caries

- Fermentable carbohydrates causes loss of caries resistance.
- Caries process is an interplay between oral bacteria, local carbohydrates & tooth surface

Bacteria + Sugars+ Teeth ⟶ Organic acids

Role of carbohydrates in periodontal disease

Abnormal glucose metabolism

↓

Diabetes Mellitus

↓

Excessive carbohydrate intake

↓

Obesity

↓

RECENT CLINICAL ISSUES RELATED TO CARBOHYDRATES METABOLISM

Cystic Fibrosis

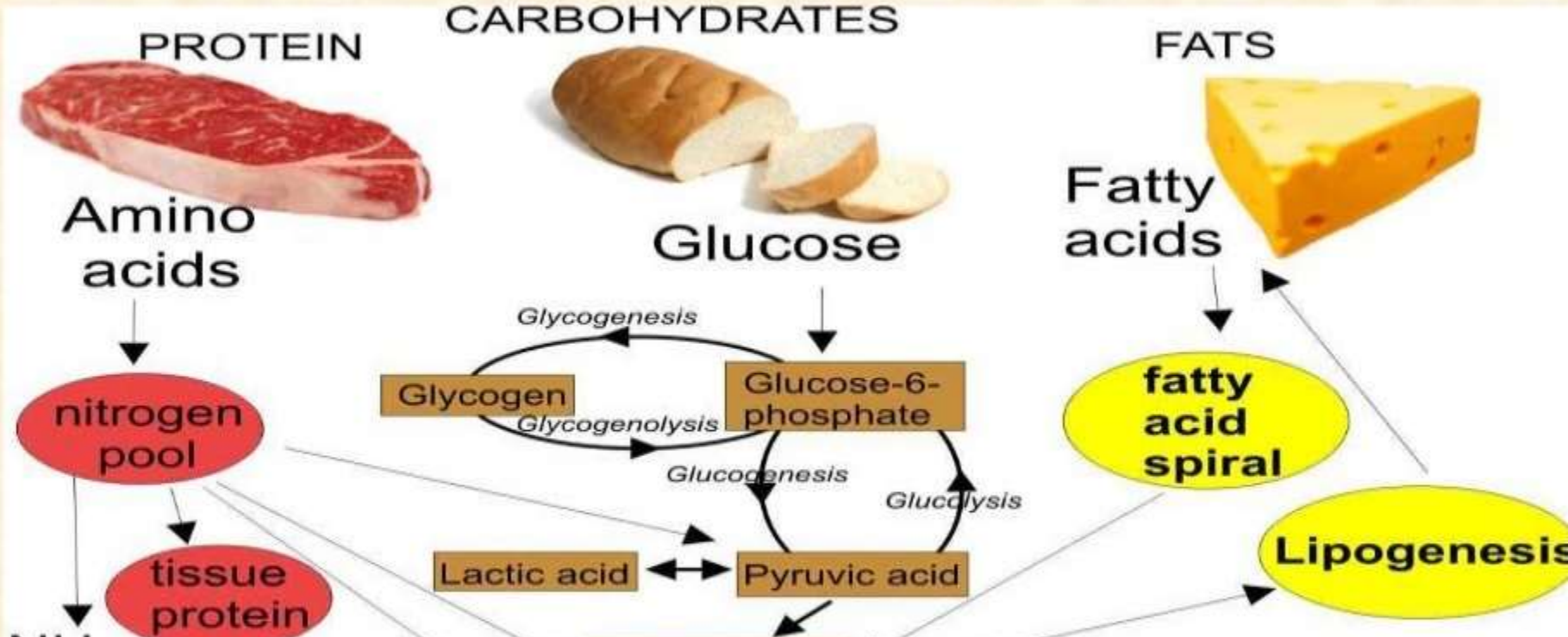
- CMD in Cystic Fibrosis is characterized by its high rates and latent course.
- The patients with CMD have retarded physical development, more pronounced morphofunctional disorders in the bronchopulmonary system, lower lung

CMD in Gout

- OGTT causes a 34% increase in the detection rate of T2D in patients with gout.

- Carbohydrate metabolic disturbances are revealed in the majority of patients with gout and associated with

SUMMARY OF CARBOHYDRATE METABOLISM



PER DAY INTAKE OF CARBOHYDRATE

- Carbohydrate Calculator http://www.calculator.net/carbohydrate-calculator.html?ctype=metric&cage=25&csex=f&cheightfeet=5&cheightinch=10&cpound=160&cheightmeter=163&ckg=74&cactivity=1.375&x=85&y=10#

CONCLUSION

- Carbohydrate are the measure source of energy for the living cells. Glucose is the central molecule in carbohydrate metabolism, actively participating in a number of metabolic pathway.
- One component of etiology of dental caries is

REFERENCES

1) Biochemistry – U.Satyanarayana-3rd Ed.

2) Textbook of Biochemistry- D.M.Vasudevan -14th Ed.

3) Textbook of Medical Biochemistry – M.N.Chattergy – 17th Ed.

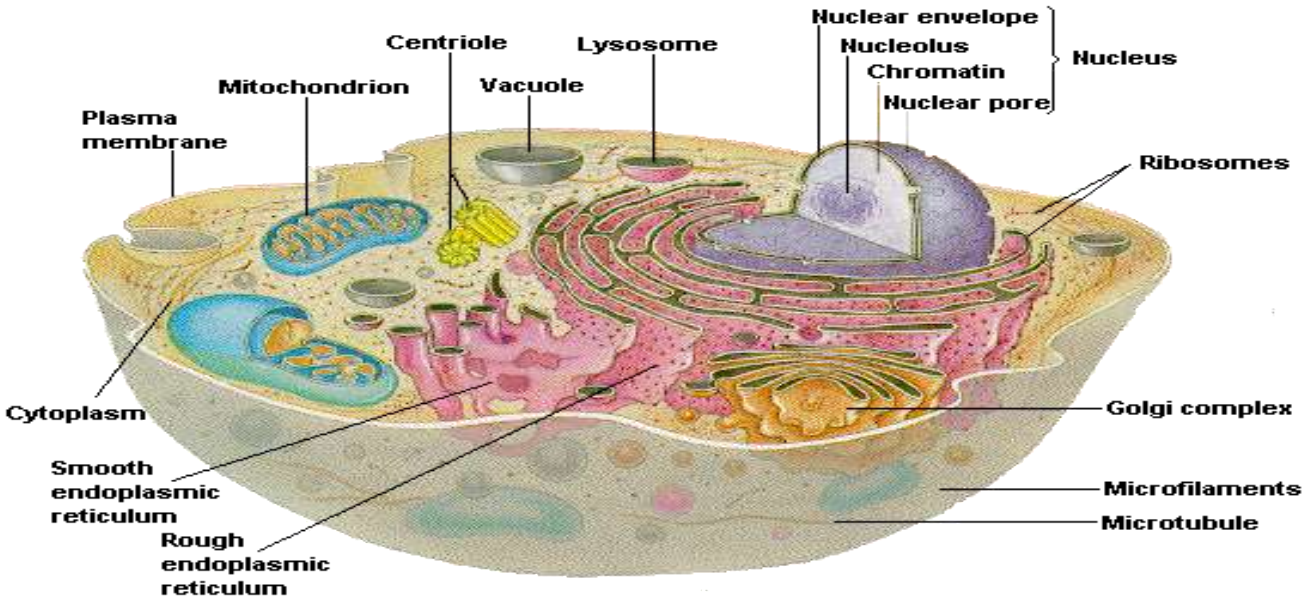
4) Text book of Physiology –Ganong – 24th Ed.

THANK
YOU

Unit-I

Cells:

The Basic Units of Life



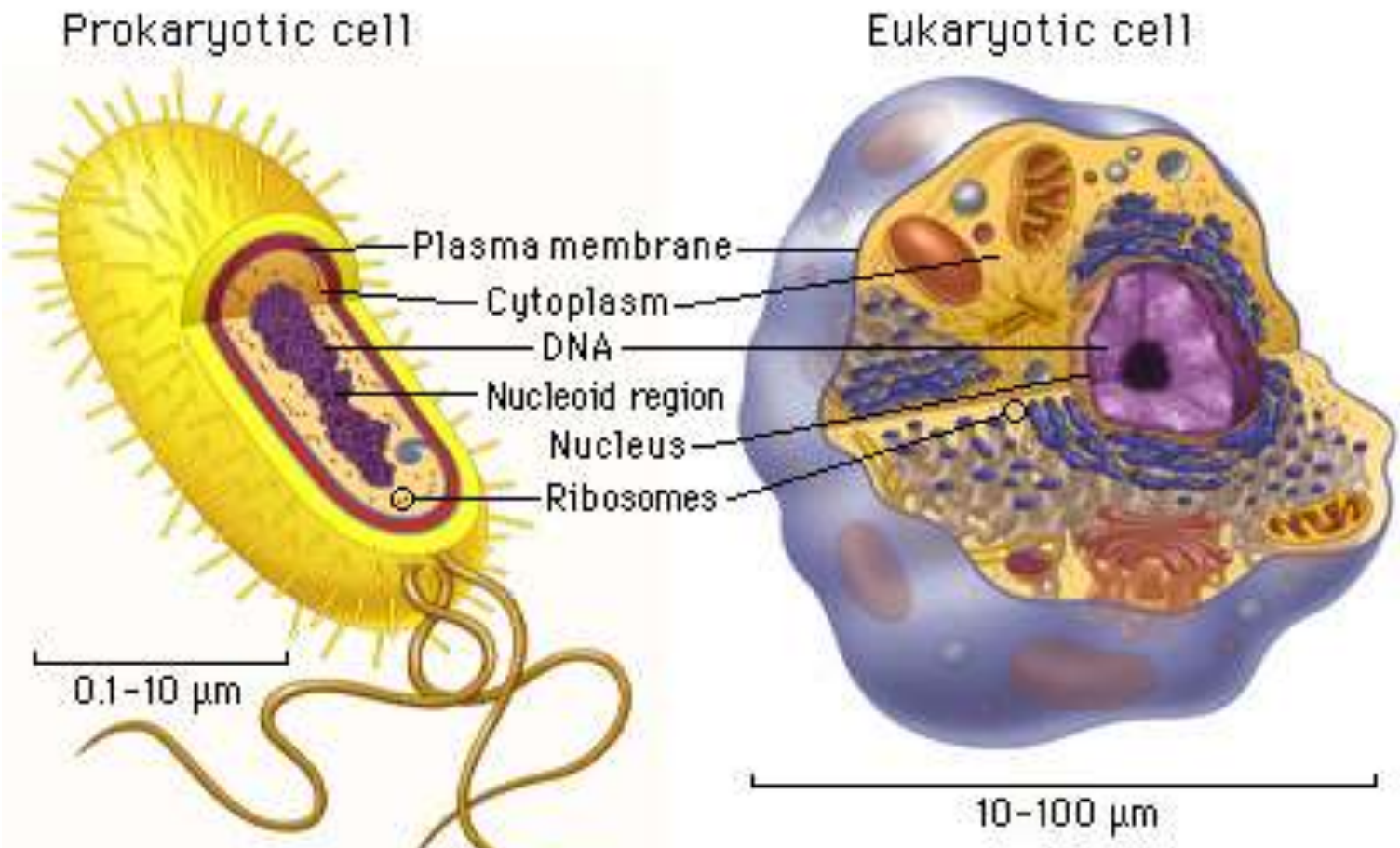
Presented By
Mr. Krunal Kanase
HOD , Department of Pharmacology
PDEA's Shankarrao Ursal College of Pharmaceutical Sciences & Research Center, Kharadi.

The Cell Theory

1. All organisms are made of cells.
2. The cell is the basic unit of life in all living things.
3. All cells come from existing cells.

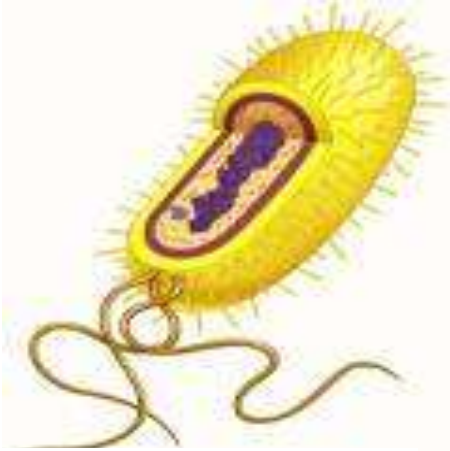
THIS IS IMPORTANT BECAUSE IT SHOWS THAT ALL LIVING THINGS SHARE A SIMILAR STRUCTURE

Two Types of Cells



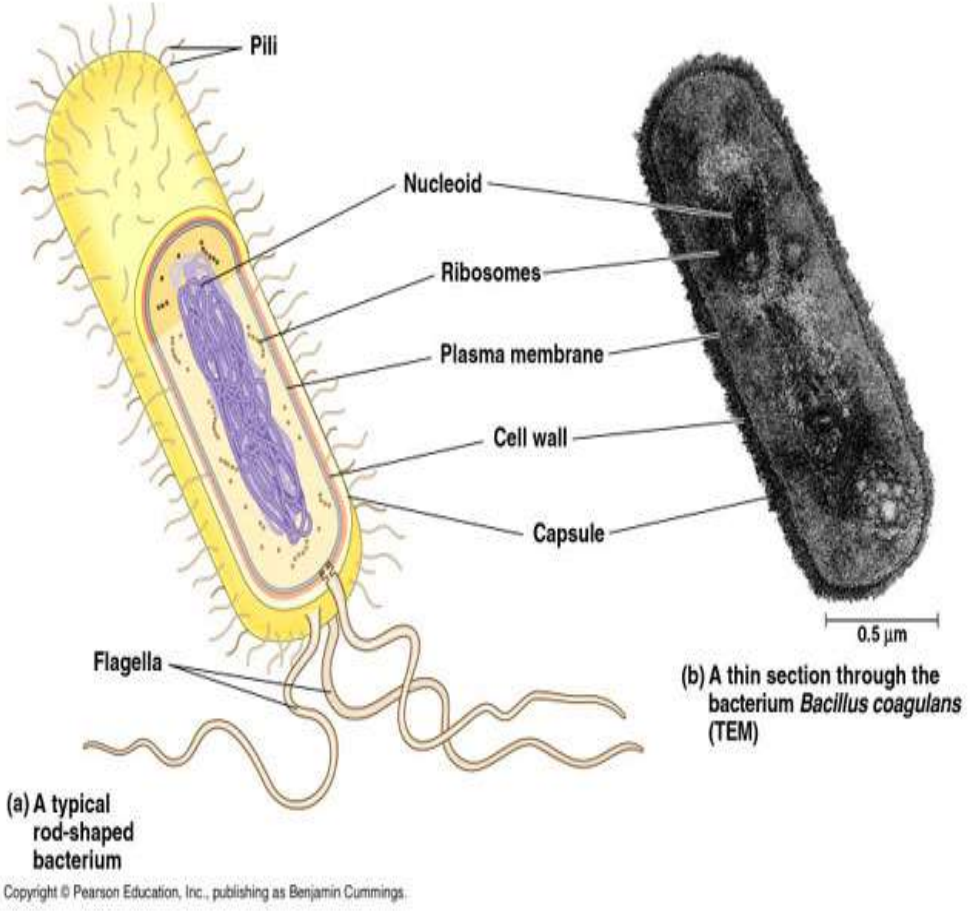
All cells, whether they are prokaryotic or eukaryotic, have some common features

Two Types of Cells



Prokaryotic Cells:

- Have no membrane covered nucleus
- Have no membrane - covered organelles
- Have circular DNA
- Are bacteria



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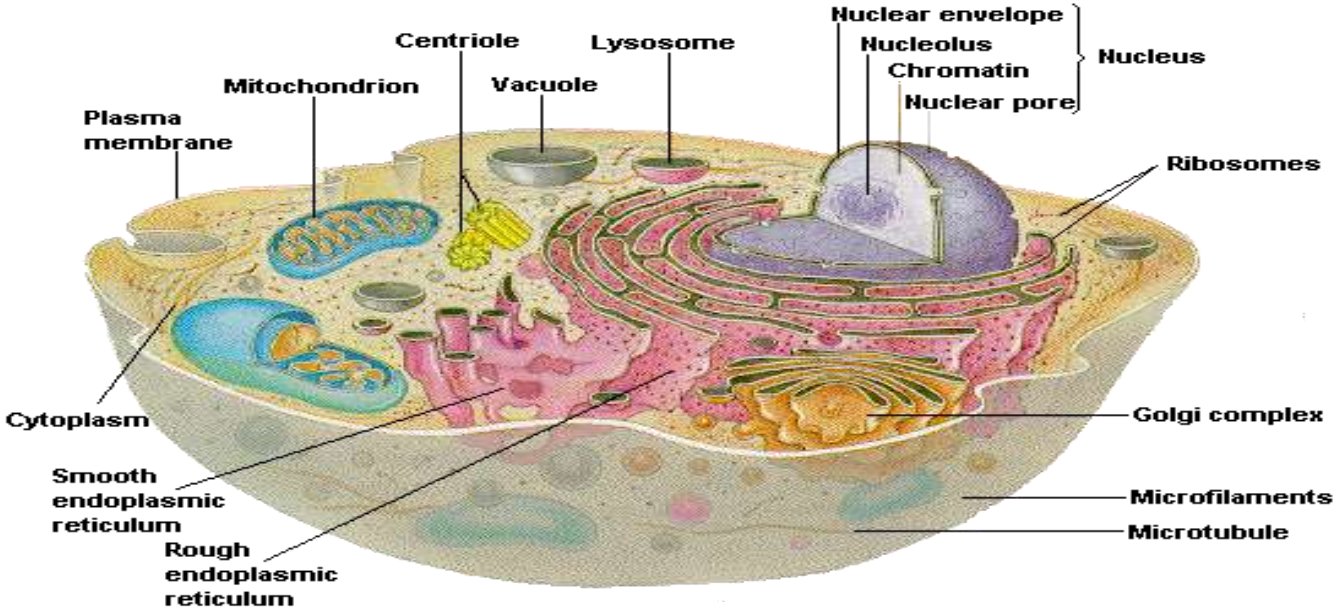
Two Types of Cells

Eukaryotic Cells:

- Have a nucleus
- Have a membrane - covered organelles
- Have linear DNA
- Are all other cells

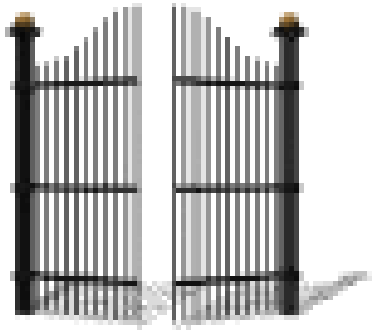
Organelles

Organelles are structures that enable the cell to live, grow and reproduce.

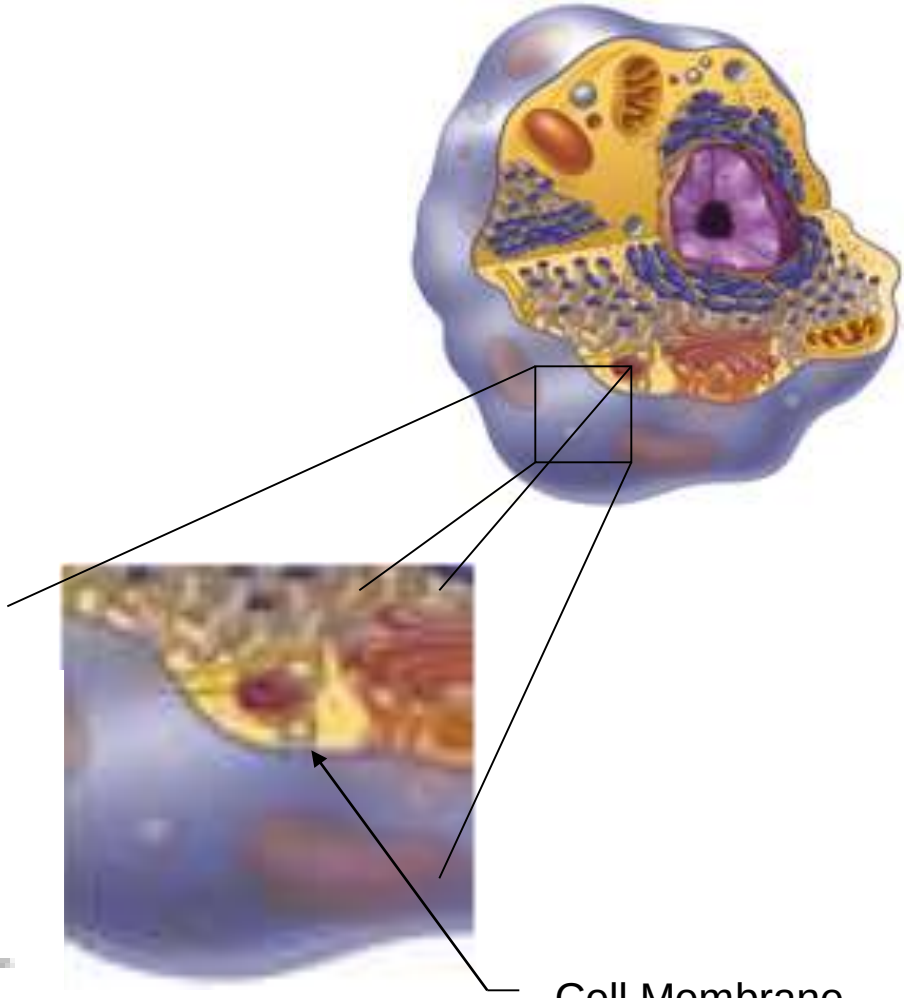


Cell Membrane

- Outer layer of cell
- Allows nutrients into the cell and wastes outside of the cell

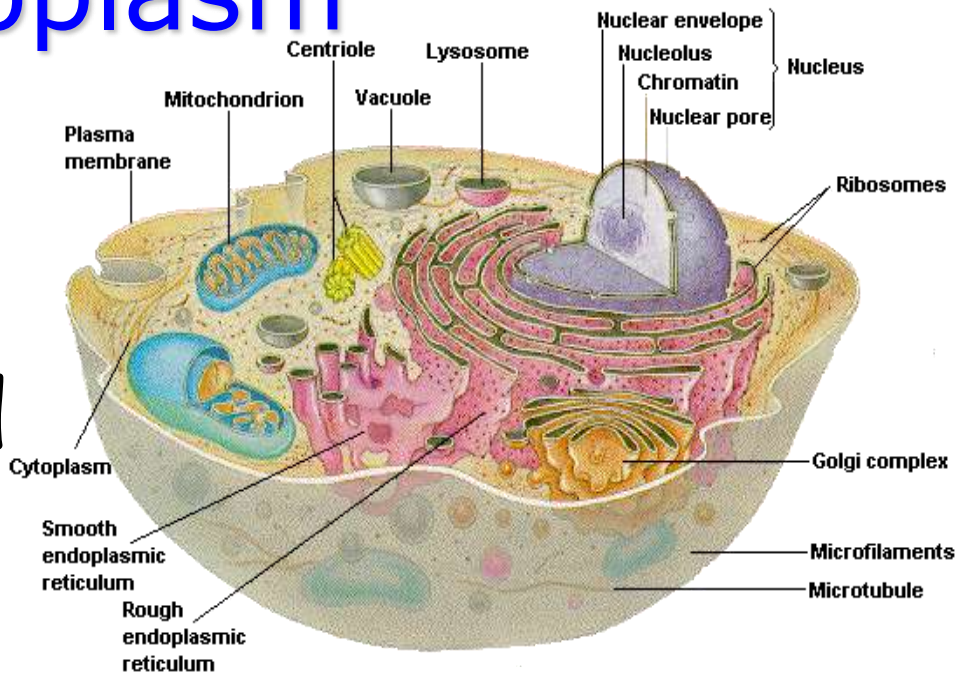


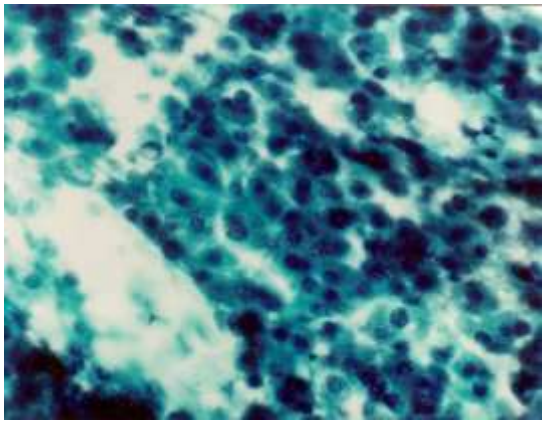
"Gate into the city"



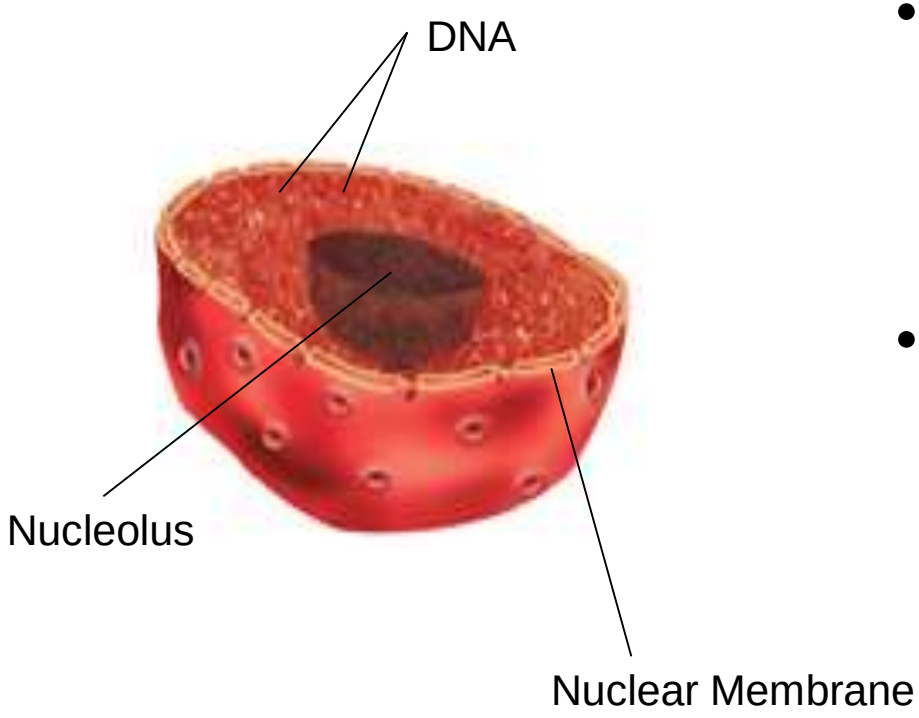
Cytoplasm

- Cytoplasm a jelly-like fluid contained in the cell that holds the organelles.





The Nucleus



- The control center of the cell
- Contains the Cell's DNA

"Mayor's office"

Mitochondria

- Power center of cell
- Provides the energy the cell needs to move, divide, etc.

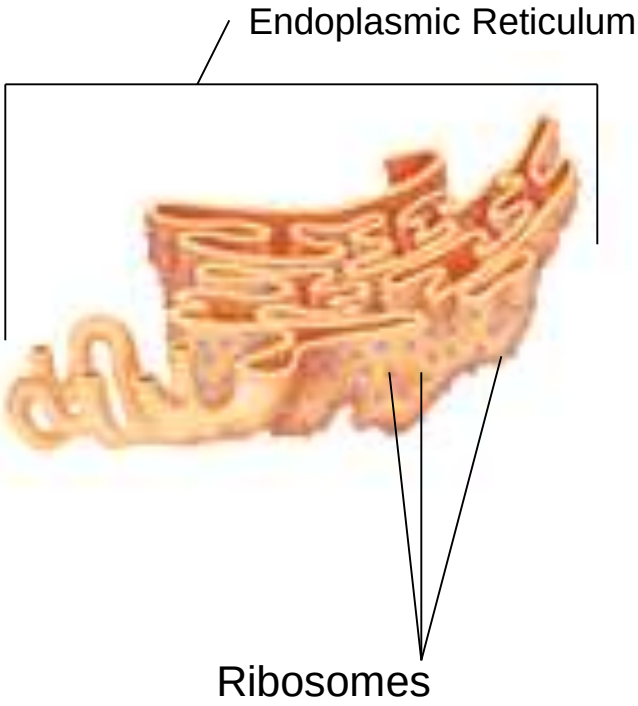
"Electric company of the cell"

Ribosomes

- Site where proteins are made
- Cell parts are made of proteins

"Factories of the cell"

Endoplasmic Reticulum



- **Transportation system of cell**
- **Rough ER- ribosome's attached**
- **Smooth ER- no ribosome's**

"Roadways of the cell"

Golgi Complex

- **Packaging house of cell**
- **Packages, processes, and ships out the stuff the cell makes**

"UPS of the cell"

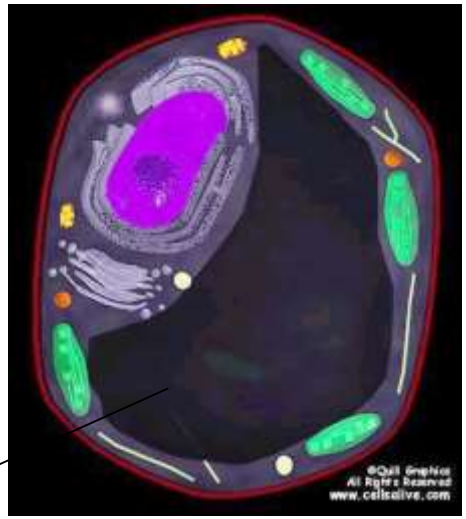
Lysosomes

- Digests food particles and cell parts
  - "Garbage men"
- Protects cell by digesting foreign invaders
  - "Police men

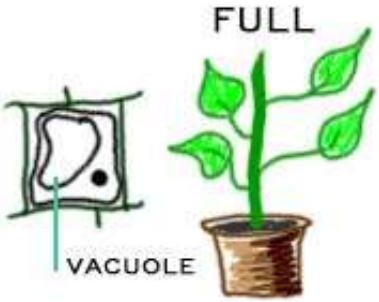
Vacuole

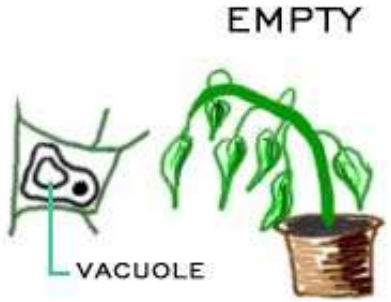
Vacuole

- Stores water, food & wastes



Vacuole is largest organelle in plant cell





Cell Wall

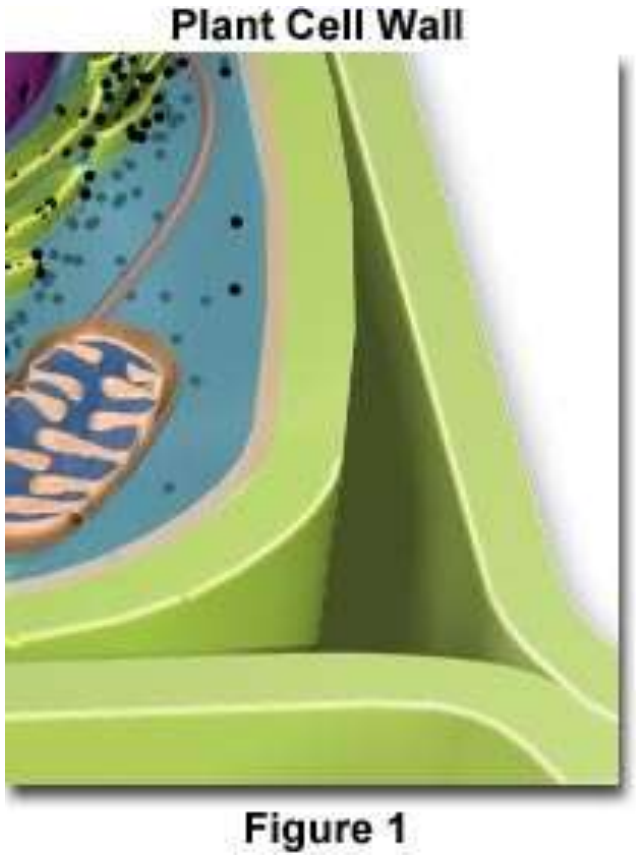
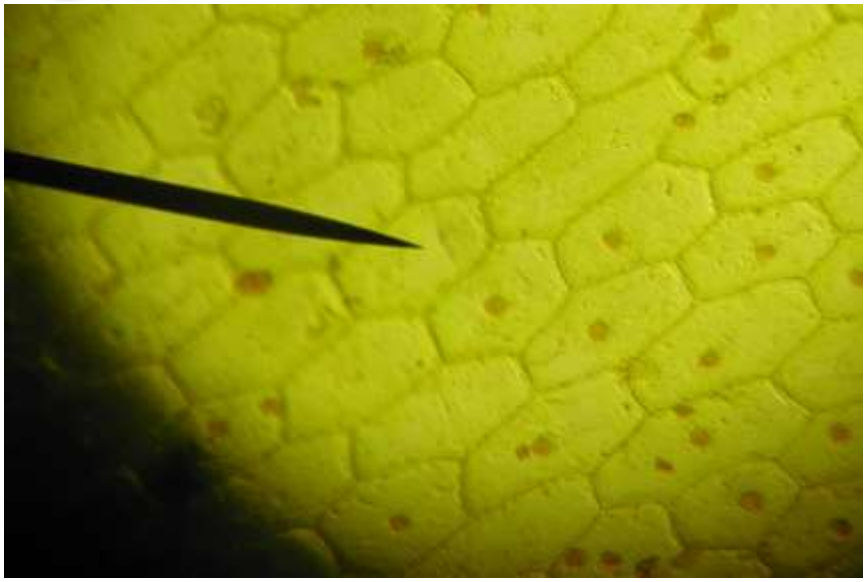
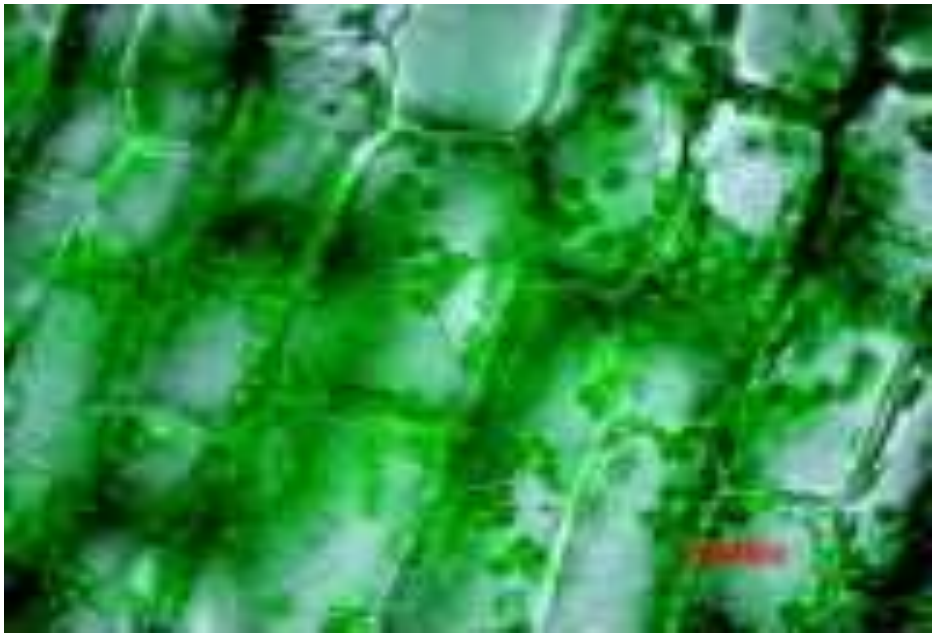


Figure 1

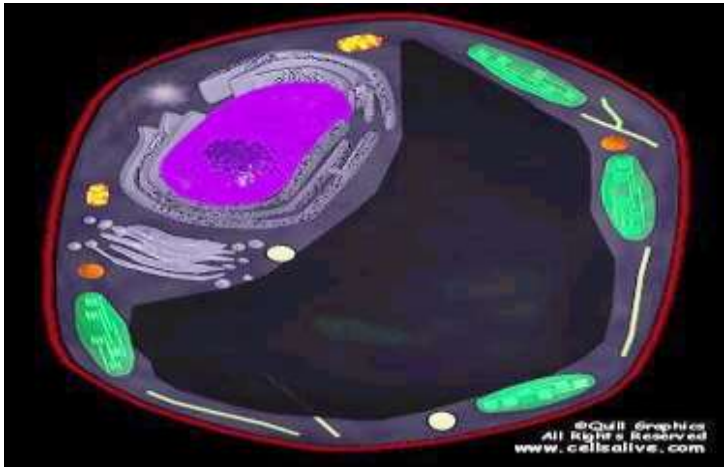


- Found only in plant cells
- Protects and supports the cell

Chloroplasts



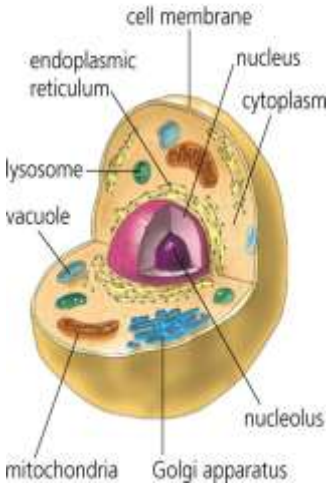
- Found only in plant cells
- Contains chlorophyll (makes plants green)
- Where photosynthesis takes place



Plant or Animal Cell?

Found in Plant and Animal cells:

- Nucleus
- Golgi Complex
- Mitochondrion
- Lyosomes
- Endoplasmic Reticulum
- Cell Membrane
- Ribosomes
- Vacuoles



Found only in Plant Cells:

- Chloroplasts
- Cell Wall

Classification of Microorganisms

Presented by

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Types of Classification

Cell Nature

Prokaryotic

Nucleus and cell organelles are not demarcated

Eukaryotic

Clear nucleus and membrane-bound cell organelles

Cell Occurrence

Unicellular

Made up of single cell

Multicellular

Multiple cells in clusters to form specialized tissue and organs

Mode of Nutrition

Autotrophs

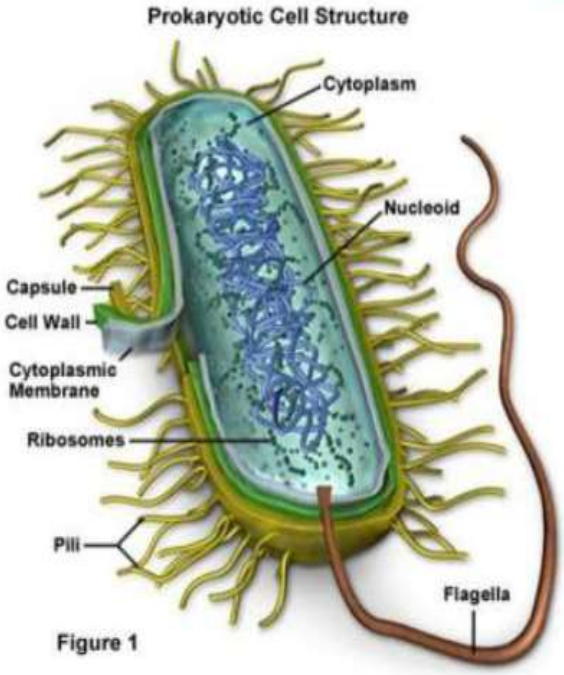
Make their own food

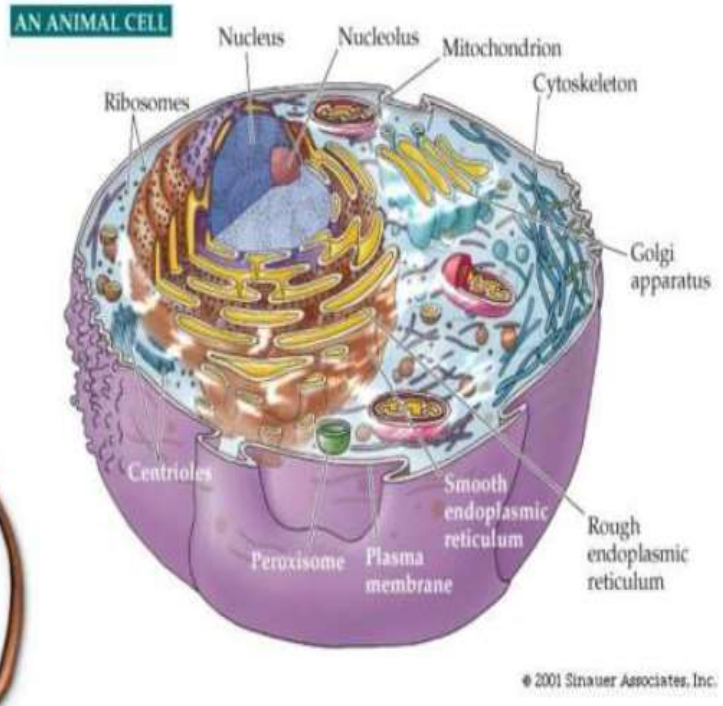
Heterotrophs

Depend on others for food

Level of Body Organization

Prokaryotic vs Eukaryotic Cells





Basic structural and functional unit of life: Cell

	Prokariyotic cell	Eukariyotic cell
Organisms	Bacteria	Algae, Fungi, Protozoa, Plants, Animals
Size	1-4 µm	>5 µm
Nucleus	Nuclear membrane absent, Nucleolus absent	Nucleus bounded by nuclear membrane, Nucleolus present
Cell wall	Peptidoglycan present	Peptidoglycan absent
Plasma membrane	Sterols absent, Cytoplasmic streaming absent	Sterols present, Cytoplasmic streaming present
Membrane-enclosed organelles	Absent	Present
Ribosome	70 S	80 S

Classification of Micro-organisms

Taxonomy: It is the science that deal with logical arrangement of living thing into categories .

1)Aristotle classified living thing into plant and animals

2)Karls linaus classified living thing into kingdom Plantae and Animalia.

He also devised binomial (genus and species) scheme of nomenclature.

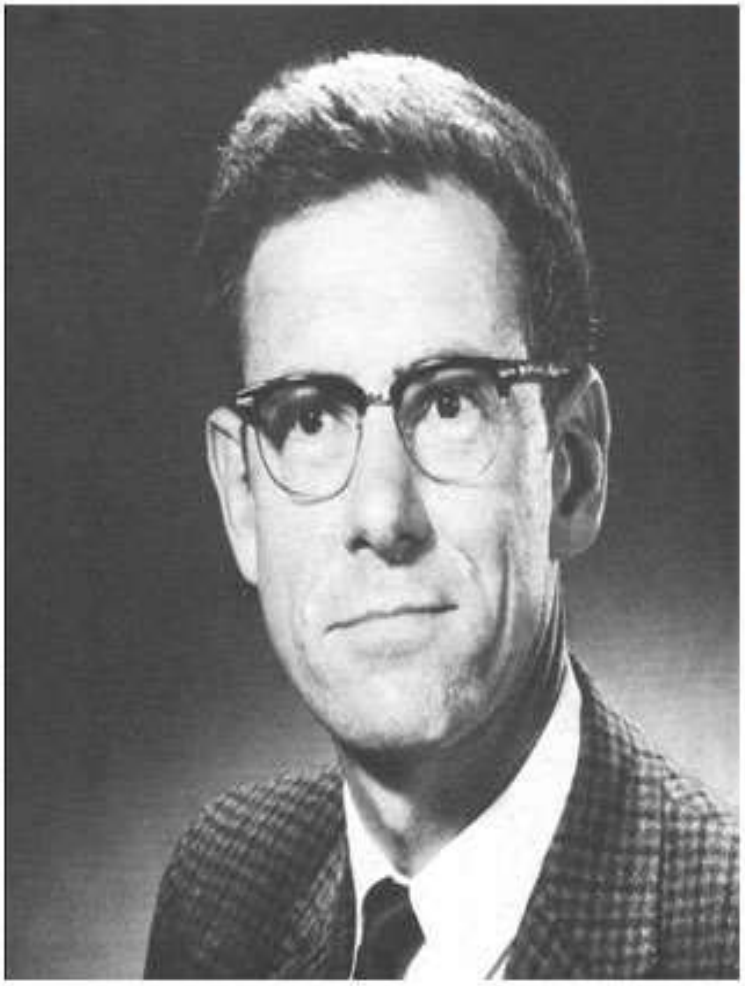
3) In 18th century the German zoologist E.H. Haeckel proposed 3rd kingdom named as the protista.

- Include unicellular m.o. typically they are neither plant nor animal.
- Protista, include bacteria, fungi, algae, protozoa but virus not classified as protista.
- Bacteria is classified as a lower protista and fungi, algae, protozoa classified as a higher protista.

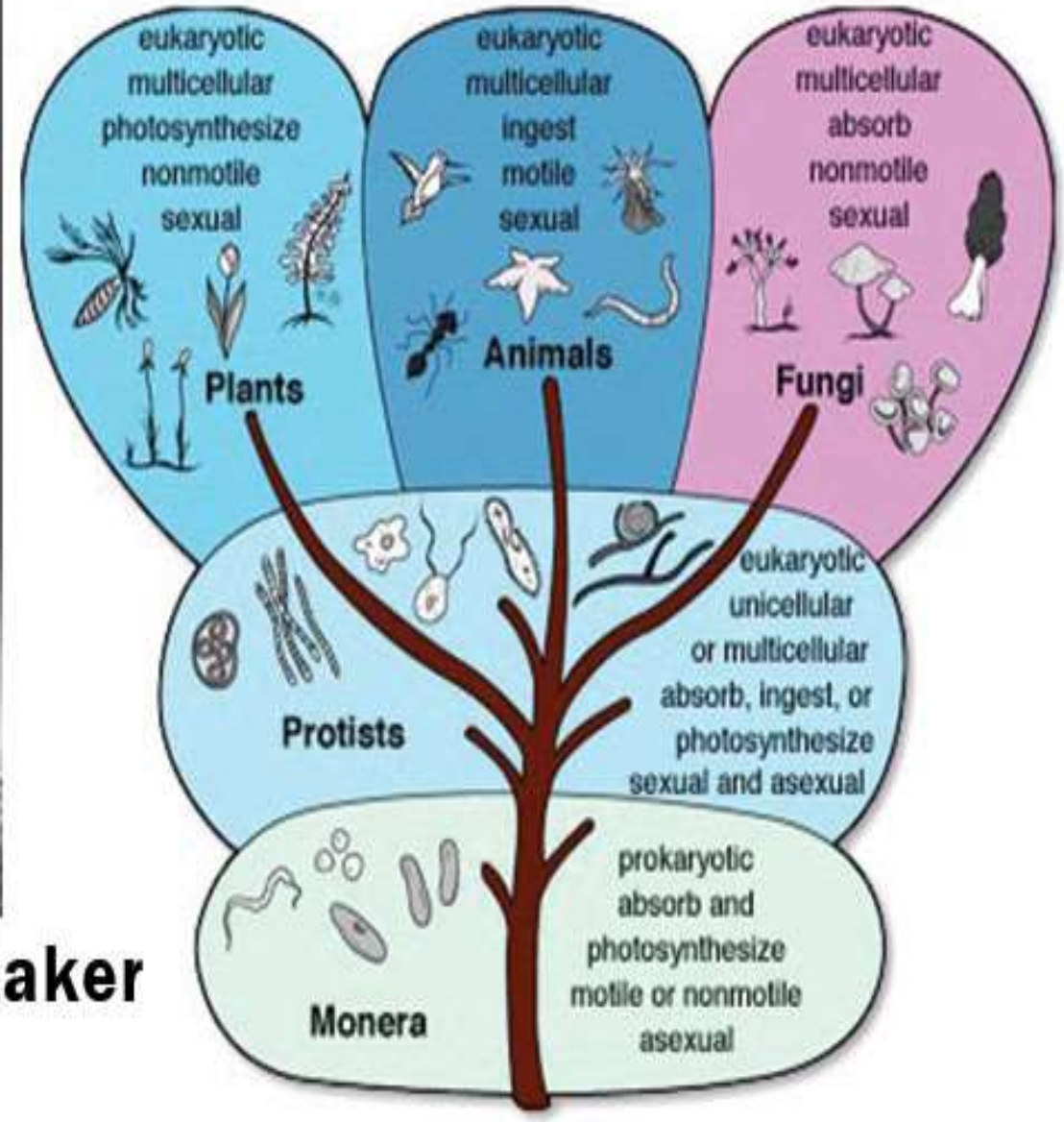
- In 1940 it was discovered in some cell e.g typical bacteria, the genetic material was not enclosed by nuclear membrane.
- In other cells i.e (algae, fungi, protozoa) the nucleus was enclosed in membrane this resulted into division of organism Prokaryotes Eukaryotes
- Bacteria are prokaryotic microbes (absence of nuclear membrane)
- Fungi, algae, protozoa, plant and animal cell are eukaryotic microbes (presence of nuclear membrane)

Table 2.1. Summary of the equivalences in Kingdom classification

<table>
<tr><th>Linnaeus
1735
2 kingdoms</th><th>Haeckel
1866
3 kingdoms</th><th>Chatton
1937
2 empires</th><th>Copeland
1956
4 kingdoms</th><th>Whittaker
1969
5 kingdoms</th><th>Woese et al.
1977
6 kingdoms</th><th>Woese et al.
1990
3 domains</th></tr>
<tr><td rowspan="2">(not treated)</td><td rowspan="2">Protista</td><td rowspan="2">Prokaryota</td><td rowspan="2">Monera</td><td rowspan="2">Monera</td><td>Eubacteria</td><td>Bacteria</td></tr>
<tr><td>Archaebacteria</td><td rowspan="3">Archaea</td></tr>
<tr><td rowspan="3">Vegetabilia</td><td rowspan="3">Plantae</td><td rowspan="4">Eukaryota</td><td rowspan="2">Protoctista</td><td>Protista</td><td>Prostista</td></tr>
<tr><td>Fungi</td><td>Fungi</td></tr>
<tr><td>Plantae</td><td>Plantae</td><td>Plantae</td><td>Eukarya</td></tr>
<tr><td>Animalia</td><td>Animalia</td><td>Animalia</td><td>Animalia</td><td>Animalia</td><td></td></tr>
</table>



Robert Harding Whittaker
(1920 – 1980)



The Three Domains of Life
(Carl Woese, 1990)

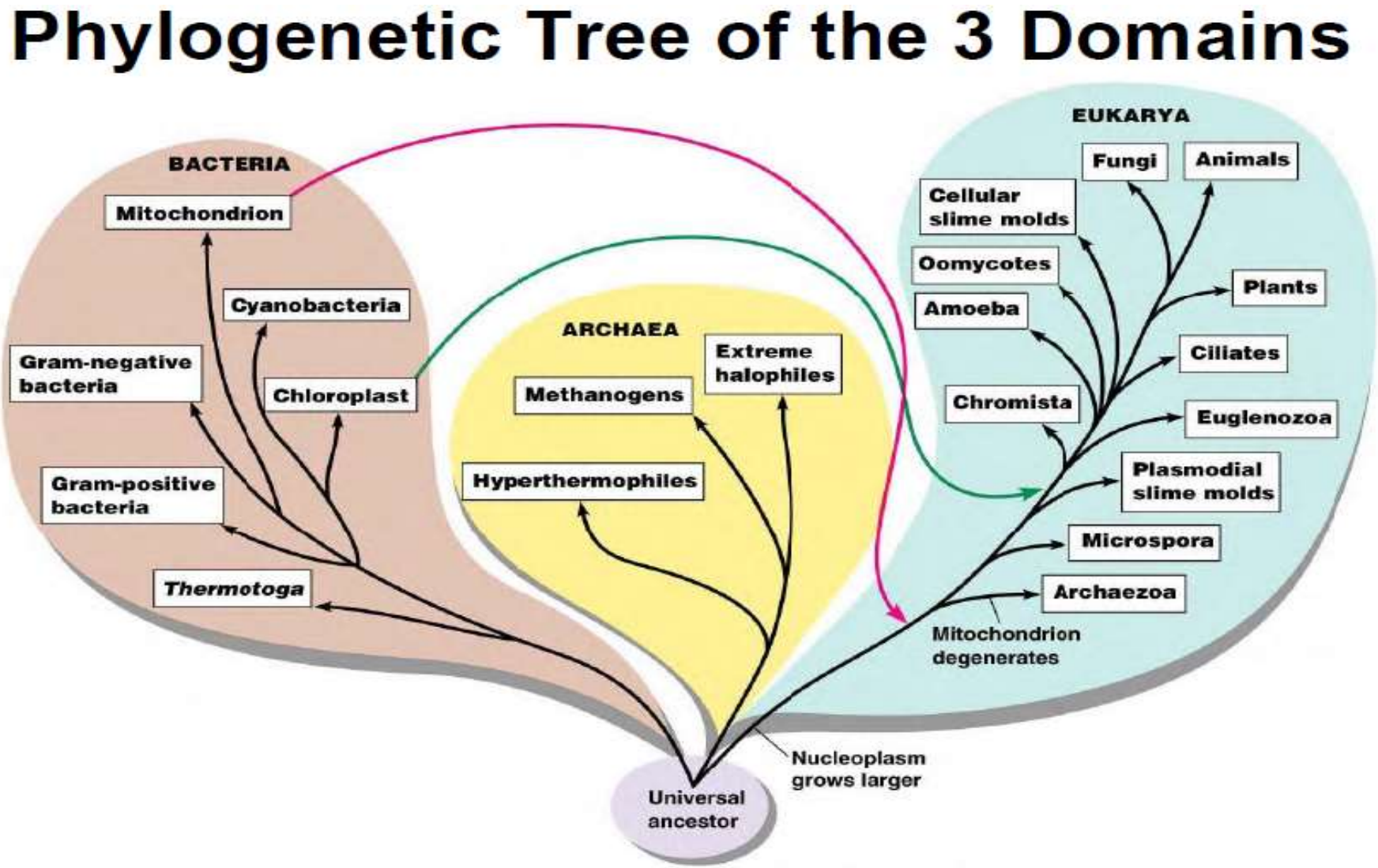
Starting in the early 1970s, Dr. Carl Woese, began to find evidence for a previously unknown group of prokaryotic organisms.

These organisms lived in **extreme** environments **-** deep sea hydrothermal vents, "black smokers", hot springs, the **Dead Sea, acid lakes, salt evaporation ponds -** environments that scientists had never suspected would contain a profusion of life!

Because they appeared prokaryotic, they were considered bacteria and named "**archaebacteria**" ('ancient' bacteria).

However, became obvious from biochemical characteristics and DNA sequence analysis (Genetics) that there were **numerous** differences between these archaebacteria and other bacteria. Before long, it was realized that these archaebacteria were **more closely related to the eukaryotes** than to bacteria. Today, these bacteria have been renamed **Archaea**.

The three-domain system is a biological classification introduced by Carl Woese



	ARCHAEA	BACTERIA	EUKARYA
CELL TYPE	PROKARYOTIC	PROKARYOTIC	EUKARYOTIC
CELL WALL	• VARIES IN COMPOSITION. • CONTAINS NO PEPTIDOGLYCAN	• CONTAINS PEPTIDOGLYCAN	• VARIES IN COMPOSITION • CONTAINS CARBOHYDRATES
MEMBRANE LIPIDS	COMPOSED OF BRANCHED CARBON CHAINS ATTACHED TO GLYCEROL BY ETHER LINKAGE	• COMPOSED OF STRAIGHT CARBON CHAINS ATTACHED TO GLYCEROL BY ESTER LINKAGE	• COMPOSED OF STRAIGHT CARBON CHAINS ATTACHED TO GLYCEROL BY ESTER LINKAGE
FIRST AMINO ACID IN PROTEIN SYNTHESIS	METHIONINE	FORMYLMETHIONINE	METHIONINE
T RNA LOOP	LACKING	PRESENT	LACKING

Major Groups of Microorganisms

1. Bacteria
2. Viruses
3. Fungi
4. Protozoa
5. Algae

Bacteria

Size:

- Most bacteria range from 0.2–5.0 µm (micrometers) in diameter.

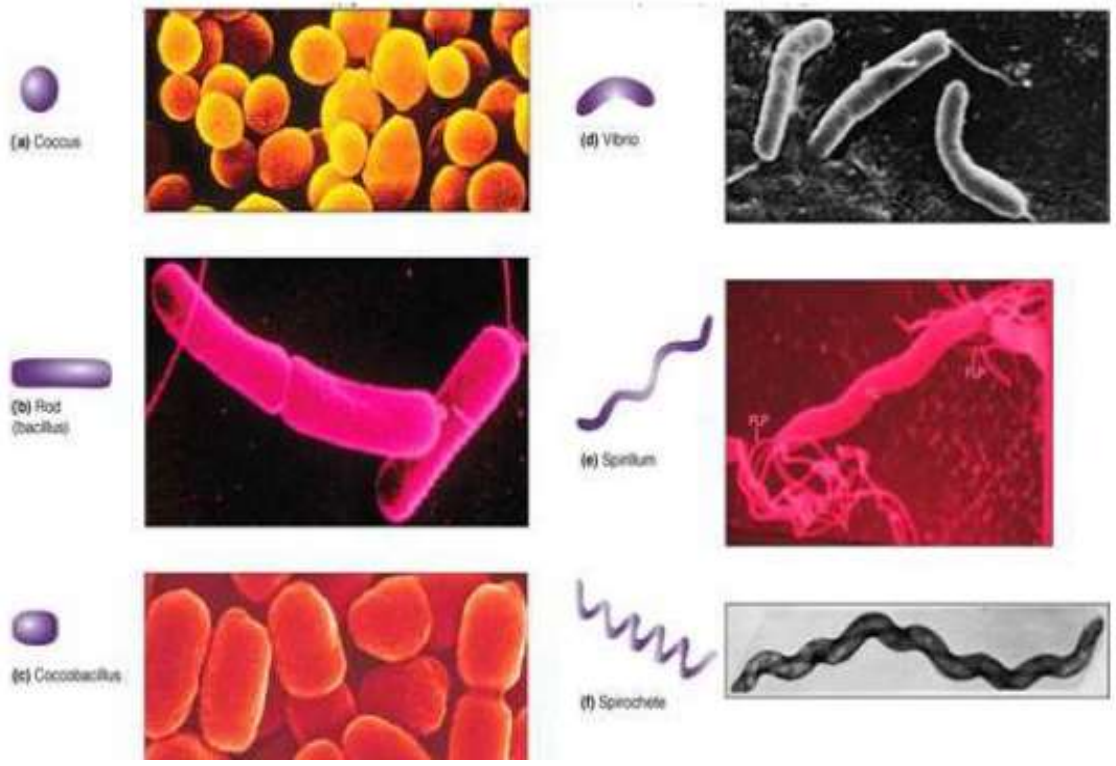
Important Characteristics:

- Prokaryotic
- Unicellular
- Simple Internal structure
- Grow on artificial laboratory media
- Reproduction asexual (mostly simple cell division, binary fission)

Practical significance:

- Some cause diseases
- Some perform role in natural cycling of elements and increase soil fertility
- Manufacture of valuable compounds in Industry

bacillus (rod)
coccus (spherical)
spirillum (spiral)
spirochaete (corkscrew)
vibrio (comma)
chain of cocci
cluster of cocci
pair of cocci
chain of bacilli


(a) Coccus
(d) Vibrio
(b) Rod (bacillus)
(e) Spirillum
(c) Coccobacillus
(f) Spirochete

Viruses

Size:

0.015-0.2 µm

Important Characteristics:

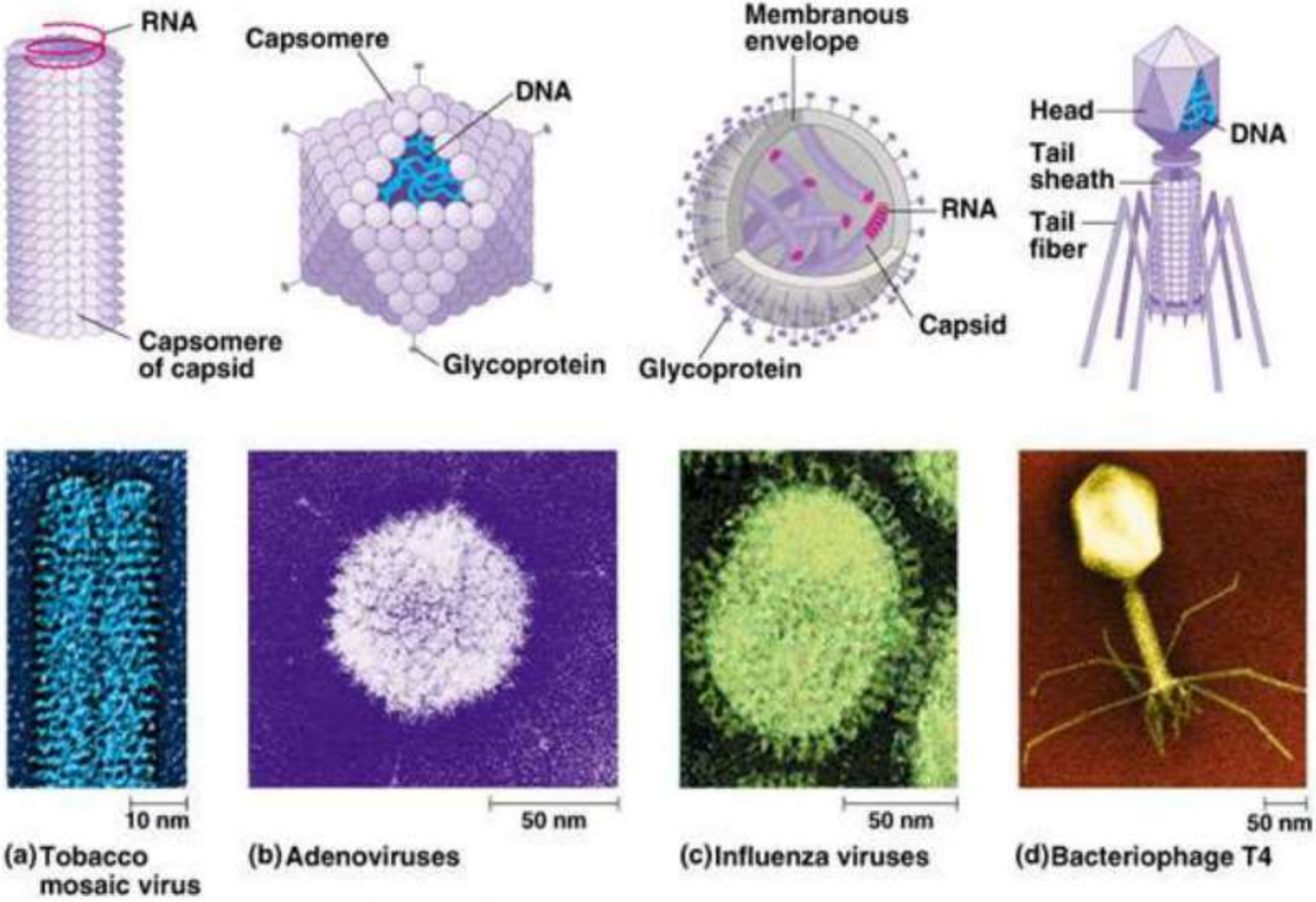
- Do not grow on artificial media

require living cells within which they reproduce

- Obligate intracellular parasites
- Electron microscopy required to observe

Practical significance:

- Cause diseases in humans animals & plants
- Also infect microorganisms


RNA
Capsomere of capsid
Capsomere
DNA
Glycoprotein
Membranous envelope
RNA
Capsid
Glycoprotein
Head
DNA
Tail sheath
Tail fiber
10 nm
(a) Tobacco mosaic virus
50 nm
(b) Adenoviruses
50 nm
(c) Influenza viruses
50 nm
(d) Bacteriophage T4

Fungi (Yeasts)

Size:

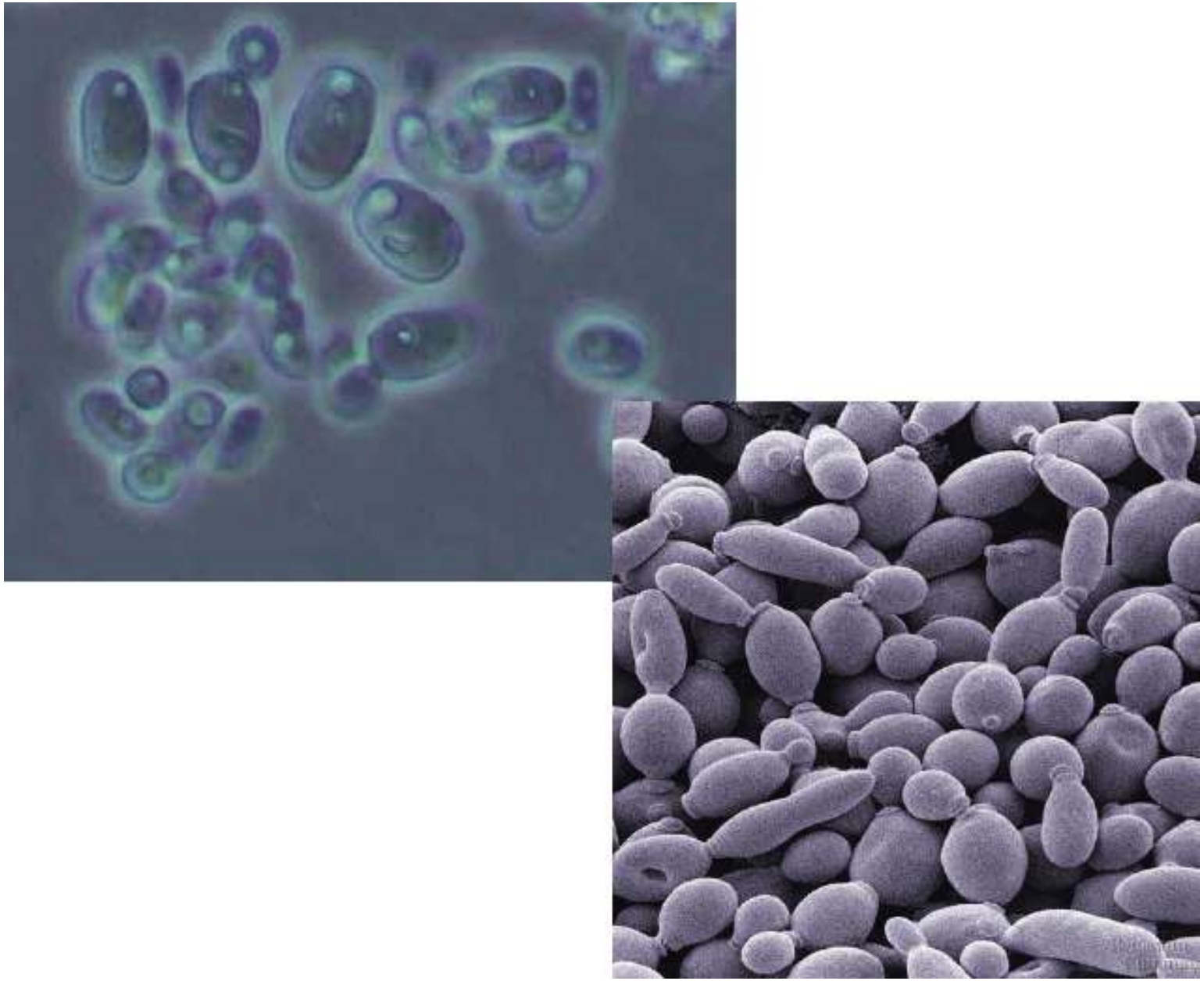
5.0-10.0 µm

Important Characteristics:

- Eukaryotic
- **Unicellular**
- Grow on artificial laboratory media
- Reproduction asexual (cell division/ budding) or sexual

Practical significance:

- Some cause diseases
- Some are used as food supplements
- Manufacture of alcoholic beverages



Fungi (Molds)

Size:

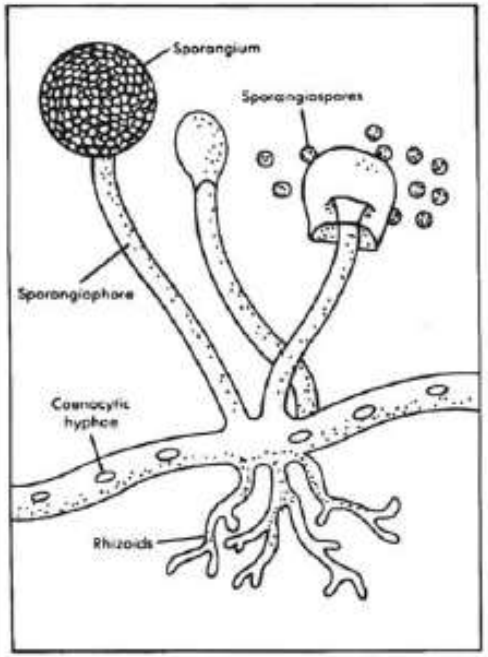
2.0-10.0 µm by several mm

Important Characteristics:

- Eukaryotic
- **Multicellular**
- Many distinctive structural features
- Cultivated on artificial laboratory media
- Reproduction asexual or sexual

Practical significance:

- Decomposition of many materials
- Industrial production of many chemicals like antibiotics
- Can cause diseases


Sporangium
Sporangiospores
Sporangiophore
Coenocytic hyphae
Rhizoids

Courtesy of
The Geraldine Kaminski Medical Mycology Library
Produced by: David Ellis and Roland Hermanis
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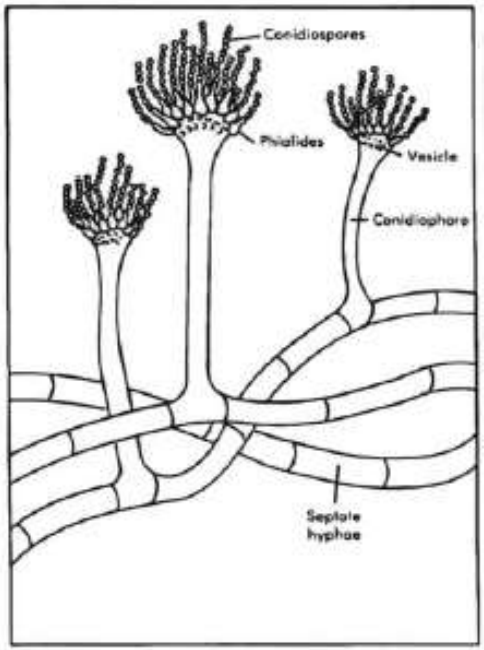

Conidiospores
Phialides
Vesicle
Conidiophore
Septate hyphae

Protozoa

Size:

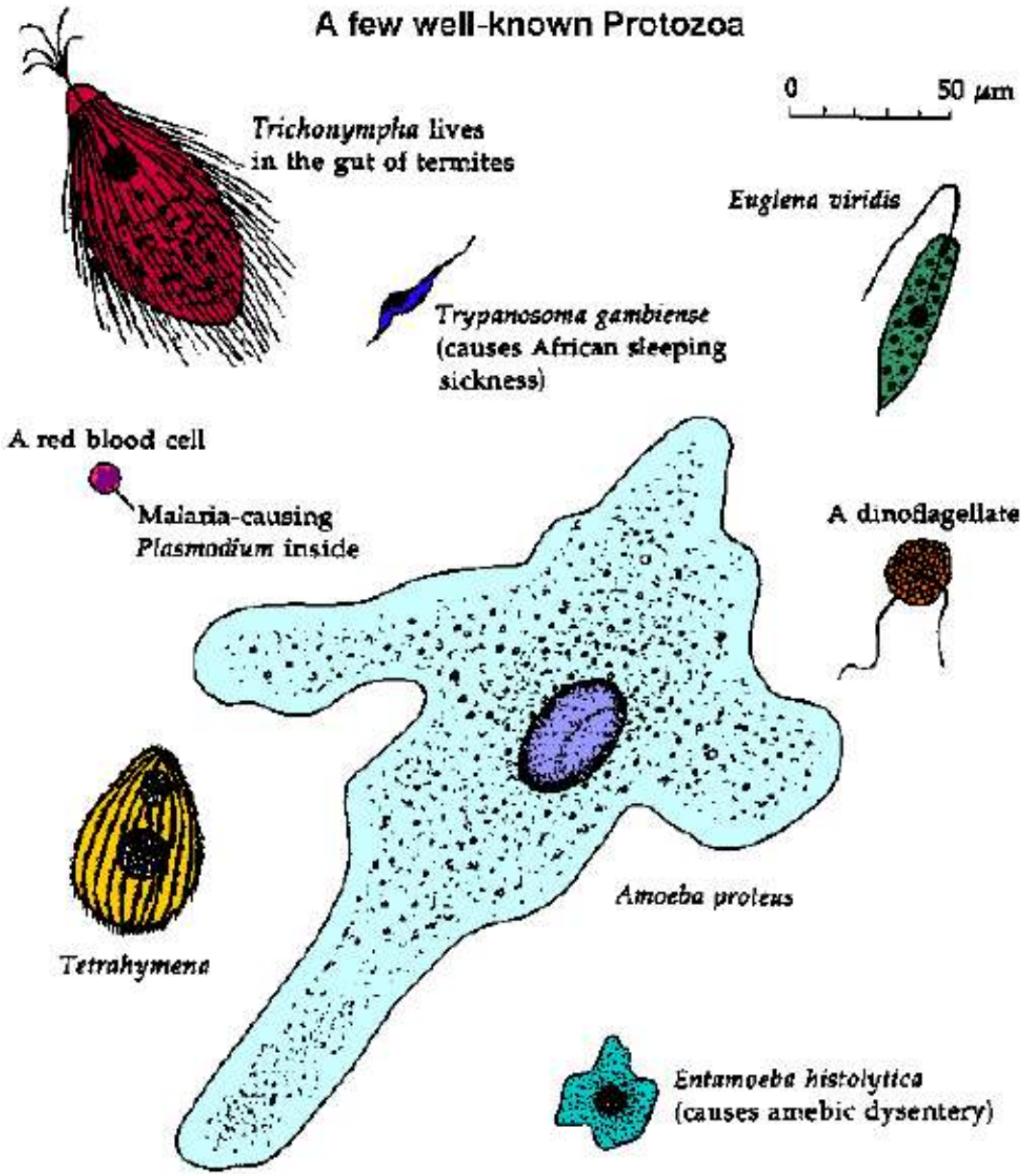
2.0-200 µm

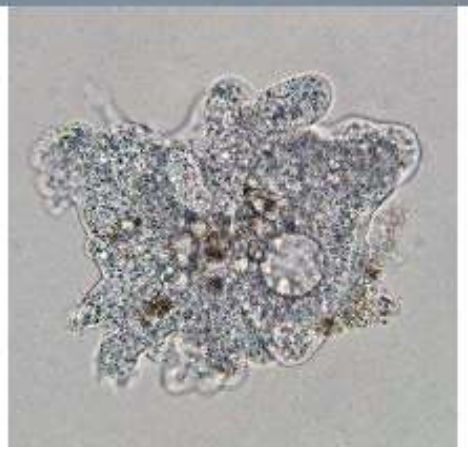
Important Characteristics:

- Eukaryotic
- Unicellular
- Some cultivated on laboratory media while some are intracellular parasites
- **Reproduction asexual or sexual**

Practical significance:

- Some cause diseases
- Food for aquatic animals


A few well-known Protozoa
0
50 μm
Trichonympha lives in the gut of termites
Euglena viridis
Trypanosoma gambiense (causes African sleeping sickness)
A red blood cell
Malaria-causing Plasmodium inside
A dinoflagellate
Amoeba proteus
Tetrahymena
Entamoeba histolytica (causes amebic dysentery)



Algae

Size:

1.0 µm to several centimeters

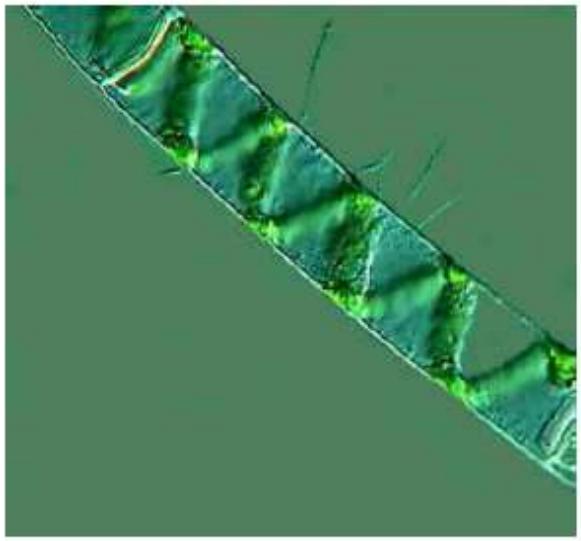
Important Characteristics:

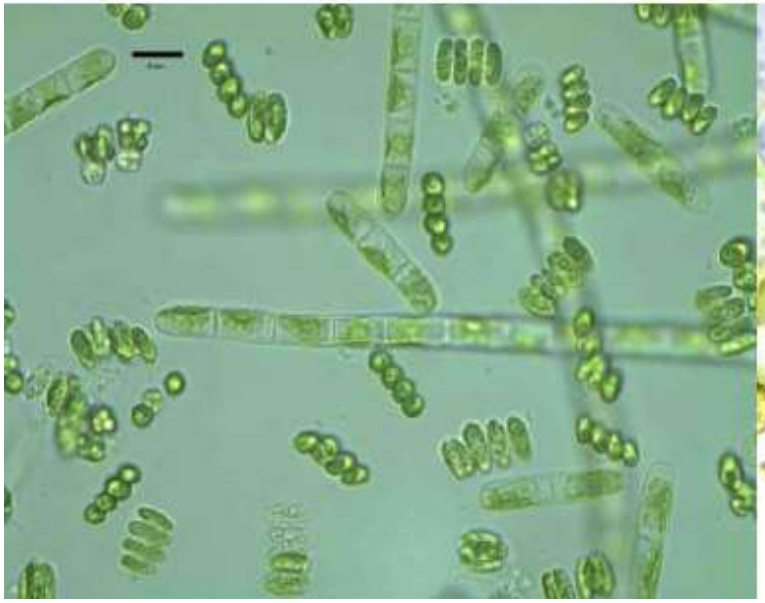
- Eukaryotic
- Unicellular or Multicellular
- Photosynthetic
- Most occur in aquatic environments
- Reproduction asexual or Sexual

Practical significance:

- Production of food in aquatic environments
- Source of food and in Pharmaceuticals
- Some produce toxic substances

10.0 µm





Thank you

Clinical Research

Presented By
Mr. Krunal Kanase
HOD , Department of Pharmacology
PDEA's Shankarrao Ursal College of Pharmaceutical Sciences & Research Center, Kharadi.

Clinical Research

✓It is branch of medical science

✓A systematic observational and experimental biomedical studies performed in human subjects to test new drugs or combination of drugs or devices or biologics for its safety and therapeutic uses or new approaches to surgery or radiotherapy or procedures to improve the diagnosis of disease and the Quality life of the patient.

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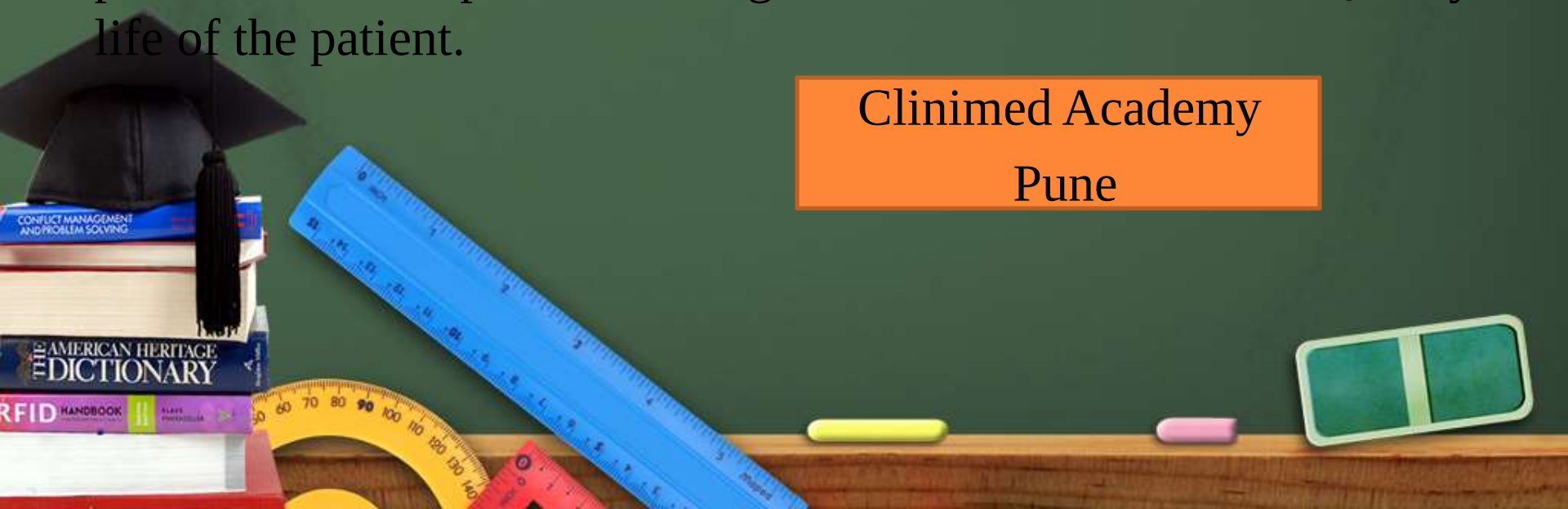
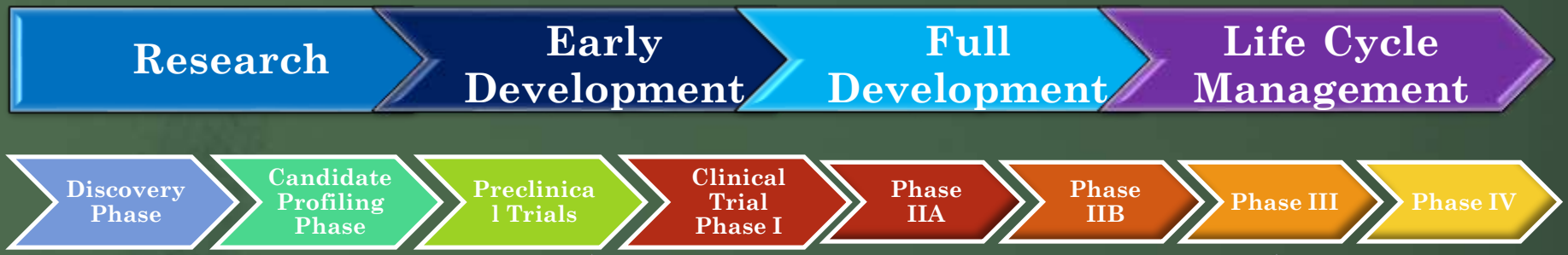


New Drug Development Process



Candidate Selection Point

Selected for Proof of Concept

IND

Development Decision Point

Full Development Point

Phase III Check Point

NDA

Submission Decision Point

R&D

- Research Target
- Discovery of Lead Compound
- Selection of Suitable Candidate

Preclinical

- Lab testing
- Animal Studies
- Toxicology Studies etc.,.

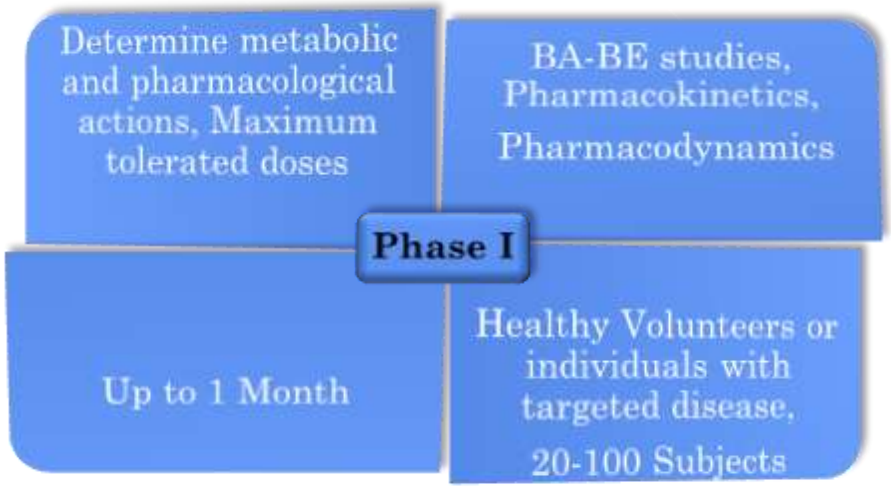
Clinical (I & II)

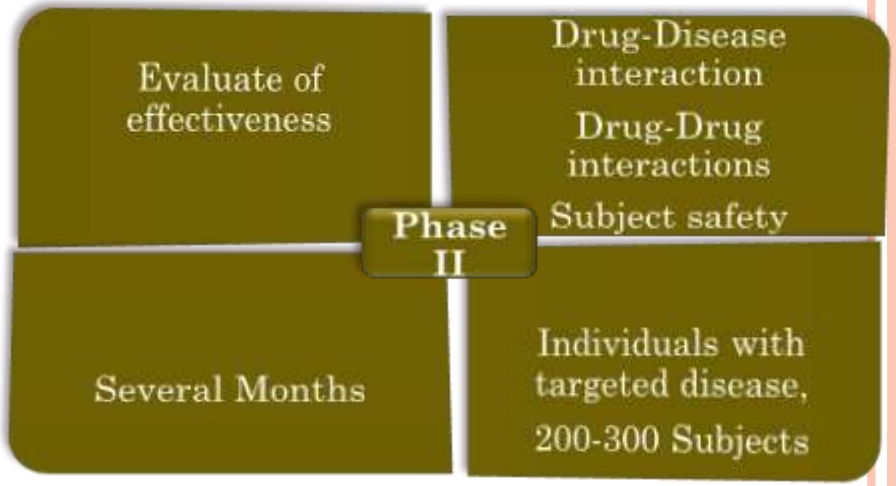
- Biological Tests and Pharmacological test in humans
- Regulatory Clearance

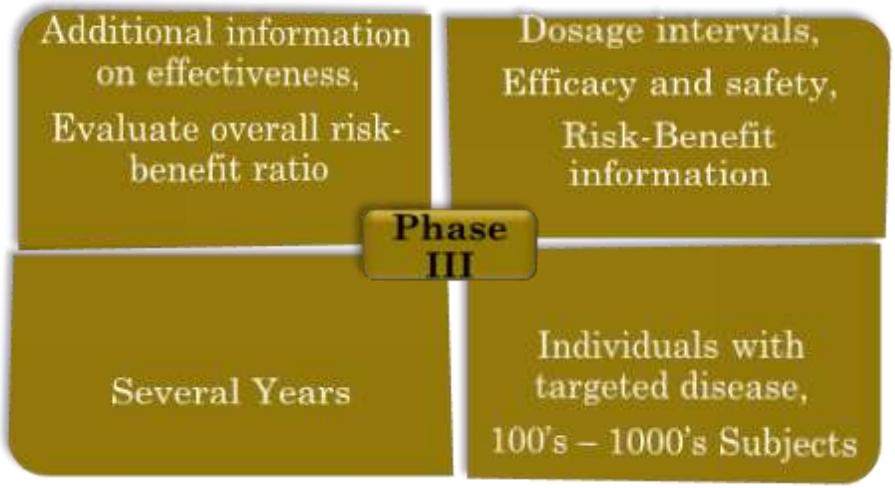
Clinical (III&IV)

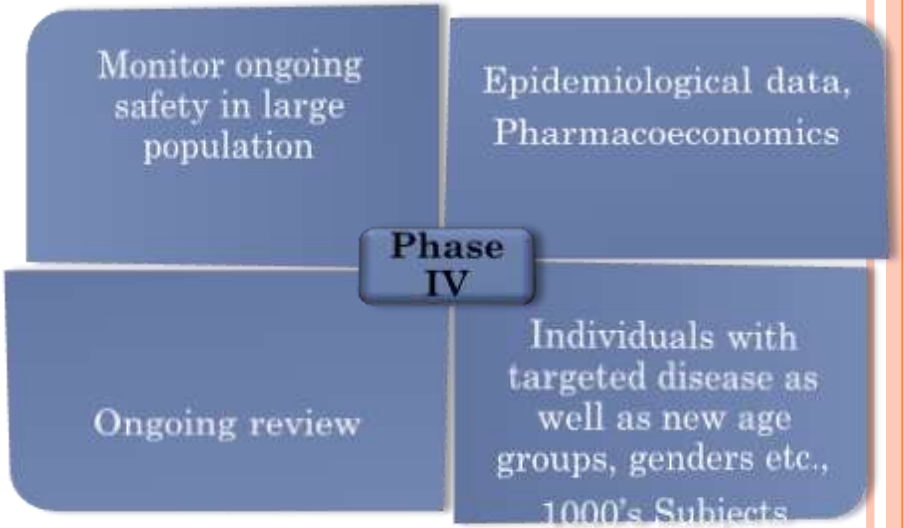
- Registration with Health Authorities
- Preparation for Launch
- Launch and Sales

Clinical Research - Phases









Clinical Trial

- One form of Clinical research
- Systematic experimental Biomedical studies.
- To evaluate the effectiveness and safety of medications or medical devices or biologics etc.,.

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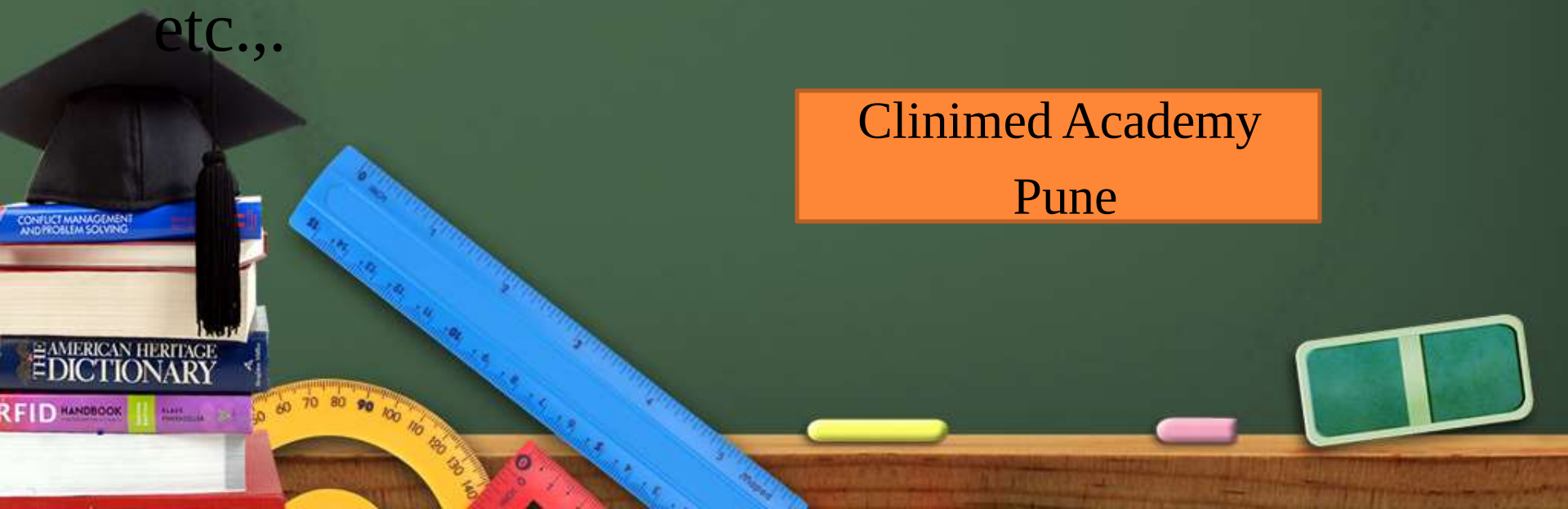

Phases of Clinical Trial

Phase O → Phase I → Phase II → Phase III → Phase IV

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Phase 0

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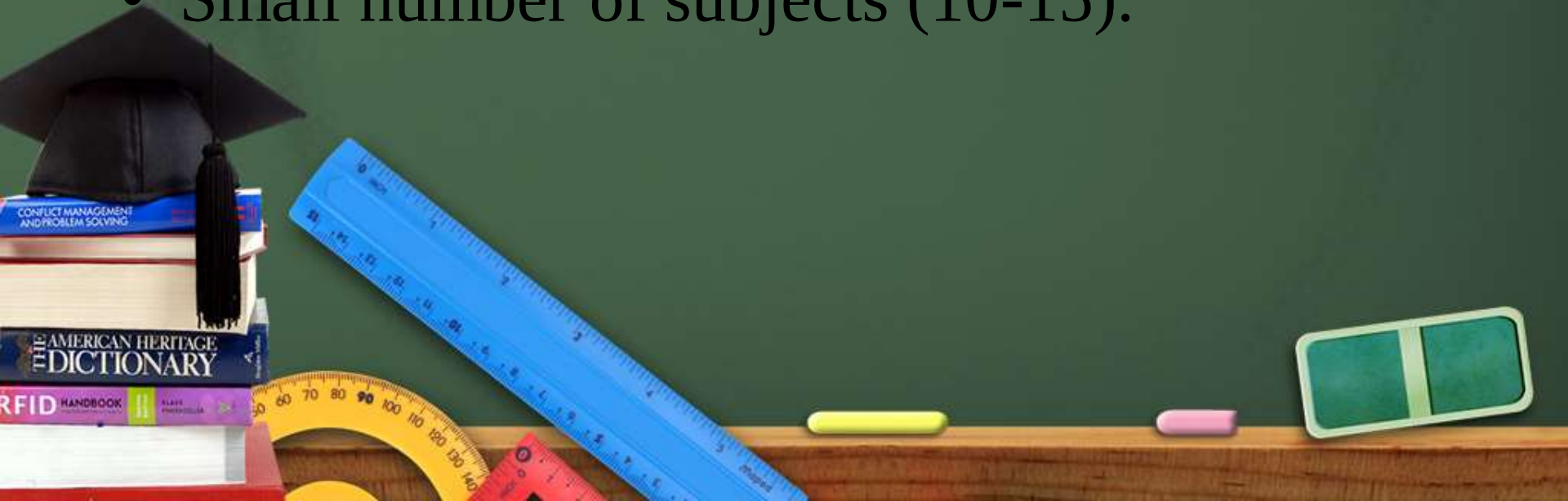
- Also called Human Micro-dosing studies.
- Gathers preliminary data Pharmacodynamics and Pharmacokinetics.
- Gives no data on safety or efficacy.
- Small number of subjects (10-15).



Phase I- Human P'cology Study

- First stage of testing in human subjects.
- Normally, a small (20-100) group of healthy volunteers will be selected.
- Designed to assess the safety (Pharmacovigilance), tolerability, Pharmacokinetic and Pharmacodynamic of a drug.

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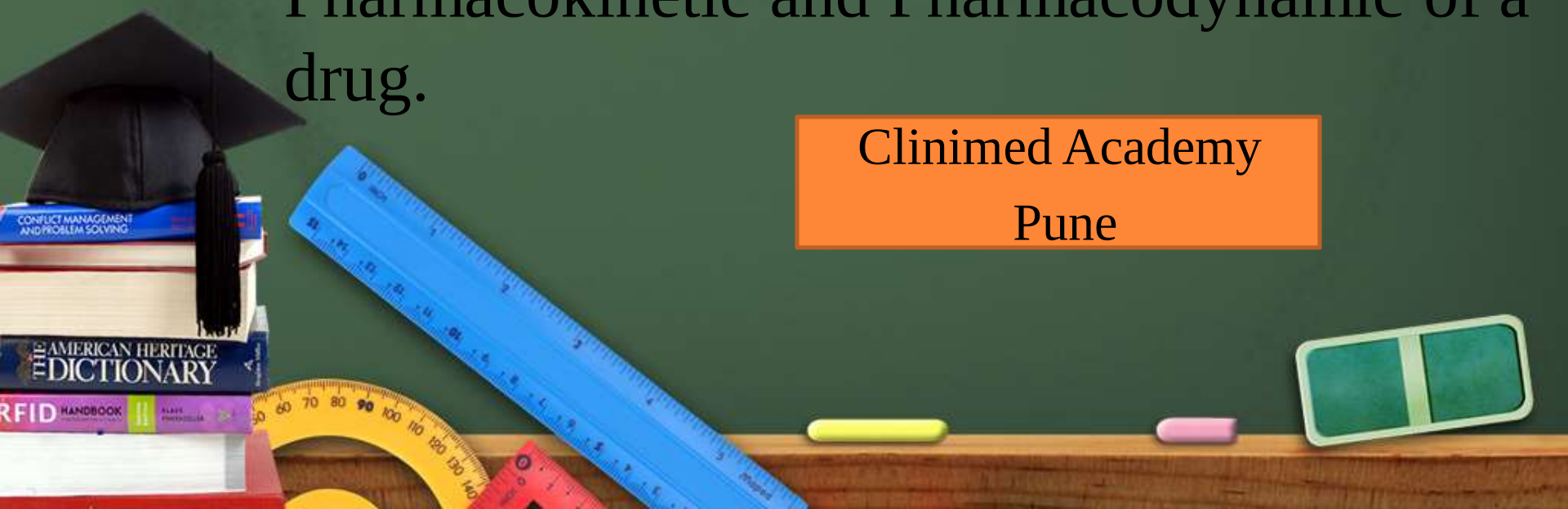

Phase I-

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- Phase I trials are the first stage of testing in human subjects.
- Normally, a small (20-100) group of healthy volunteers will be selected.
- Designed to assess the safety (Pharmacovigilance), tolerability, Pharmacokinetic and Pharmacodynamic of a drug.

Phase I

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- These trials are often conducted in an inpatient clinic, where the subject can be observed by full-time staff.
- Phase I trials most often include healthy volunteers. Howaver, there are some circumstances when real patients are used, such as patients who have terminal cancer or HIV and lack other treatment options.
- Volunteers are paid an inconvenience fee for their time spent in the volunteer center. Pay ranges from small amount of money for a short period of residence, to a larger amount of up to approx $ 6000 depending on length of participation.

Phase I- SAD Studies

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- Single Ascending Dose studies are those in which small groups of subjects are given a single dose of the drug while they are observed and tested for a period of time.
- If they do not exhibit any adverse side effects, and the pharmacokinetic data is roughly in line with predicted safe values, the dose is escalated, and a new group of subjects is then given a higher dose.
- This is continued until pre-calculated pharmacokinetic saftey levels are reached, or intolerable side effects start showing up at which point the drug is said to have reached the Maximum Tolerated Dose(MTD).

Phase I- MAD Studies

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- Multiple Ascending Dose studied are conducted to better understand the pharmacokinetics & pharmacodynamics of multiple doses of the drug.
- A group of patients receives a low drug dose, which is then escalated to a predetermined level. Blodd and fluid samples are collected and analysed to see how the body is processing the drug.

Phase I- Food Effect Studies

- These studies are usually run as a crossover study, with volunteers being given two identical doses of the drug on different occasions, one while fasted and one after being fed.

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Phase II- THERAPEUTIC EXPLORATORY TRIAL

- Once the initial saftey of the study drug has been confirmed in Phase I trials, Phase II trials are performed on larger group (100-300) and are designed to assess how well the drug works (efficacy), as well as to countinue Phase I saftey assessments in a larger group of patients.
- When the development processfor a new drug fails, this usually occurs during Phase II trials when the drug is discovered not to work as planned or to have toxic effects.
- Efficacy in patients (primary objective)
- Safety issues (secondary objective)

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Phase II -Subtypes

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- Phase IIa studies focus on proving the hypothesized mechanism of action-often called ‘proof of concept’ or ‘proof of mechanism’ studies and tend to be with fewer numbers of patients than Phase IIb studies.
- Having confirmed the hypothesis, Phase IIb studies look to find the optimum dose at which the drug shows biological activity with minimal side effects often called ‘dose ranging studies’.

Phase III-Therapeutic Confirmatory Studies

- Phase III studies are randomized controlled multicenter trials on large patient groups (300-3000) or more depending upon the disease/medical condition studied.
- Aimed at being the definitive assessment of how effective the drug is, in comparison with current ‘gold standard’ treatments.

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PHASE-III

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- Other reasons for performing trials at this stage includes attempts by the sponsor at 'Label Expansion' (to show the drug works for additional types of patients/diseases beyond the original use for which the drug was approved for marketing), to obtain additional saftey data or to support marketing claim for the drug.

Phase III

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- Phase IIIa-
- Focused on regulatory approval issues and involve numerous sites and generally last two to three years. After the successful completion of Phase IIIa the sponsor of new drug submits a registraion dossier containing all preclinical, pharmacologic, efficacy and saftey data information about the drug's composition and the sponsor's plans for producing, packaging and labeling the drug. The regulatory review process can take up to 30 months or longery type of drug and other factors.

PHASE II

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- Phase IIIb-
- Trials usually begins after submission of the registration dossier and prior to regulatory approvals. These studies which also involves large numbers of patients, generally focus on issues such as the **study drug's cost – effectiveness or its relative efficacy compared with approved drugs** in the same therapeutic class or those that are used to treat the same disease.

Phase IV

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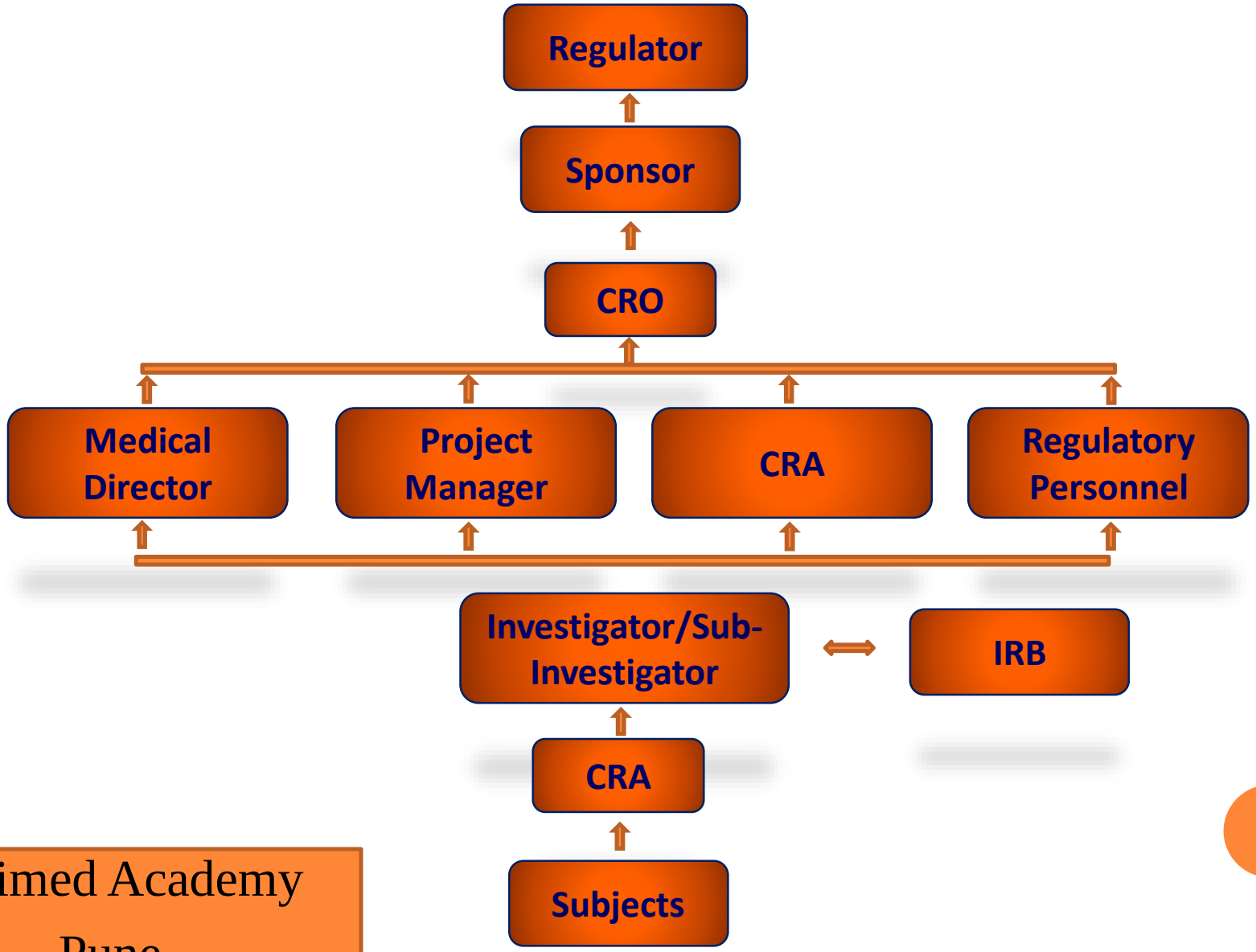
- Phase IV trial is also known as Post Marketing Surveillance Trial. Phase IV involves the safty surveillance and ongoing technical support of a drug after it receives permission to be sold.
- The safety surveillance is designed to detect any rare or long term adverse effects over a much larger patient population and longer time period than was possible during the Phase I-III clinical trials.
- Harmful effects discovered by Phase IV trials may result in drug being no longer sold or restricted to certain uses.

Players in Clinical Trial



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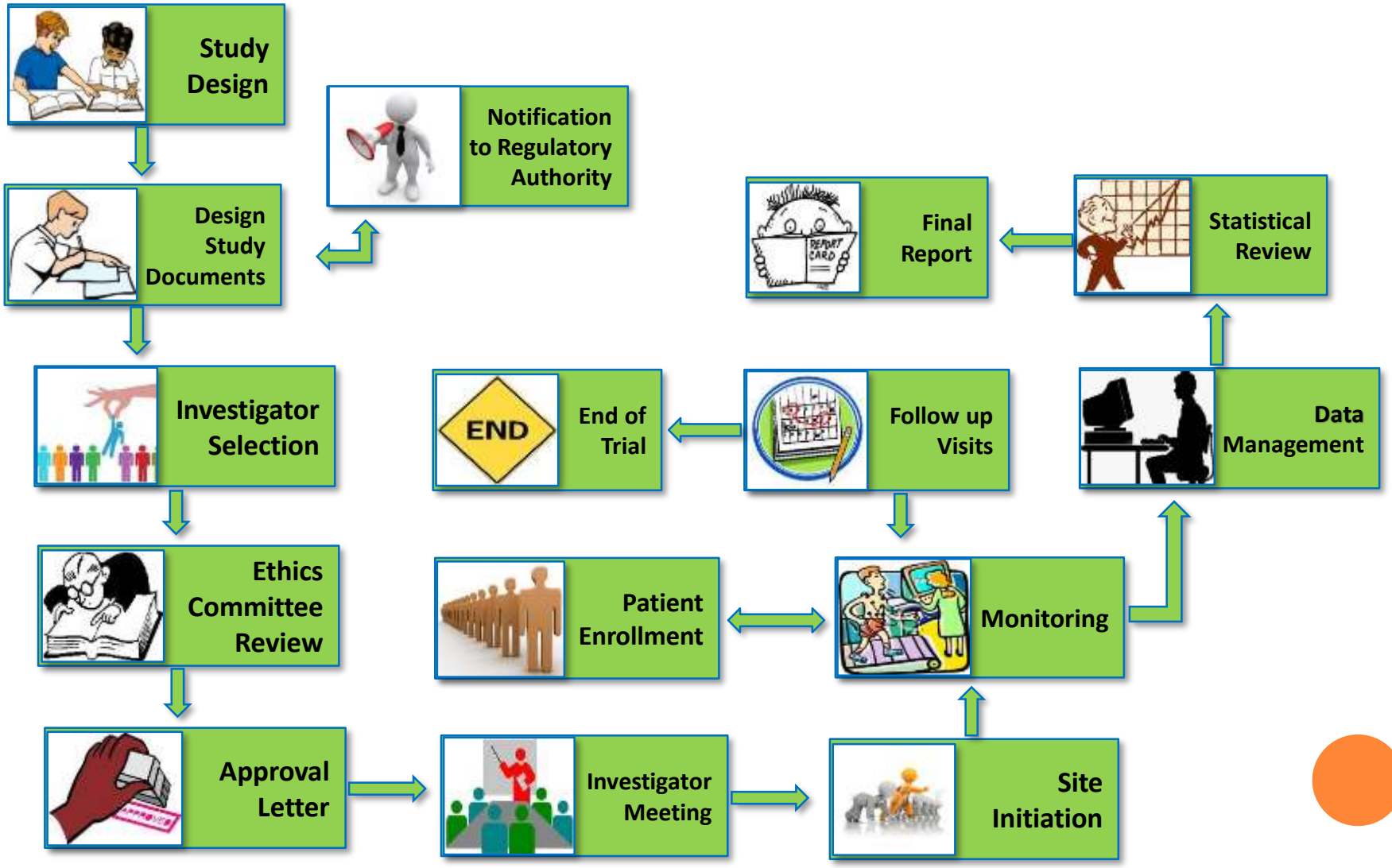
Overview of Clinical Trial



What Happens During the Clinical Trial

- Depends on the kind of trial being conducted.
- Includes Doctors (Investigators), nurses, health care professionals etc.,
- Monitors the participants carefully during the trial and stay in touch after the trial is completed.
- CT, successful when the protocol is carefully followed.
- Some trials involves interviews, questionnaires or surveys of interventional treatment.

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SUMMARY

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- Clinical Research involves processes through which the testing article is tested for its effectiveness and safety for human use.
- Ensures complete safety of volunteers.
- Highly monitored and regulated.

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BP 403 T. PHYSICAL PHARMACEUTICS-II (Theory)

Unit-I
Colloidal dispersions

Subject Teacher:
Dr. Prashant Khade

- **Thomas Graham (1861)** studied the ability of dissolved substances to diffuse into water across a permeable membrane. He observed that crystalline substances such as sugar, urea, and sodium chloride passed through the membrane, while others like glue, gelatin and gum arabic did not. The former he called **crystalloids and the latter colloids.**
- The term colloid has been derived from two terms, namely **kolla and eidos. 'kolla' means glue and 'eidos' means like i.e. glue-like**. The size of the particles in colloidal solutions is bigger than the size of particles present in crystalloid solutions such as sugar or salt in water.

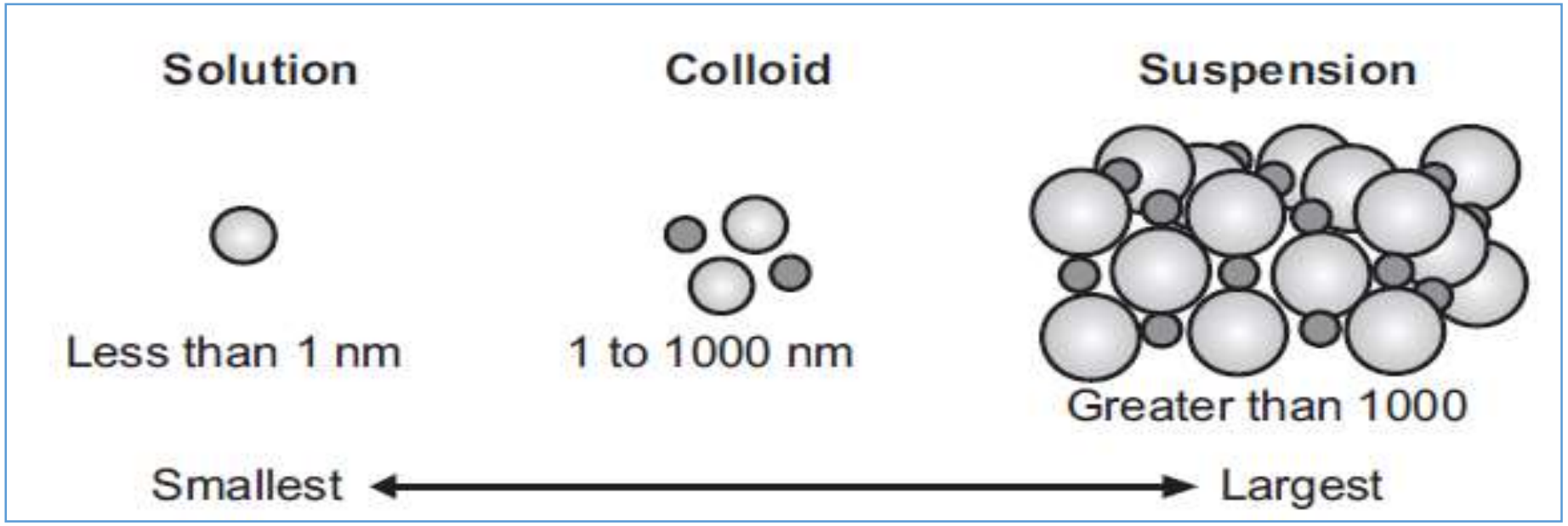


Table 5.1: Some important properties of true solutions, colloid and suspensions

Sr. No.	Name of Property	True Solution	Colloid Solution	Suspension
1.	Size	Size of properties is less than 1 nm.	Size of particles is between 1 nm and 1000 nm.	Size of particles is greater than 1000 nm.
2.	Filterability	Pass through ordinary filter paper and also through animal membrane.	Pass through ordinary filter paper but not through animal membrane.	Do not pass through filter paper or animal membrane.
3.	Settling	Particles do not settle down on keeping.	Particles do not settle down on their own but can be made to settle down by centrifugation.	Particles settle down on their own under gravity.
4.	Visibility	Particles are invisible to the naked eye as well as under a microscope.	Particles are invisible to the naked eye but their scattering effect can be observed with the help of a microscope.	Particles are visible to the naked eye.
5.	Separation	The solute and solvent cannot be separated by ordinary filteration or by ultra filteration.	The solute and solvent cannot be separated by ordinary filteration but can be separated by ultra-filteration.	The solute and solvent can be separated by ordinary filteration.
6.	Diffusion	Diffuse quickly.	Diffuse slowly.	Do not diffuse.

Table 5.2: Types of colloidal solutions

Sr. No.	Dispersed Phase	Dispersion Medium	Type of Colloidal Solution	Examples
1.	Solid	Solid	Solid solution	Gemstones
2.	Solid	Liquid	Sol	Paints, muddy water, gold sol, starch sol, arsenious sulphide sol.
3.	Solid	Gas	Aerosol of solids	Smoke, dust in air
4.	Liquid	Solid	Gel	Jellies, Cheese
5.	Liquid	Liquid	Emulsion	Milk, Cream
6.	Liquid	Gas	Aerosol	Mist, fog, cloud
7.	Gas	Solid	Solid foam	Foam rubber, pumice stone
8.	Gas	Liquid	Foam	Forth, whipped cream

Table 5.3: Distinguish between types of colloidal dispersion

Property	Lyophilic Colloids	Lyophobic Colloids	Association Colloids
Dispersed phase	Mostly organic molecules	Largely inorganic particles	Aggregation of surface active agents
Nature of interaction	Stronger, solvent sheath around particle	Little interaction	Aggregates are solvated
Presence of charge	Less charged, but solvated	High charged	Charged micelles, but solvated
Method of preparation	Readily form sol	Special methods are required	Readily form when concentration is equal to CMC
Viscosity of the dispersion	Higher than that of medium alone	Nearly same as the dispersion medium	Viscosity increases, but not appreciably
Presence of electrolytes Low concen-trations High concen-trations	 Stable Coacervation precipitates	 Unstable (coagulation) stable	 Stable precipitates
Reversibility	Reversible	Irreversible	Reversible

5.4.3 Associated Colloids

Associated or amphiphilic colloids form the third group in this classification. Associated colloids is the solution of surfactant or amphiphilic molecules. As we have seen in Chapter 1, in Article Surfactant (Refer Article 1.5.1, from Chapter 1). In this it includes structure of surfactant, CMC and micelle formation.

Example:

Soap is an example. Soap is sodium salt of long chain fatty acid RCOONa. When put in water, soap forms $RCOO^{-}$ (insoluble tail) and Na^{+} (hydrophilic head). These $RCOO^{-}$ ions associate themselves around dirt particles as shown below forming a micelle (Fig. 5.2).

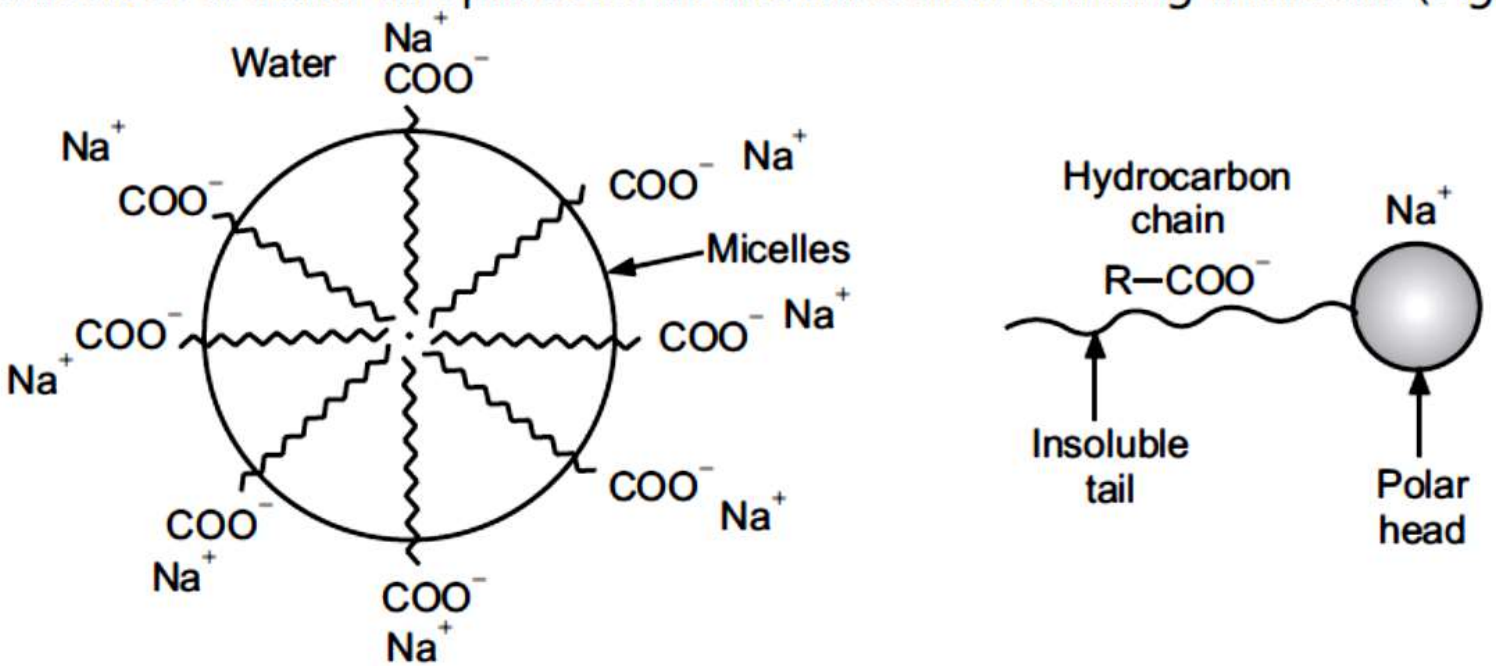
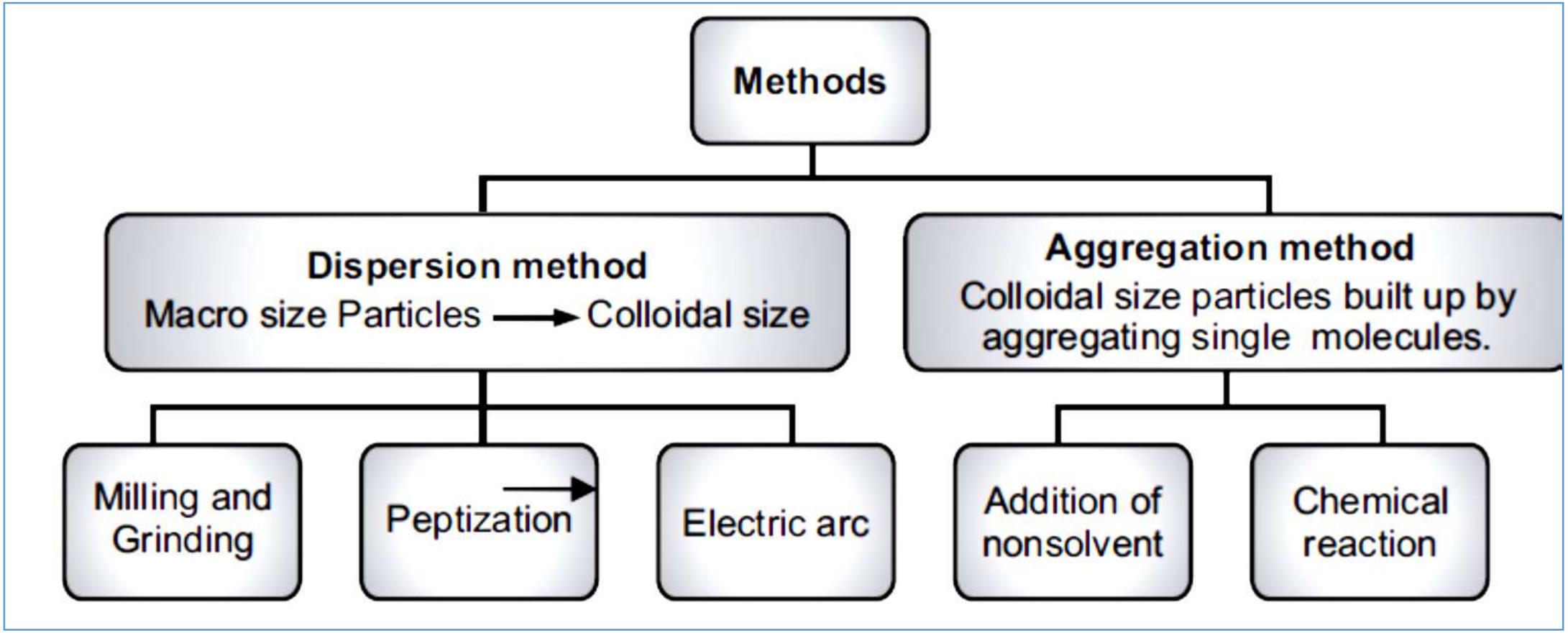


Fig. 5.2: Aggregation of $RCOO^{-}$ ions to from micelles

METHODS OF PREPARATION



Milling and Grinding (Colloidal Mill)

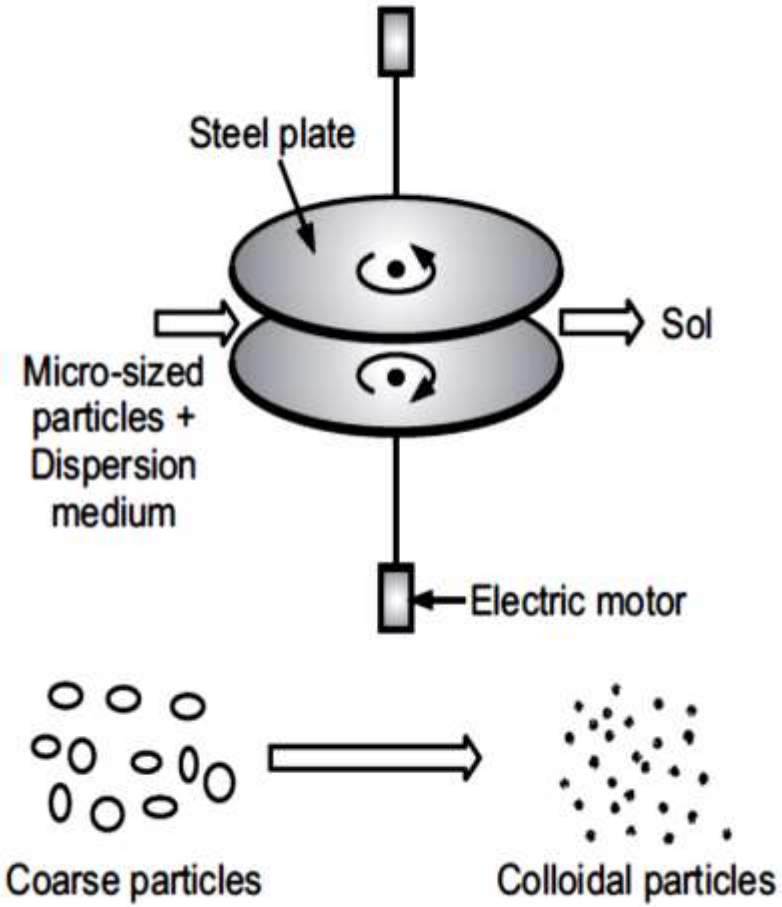
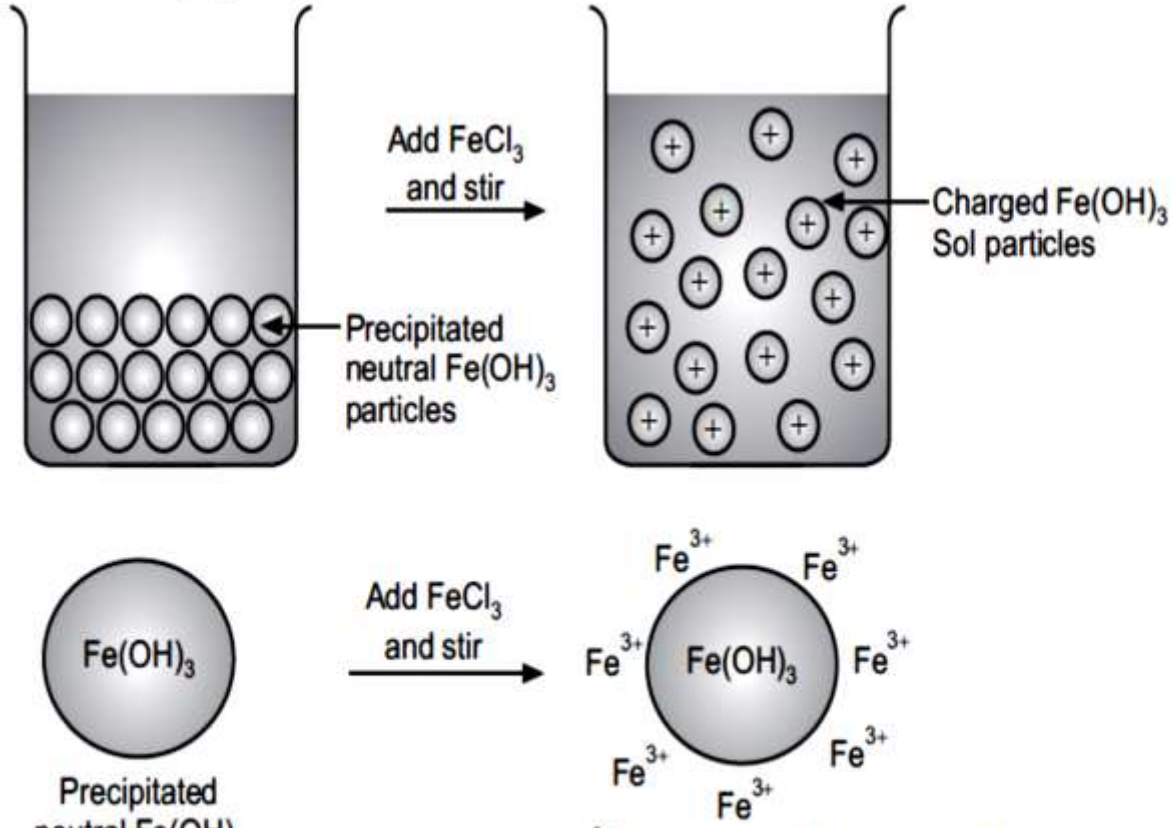


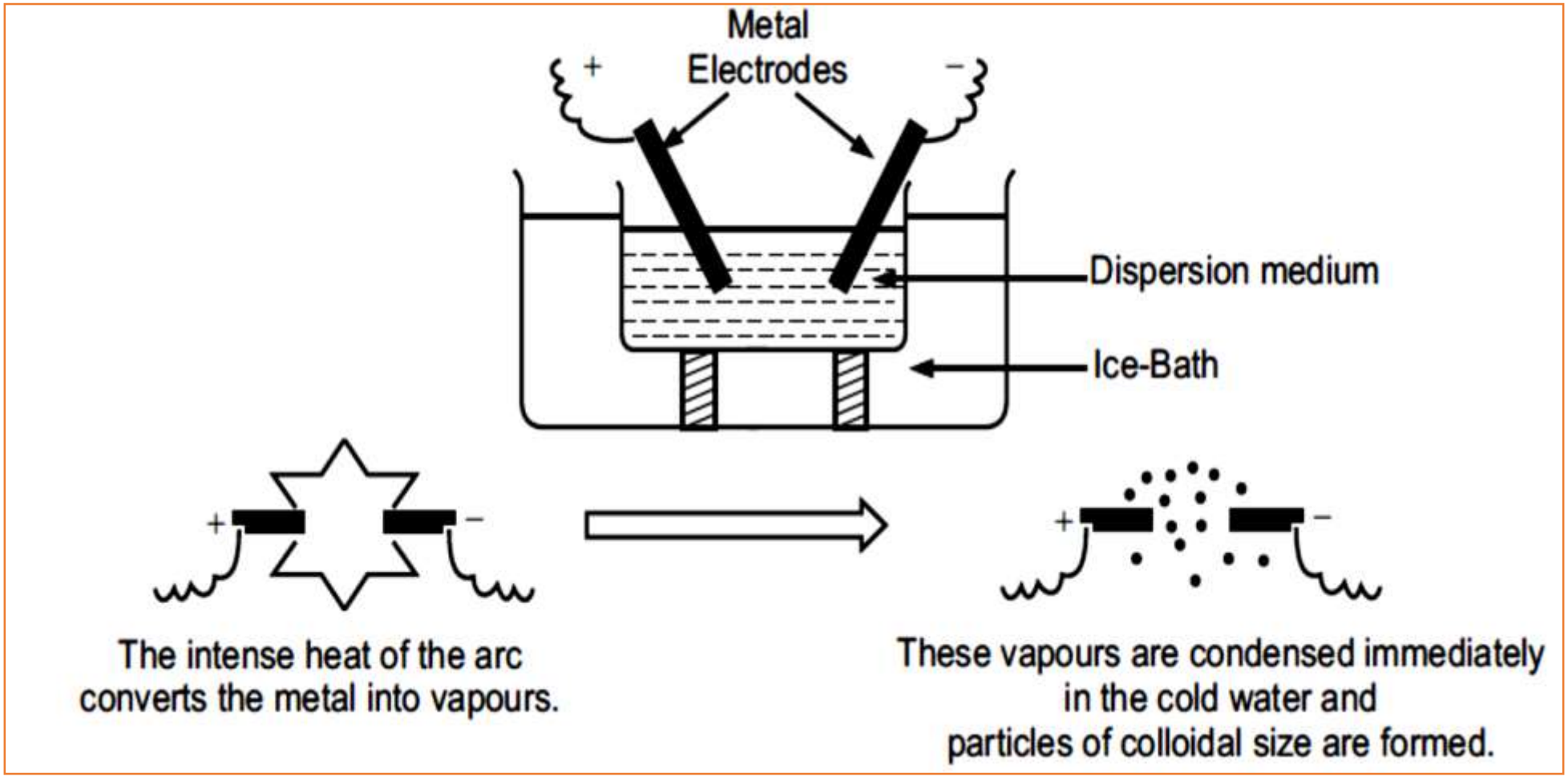
Fig. 5.3: Colloidal mill

Peptization



Fe^{3+} Ions are adsorbed by $Fe(OH)_3$ particles.
$Fe(OH)_3$ particles acquire +ve charge,
start repelling each other forming a colloidal solution.

Electric Arc



Addition of Nonsolvent

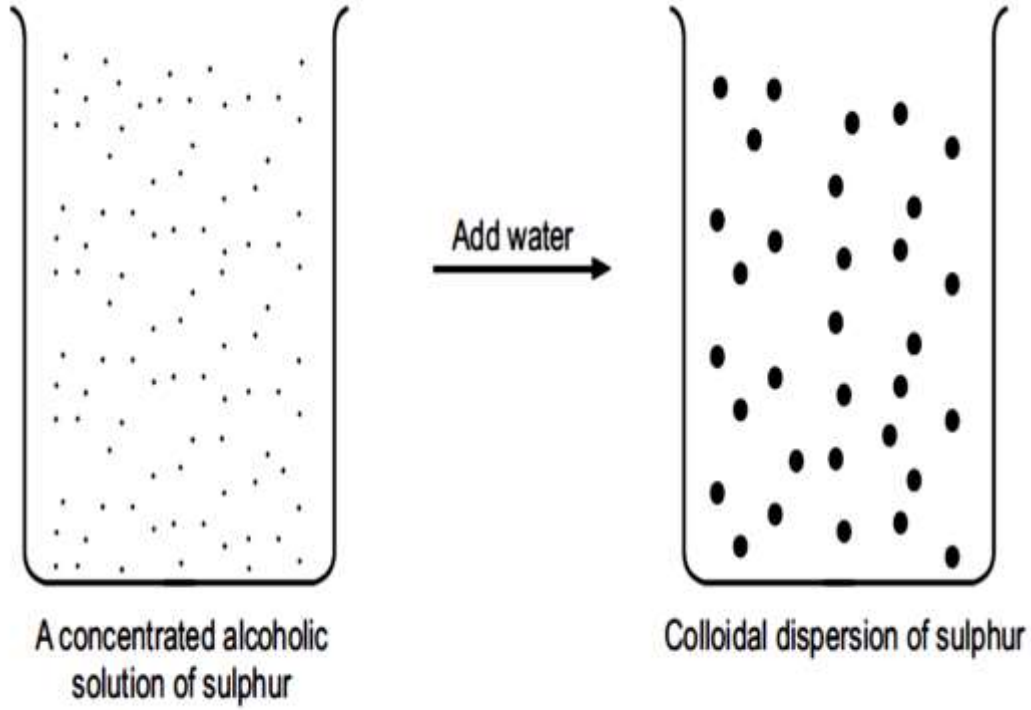


Fig. 5.6: Preparation of sulphur solution

Chemical Reaction

(a) Double Decomposition: An arsenic sulphide (As_2S_3) sol is prepared by passing a slow stream of hydrogen sulphide gas through a cold solution of arsenious oxide (As_2O_3). This is continued till the yellow colour of the sol attains maximum intensity.

$$As_2O_3 + 3H_2S \longrightarrow As_2S_3\ (sol) + 3H_2O$$

Excess hydrogen sulphide (electrolyte) is removed by passing in a stream of hydrogen.

(b) Reduction: Silver sols and gold sols can be obtained by treating dilute solutions of silver nitrate or gold chloride with organic reducing agents like tannic acid or methanal (HCHO).

$$AgNO_3 + \text{Tannic acid} \longrightarrow \text{Ag sol}$$

$$AuCl_3 + \text{Tannic acid} \longrightarrow \text{Au sol}$$

(c) Oxidation: A sol of sulphur is produced by passing hydrogen sulphide into a solution of sulphur dioxide.

$$2H_2S + SO_2 \longrightarrow 2H_2O + S\downarrow$$

(d) Hydrolysis: Sols of the hydroxides of iron, chromium and aluminium are readily prepared by the hydrolysis of salts of the respective metals. In order to obtain a red sol of ferric hydroxide, a few drops of 30% ferric chloride solution is added to a large volume of almost boiling water and stirred with a glass rod.

$$FeCl_3 + 3H_2O \longrightarrow Fe(OH)_3 + 3HCl$$

Red sol

PURIFICATION OF COLLOIDAL SOLUTION

1. Dialysis

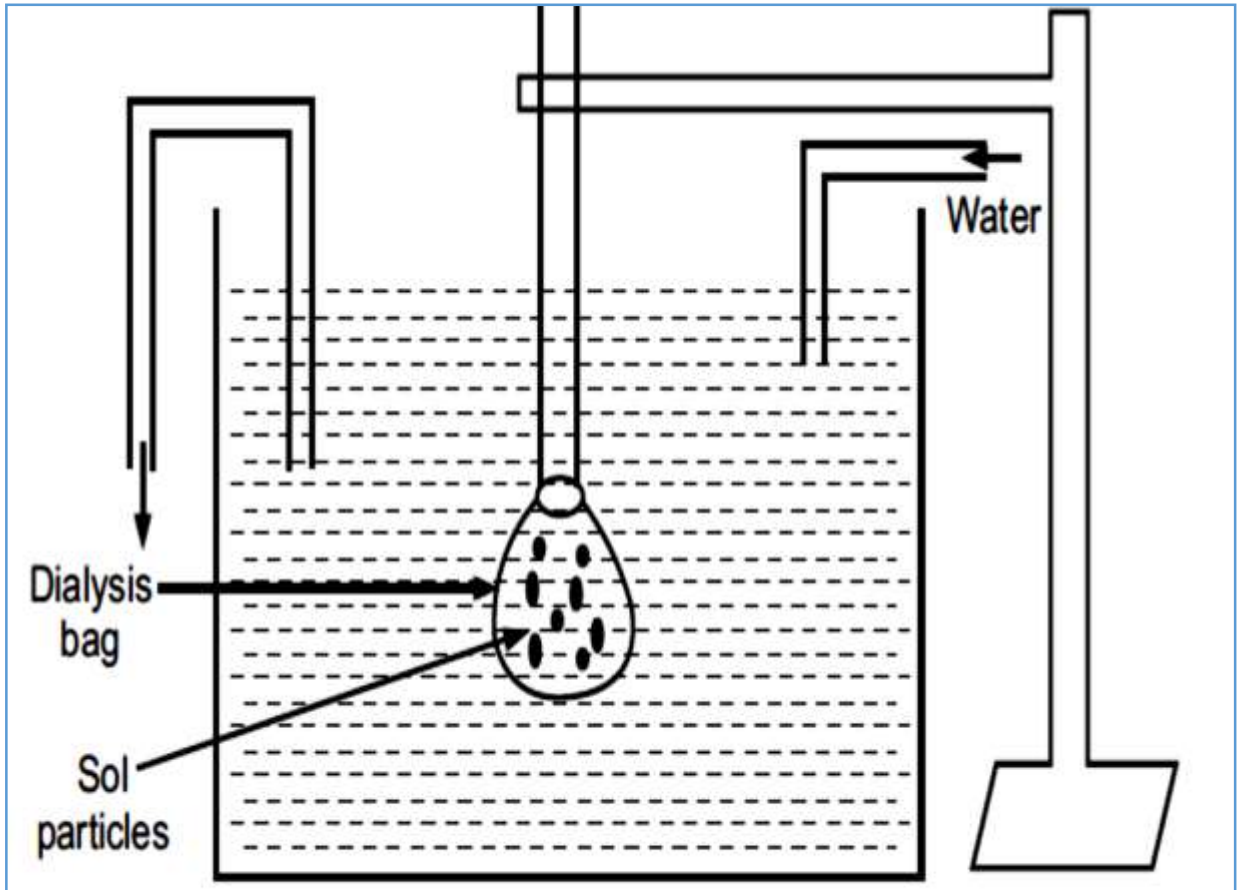


Fig. 5.7: A dialyser

2. Electrodialysis

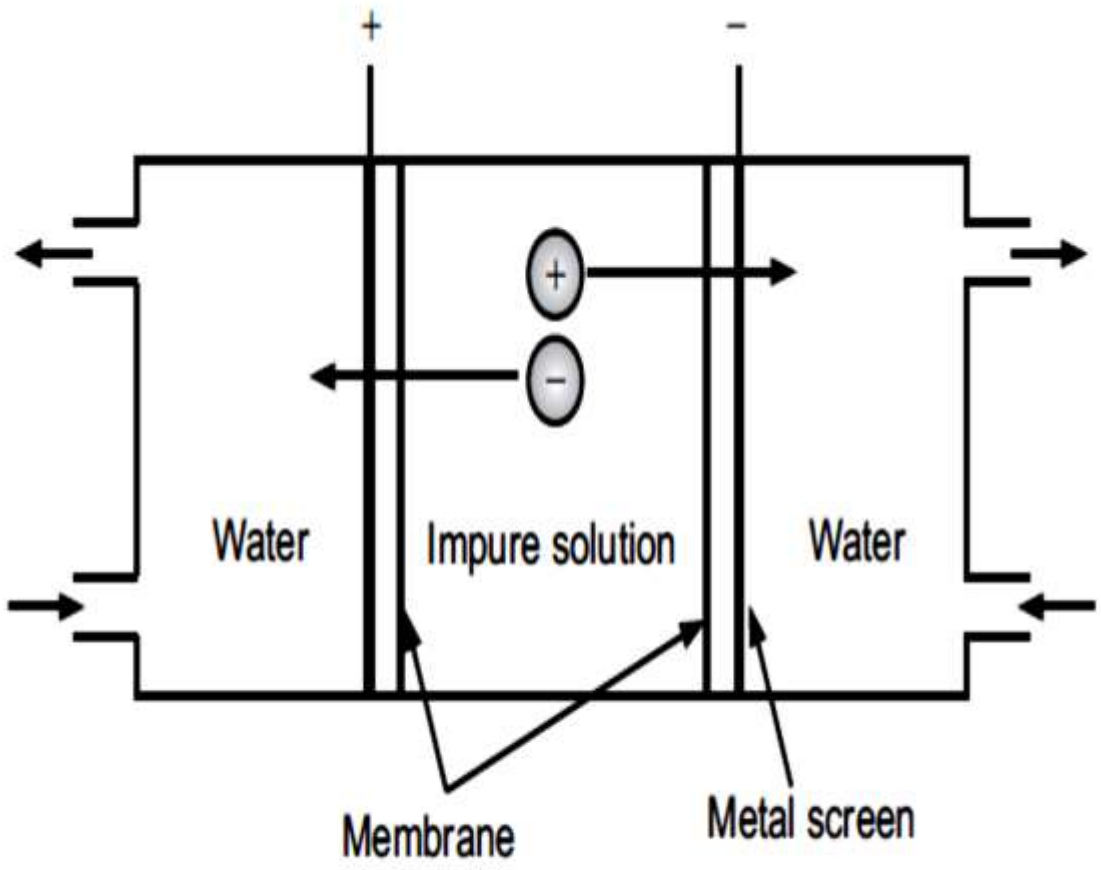


Fig. 5.8: Electrodialysis

3. Ultrafiltration

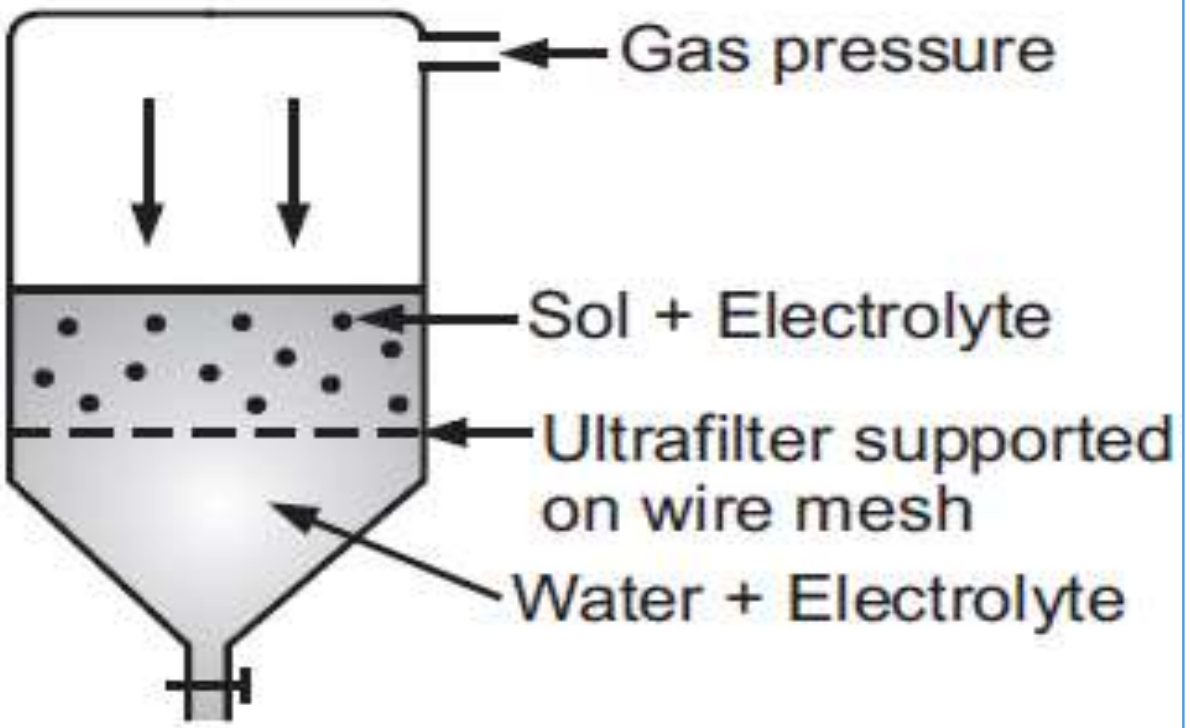


Fig. 5.9: Ultrafiltration

OPTICAL PROPERTIES OF COLLOIDAL SOLUTION

1. Tyndall Effect

Tyndall in 1869, observed that if a strong beam of light is passed through a colloidal solution placed in a dark place and viewed at right angles to the direction of light beam, the path of light shows up as a hazy beam or cone. This is due to the fact that sol particles absorb light energy and then emit it in all directions in space. This emission of light is known as **Scattering of Light.**

"The phenomenon of scattering of light by the sol particles is called **Tyndall Effect".**

The illuminated beam or cone formed by the scattering of light by the sol particles is often referred as **Tyndall Beam or Tyndall Cone.**

True solutions do not show Tyndall effect. Since ions or solute molecules are too small to scatter light, the beam of light passing through a true solution is not visible when viewed from the side. Thus, Tyndall effect can be used to distinguish a colloidal solution from a true solution.

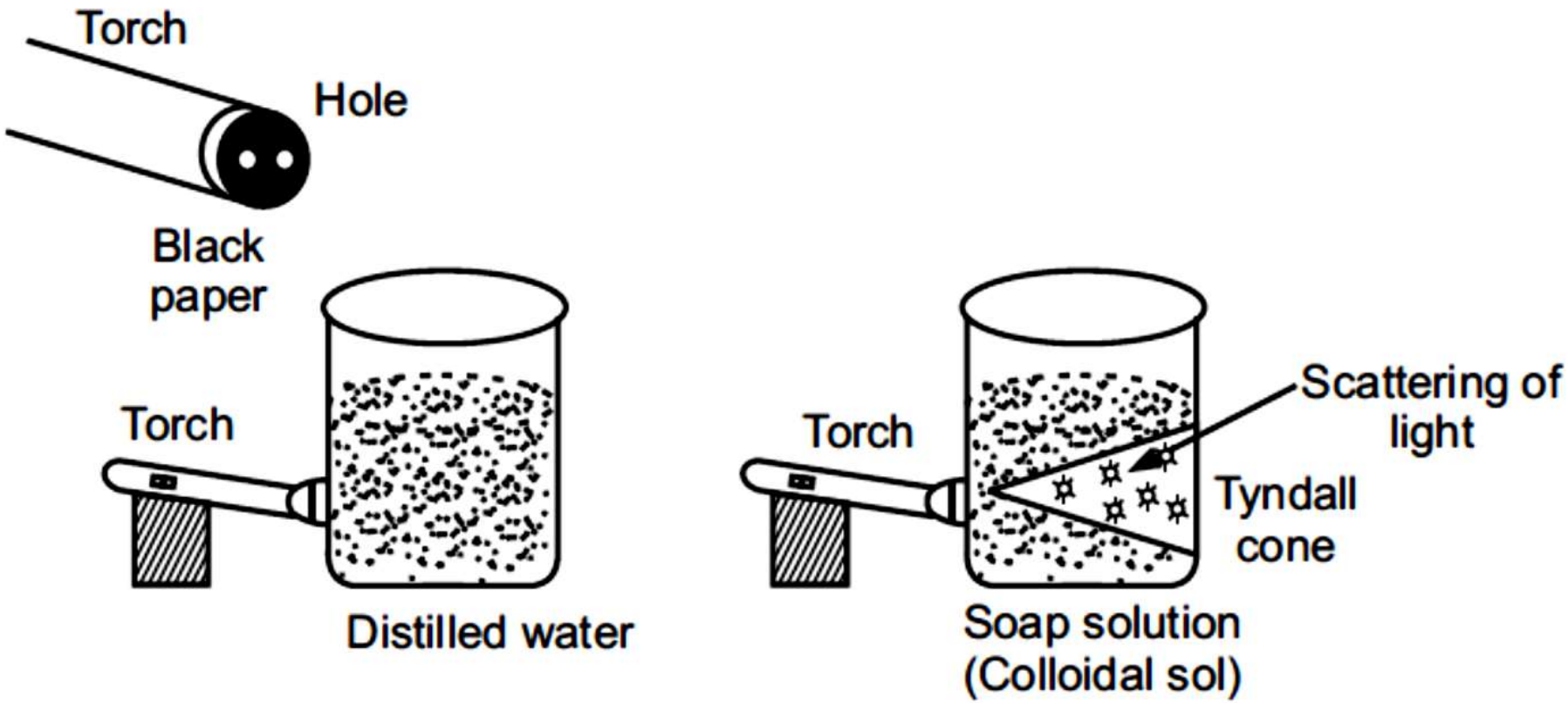


Fig. 5.10: Scattering of light

Ultramicroscope Shows the Presence of Individual Particles

- Colloidal particles cannot be seen with a microscope.
- Zsigmondy (1903) used the Tyndall phenomenon to set up an apparatus named as the ultramicroscope. An intense beam of light is focused on a sol contained in a glass vessel.
- The focus of light is then observed with a microscope at right angles to the beam. Individual sol particles appear as bright specks of light against a dark background (dispersion medium). It may be noted that under the ultramicroscope, the actual particles are not visible.
- These are the larger halos of scattered light around the particles that are visible.
- Thus, an ultramicroscope does not give any information regarding the shape and size of the sol particles.

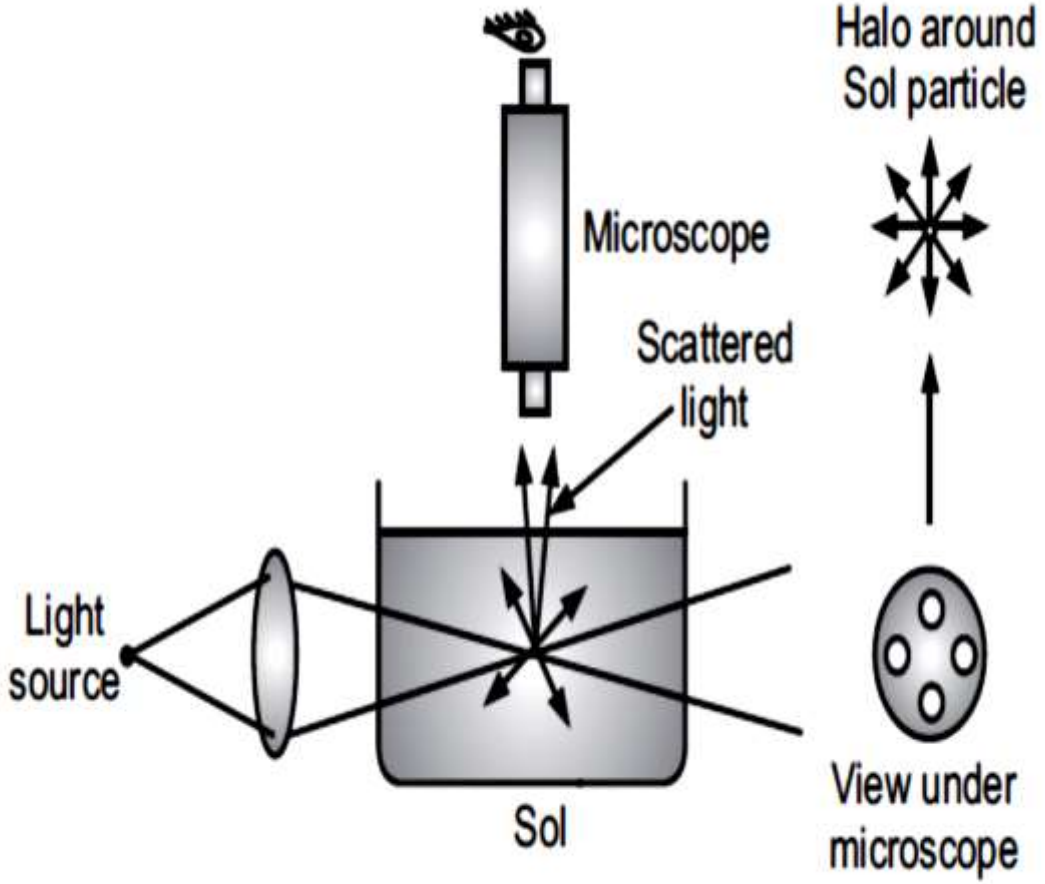


Fig. 5.11: Ultramicroscope

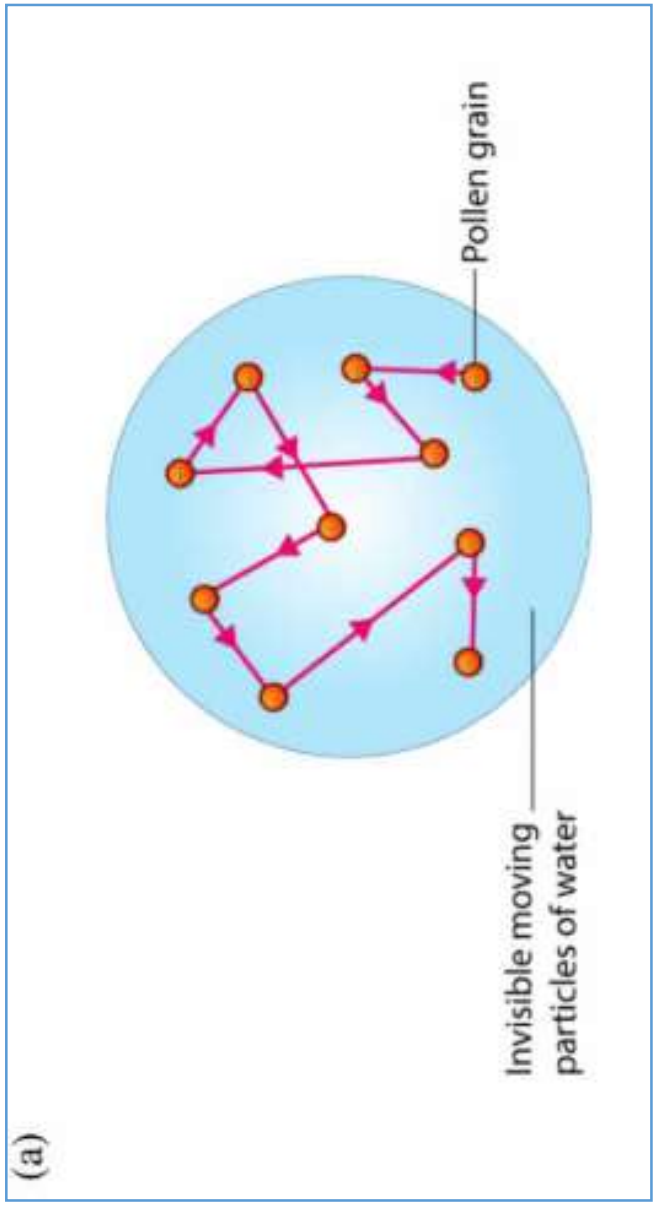
Solution Particles can be seen with an Electron Microscope

- In an electron microscope, beam of electrons is focused by electric and magnetic fields on to a photographic plate.
- This focused beam is allowed to pass through a film of sol particles.
- Thus, it is possible to get a picture of the individual particles showing a magnification of the order of 10,000.
- With the help of this instrument, we can have an idea of the size and shape of several sol particles including paint pigments, viruses and bacteria.
- These particles have been found to be spheriod, rod-like, disc-like or long filaments.

KINETIC PROPERTIES OF COLLOIDS

1. Brownian Movement

1. This phenomenon is so named after Sir Robert Brown who discovered it in 1827.
2. He was observed this phenomenon with pollen grains suspended in water.
3. When a sol is examined with an ultramicroscope, the suspended particles are seen as shining specks of light.
4. By following an individual particle it is observed that the particle is undergoing a constant rapid motion.
5. It moves in a series of short straight-line paths in the medium, changing directions abruptly.
6. Brownian movement may be exhibited by particle as large as about 5 nm.
7. They bombard with each other and collide with the wall of the container.
8. Also, colloidal particles cannot sediment.
9. Brownian movement works against the gravitational force.



2. Diffusion

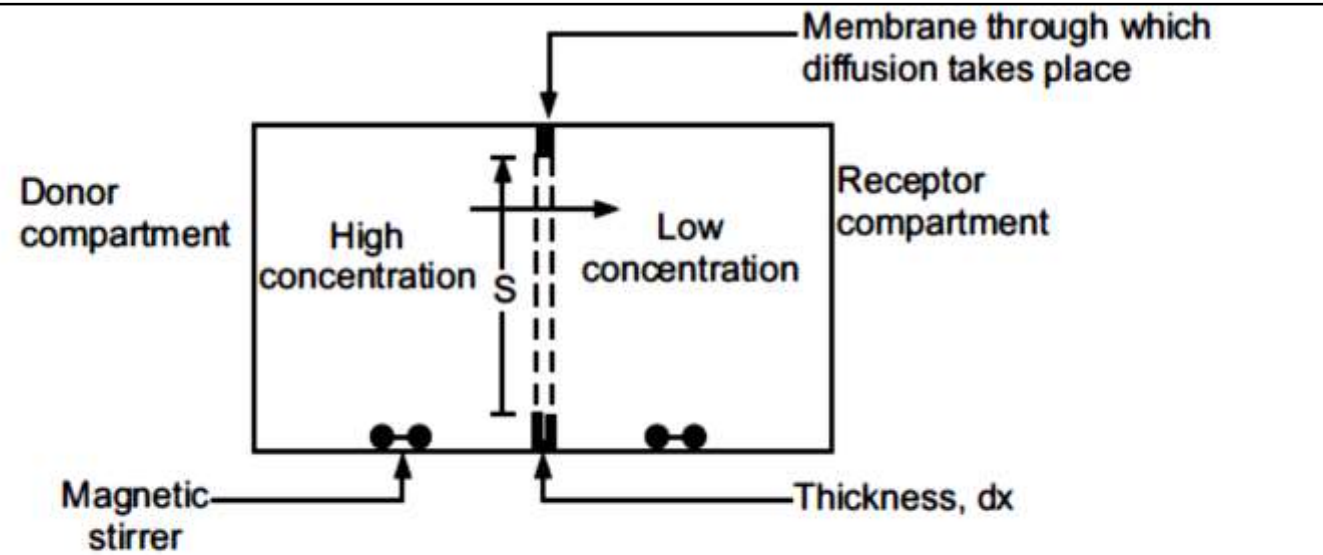


Fig. 5.14: Diffusion of colloids in high concentration to low concentration

Fick's law is written as,

$$d_q = -DS\frac{dc}{dx}\,dt \qquad \ldots (5.5)$$

Where,

D : The diffusion coefficient,

$\frac{dc}{dx}$: The amount of material diffusing per unit time across a unit area, called the concentration gradient.

3. Osmotic Pressure

For example, the depression of freezing point of a 1% w/v solution of a macromolecule of molecular weight 70000 Da is only 0.0026 K, far too small to be measured with sufficient accuracy by conventional methods and also very sensitive to the presence of low molecular weight impurities.

In contrast, the osmotic pressure of this solution at 20°C would be 350 Nm^{-2} or about 35 mm of water. Not only does the osmotic pressure provide an effect that is measurable, but also the effect of any low molecular weight material, which can pass through the membrane, is virtually eliminated.

$$\frac{\pi}{C} = \frac{RT}{M} + BC \qquad \ldots (5.6)$$

Where,

π : Osmotic pressure,

C : The concentration of the solution,

M : The molecular weight of the solute,

R : Gas constant,

T : Absolute temperature,

B : A constant depending on the degree of interaction between the solvent and solute molecules.

4. Sedimentation

Stoke's law:

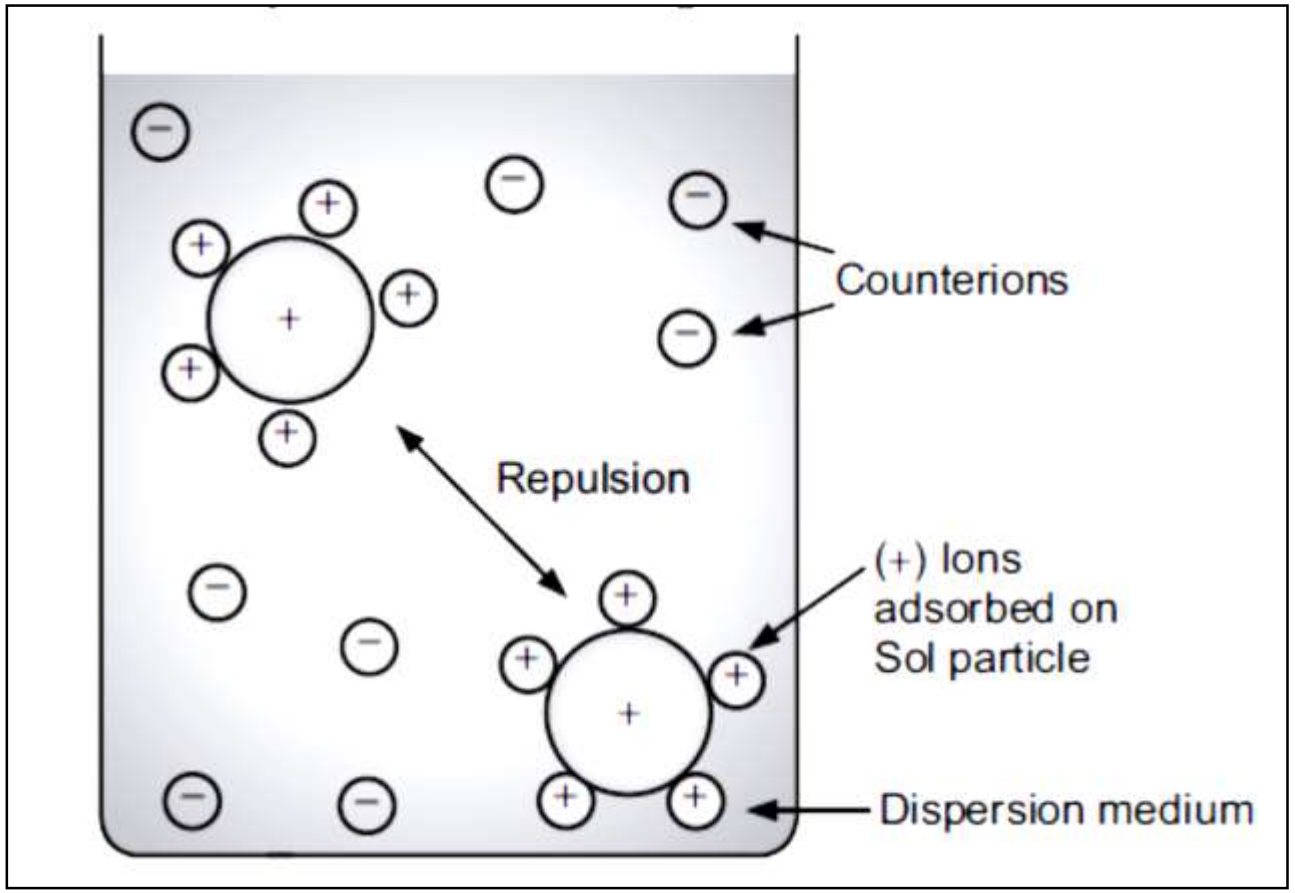
$$v = \frac{2r^2 (\rho - \rho_0) g}{9 \eta_0}$$

Ultra-centrifuge:

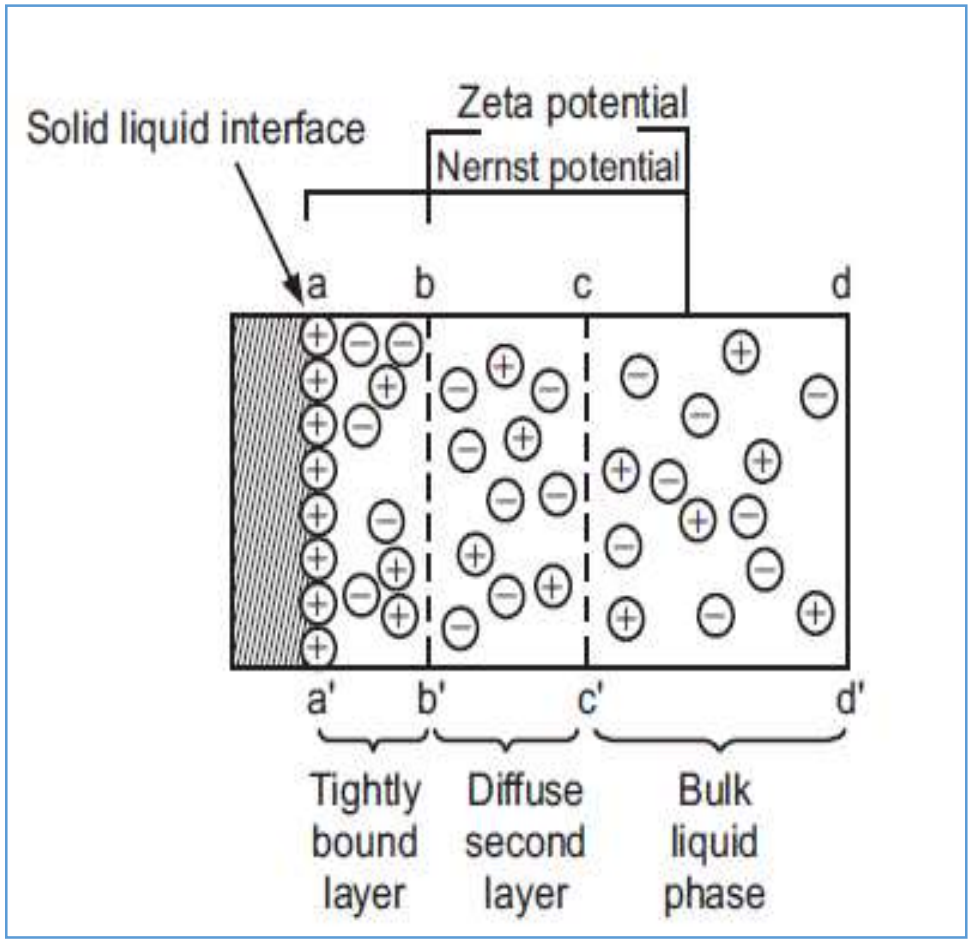
$$v = \frac{dx}{dt} = \frac{2r^2 (\rho - \rho_0) \omega^2 x}{9 \eta_0}$$

ELECTRIC PROPERTIES

1. The Sol Particles carry an Electric Charge



Electric Double Layer



Nernst potential:

It is the potential of the solid surface itself, aa' owing to the presence of potential determining ions.

Nernst potential, E, or electrothermodynamic potential is defined as 'the difference in potential between the actual surface and the electroneutral region of the solution'.

Zeta potential:

It is the potential observed at the shear plane i.e. bb' line (Fig. 5.17).

Zeta potential, ζ, or electrokinetic potential is defined as 'the difference in the potential between the surface of the tightly bound layer and the electroneutral region of the solution'.

Zeta potential can also be defined as 'the work required bringing a unit charge from infinity to the surface of the particles'.

Electrophoresis

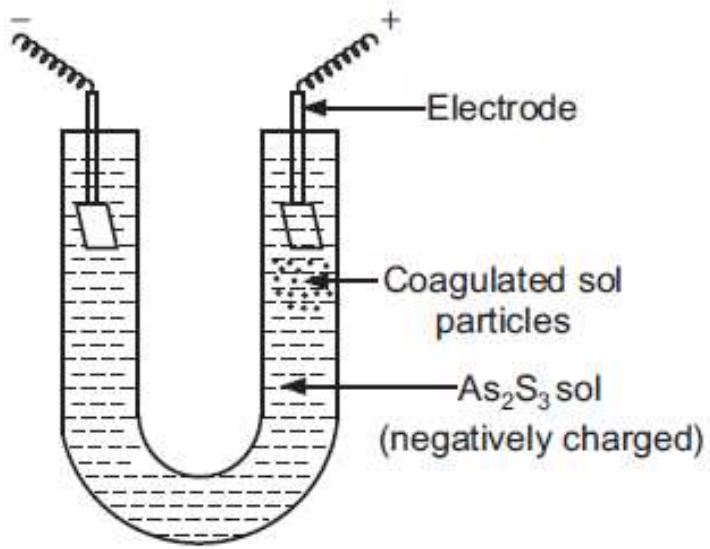


Fig. 5.18: Demonstration of electrophoresis

Electro-Osmosis

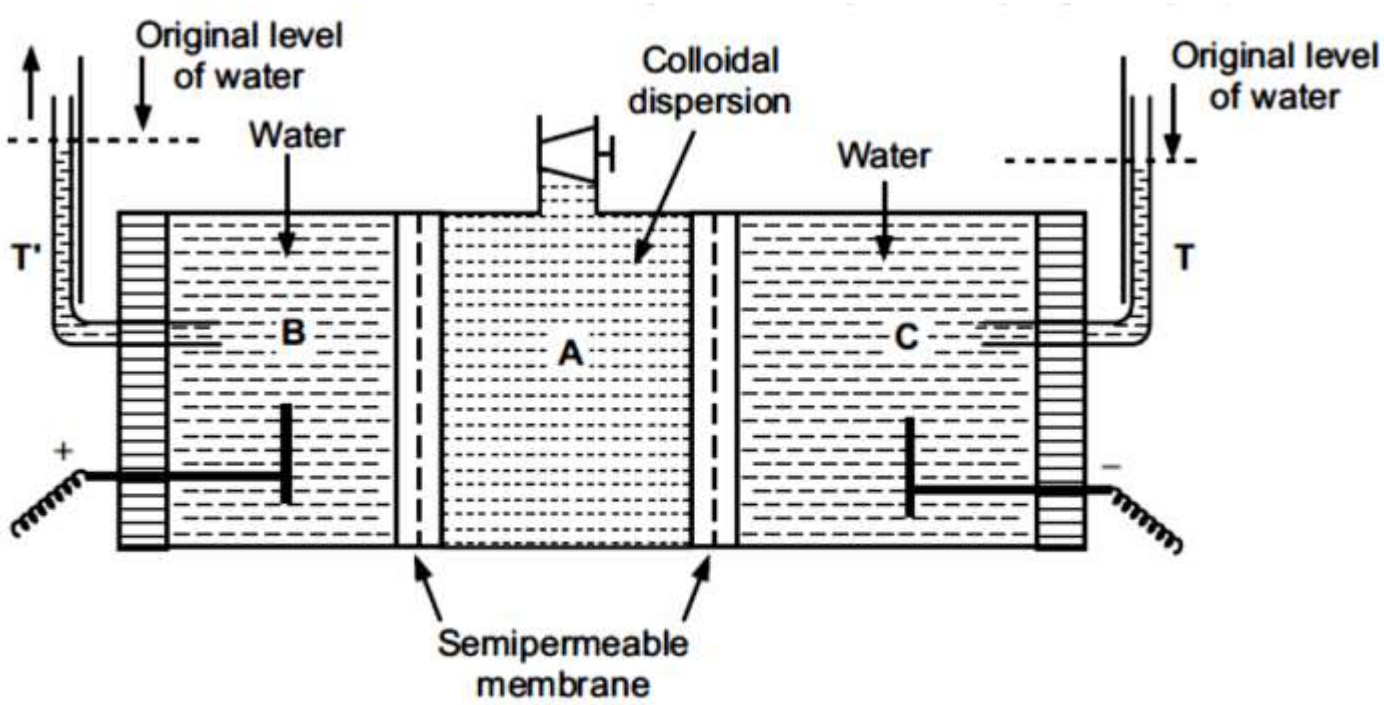


Fig. 5.19: Demonstration of electro-osmosis

STABILIZATION OF COLLOIDS

1. Provide electric charge on the surface of the dispersed particle

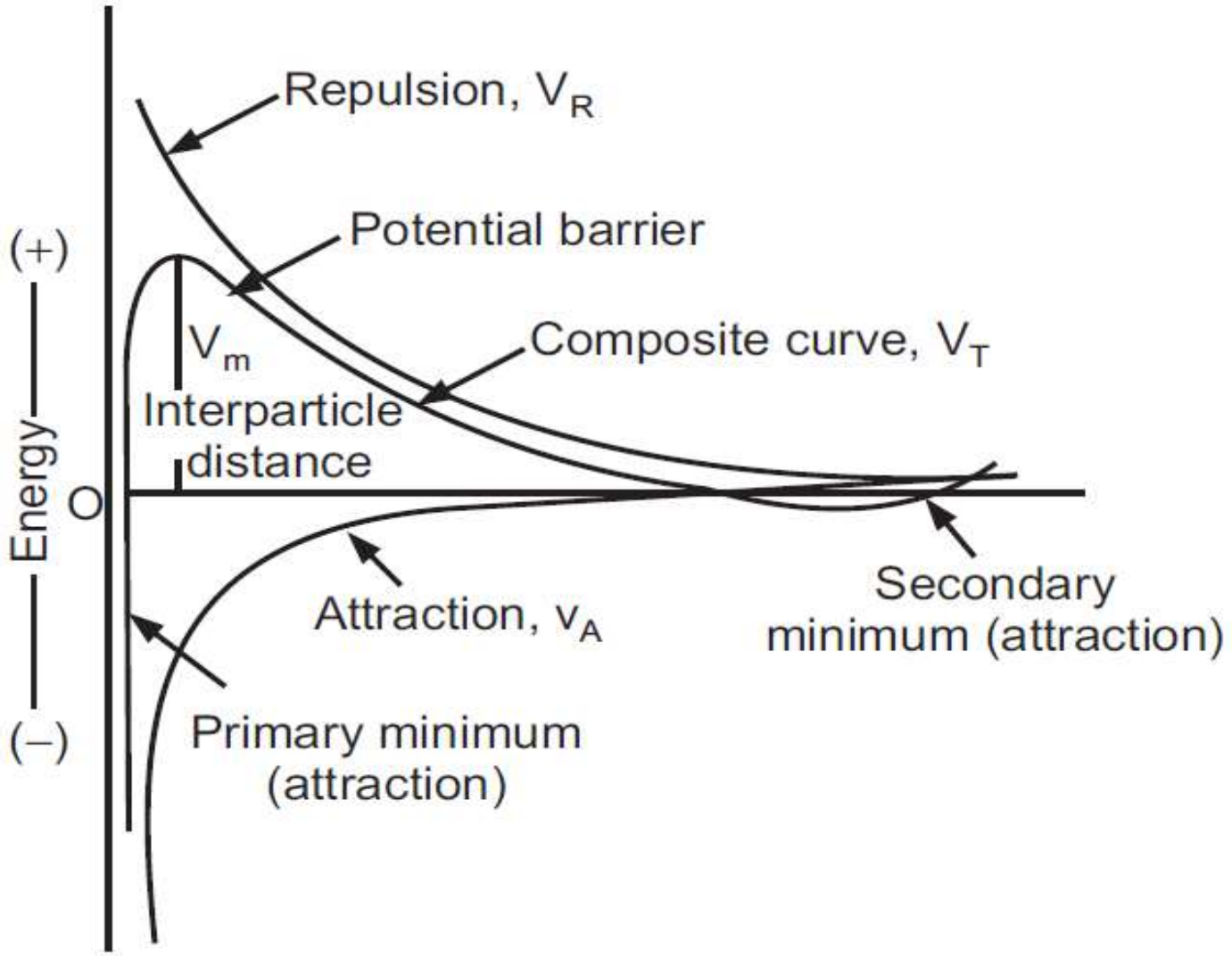
2. Maintain a solvent sheath around the particle:

The following factors sensitize the dispersed phase, which results in precipitation (coagulation or flocculation) of the particles. Lyophobic colloids are more prone to precipitation.

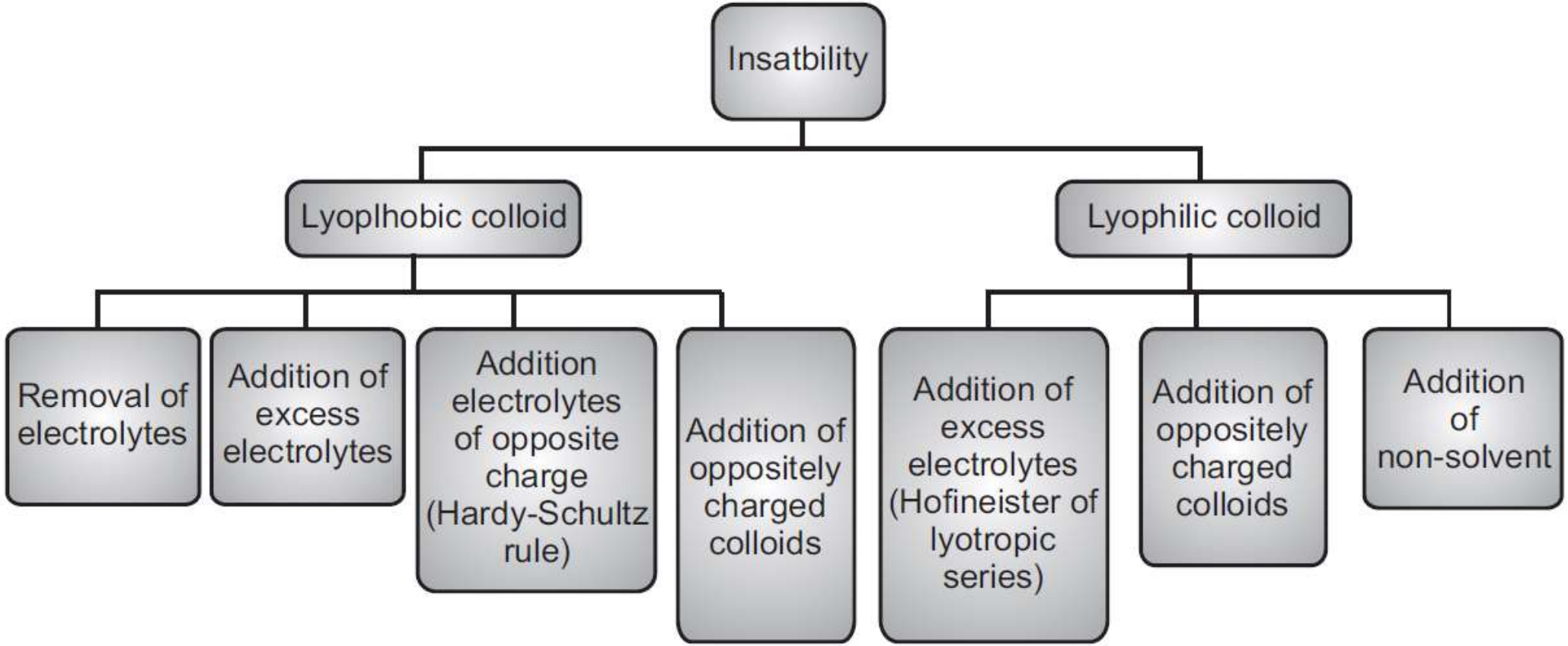
Formulation: Ingredients, electrolytes etc.

Environmental: Temperature, agitation during handling etc.

DLVO Theory: (Derjaguin, Landau, Verway and Overbeek Theory)

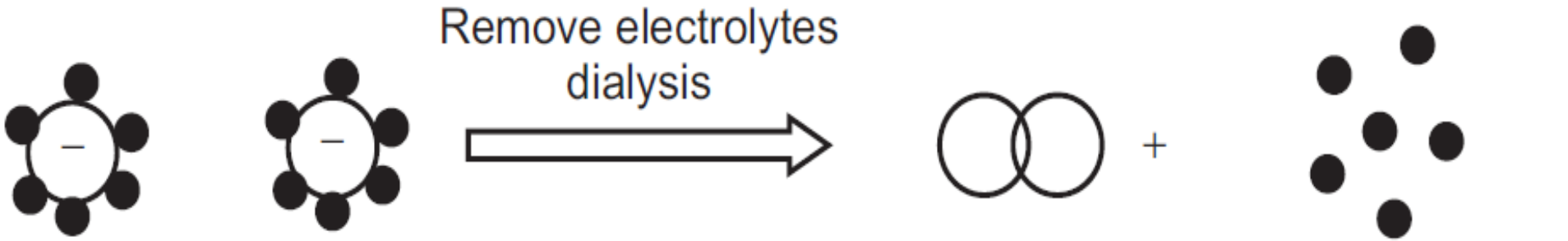


Factors Affecting Stability of Colloidal Dispersion



Instability of Lyophobic Colloids

(A) Removal of Electrolytes:

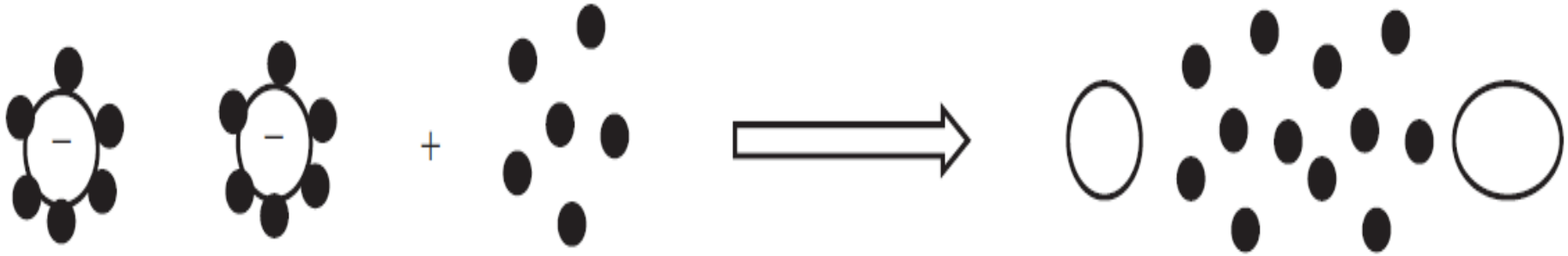


– ve sign colloidal particles stable because surface cover by electrolytes

Charge loss, coagulation, unstable, primary minimum

Electrolytes

(B) Addition of excess electrolytes:



Lyophobic sol

Excess of electrolytes

Flocculation, unstable, accumlation of electrolytes (Secondary minimum)

(C) Addition of opposite charge Electrolytes (Hardy-Schultz Rule):

They gave a generalisation known as Hardy – Schultz Law, which states, **"Greater the valency of oppositely charged ions of the electrolyte being added, the faster is coagulation".**

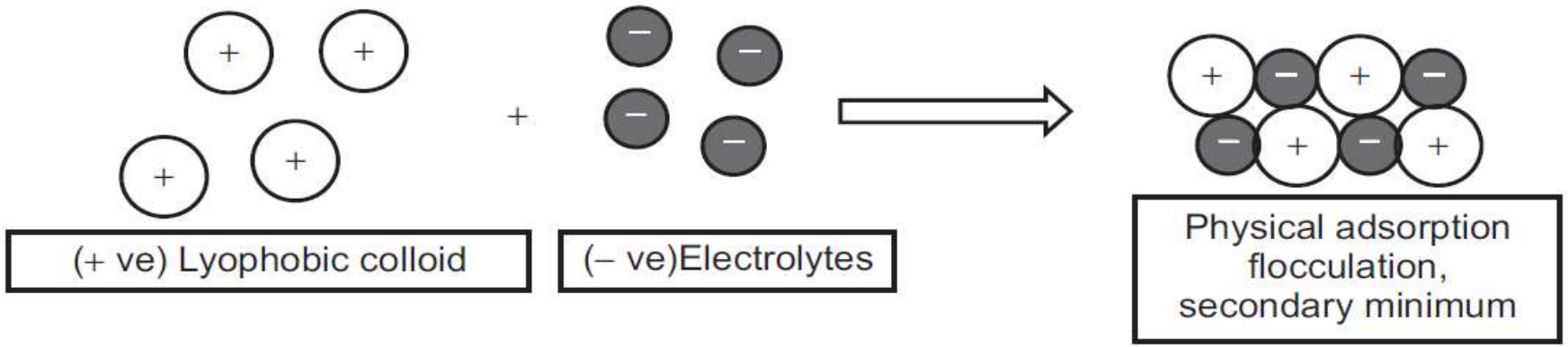
So, for coagulation of sols carrying negative charge Al^{3+} ion is more effective than Ba^{2+} ions or Na^{+} ions.

Thus in case of positively charged sol, the coagulating power of anions is in the order of

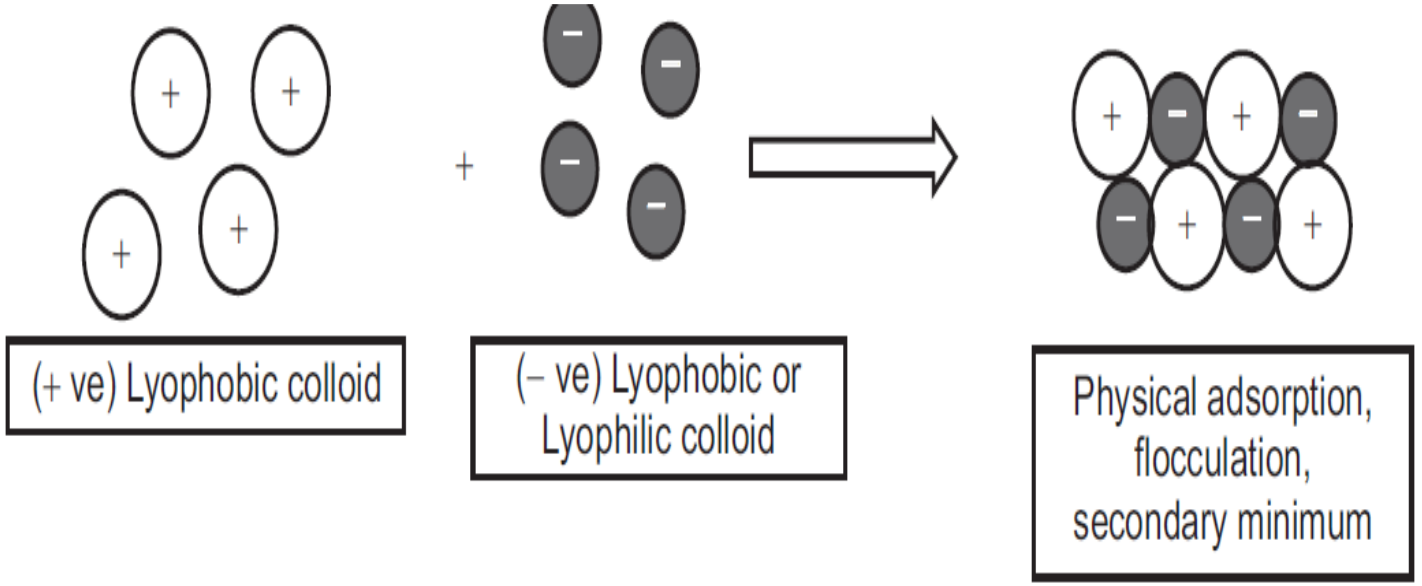
$$[Fe(CN)_4]^{4-} > PO_4^{3-} > SO_4^{2-} > Cl^{-}$$

and in case of negatively charged sols, the coagulating power of cations is in the order of

$$Al^{3+} > Ba^{2+} > Na^{+}$$



(D) Addition of oppositely charged colloids:



Instability of Lyophilic Colloids

(A) Addition of excess electrolytes (Hofineister of lyotropic series):

Hofineister of lyotropic series:

The coagulating power of anions or cations on hydrophilic colloids is arranged by Hofineister of lyotropic series.

Hofmeister rank order states that "the precipitating power of an ion is directly related to the ability of that ion to separate water molecules from the colloidal particles".

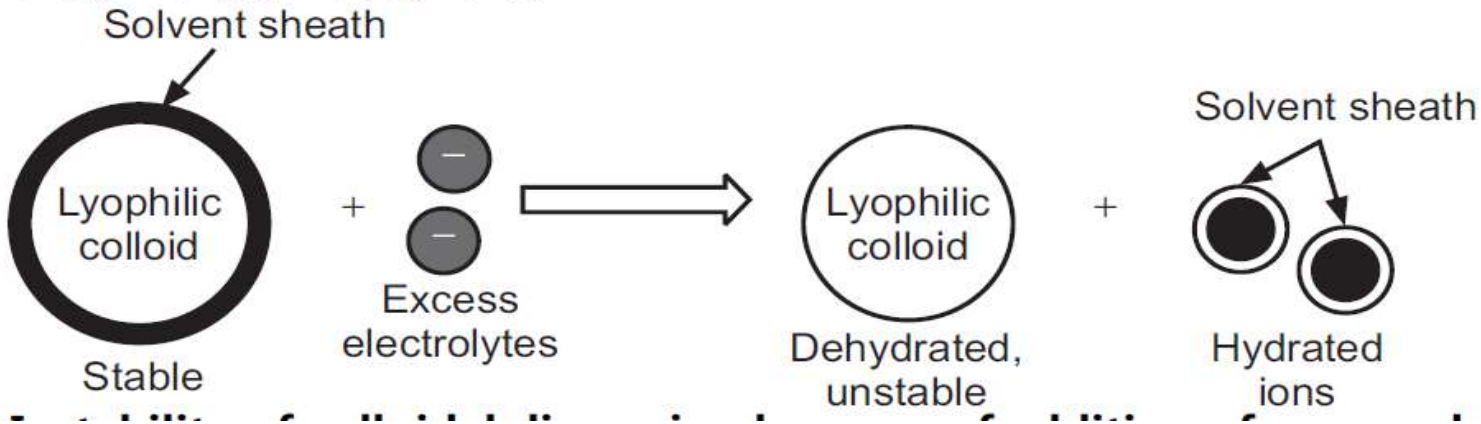
According to this statement, anions or cations are arranged in a series listed as follows:

Anions:

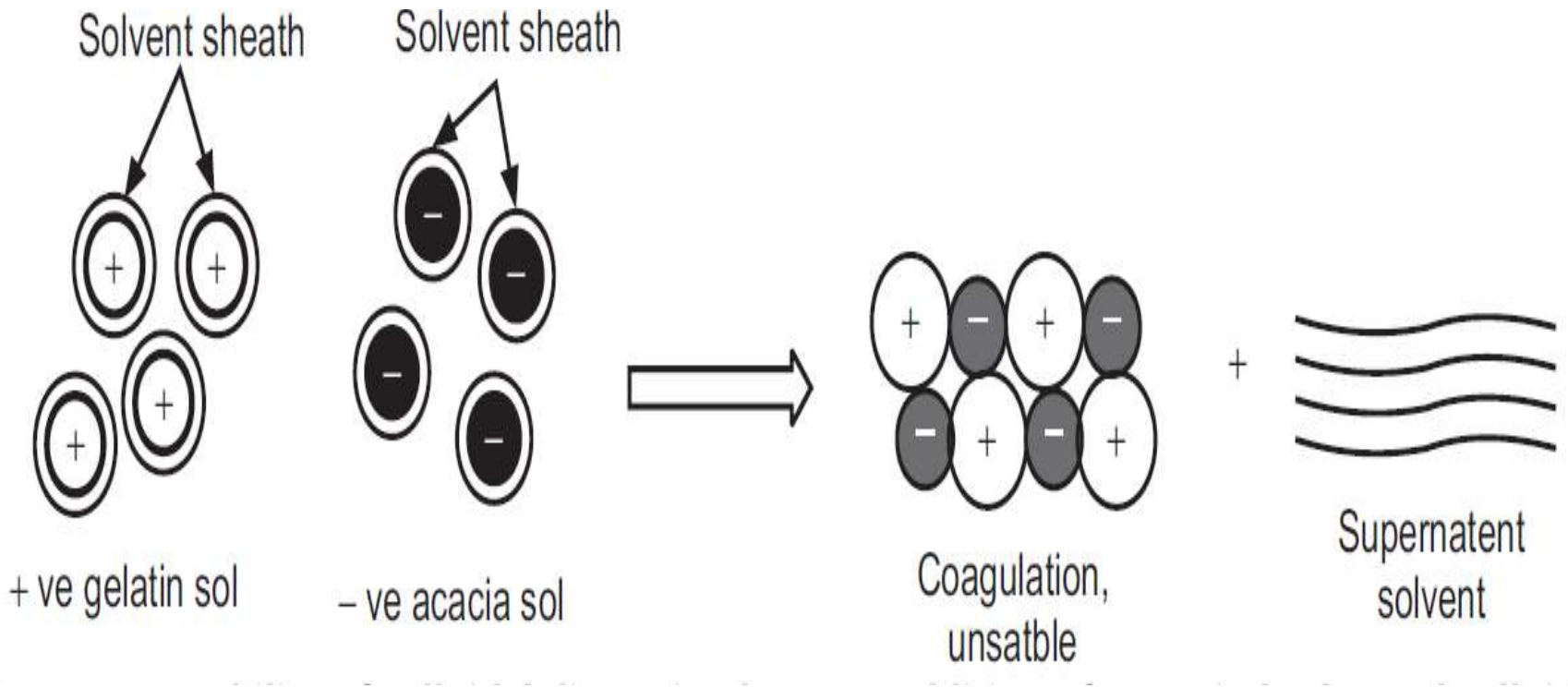
citrates > tartrates > sulfates > acetates > chlorides > nitrates > bromides > iodides

Cations:

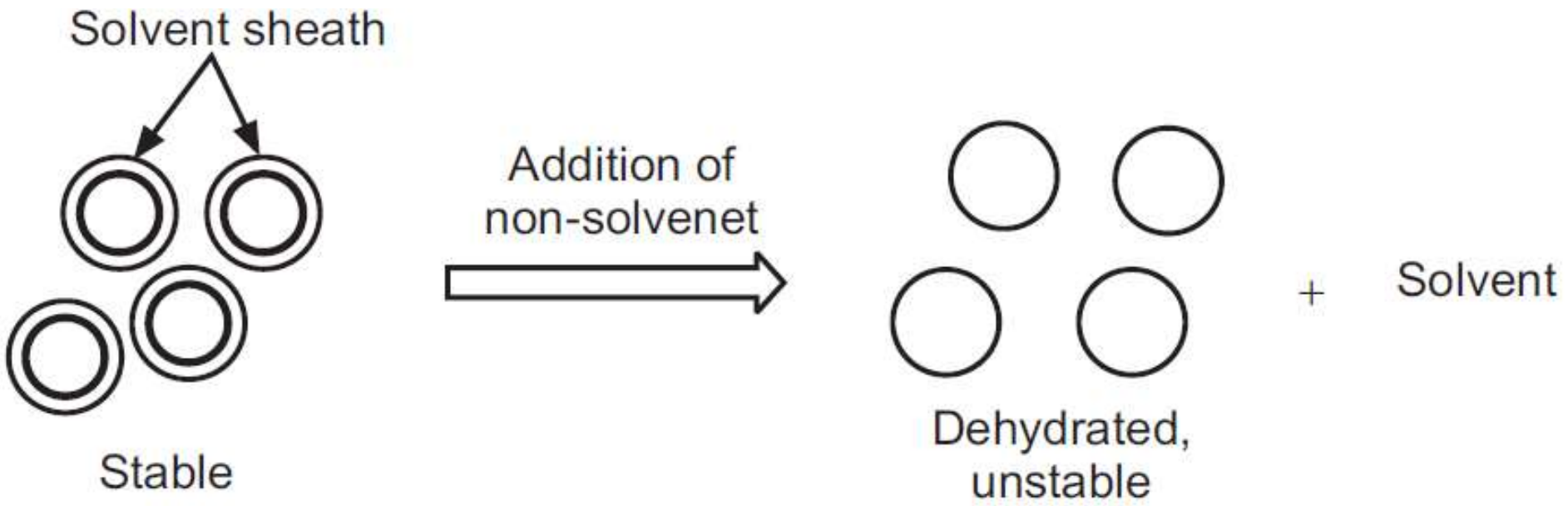
$Mg^{2+} > Ca^{2+} > Ba^{2+} > Na^{+} > K^{+}$



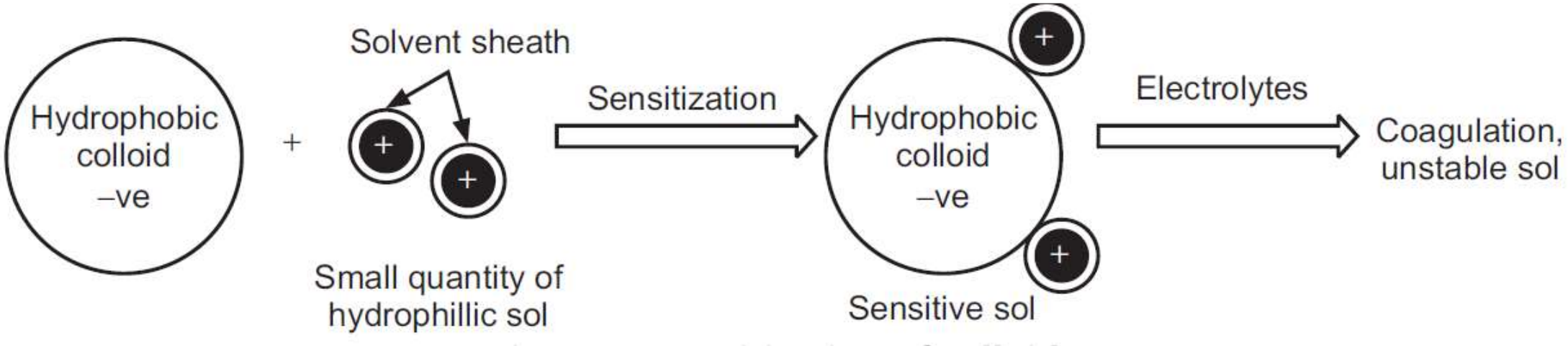
(B) Addition of oppositely charged colloids:



(C) Addition of Nonsolvent



SENSITIZATION OF COLLOIDS



GOLD NUMBER AND PROTECTION OF COLLOIDS

Gold number: Zsigmondy introduced a term called gold number to describe the protective power of different colloids. This is defined as, "weight of the dried protective agent in milligrams, which when added to 10 ml of a standard gold sol (0.0053 to 0.0058%) is just sufficient to prevent a colour change from red to blue on the addition of 1 ml of 10 % sodium chloride solution, is equal to the gold number of that protective colloid."

Thus, smaller is the gold number; higher is the protective action of the protective agent.

$$\text{Protective power} \propto \frac{1}{\text{Gold number}} \quad \ldots$$

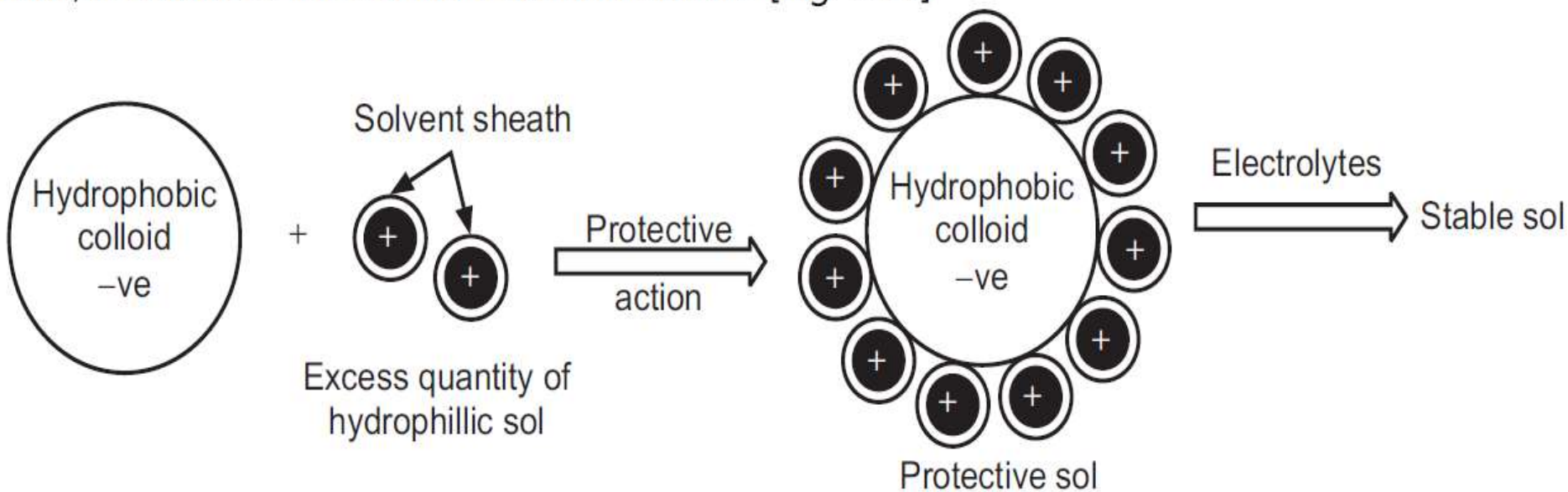
Table: 5.4: Gold numbers of some hydrophilic substances

Hydrophilic substance	Gold number	Hydrophilic substance	Gold number
Gelatin	0.005 - 0.01	Sodium oleate	0.4 - 1.0
Sodium caseinate	0.01	Gum tragacanth	2
Hamoglobin	0.03 - 0.07	Potato starch	25
Gum arabic	0.15 - 0.25		

Congo rubin number: Ostwald introduced congo rubin number to account for protective nature of colloids. It is defined as "the amount of protective colloid in milligrams which prevents colour change in 100 ml of 0.01 % congo rubin dye to which 0.16 g equivalent of KCl is added".

Mechanism of Solution Protection

The actual mechanism of sol protection is very complex. However, it may be due to the adsorption of the protective colloid on the lyophobic sol particles, followed by its solvation. Thus, it stabilises the sol via solvation effects. [Fig. 5.33]



DENTAL PRODUCTS

By

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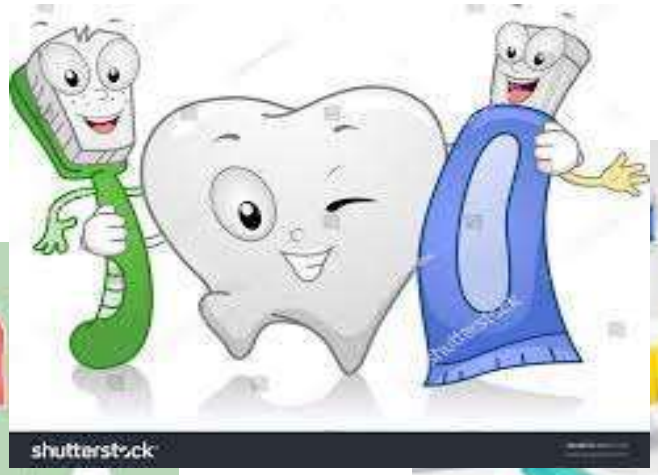
Dental Products



Introduction

- Dental hygiene is very important.
- A large no.of inorganic chemicals and their preparations find application in the practice of dental and oral disorders
  - **Dental products include-**

✓ **Anti-caries agents**

✓ **Cleaning agents/ Dentifrices**

✓ **Polishing Agents**

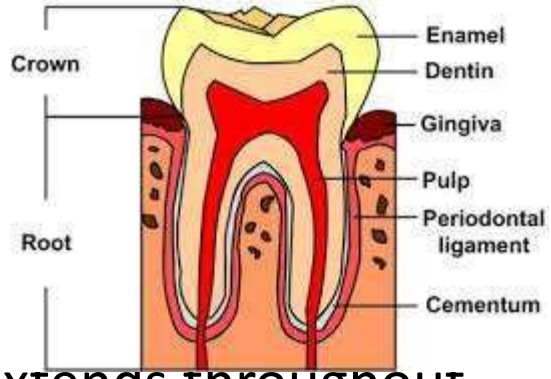
✓ **Desensitizing agents**

✓ **Oral antiseptics and astringents**

✓ **Mouthwashes**

✓ **Cements and Fillers**

Tooth



- Tooth consists of 3 layers

1. Dentine- It surrounds the pulp cavity and extends throughout the entire portion of tooth. 75% mineral
2. Cementum- It is the layer covering the portion of the lying buried in the gum
3. Enamel- white, hard material covering the portion of tooth projecting above the gum.98% mineral-hardest part of the body

- Vit A , C and D are necessary for proper tooth formation.
- Vit A deficiency causes hypoplastic enamel (imperfectlycalcified)
- Vit C deficiency affects calcification of dentine
- Vit D is important for absorption of Calcium from GIT and proper deposition of calcium and phosphorus in tooth

Anti caries Agents

- Dental caries or tooth decay is caused by acid produced by the action of microorganism or carbohydrates- involving decalcification of tooth accompanied by foul odour.
- Exact cause and mechanism not known
- Proposed mechanism-

1. Dental caries starts on the surface of the teeth
2. Acids produced by bacterial metabolism of fermenting carbohydrates act on teeth and produce lesions where bacteria get localised.
3. Demineralisation of enamel takes place (which initially appears as a white , chalky area and eventually becomes brown or yellow)

Dental caries if not treated , then micro-organisms may invade the pulp causing inflammation and infection

Prevention of dental caries

- Maintaining dental hygeine with the help of dentrifices- Dentrifices enhance removal of dental plaque and stains
- Flossing and brushing regularly
- Administration of Fluoride

(Anti caries agents- Sodium Fluoride, Stannous Fluoride, Sodium Monofluorophosphate U.S.P)

Role of Fluoride

- Role of fluoride in preventing dental caries is well accepted.
- Administration of traces of fluoride having salts or their use topically to the teeth have reported encouraging results
- Fluoride ion is a trace element which occurs in the body.

- Water fluoridation as well as topical fluoride applications (e.g. fluoridated toothpaste or varnish) appears to prevent caries, primarily on permanent dentition.
- Topical fluoride sustains the fluoride levels in the oral cavity and helps to prevent caries, with reduced systemic availability.
- Fluoride can affect both the inorganic tooth structure & the bacterial metabolism in plaque, several

- The main in organic constituent of tooth and bone is hydroxy apatite (HAP).
- Hydroxy apatite on addition of fluorine results in the formation of flour apatite (FAP) or fluoridated hydroxy apatite because not all the hydroxyl groups are replaced by fluorides.
- A pure fluorapatite crystal would contain 38,000 ppm F but enamel form a fluoridated area contain only 500 to 2000 ppm.
- This leads to speculation on several possible mechanims of action of systemically ingested fluoride improved crystalinity, the void theory, FAP V/s HAP solubility in acid & improved tooth morphology

Proposed Mechanism of action of fluorides

- **Reduced enamel solubility**-decreased solubility of fluoridated enamel is that fluorapatite (with a solubility product constant of 10-60) is less soluble than hydroxyapatite
- **Improved crystallinity**- Fluoride increases the crystal size and produces less strain in crystal lattice.
- **Promotion of reminerlization**- Dissolved enamel Minerals of tooth enamel are continuously inexchange with the minerals of saliva and thusthe balance is maintained. This Equilibrium can get disturbed with the organic acidproduced by the metabolism of fermentable carbohydrates by the microorganism. Thisleads to drop in PH. of the plaque on the enamel surface and in the sub surface.Minerals, particularly calcium and phosphate leave the dissolved enamel in their ionic form an entrace the plaque fluid. This process iscalled demineriliza tion this get reverrse with the factor like fluoride and is terms reminerilization.

- **Lower free surface energy**- void in the crystals decreases the stability and increases chemical reactivity. If fluoride fills these void in the hydroxy apetite crystals it will attain stable from with formation of more and stronger hydrogen bonds. Greater stability will leads to lower solubility and hence greater resistance to dissolution in acids.
- **Desorption of protein and bacteria**
- **Reduced cariogenic flora** - . fluoride is a potent suppressor of the bacterial growth because it oxidizes the thiol group present in bacteria thus inhibiting bacterial matabolism. The concentration of fluoride above 2 ppm in solution progressively decrease the transport of uptake of glucose into Cells of streptococci and also reduces ATP synthesis.(Anti bacterial action)

  The primary assumption in this theory is that dental caries results from a specific pathogen, S. mutans. Thus the elimination or reduction of this pathogen with provide a lasting cariostatic effect.
- **Inhibition of bacterial enzymes systems**-Fluoride has enolase inhibition effect and it also inhibits glucose transport, enolase is a metallo enzyme that requires adjavalent cation for tis activity., fluoride due to its increased reactivity forms a complex with this cation. Thus inhibiting the enzyme. It also inhibits non-metallo enzyme like phosphatage thus leading to reduce acid production

Monographs of Sodium Fluoride

Title: Sodium Fluoride
Molecular formula: NaF
Mol. Wt.: 42.0
Standard: Sodium Fluoride contains not less than 98.5 per cent and not more than 100.5 per cent ofNaF, calculated on the dried basis.
Category. Preventive for dental caries.
Description. A white powder or colourless crystals.
Identification

A. Dissolve 2.5 g in sufficient carbon dioxide free water without heating to produce 100 ml (solution A). To 2 ml of solutionAadd 0.5 ml of calcium chloride solution; a gelatinous white precipitate is produced which dissolves on adding 5 ml of ferric chloride solution.
B. Add about 4 mg to a mixture of 0.1 ml of alizarin red S solution and 0.1 ml of zirconyl nitrate solution and mix; the colour changes to yellow.
C. Gives reaction A of sodium salts

Test: Appearance of solution (clear and colourless) , Acidity or alkalinity, Chlorides (NMT 250 ppm), Fluorosilicate (Absent) , Sulphates (NMT 10 ppm) , Loss on drying (NMT 0.5 %)

Assay: Non aqueous titration with perchloric acid using crystal violet solution as indicator, until a green colour is produced.

Storage. Store protected from moisture.

USES

- 2 % aqueous solution is used topically for treatment of caries.
- Component of various anti-caries toothpastes

Dentifrices

- Dentifrice is a material which is used for cleaning of teeth and adjacent gums.
- The cleaning is dependent on abrasive property and the rubbing force used.
- They may be applied as pastes or powders with the help of fingers or toothbrush.
- Flavors and colors are usually added to dentifrice formulations to improve their acceptance

- Dentifrices are agents used along with a toothbrush to clean and polish natural teeth. They are supplied in paste, powder, gel or liquid form. The most essential dentifrice recommended by dentists is toothpaste which is used in conjunction with a toothbrush to help remove food debris and dental plaque.
- A good cleaning agent must remove stains from teeth and to achieve this suitable abrasiveness in essential.
- The main drawback is that it will not be able to clean surfaces inside cavities and cervices between teeth.

Types of Dentifrices

- **Toothpaste**-Toothpaste is a dentifrice used in conjunction with a toothbrush to help maintain oral hygiene. The essential components are an abrasive, binder, surfactant and humectant. Other ingredients are also used. The main purpose of the paste is to help remove debris and plaque with some marketed to serve accessory functions such as breath freshening and teeth whitening.
- **Toothpowder**-Tooth powder is an alternative to toothpaste. It comes in both fluoride and non-fluoride versions.

- **Mouthwash**-Mouthwashes come in a variety of compositions, many claiming to kill bacteria that make up plaque or to freshen breath.In their basic form, they are usually recommended for use after brushing but some manufacturers recommend pre-brush rinsing. Dental research has recommended that mouthwash should be used as an aid to brushing rather than a replacement, because the sticky resistant nature of plaque prevents it from being actively removed by chemicals alone, and physical detachment of the sticky proteins is required.
- Tooth soap-Tooth soap cleans gums as well as fissures and pits in teeth using soap. The soap helps remove oils, residue and other contaminants. It is available in hard, liquid and gel.

The functions of toothpaste in conjunction with tooth brushing are:

- ✓ **Minimizing plaque build up**
- ✓ **Anti-caries action**
- ✓ **Removal of stains**
- ✓ **Mouth freshening/odorising**

Examples of dentifrices

- Calcium carbonate
- Dibasic calcium phosphate
- Calcium phosphate
- Sodium metaphosphate
- Pumice

Calcium carbonate

Synonym :Precipitated Chalk

Molecular Formula: $CaCO_3$

Molecular weight: 100.1

Standard: Calcium Carbonate contains not less than 98.0 per cent and not more than 100.5 per cent of CaC03, calculated on the dried basis

Dose. 1 to 5 g.

Description. A fine, white, microcrystalline powder.

Tests:

Substances insoluble in acetic acid: NMT 10 mg

Arsenic: NMT 4 ppm

Heavy metals: NMT 20 ppm (Method A)

Barium

Iron: NMT 200 ppm

Chloride: NMT 250 ppm

Sulphates: NMT 0.3 %

Loss on drying: NMT 2 %

Assay: Complexometric titration

Titrant: 0.05 M Disodium edetate

Indicator: Calcon mixture

End point: Pink to full blue colour

- **Calcium phosphate**: Also known as tribasic calcium phosphate/tricalcium phosphate

Synonym: Calcium Hydroxide Phosphate; Calcium Phosphate

Tribasic Calcium Phosphate consists mainly of tricalcium diorthophosphate

together with calcium phosphates of more acidic or basic character

Tribasic Calcium Phosphate contains not less than 90.0 per cent and not more than 100.5 per cent of calcium phosphates, calculated as Ca3(P04)2

Category. Pharmaceutical aid (excipient).

Description. Awhite, amorphous powder; odourless or almost odourless.

- **Dibasic Calcium Phosphate**:

Synonym: Calcium Hydrogen Phosphate

CaHP04 Mol. Wt. 136.1 (anhydrous)

CaHP04,2H20 Mol. Wt. 172.1 (dihydrate)

Dibasic Calcium Phosphate is anhydrous or contains two

molecules of water of hydration.

Dibasic Calcium Phosphate contains not less than 98.0 per

cent and not more than 105.0 per cent ofCaHP04(for anhydrous material) or ofCaHP$0_{4,}2H_20$ (for the dihydrate).

- **Pumice**: It is a substance of variable composition consisting of complex silicates of aluminium, potassium and sodium, having volcanic origin.

  It is odourless, tasteless, very light, grayish white powder with fineness. It is grity in nature. On sifting it is categorised into superfine, fine and coarse pumice powder.

Composition of dentifrice formulations

AGENTS	MATERIAL USED	FUNCTIONS
1.Polishing/Abrasives agents	Calcium carbonate Dicalcium phosphate dihydrate Alumina Silica	These agents have a mild abrasive action, which aids in eliminating plaque and removing stains from tooth surface.
2.Binding/Thickening agents	Water soluble agents a. Alginates b. Sodium carboxymethyl celluose Water insoluble a. Magnesium aluminium silicate b.Colloidal silica c.Sodium magnesium silicate	Agents which controls stability and consistency of a tooth paste.
3. Detergent/Surfactans	Sodium lauryl surface	Produce the foam which aids in the removal of food debris and also despersion of product within mouth.
4. Humectants	Sorbitol Glycerin Polyethylene glycol	Aids in reducing loss of moisture from toothpaste.

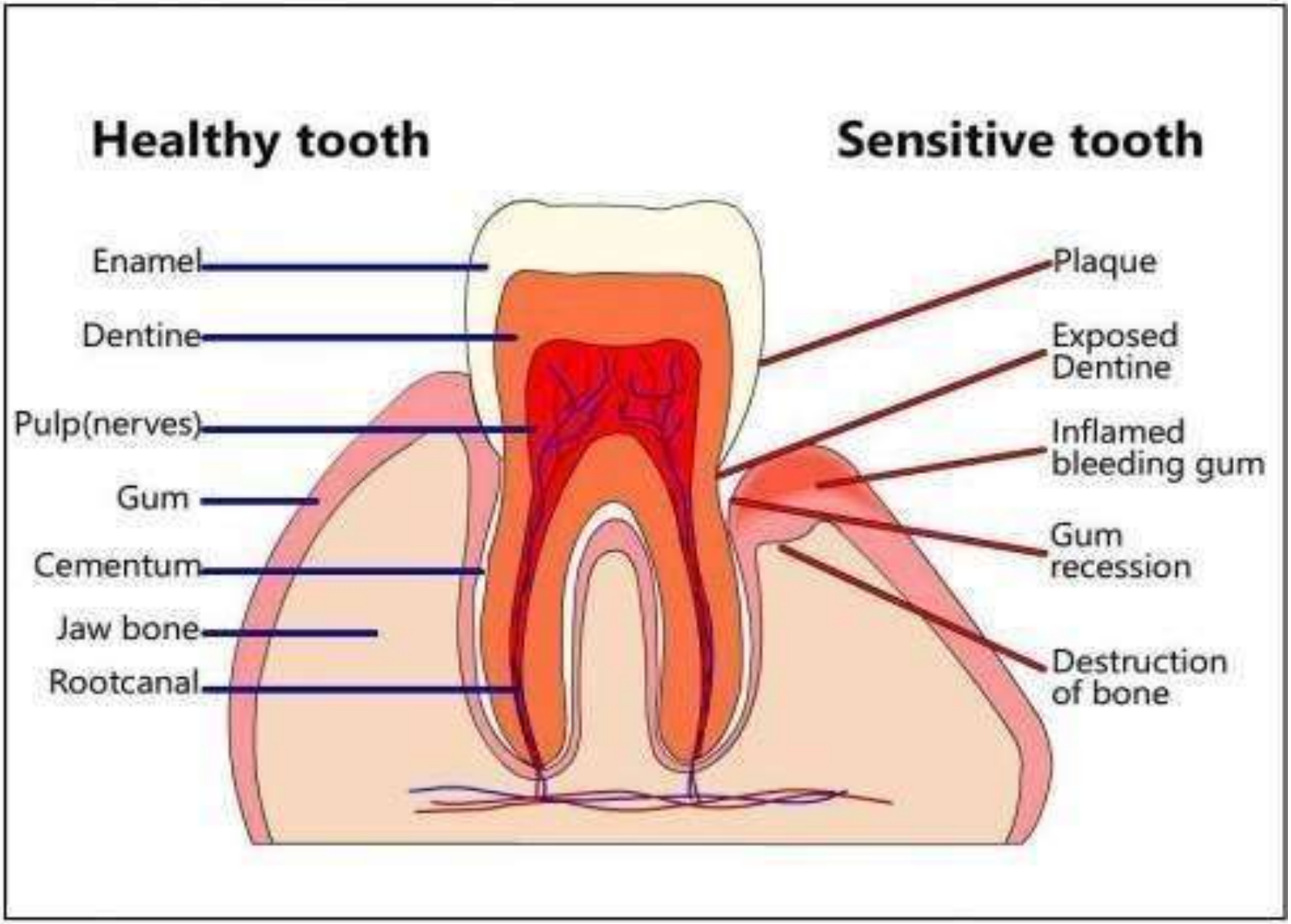
5. Flavouring agents	Peppermint oil Spearmint oil Oil of wintergreen	They render the product pleasant to use and leaves a fresh taste in mouth after use.
6. Sweeteners and Coloring agents	Saccharin	Sweetener
7. Antibacterial agents	Ticlosan Delmopinol Metallic ions Zinc citrase trihydrate	
8. Anticalculus agents	Pyrophosphate Zinc citrate Zinc chloride Gantrez acid(copolymer of methyl vinyl ether and maleic anhydride)	Anticalculus agents are mostly designed to inhibit the mineralization of plaque. They are also known as crystal growth inhibitors.
9. Anticaries agents	Sodium monoflurophosphate Sodium flouride Stannous flouride	
10. Diesensitizing agents	Sodium flouride Potassium nitrate Strontium chloride	

Monograph

- Calcium Carbonate

Desensitizing agents

- DENTIN HYPERSENSITIVITY is characterized by short sharp pain arising from exposed dentin in response to stimuli—typically thermal, evaporative, tactile, osmotic or chemical—that cannot be ascribed to any other dental defect or disease.
- Dentine hypersensitivity is sensation felt when the nerves inside the dentin are exposed to the environment
- The sensation can range from irritation all the way to intense, shooting pain.
- This sensitivity can be caused by several factors, including wear , decaying teeth or exposed tooth roots.


Healthy tooth
Sensitive tooth
Enamel
Dentine
Pulp(nerves)
Gum
Cementum
Jaw bone
Rootcanal
Plaque
Exposed Dentine
Inflamed bleeding gum
Gum recession
Destruction of bone

Causes of sensitivity

- Gastroesophageal reflux disease (GERD)
- Conditions in which person frequently vomits. I.e. Gatrioparesis or bulimia
- Gum recession
- Tooth decay
- Injured tooth
- Broken tooth
- Chipped tooth
- Worn down fillings /crowns

  Sometimes, it is temporary following dental treatments such as filling, crowning and bleaching

Treatment of dental sensitivity

- **Mode of administration**
  - At home desensitizing agents
  - In-office treatment
- **On the basis of mechanism of action**
- Nerve desensitization
  - Potassium nitrate
- **Protein precipitation**
  - Gluteraldehyde
  - Silver nitrate
  - Zinc chloride
  - Strontium chloride hexahydrate
- **Plugging dentinal tubules**
  - Sodium fluoride
  - Stannous fluoride
  - Strontium chloride
  - Potassium oxalate
  - Calcium phosphate
  - Calcium carbonate
- Dentine adhesive sealers
  - Fluoride varnishes Oxalic acid and resin Glass ionomer cements
  - Composites
  - Dentin bonding agents
- Lasers
  - Neodymium:yttrium aluminum garnet (Nd-YAG) laser
  - GaAlAs (galium-aluminium-arsenide laser)
  - Erbium-YAG laser
- Homeopathic medication
  - Propolis

Zinc oxide eugenol cement

- Zinc oxide eugenol (ZOE) is a material created by the combination of zinc oxide and eugenol contained in oil of cloves.
- An acid-base reaction takes place with the formation of zinc eugenolate chelate. The reaction is catalysed by water and is accelerated by the presence of metal salts.
- It has anaesthetic, anti-bacterial properties.

Composition of ZOE

Component	Approximate w/w %	Function
Solids		
Zinc Oxide	69 %	Principal ingredient
White Rosin	29.3%	Reduce brittleness of set cement and maintain homogeneity
Zinc acetate	1.0%	Accelerator, improve strength
Zinc stearate	0.7%	Accelerator, plasticizer
Liquids		
Eugenol	85%	Reacts with ZnO, act as anaesthetic
Olive Oil	15%	Plasticizer

Classification of Zinc oxide Eugenol (ZOE)

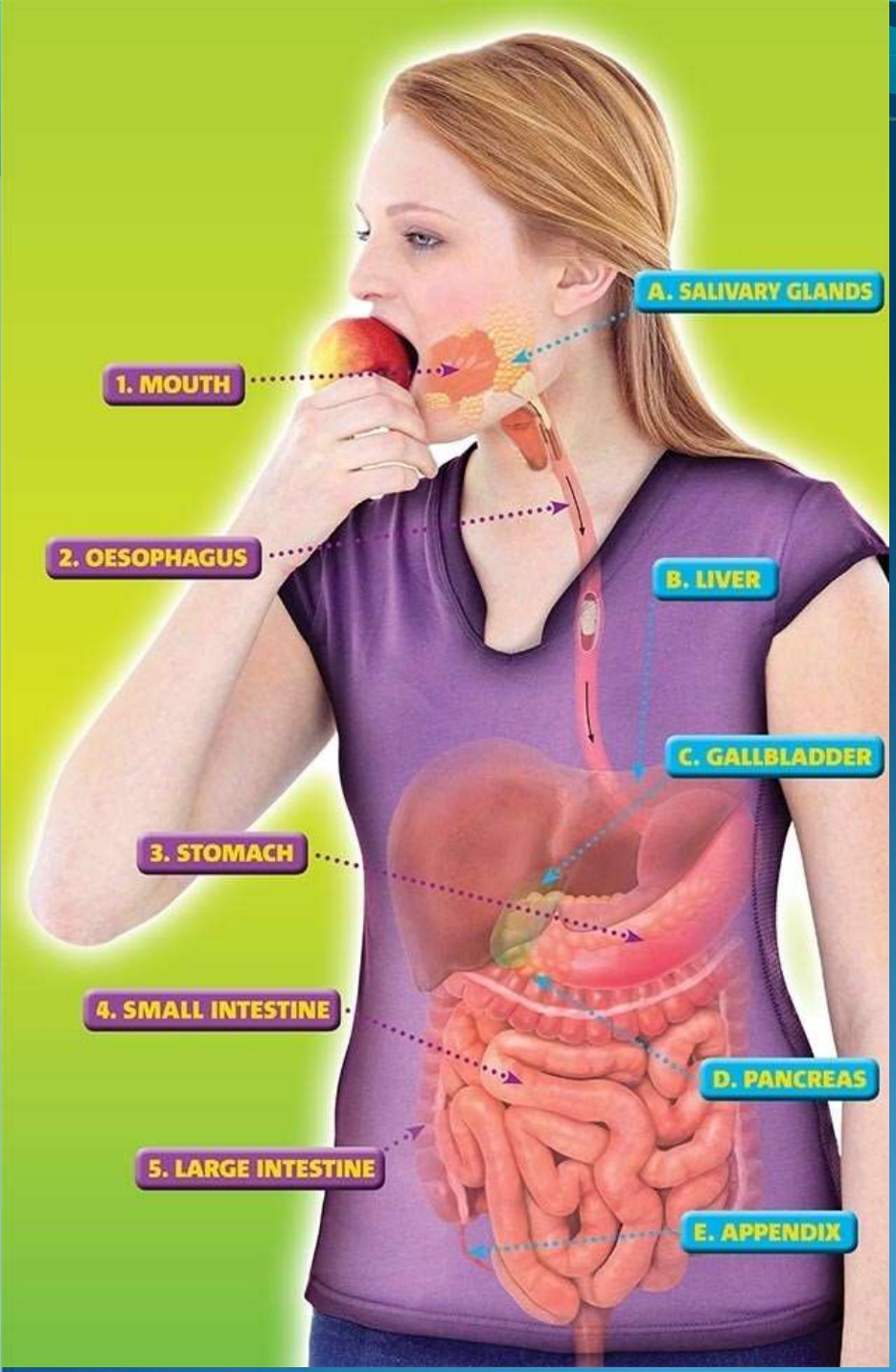
- Type –I ZOE: for temporary cementation
- Type –II ZOE: for permanent cementation
- Type III ZOE: for temporary filling and thermal base
- Type IV ZOE: cavity liner

Uses:

1. **ZOE can be used as a dental filling material or dental cement in dentistry.**
   It is often used in dentistry when the decay is very deep or very close to the nerve or pulp chamber. Because the tissue inside the tooth, i.e. the pulp, reacts badly to the drilling stimulus (heat and vibration), it frequently becomes severely inflamed and precipitates a condition called **acute or chronic pulpitis**. (This condition usually leads to severe chronic tooth sensitivity or actual toothache and can then only be treated with the removal of the nerve (pulp) called root canal therapy.)
2. The placement of a ZOE "temporary" for a few to several days prior to the placement of the final filling can help to sedate the pulp.
3. ZOE is used in mucostatic in a technique of taking impressions of gum and teeth
4. It is used as **pulp capping agent**.
5. Commonly used as cavity liner under dental amalgams or as temporary filling material.

Important Questions

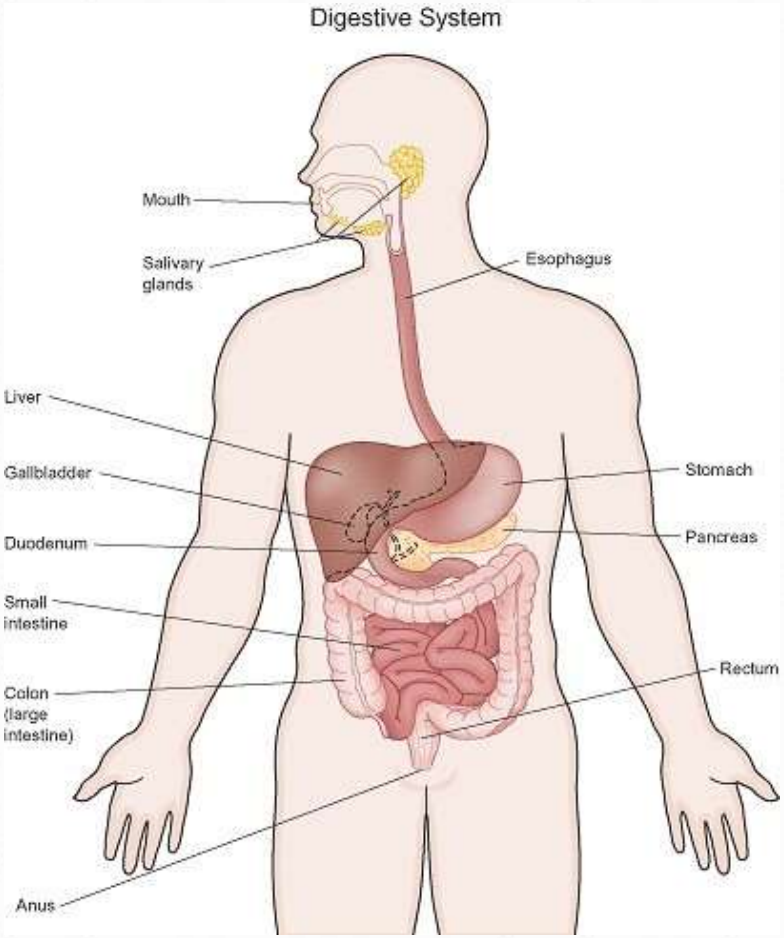
- Discuss the role of fluoride in Dental caries
- Define Dentifrices, Anti-caries agents, Desensitizing agents with examples.
- Monograph- Calcium carbonate, Sodium Fluoride, Zinc Eugenol cement
- What are dental products? Discuss their composition and role.
- What are anti caries agents? Discuss in detail.



The Digestive System

Introduction

- The digestive system is used for breaking down food into nutrients which then pass into the circulatory system and are taken to where they are needed in the body.



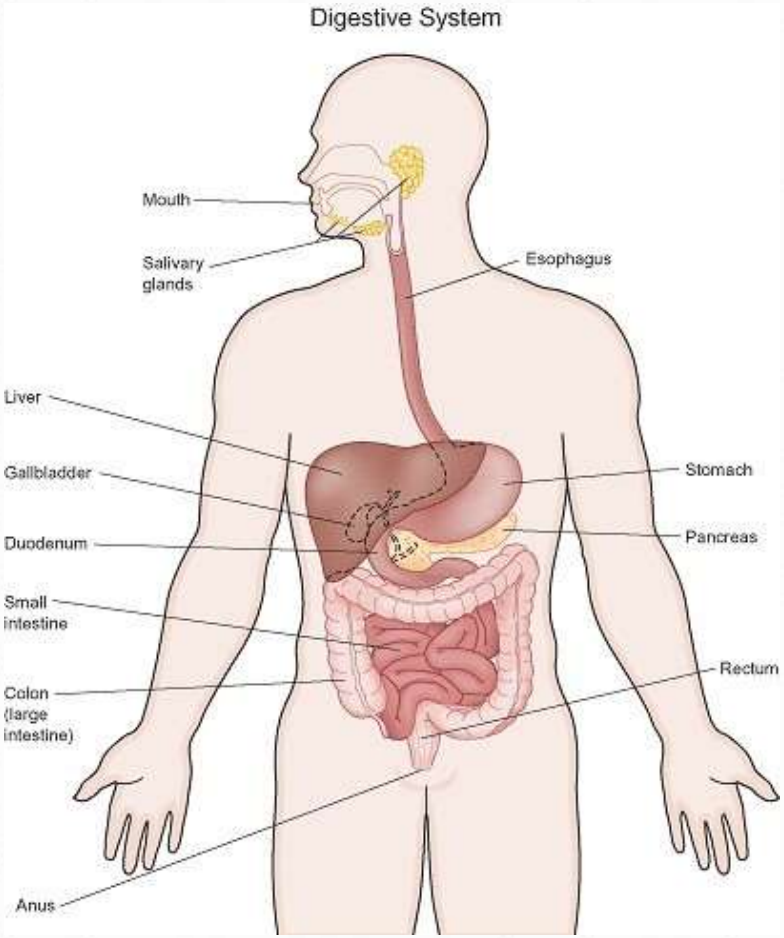
Functions of the Digestive System

- Ingest food
- Break down food into nutrient molecules
- Absorb molecules into the bloodstream
- Rid the body of indigestible remains

Introduction

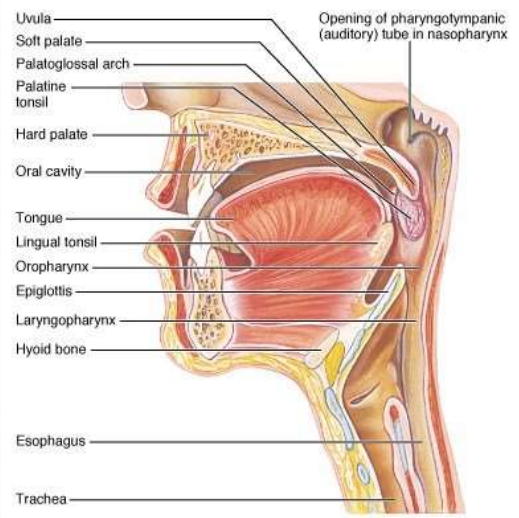
- There are four stages to food processing:

1. Ingestion: taking in food
2. Digestion: breaking down food into nutrients
3. Absorption: taking in nutrients by cells
4. Egestion: removing any leftover wastes

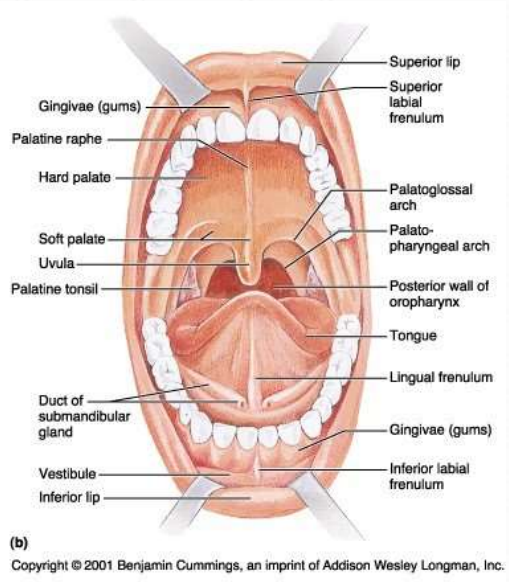


Mouth

- Teeth mechanically break down food into small pieces. Tongue mixes food with saliva (contains amylase, which helps break down starch).

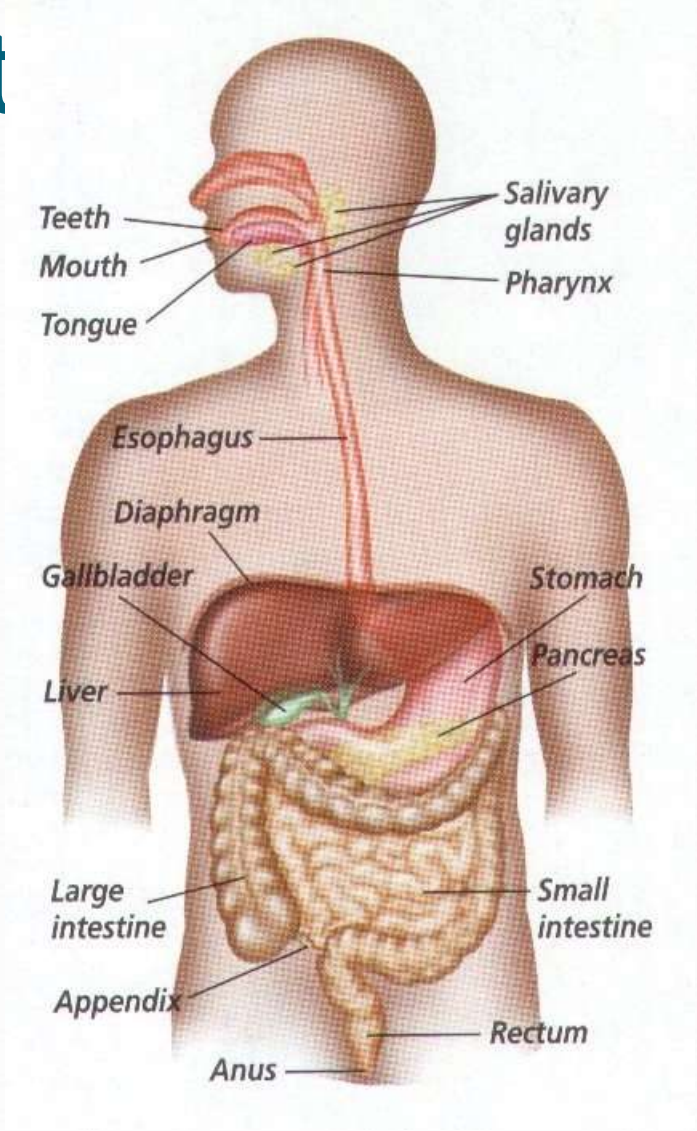


- Epiglottis is a flap-like structure at the back of the throat that closes over the trachea preventing food from entering it.



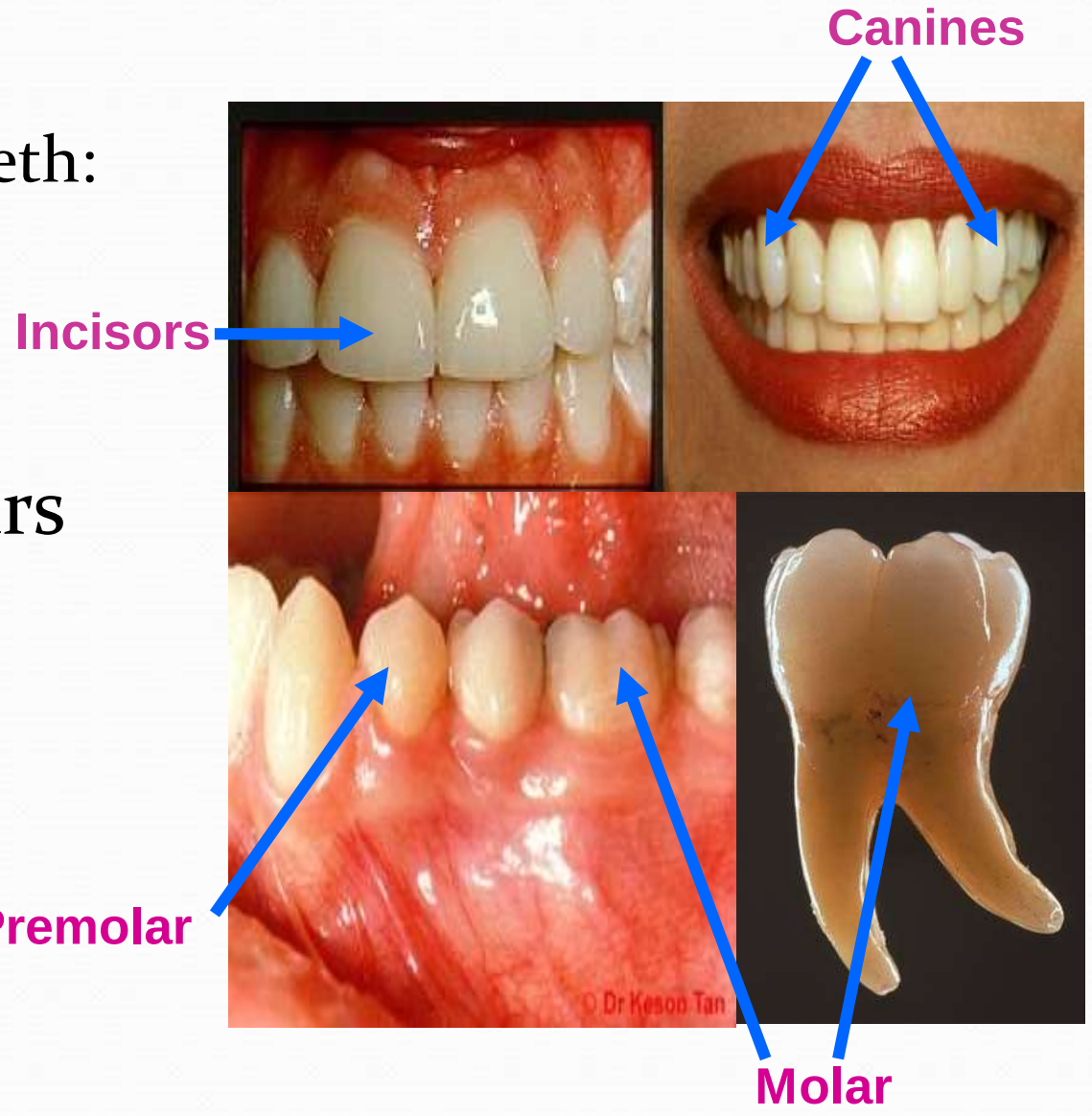
Parts of the Digest

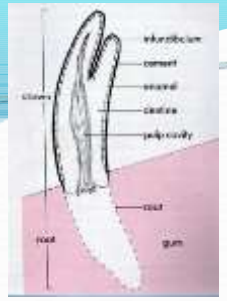
- **Teeth:** Grinds your food.
- **Salivary Glands:** Produce the saliva in your mouth.
- **Tongue:** Helps to push food into your esophagus.



Teeth

- Four types of teeth:
  1. Incisors
  2. Canines
  3. Pre – Molars
  4. Molars



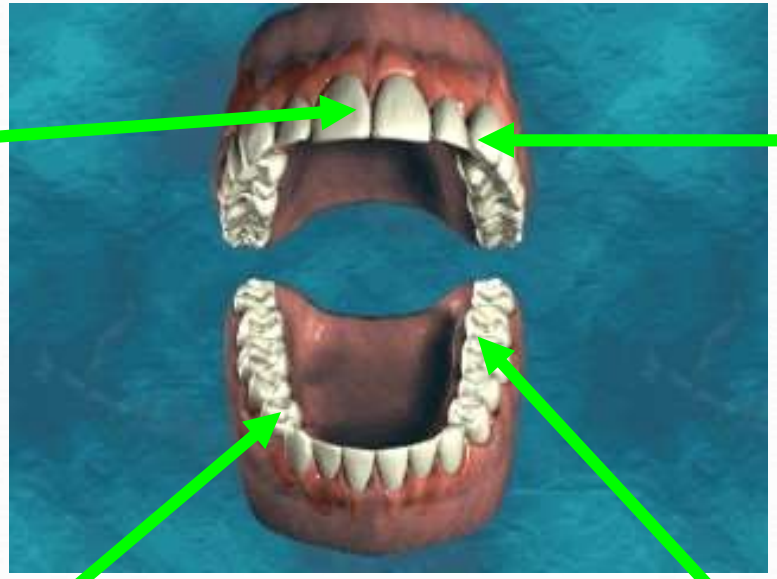


Biting and Cutting

Incisor

Grasping and Tearing

Canine



Grinding and Crushing

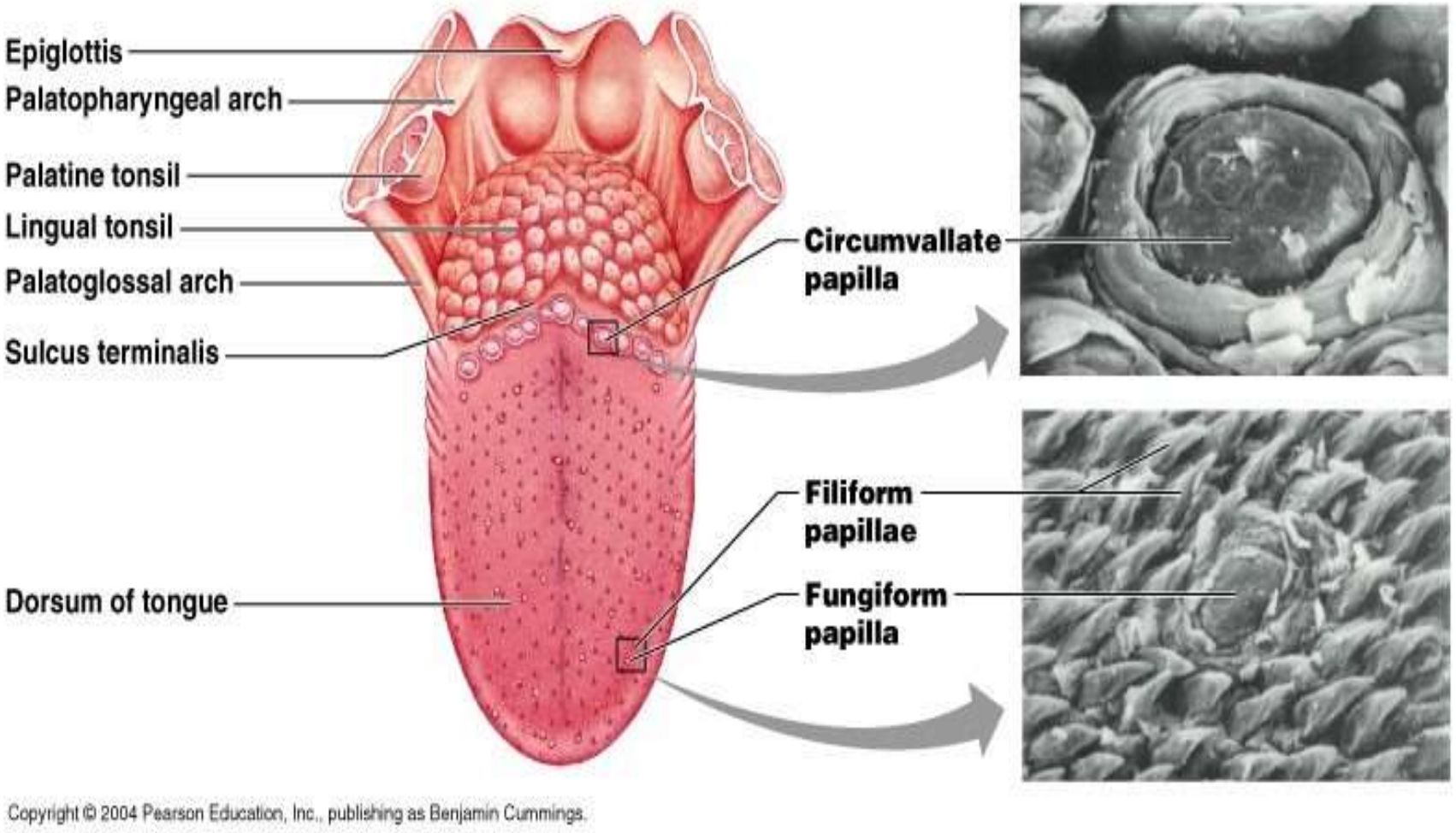
Pre - Molar

Grinding and Crushing

Molar

- The tongue is not only one muscle.
- It is a group of muscles, and it is also what we use to taste food with.

Anatomy of the Tongue



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Taste

The tongue can detect five basic tastes:

- *bitter;*
- *salt;*
- *sour;*
- *sweet;*
- *umami.*

Taste may be described by association with a particular food, e.g. meaty, minty or fruity.

The intensity can also be recorded, e.g. mild or strong Cheddar.

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Papillae have 2 purposes. Describe them.

- The purpose of the papillae is to grip the food and with the taste buds they contain.
- They also “taste” the food.

ZIDBITS

Why won't you eat it?

Science behind picky eaters

A person with an increased number of tastebuds is able to taste foods with greater intensity. These supertasters can distinguish even the slightest taste variation and their sensitivity to taste is often misread as picky eating.

Normal

Supertaster

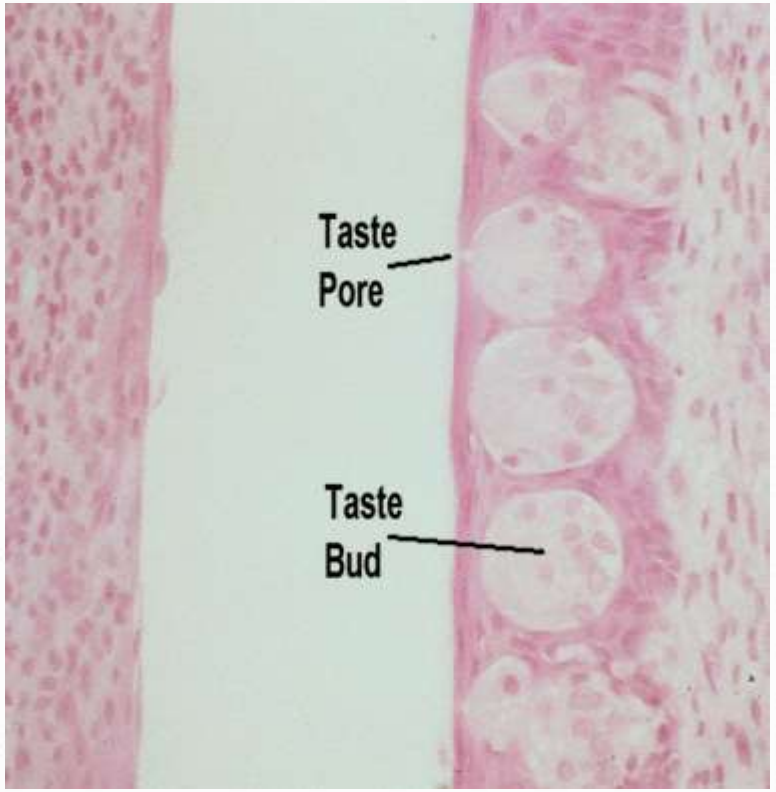
Problematic Foods for Supertasters

What is the difference in the structure between a younger person's tongue and an older person's tongue? How does this affect the sense of taste?

- A younger person has more taste buds than an older person, about 2 times as much.
- The reason for this is because as a person gets older the taste cells don't get replaced like they do when you are younger.

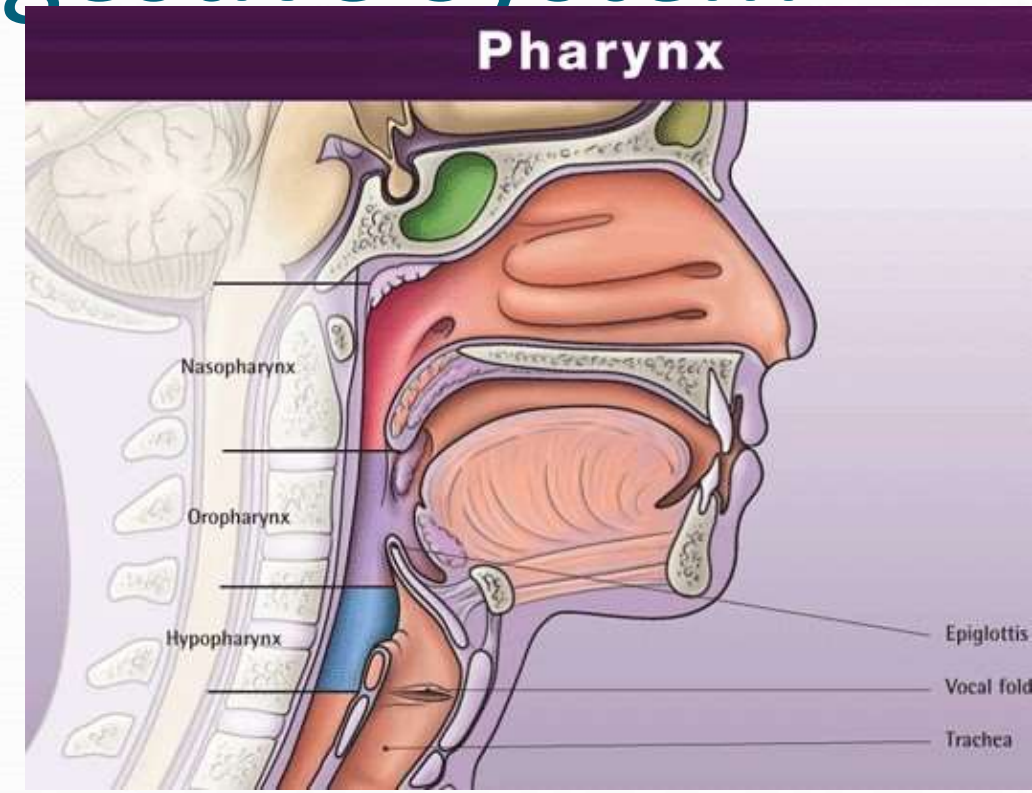
So, can you actually see taste buds with the naked eye? Explain.

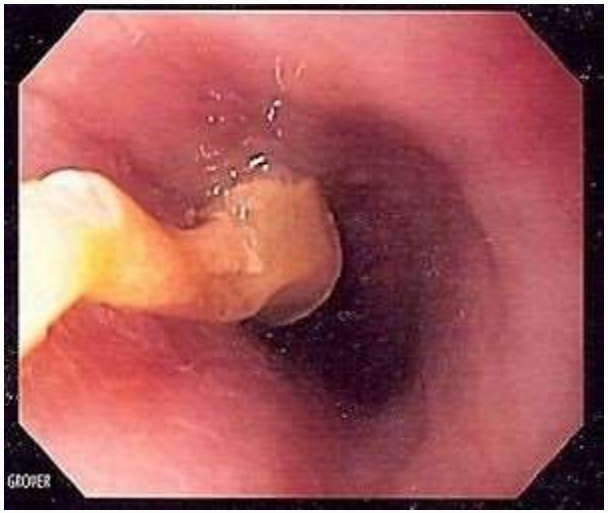
- No, you see the papillae, and within the papillae are the
  taste bubs, and
  about 100 receptors
  or "taste" cells make
  up each taste bud,
  so you actually see
  many in one papillae.



The Human Digestive System

- The tongue pushes food around until it forms a ball called a bolus.
- The bolus is passed to the pharynx (throat) and the epiglottis makes sure the bolus passes into the esophagus and not down the windpipe!





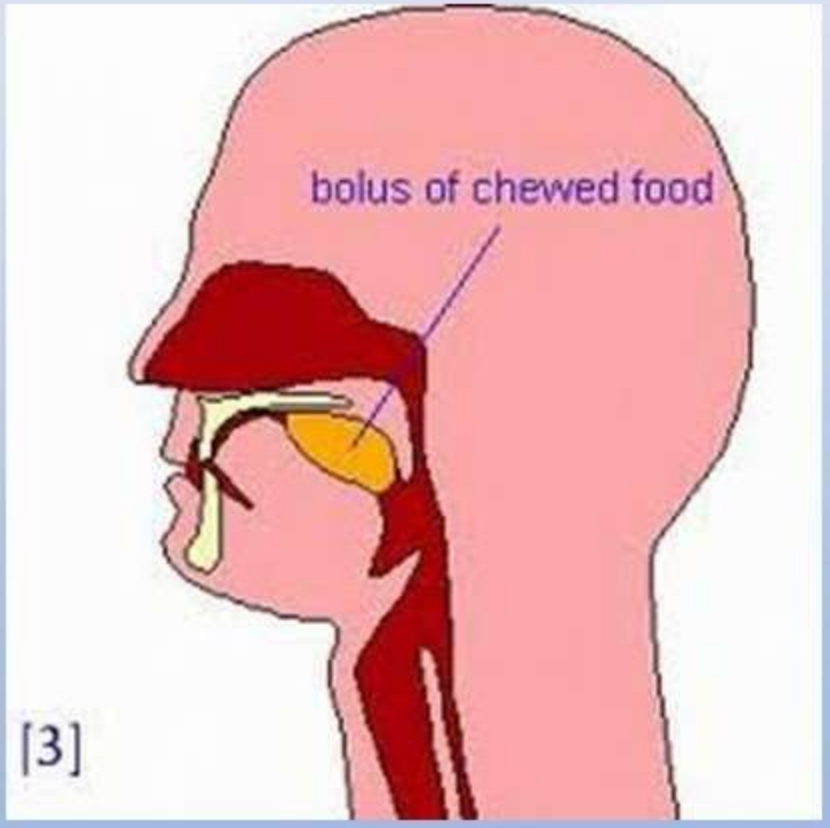
Functions of Saliva

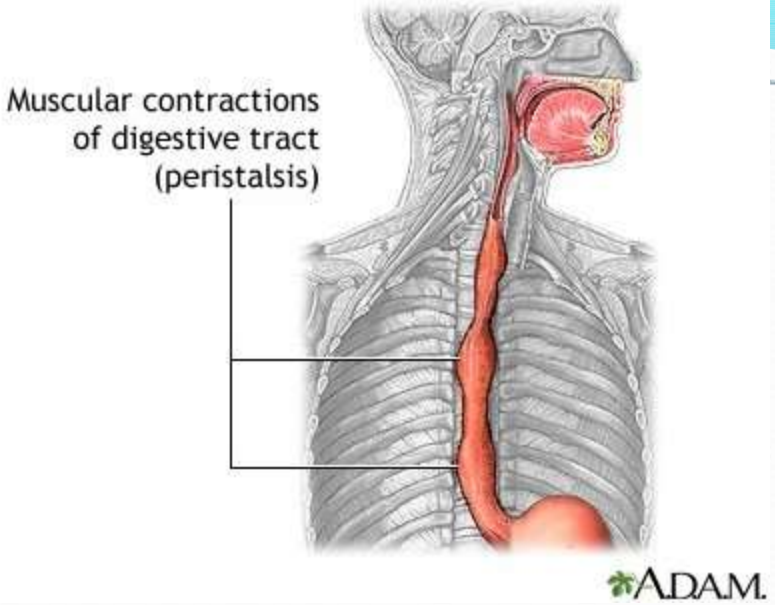
- Saliva helps prevent the deteriorative processes in the mouth in several ways:

1. It moistens food
2. It begins digestion
3. It adjusts salt appetite
4. The flow of saliva helps wash away pathogenic bacteria.

The Esophagus

- The muscular tube through which food passes from the mouth to the stomach
- As you chew, your tongue helps mould your food into a mass called a bolus that then gets pushed to the back of your mouth
- When you swallow, the bolus enters the top of the esophagus
- The opening of the esophagus lies next to the opening of your windpipe
- To prevent food from going down the wrong tube, the opening of your trachea is closed by the epiglottis (valve) when you swallow





The Human Digestive System

- The bolus passing down the esophagus by peristalsis.
- Peristalsis is a wave of muscular contractions that push the bolus down towards the stomach.

Peristalsis Waves

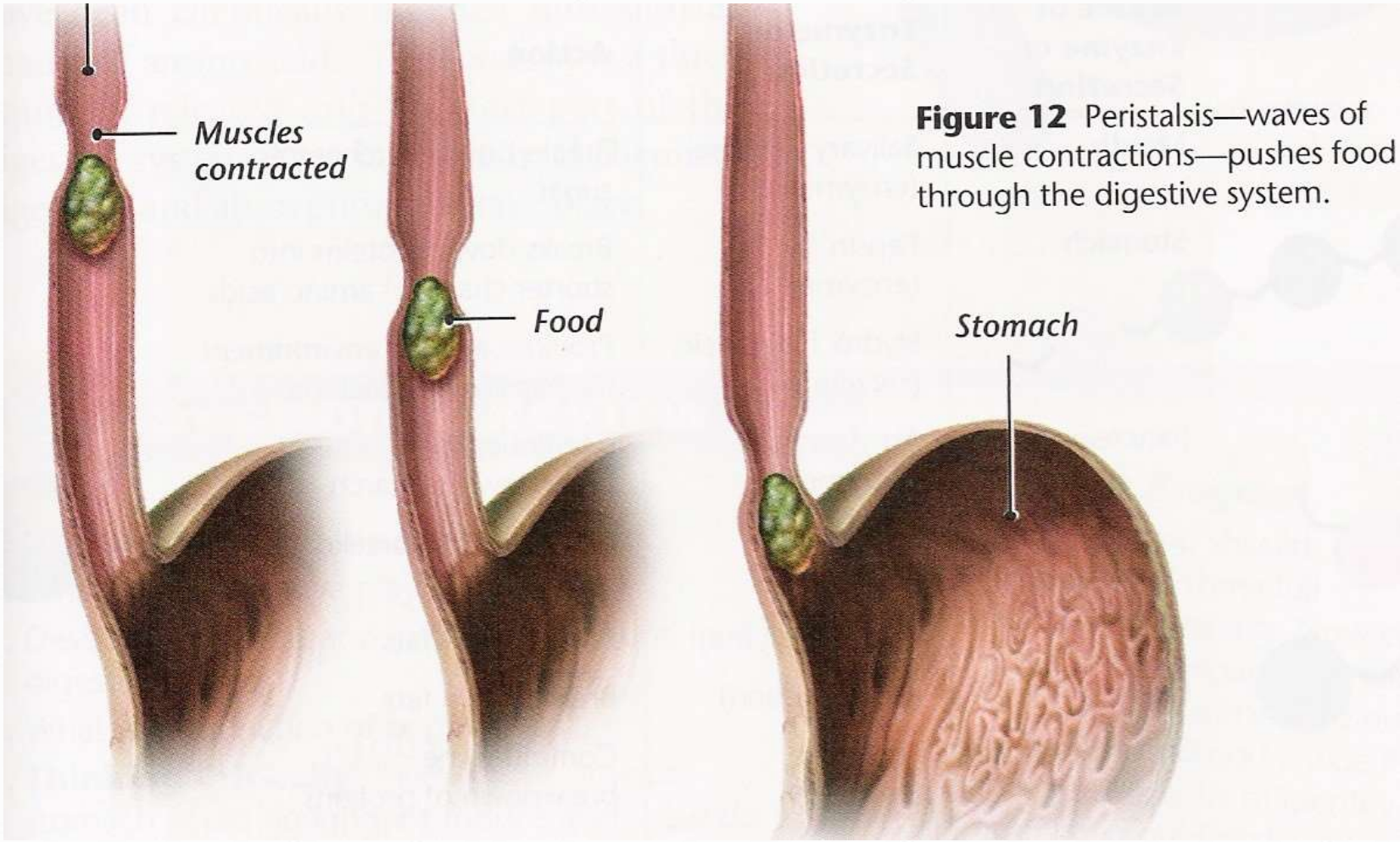
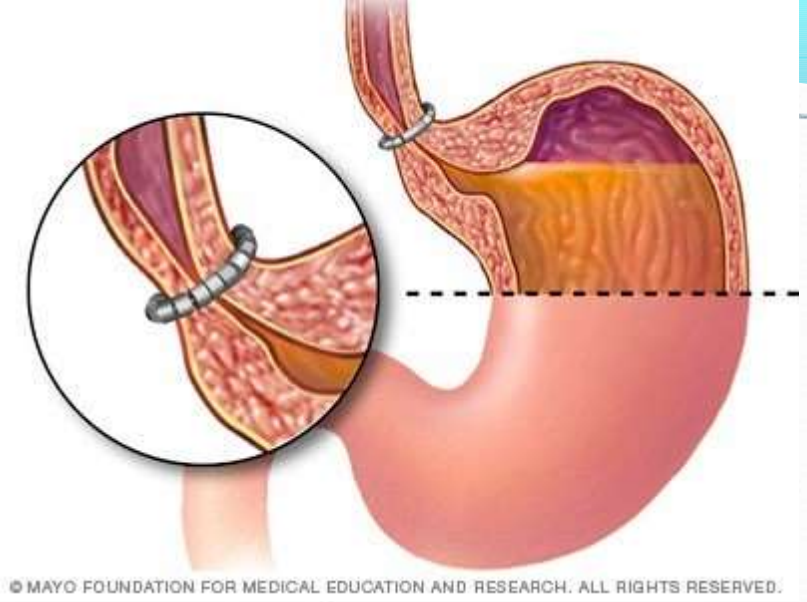


Figure 12 Peristalsis—waves of muscle contractions—pushes food through the digestive system.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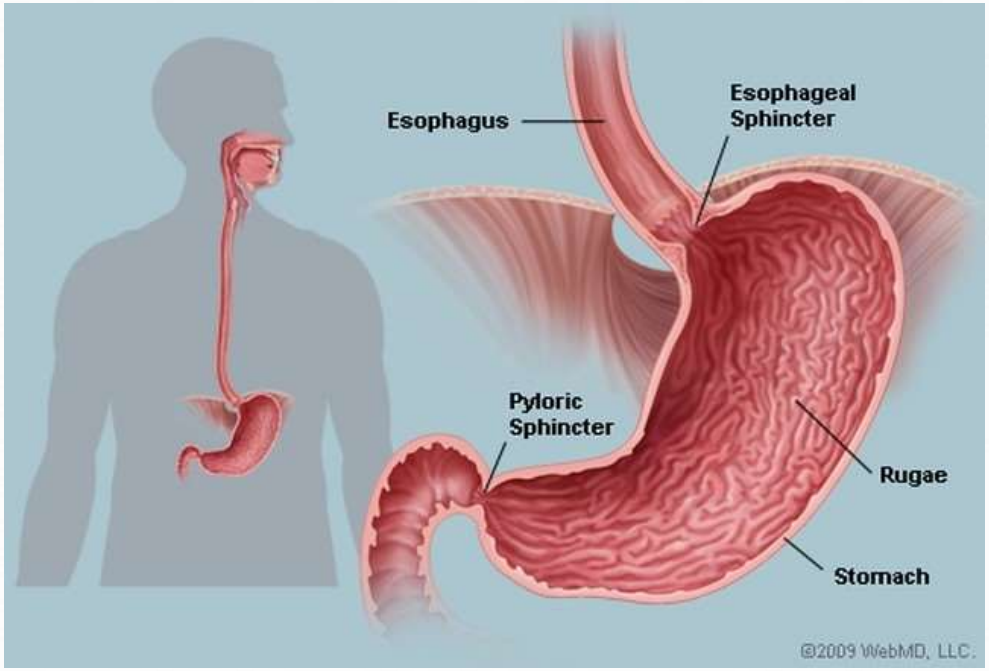
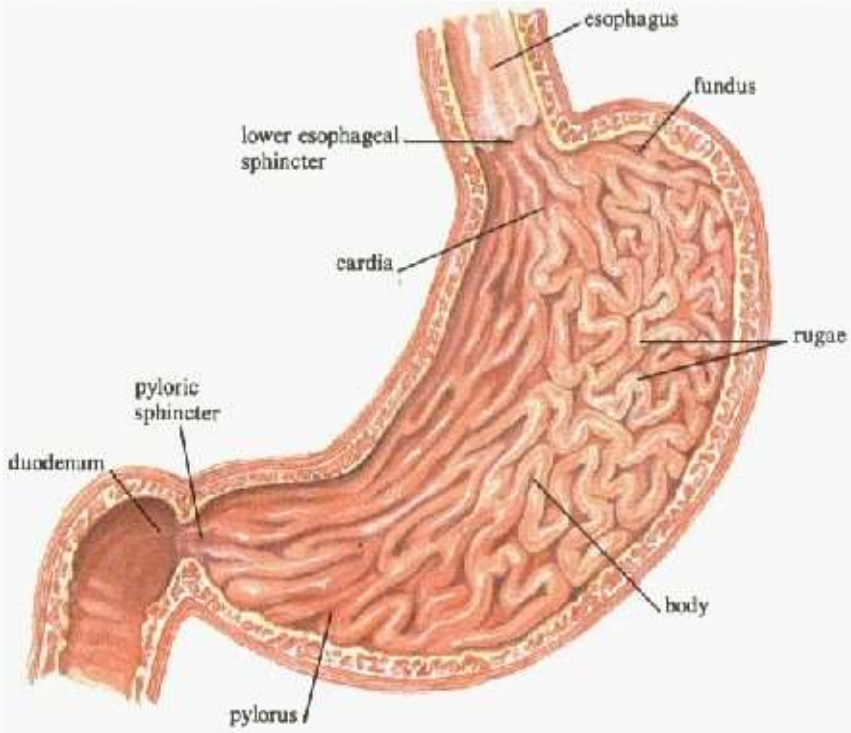
- To enter the stomach, the bolus must pass through the esophageal sphincter, a tight muscle that keeps stomach acid out of the esophagus.



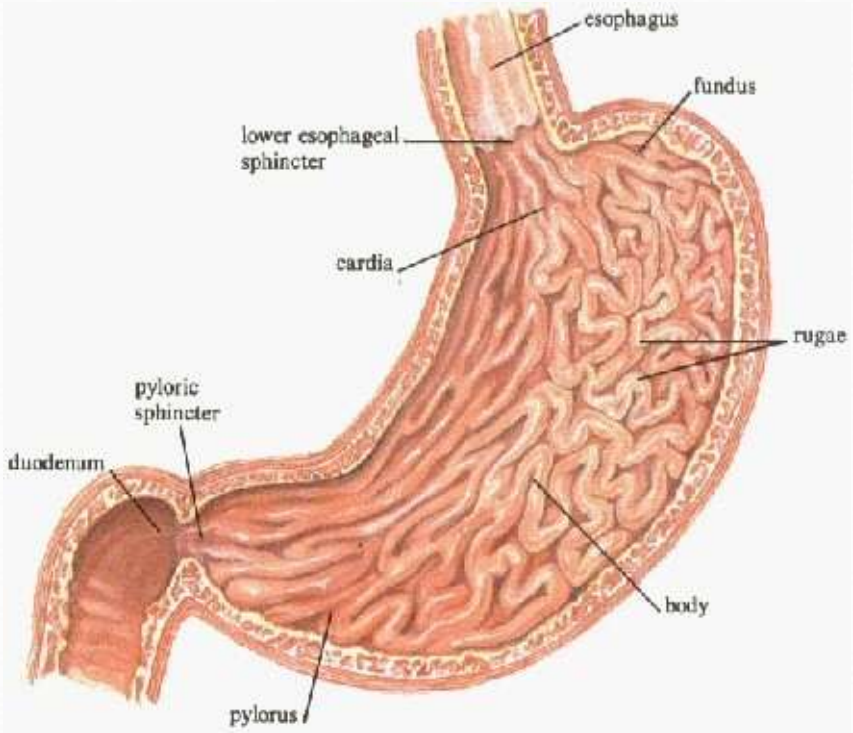
The Human Digestive System

- The stomach has folds called and is a big muscular pouch which turns the bolus and mixes it with gastric juice, a mixture of stomach acid, mucus and enzymes.



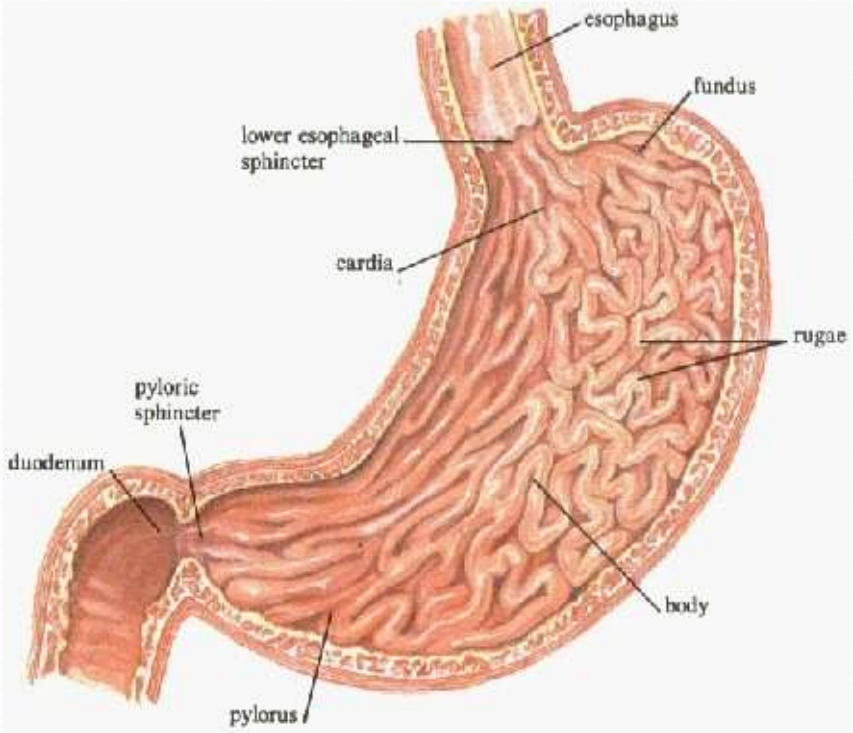
The Human Digestive System

- The acid kills off any invading bacteria or viruses.
- The enzymes help break down proteins and lipids.
- The mucus protects the lining of the stomach from being eaten away by the acid.



The Human Digestive System

- The stomach does do some absorption too.
- Some medicines (i.e. aspirin), water and alcohol are all absorbed through the stomach.
- The digested bolus is now called chyme and it leaves the stomach by passing through the pyloric sphincter.



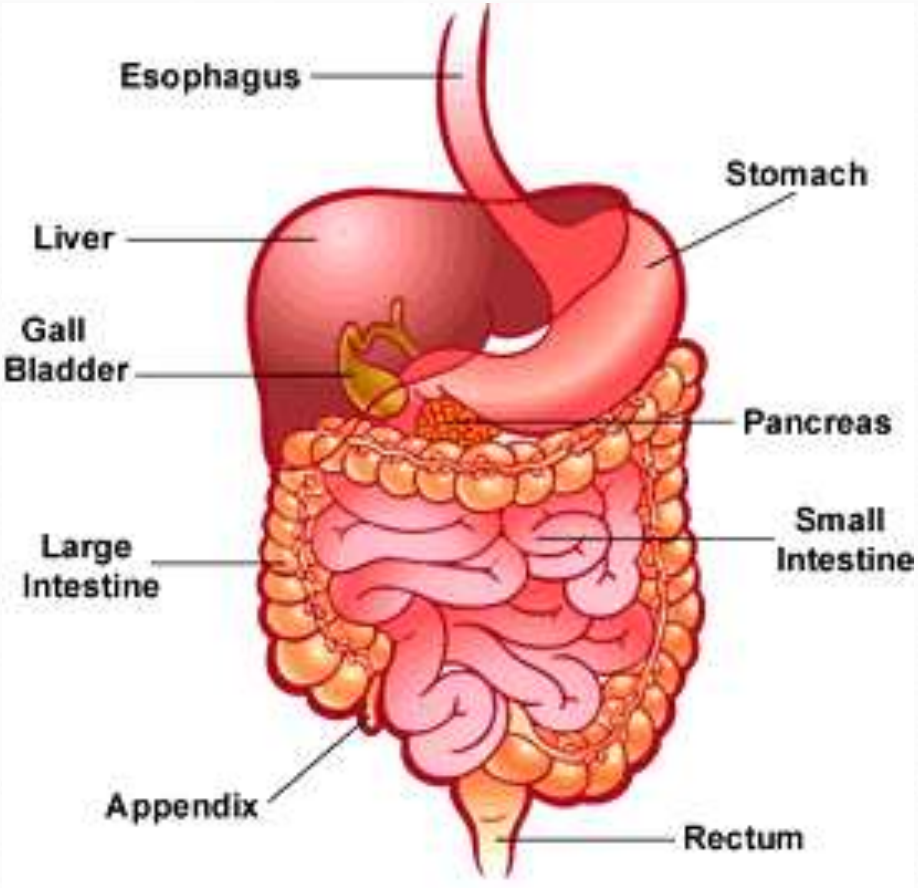
The Human Digestive System

- Chyme is now in the small intestine.

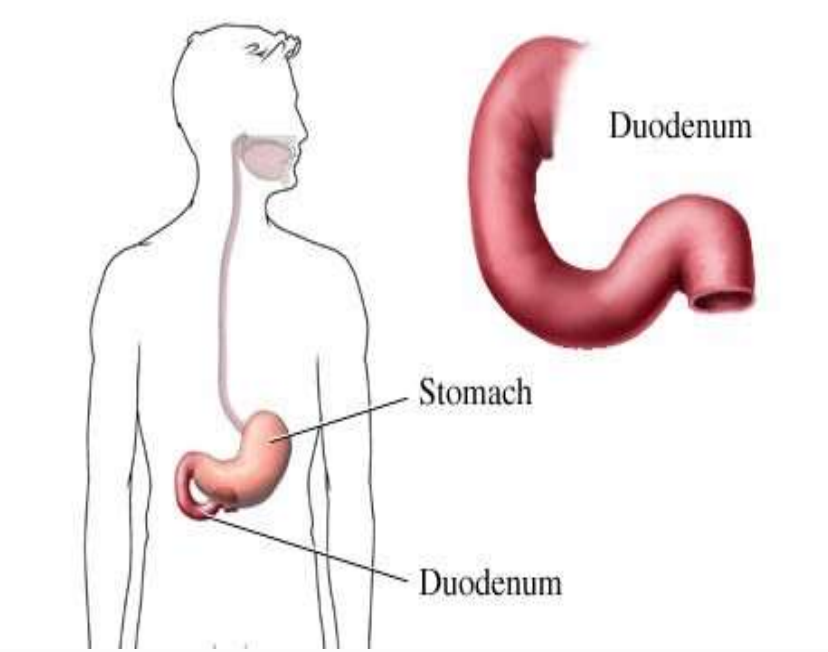
90% of absorption occurs here.

- The liver and pancreas help the small intestine to maximize absorption.

- The small intestine is broken down into three parts:



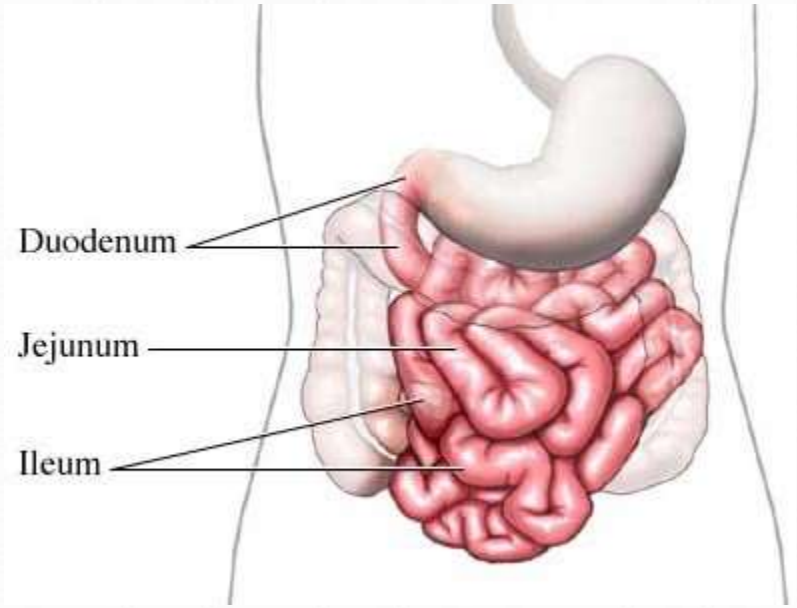
- **Duodenum:** The tube that runs from the stomach to the small intestine.



The Human Digestive System

2. Jejunum

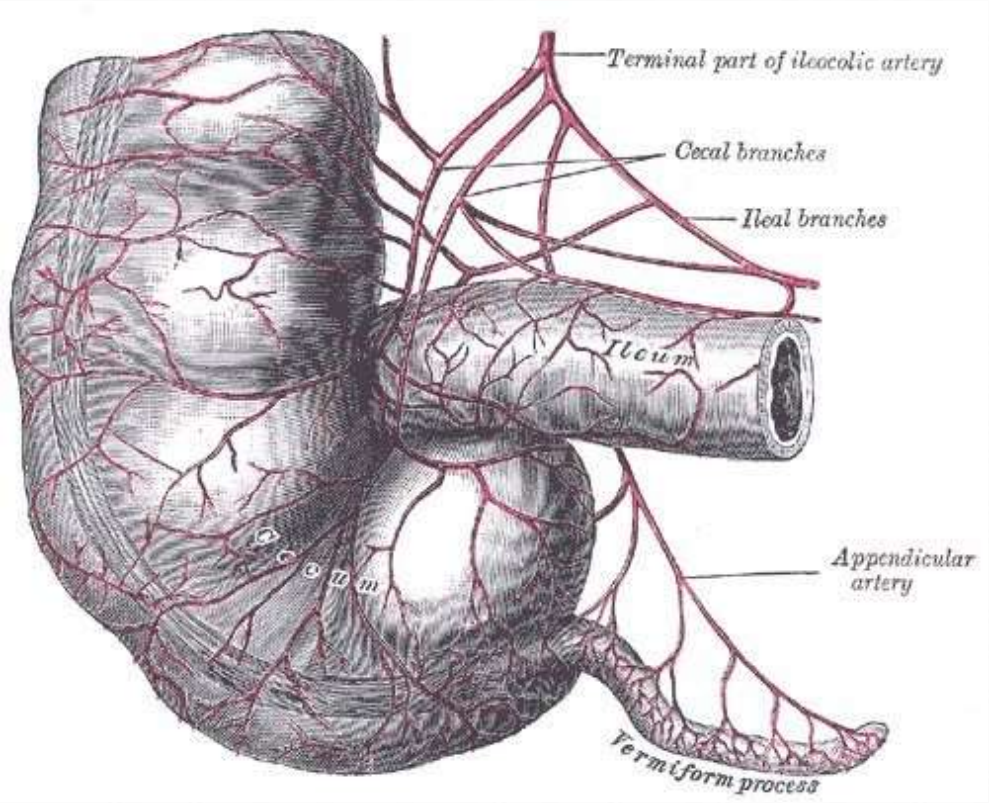
- The jejunum is where the majority of absorption takes place.



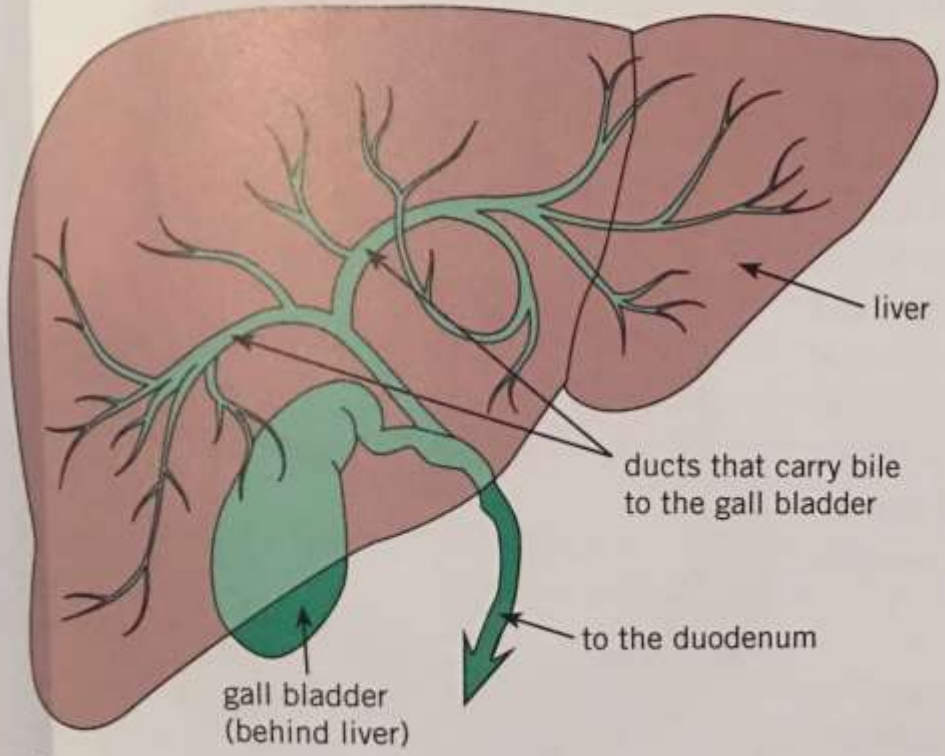
The Human Digestive System

3. Ileum

- The last portion of the small intestine is the ileum, compacts the leftovers to pass through into the large intestine.



Take a moment to tell someone what you learned about the stomach and the small intestine.



Your liver has many functions, but one that is directly related to digestion is the making of bile, which is stored in the gall bladder.

Living Liver

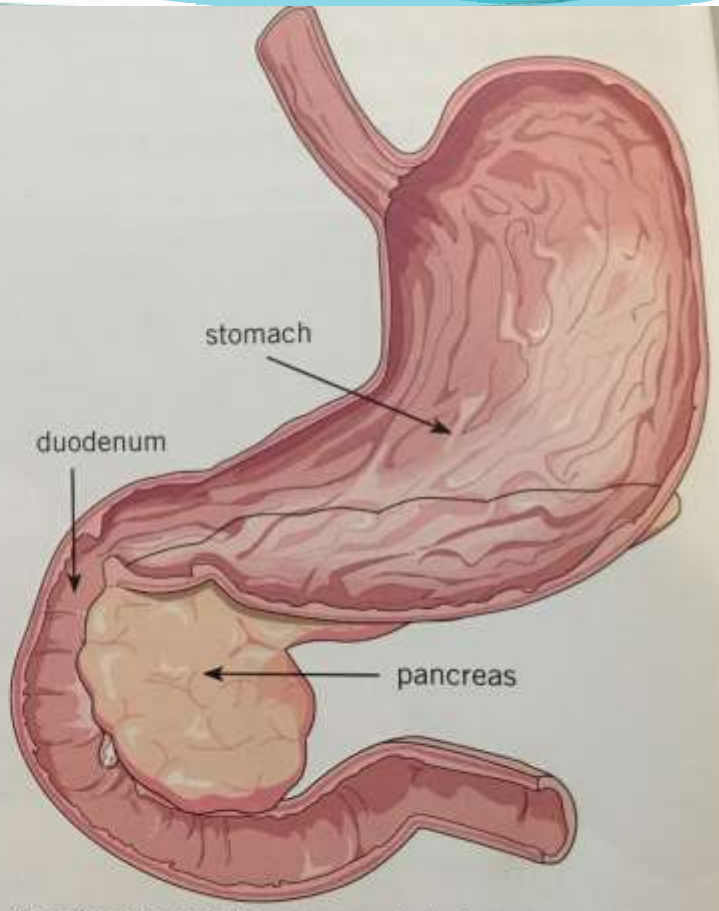
Once the nutrients are absorbed into the bloodstream, they pass through the **liver**. The liver has many important roles in the body. In the liver, nutrients are processed and then either stored or distributed throughout the body. The main thing the liver stores is glucose. This is the little sugar molecule your cells (especially your brain cells) rely on for energy. If you have a lot of glucose in your body, your liver strings the little glucose molecules together to make **glycogen** (gly' kuh jen). When your body runs low on glucose (like between meals), the liver breaks the glycogen back down into many glucose molecules and sends them into your bloodstream. The liver also stores various vitamins and minerals, including iron.

The liver is like a huge chemical factory. One of the chemicals it makes is called **bile**, and it is a very important chemical for the digestive process. After being made in the liver, it is delivered to the gall bladder so it can be stored and concentrated. As the chyme goes into the small intestine, the **gall bladder** then releases concentrated bile into the duodenum. What does bile do for you? Well, bile helps break down fats! Fats don't mix well with water, so in a watery mixture like chyme, they form large clumps. Bile breaks those large clumps into smaller clumps that are easier for your small intestine to digest. You can do the same thing!

Pancreas Potential

Another organ used for digestion is your **pancreas** (pan' kree us). It produces hormones as well as digestive juices. One hormone it makes is insulin, which controls how much sugar is in your blood (we call that your blood sugar level). We'll discuss insulin in a later lesson. The pancreas also produces **pancreatic juice**, which is secreted into the small intestine when chyme arrives. This juice has two main functions. It neutralizes the acid in the chyme, making it far less harsh, and it contains important enzymes that help to digest fats and break down proteins and starches. Since much of what you eat (meats, fruits, vegetables) contains cells, you need to be able to break down the contents of these cells. Without a pancreas, your body would not be able to break down the food you eat and give the nutrients to the rest of your body. Aren't you glad God gave you a pancreas?

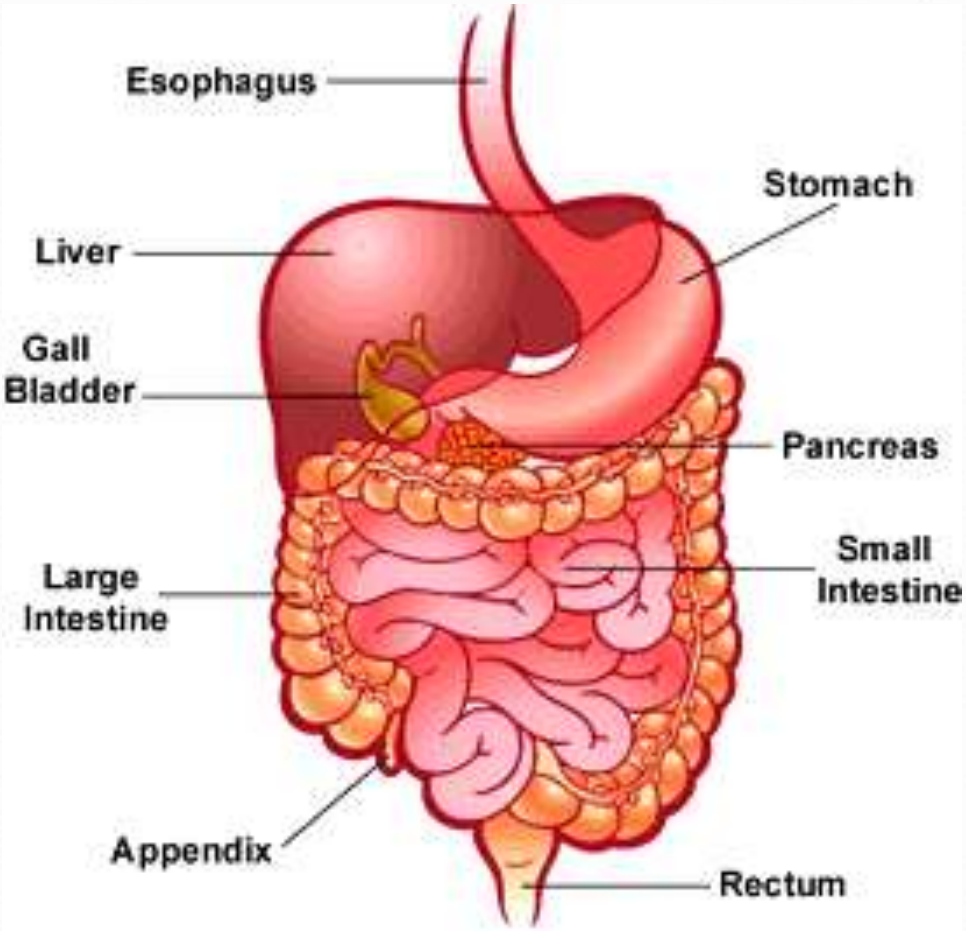
Now you've learned all about the first part of digestion. Explain what each organ you've learned about does for your body. Don't forget the teeth, saliva, stomach, small intestine, liver, and pancreas.



One function of the pancreas is to inject pancreatic juice into the duodenum.

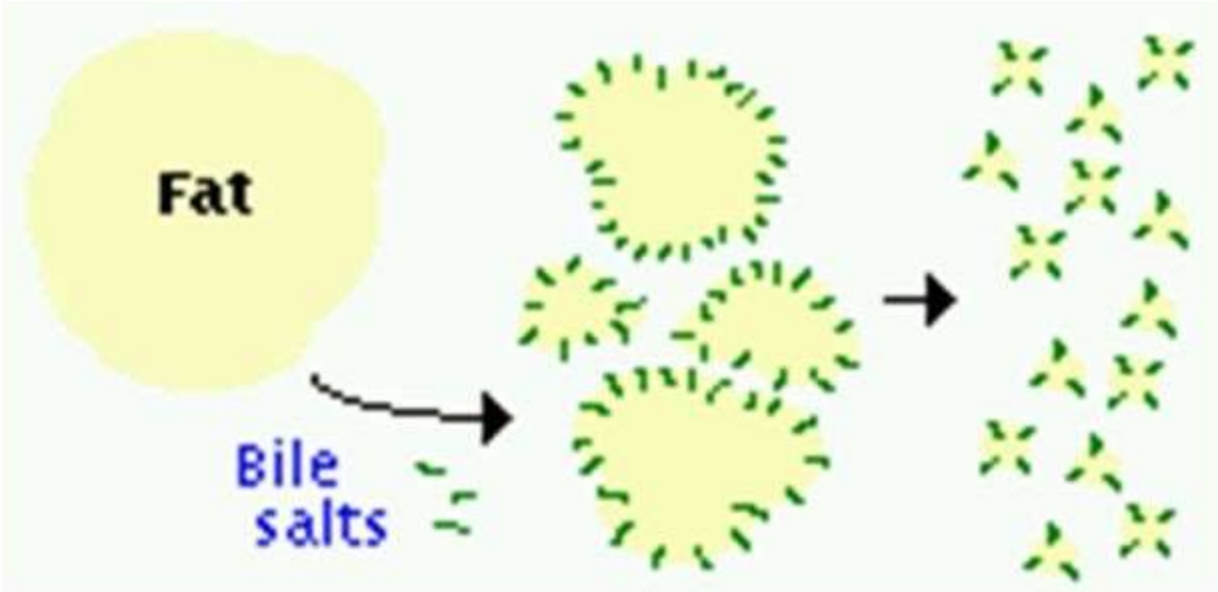
Bile, produced in the liver but stored in the gall bladder, breaks down fats.

- The pancreas secretes a juice to reduce the acidity of the chyme.



BILE

Bile contains bile acids, which are critical for digestion and absorption of fats



All Bile does is **EMULSIFICATION:** Breaks large fat globules into smaller fat droplets

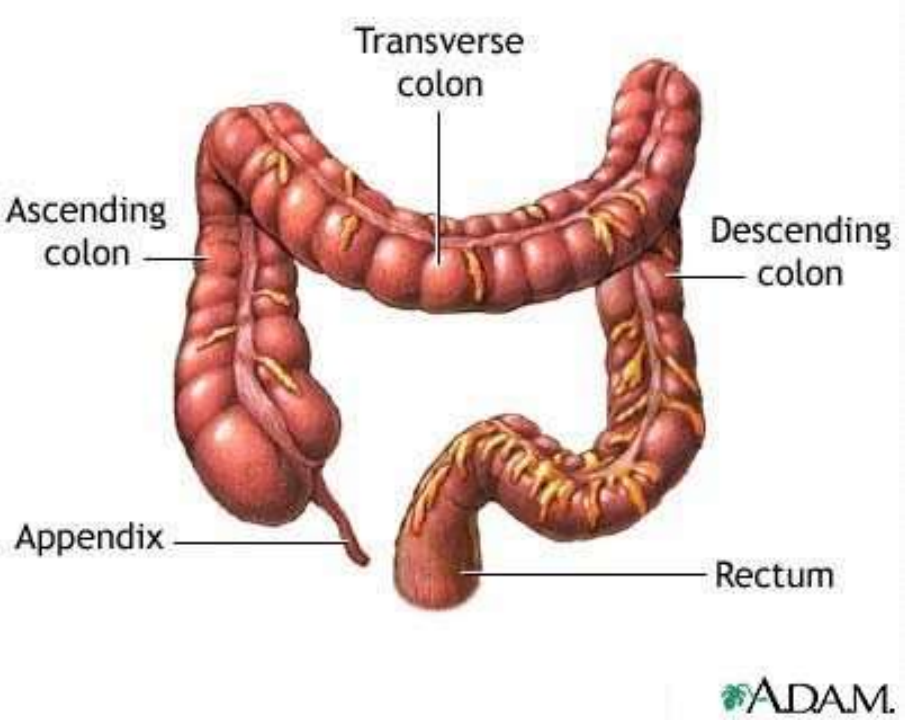
Bile does not contain enzymes: Mechanical Digestion

Try This!

Pour some cooking oil (which is just a kind of fat) into a bowl of water. Notice that there are large globs of oil floating on the water. Now, use a fork to whisk the oil a bit in the bowl. Do you see that the drops of oil are smaller? Now add some liquid dishwashing soap and whisk the oil, water, and soap together. Let the bowl sit for a while and come back every now and then to check on it. Once all the bubbles are gone, notice the difference. You should see a lot of very tiny drops of oil now, because the soap acted like bile, breaking the fat into tiny clumps that are much smaller than they were before.

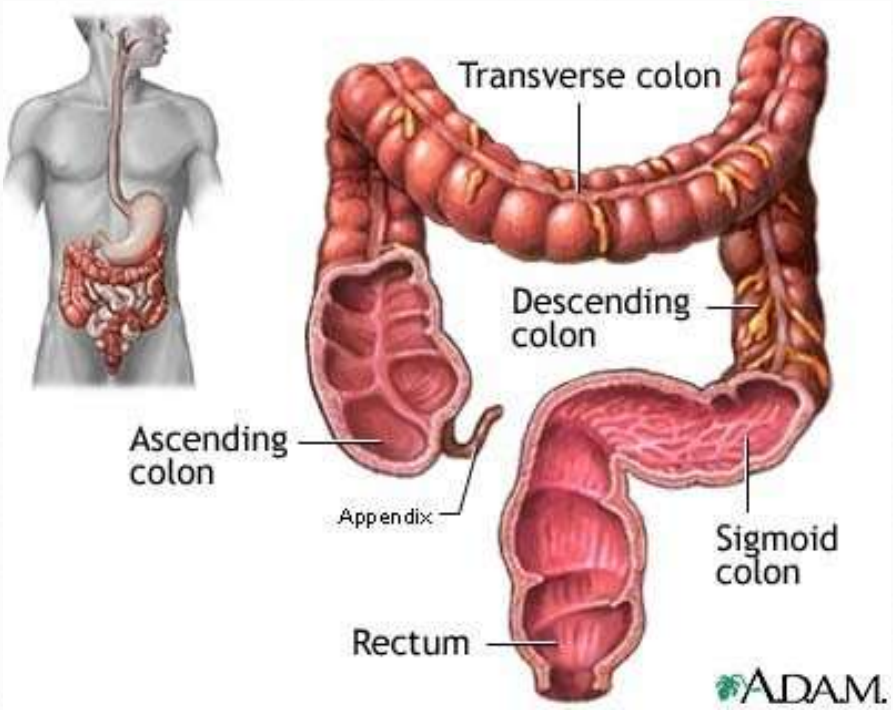
The Human Digestive System

- The large intestine (or colon) is used to absorb water from the waste material leftover and to produce vitamin K and some B vitamins using the helpful bacteria that live here.



The Human Digestive System

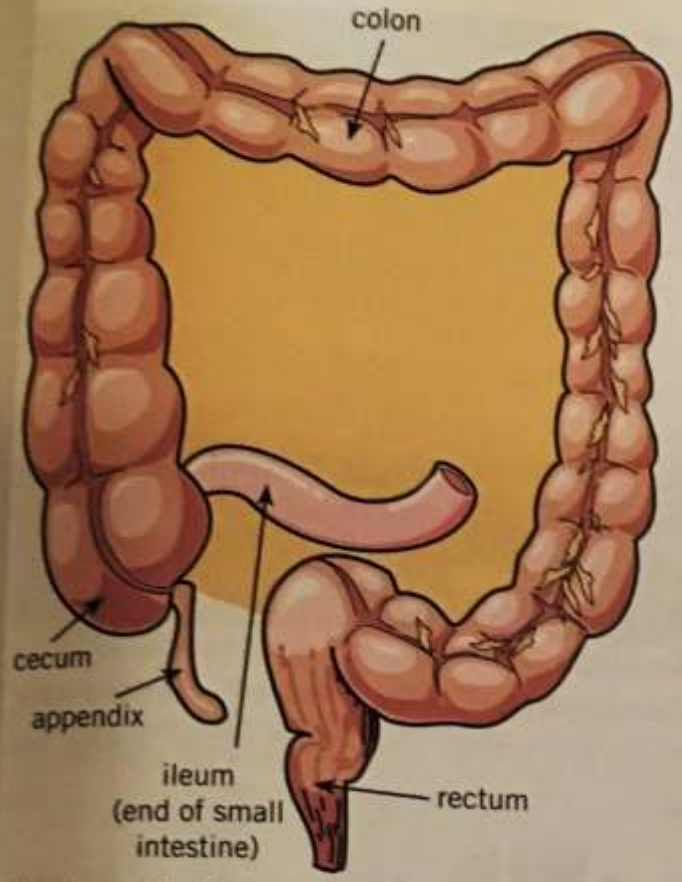
- All leftover waste is compacted and stored at the end of the large intestine called the rectum.
- When full, the anal sphincter loosens and the waste passes out of the body .



Large Intestine

So now your piece of pizza is about to leave the small intestine, but it doesn't look anything like that cheesy slice you put in your mouth about six hours ago. At the meeting point between the small and large intestines you find the **ileocecal** (ill ee oh see' kul) **sphincter**, which opens and closes to let the liquid chyme from the small intestine enter the large intestine.

The large intestine has three parts: the **cecum** (see' kum), the **colon** (kol' un), and the **rectum** (rek'



Your large intestine receives chyme from your small intestine and pulls out water and salts to put into your body.

tum). Its main job is to form the chyme into **feces** (fee' seez). Feces are the waste material that we evacuate into the toilet. In order to make feces, the large intestine removes the water from the chyme. This water is absorbed through the wall of the large intestine into the blood stream. Almost five cups of water (1 liter) are sent from the large intestine into the bloodstream every day. Now you would think that water from your large intestine would contaminate your body, but when the large intestine pulls it from the chyme, it only pulls clean water and some salts, both of which are good for your body. So basically, the large intestine is drying up your food so you can use the water that was in it!

Here is the interesting part. You see, living in the large intestine are millions upon millions of friendly bacteria. Now remember, some of these microscopic creatures cause illness, but not all of them! You have a **symbiotic** (sym bye' ah tik) relationship with some of them. If you studied zoology, you may remember what symbiotic relationships are. They are relationships where two living things work together to benefit one another. Have you ever hosted guests at your house? Well, you are hosting the friendly bacteria in your colon right now. Say hello to your guests! You both benefit from their presence in your colon. You benefit because these friendly bacteria digest some otherwise indigestible things in the chyme, and in doing so, they produce vitamins that are absorbed by

The most significant vitamin these bacteria produce is vitamin K. Without vitamin K, you would bleed to death when you cut yourself. Vitamin K helps your blood to clot. Vitamin K is also present in some foods you eat, such as broccoli and spinach. The intestinal bacteria synthesize some B vitamins as well. Not only do you benefit from this relationship, but the bacteria benefit as well, because they have a place to live with lots of food. You're a good host! Interestingly, these friendly bacteria remain friendly only if they remain in the colon. If they travel elsewhere in the body, they can cause serious illness. Fortunately, this is rare.

The wormlike tube on the cecum is called the **appendix**. For a long time, scientists didn't know what its role was in the body, and some actually thought it had none. Of course, anyone who understands that we are made by God knows that He wouldn't make something in our body that was useless! Thus, creation scientists always thought it had a job in the body. Well, in 2007 at least one of its jobs was discovered. Remember those friendly bacteria? The appendix provides a safe place for them to hide when your body gets an illness that could kill them. You see, some diseases you get are bad for your friendly bacteria. So they hide out in the appendix until the illness is gone, and then they go back to living in your intestine and making vitamins for you! This allows you to recover from those kinds of illnesses much more quickly.

Did you ever wonder why beans can cause gas to form in your intestines? It's because beans contain complex carbohydrates, which are very resistant to being broken down by your digestive system. The bacteria in your intestines can digest these carbohydrates, but in doing so they release several different gasses, which can cause some rather embarrassing side effects. We call this side effect **flatulence** (flach' uh lentz). Some people are better at digesting beans than others. I'll say no more on this subject!

As wavelike muscle movements continue to move the chyme through the large intestine, more and more water is removed. This can take anywhere from half a day to three days, depending on your diet. A diet high in fiber (plant material that you can't digest) speeds up the process. Since the chyme moves more quickly through the large intestine, less water is removed from it, so it stays reasonably soft. This means that a diet high in fiber

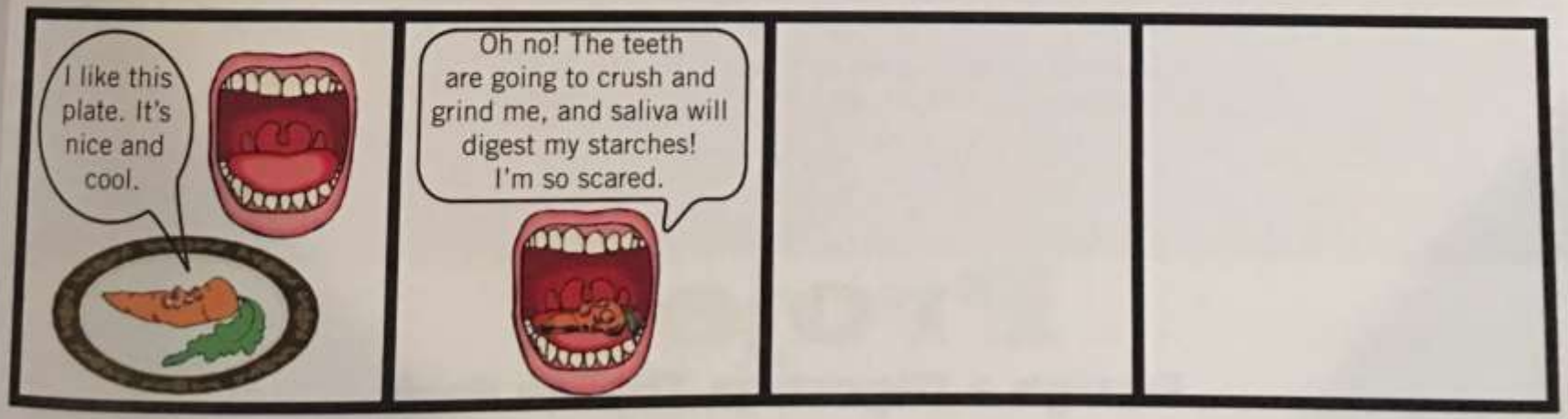
These paper cups once held sports drinks that marathon runners used to replenish their water and salts. When you run and sweat for a long time, you lose too much water and salt, which must be replenished. You can see from the number of cups that a lot of runners needed water and salts!

What Do You Remember?

What is the white outer layer of your tooth called? What is the layer right below that called? What is the hardest substance in your body? Name a few things saliva does for you. What is the name of the pipe that food goes down after you swallow it? How do your stomach and esophagus keep from getting burned by your own stomach acid? What is the food called when it enters the small intestine? What happens in the small intestine? Which organ is like a huge chemical factory? What do the kidneys do?

Notebooking Activities

In addition to writing down all the fascinating facts you learned about the digestive and renal systems, you will create a comic strip of the digestion process. A comic strip is simply a series of boxes with different actions happening in each box. You will animate, or bring to life, a piece of food. You can choose any food you wish. Follow it as it goes from your plate and through your digestive system. Imagine that the food has feelings. How does it react to each of the stages of digestion? Express all that in your comic strip.



ENZYMES

By

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Department of Pharmaceutical Chemistry
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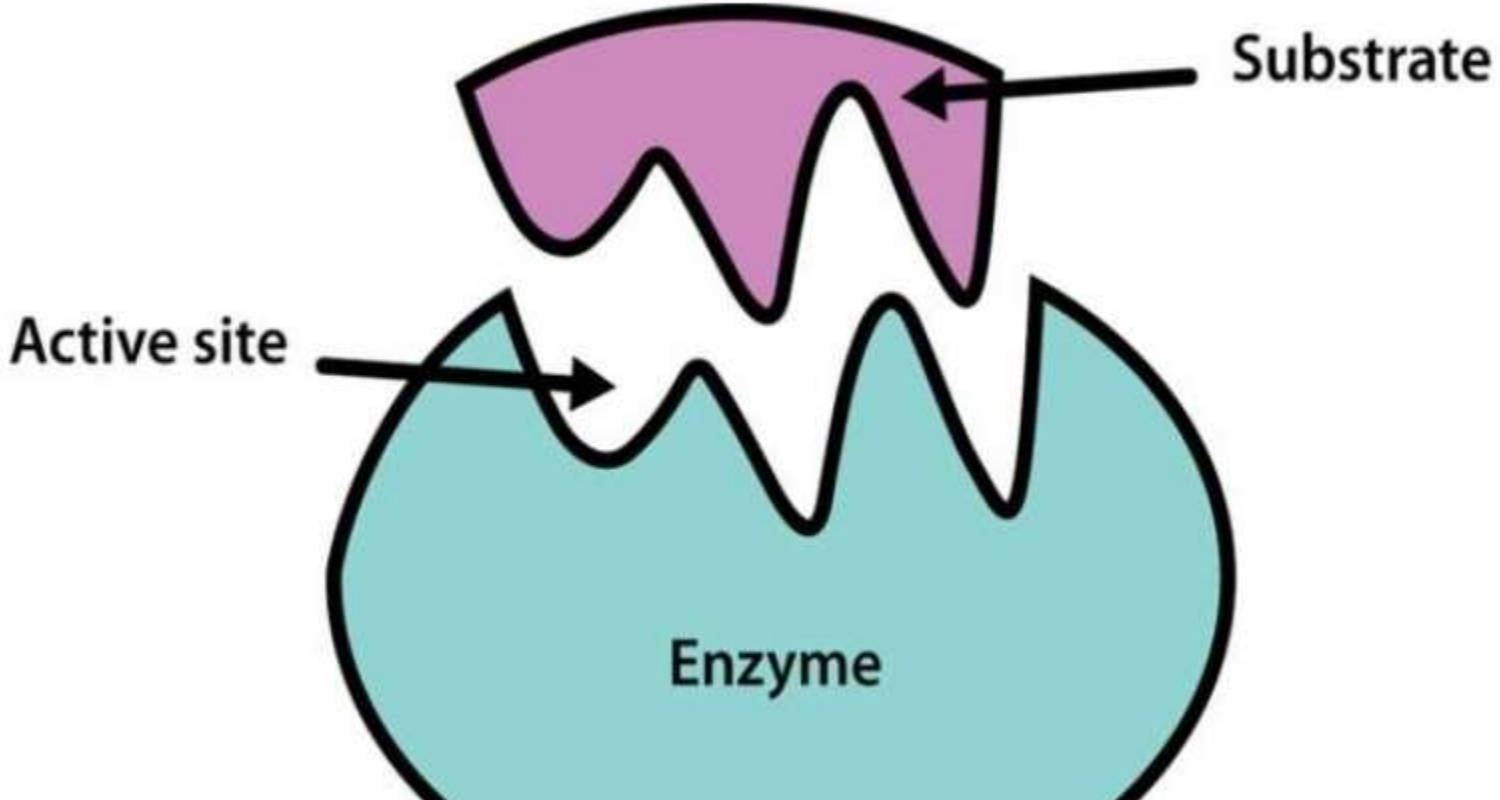
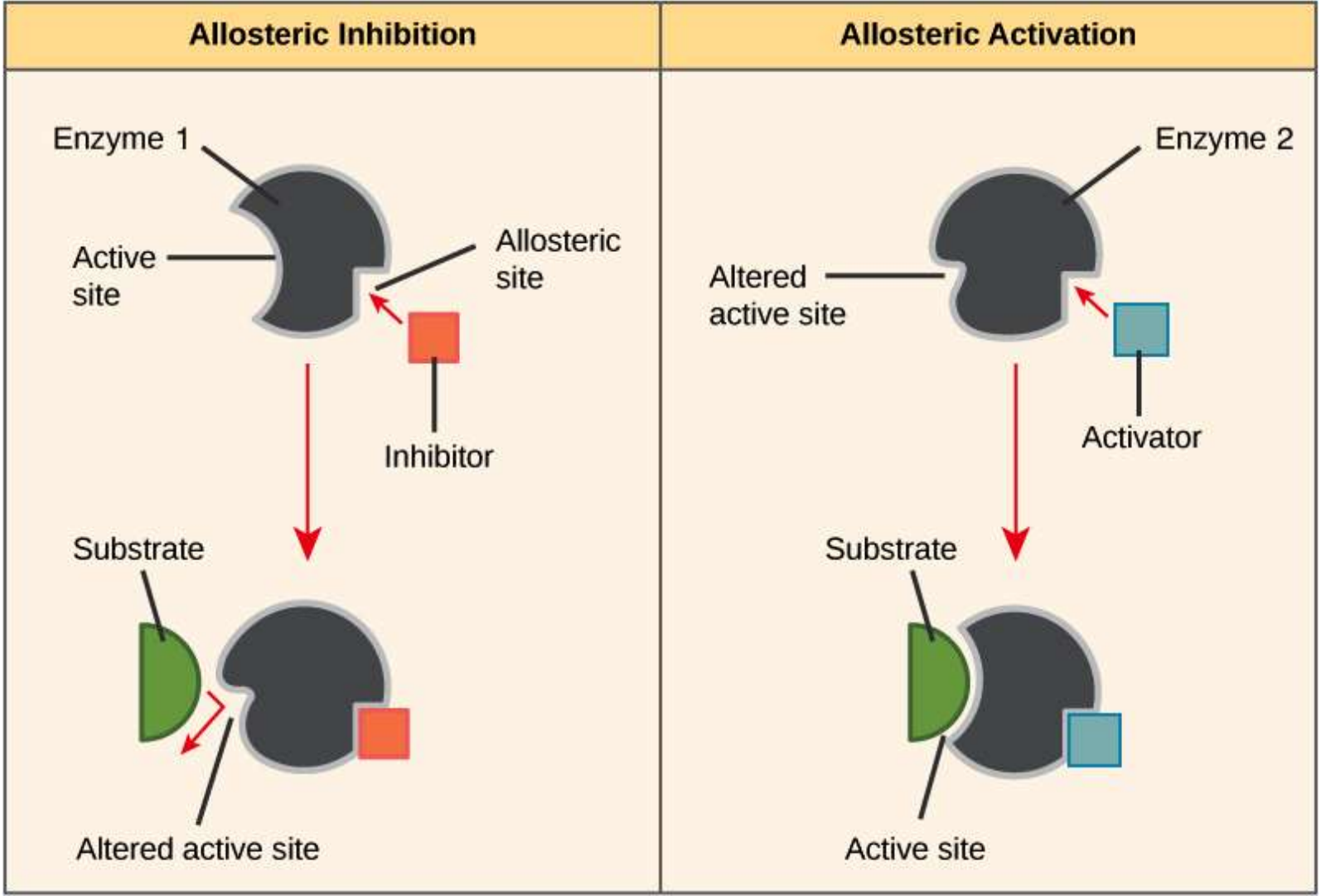

Substrate
Active site
Enzyme


Allosteric Inhibition
Allosteric Activation
Enzyme 1
Active site
Allosteric site
Inhibitor
Substrate
Altered active site
Enzyme 2
Altered active site
Activator
Substrate
Active site

INTRODUCTION

- Enzymes are the biological catalysts which are required in almost all the biochemical reactions in a living system.
- Biocatalysts are designated as enzyme since they were discovered as the substances found in yeasts (Greek- en=in, and zyme=yeast).
- Enzymes are biocatalysts-the catalysts of life.
- A good teacher is always a good catalyst in students' life!

DIFFERENCE BETWEEN AN ENZYME AND A CHEMICA CATALYST

- An enzyme catalyzed reaction has a higher reaction rate than that of the corresponding chemically catalyzed reaction.
- An enzymatic reaction occurs under relatively milder conditions, such as a temperature near 37°C, pH near neutrality and at atmospheric pressure.

HISTORICAL BACKGROUND

- Berzelius in 1836 coined the term catalysis.
- In 1878, Kuhne used the word enzyme to indicate the catalysis taking place in the biological systems.
- Isolation of enzyme system from cell-free extract of yeast was achieved in 1883 by Buchner.
- In 1926, James Sumner first achieved the isolation and crystallization of the enzyme urease

CHEMICAL NATURE OF AN ENZYME

- Most of the enzymes are protein in nature with large molecular weight, however, a few RNA molecules have been shown to function as enzymes.
- Each enzyme has its own tertiary structure and specific conformation which is very essential for its catalyti activity.

MULTIMERIC PROTEINS

- Most of the enzymes though are simple proteins having a single polypeptide chain, there are several enzymes which have more than one polypeptide chain. These are called as multimeric proteins.
- Such enzymes (multimeric proteins) may be found as either

OLIGOMERIC ENZYMES

- Enzymes with two or more subunits are called oligomeric enzymes.
- For example, phosphorylase hexokinase, lactate dehydrogenase etc.
- These occur as tetramers, i.e each enzyme has four protein subunits.
- Others like phosphofructokinase, fructose-1-bisphosphatase

Multienzyme complex

- Several enzymes occur in the form of a multienzyme complex, i.e. they have more than one enzyme activities.
- Such a complex catalyzes the conversion of a substrate, in a sequential series of reactions, to different products, e.g. pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase complex etc. Each of these complexes contains three enzymes.
- Fatty acid synthase is a multimeric complexes with six different enzyme activities.

USEFUL TERMS

- The functional unit of the enzyme is known as **holoenzyme** which is made up of apoenzyme (the protein part) and a coenzyme (non-protein organic part).

Holoenzyme ➡ Apoenzyme + Coenzyme

(Active enzyme) (protein part) (non-protein part)

- The term **prosthetic group** is used when the non-protein moiety tightly (covalently) binds with the apoenzyme.
- The coenzyme can be separated by dialysis from the enzyme while the prosthetic group cannot be.
- Some of the enzymes require the

INTRACELLULAR LOCALIZATION

- Most of the enzymes are produced by the cells of a particular tissue ar function within the cell. Such enzymes are called as intracellular enzymes, e.g. the enzymes of glycolysis, TCA cycle and fatty acid synthesis.
- On the other hand, there are certa enzymes which are produced by th cells of a particular tissue and liberated from there for use in the other tissues. Such enzymes are called as extracellular enzymes, e.g

ZYMOGEN

- Most of the intracellular enzymes are secreated in their active form, called ‘zymase’ form of the enzyme.
- Proteolytic enzymes on the other hand, are usually synthesized as somewhat larger inactive precursors, known as ‘zymogens’.
- These molecules are stored in the storage vesicles known as zymogen granules, secreted as zymogens and undergo modifications in structure, after coming in contact with certain activating agents

ACUTE PANCREATITIS

- The inactivity of zymogens is cruci because if these enzymes were synthesized in their active forms within the cell, this situation woul be potentially self-destructing.
- Acute pancreatitis, a painful and sometimes fatal condition is characterized by the premature activation of the digestive enzyme synthesized by this organ.
- The enzymes damage the pancrea and spill over into the circulation.
- Pancreatic amylase is found at ver

NOMENCLATURE OF ENZYMES

- In the early, days the enzymes were given names by their discovers in an arbitary manner.
- Enzymes are commonly named by putting the suffix '-ase' to the name of the substrate or with the catalytic action of the enzyme.
- E.g. a lipase is an enzyme which catalyses the hydrolysis of a lipid, a fatty acid synthase is an enzyme that catalyzes the synthesis of a fatty acid.
- These names of the enzymes are called as trivial

CLASSIFICATION OF ENZYMES

- For the rational naming of each enzyme, International Union of Biochemistry and Molecular Biology (IUBMB) adopted a scheme and suggested functional classification of enzymes and appointed an Enzyme Commission in 1961.
- As per IUBMB, enzymes are named with the suffix 'ase' with the name of the substrate and/or the type of the reaction it catalyzes.
- Each enzyme is assigned a name and a systematic four digit enzyme code, commonly known as Enzyme Commission number (E.C. number).
- Its first digit denotes the main class which is according to the general type of chemical reaction that an enzyme catalyzes.
- The second digit characterizes the sub-class, based on the nature of a chemical group removed or transferred, or any bond split or formed.
- The third digit indicates the sub-sub-class and is used for more detailed subdivision of the sub-class.

- It is the first enzyme of the sub-subclass 1 (NAD^+ utilizing), of the subclass 1 (dehydrogenase) and belongs to class 1 (oxidoreductase).
- Enzymes are thus classified into six major classes according to the nature of a chemical reaction they catalyze.

❑ Oxidoreductases

- Enzymes involved in oxidation-reduction reactions

❑ Transferases

- Enzymes that catalyse the transfer of functional groups.

❑ Hydrolases

- Enzymes that bring about hydrolysis of various compounds.

❑ Lyases

- Enzymes specialized in the addition or removal of water, ammonia, CO_2 etc.

❑ Isomerase

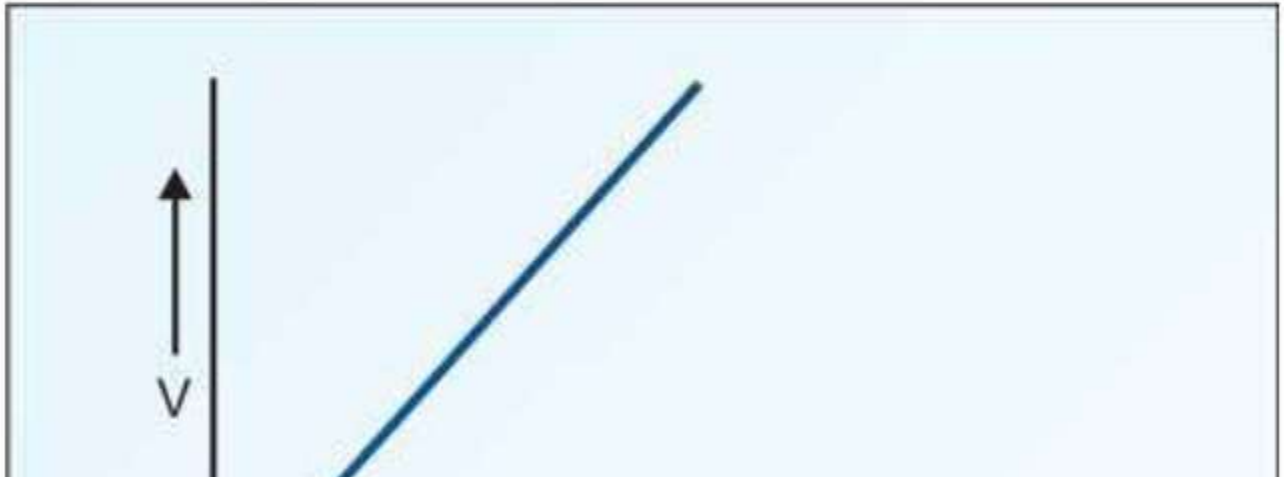
Table 66.1 Classification of enzymes

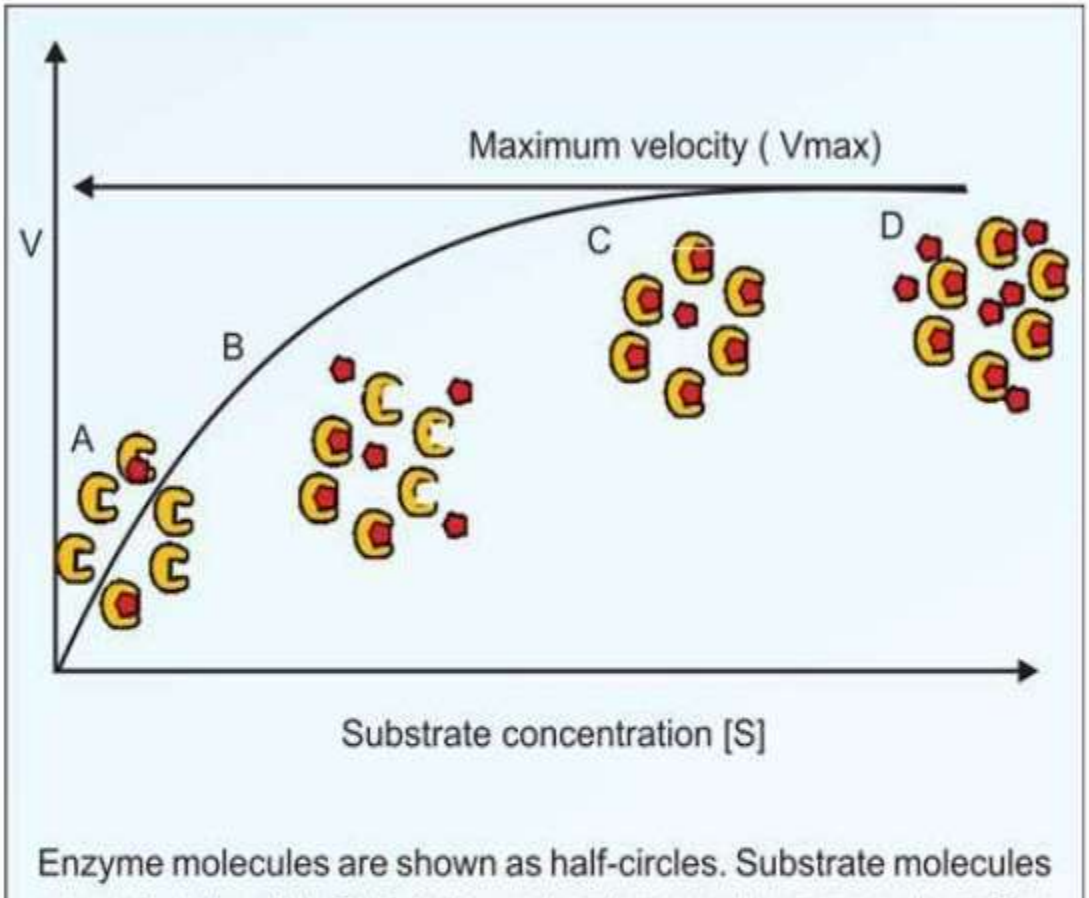
*Enzyme class with examples**	*Reaction catalysed*
1. Oxidoreductases	
Alcohol dehydrogenase (alcohol : NAD^+ oxidoreductase E. C. 1.1.1.1.), cytochrome oxidase, L- and D-amino acid oxidases	Oxidation ⟶ Reduction $AH_2 + B \longrightarrow A + BH_2$
2. Transferases	
Hexokinase (ATP : D-hexose 6-phosphotransferase, E. C. 2.7.1.1.), transaminases, transmethylases, phosphorylase	Group transfer $A - X + B \longrightarrow A + B - X$
3. Hydrolases	
Lipase (triacylglycerol acyl hydrolase E. C. 3.1.1.3), choline esterase, acid and alkaline phosphatases, pepsin, urease	Hydrolysis $A - B + H_2O \longrightarrow AH + BOH$
4. Lyases	
Aldolase (ketose 1-phosphate aldehyde lyase, E. C. 4.1.2.7), fumarase, histidase	Addition ⟶ Elimination $A - B + X - Y \longrightarrow AX - BY$
5. Isomerases	

FACTORS AFFECTING ENZYME ACTIVITY

1. concentration of enzyme

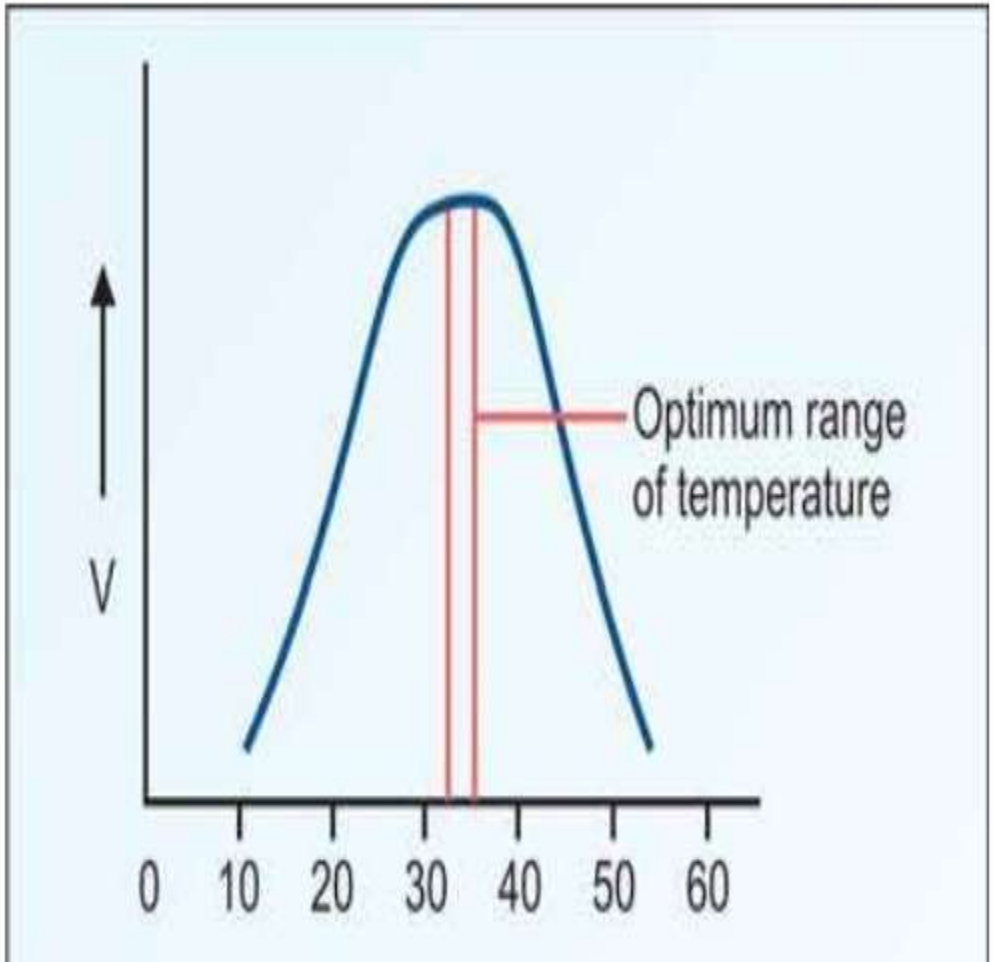
As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases.





2. Concentration of the substrate

- Increase in the substrate concentration gradually increases the velocity of enzym reaction within the limited ran of substrate levels.



3. Effect of temperature

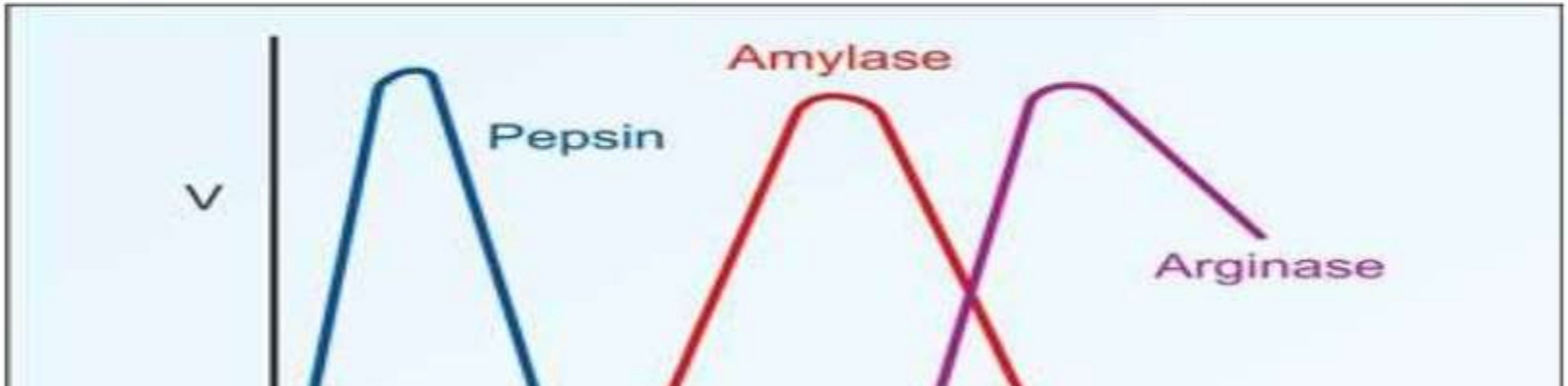
- Velocity of an enzyme reaction increases with increase in temperature up to a maximum and then declines. A bell-shaped curve is usually observed.

❑ Clinical significance

- Foods can be preserved in refrigerators (at low temperatures) due to reduced bacterial enzyme activities.
- Certain surgeries are carried out by lowering the patient's body

4. Effect of pH

The relationship between enzyme activity and pH is also represented by a bell shaped curve which has its peak at the optimum pH. This p is characteristic for each enzyme. Optimum pH for most of the intracellular enzymes is in the neutral range, i.e. around 7.0. The optimum pH for pepsin however, is about 2.0 while for the enzymes the pancreatic juice, optimum pH is nearly 8.0.



5. Effect of Product concentration

- The accumulation of reaction products generally decreases the enzyme velocity.

6. Effect of activators

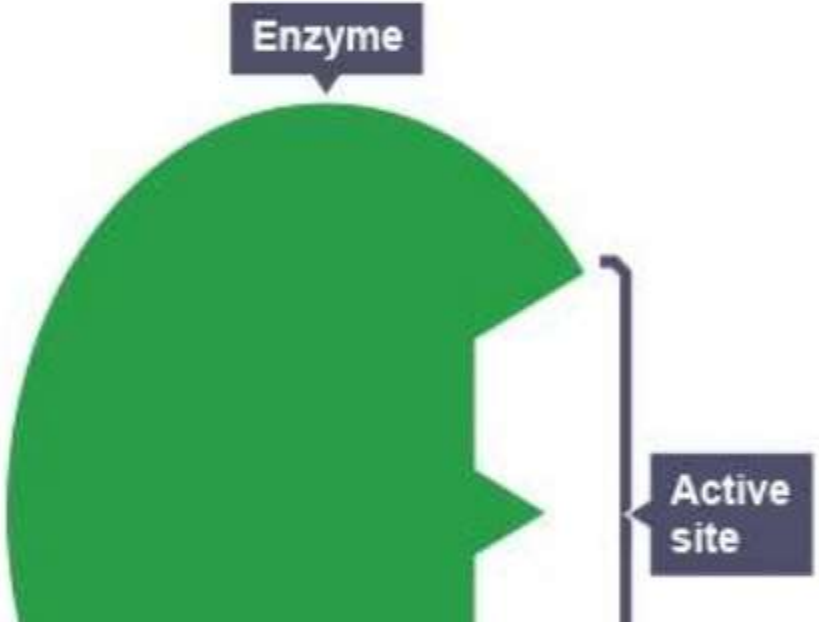
- Some of the enzymes require certain inorganic metallic cations like Mg^{2+}, Mn^{2+}, Zn^{2+} etc. for their optimum activity. Rarely, anions are also needed for enzyme activity e.g. Chloride ion (Cl^{-}) for amylase.
- Metals function as activators of enzyme velocity through various mechanisms- combining with the substrate, formation of ES-metal complex etc.

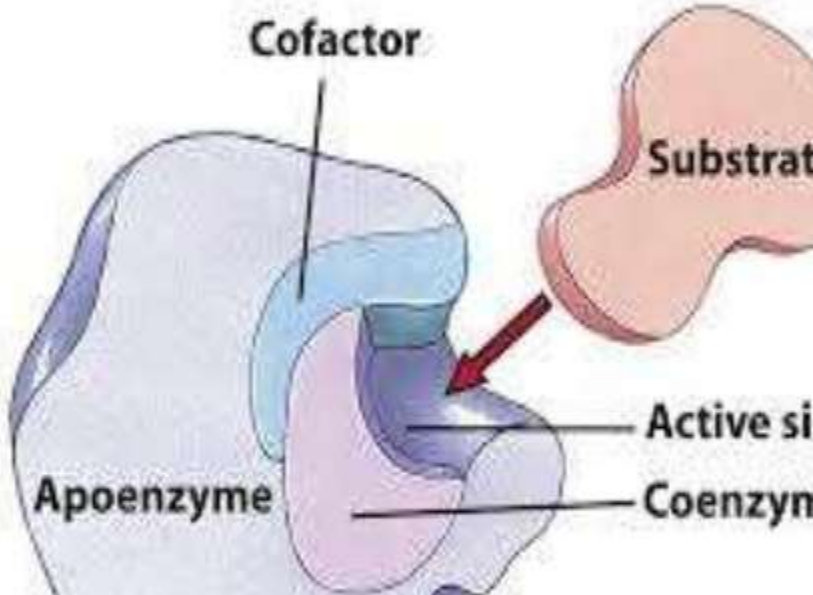
7. Effect of light and radiation

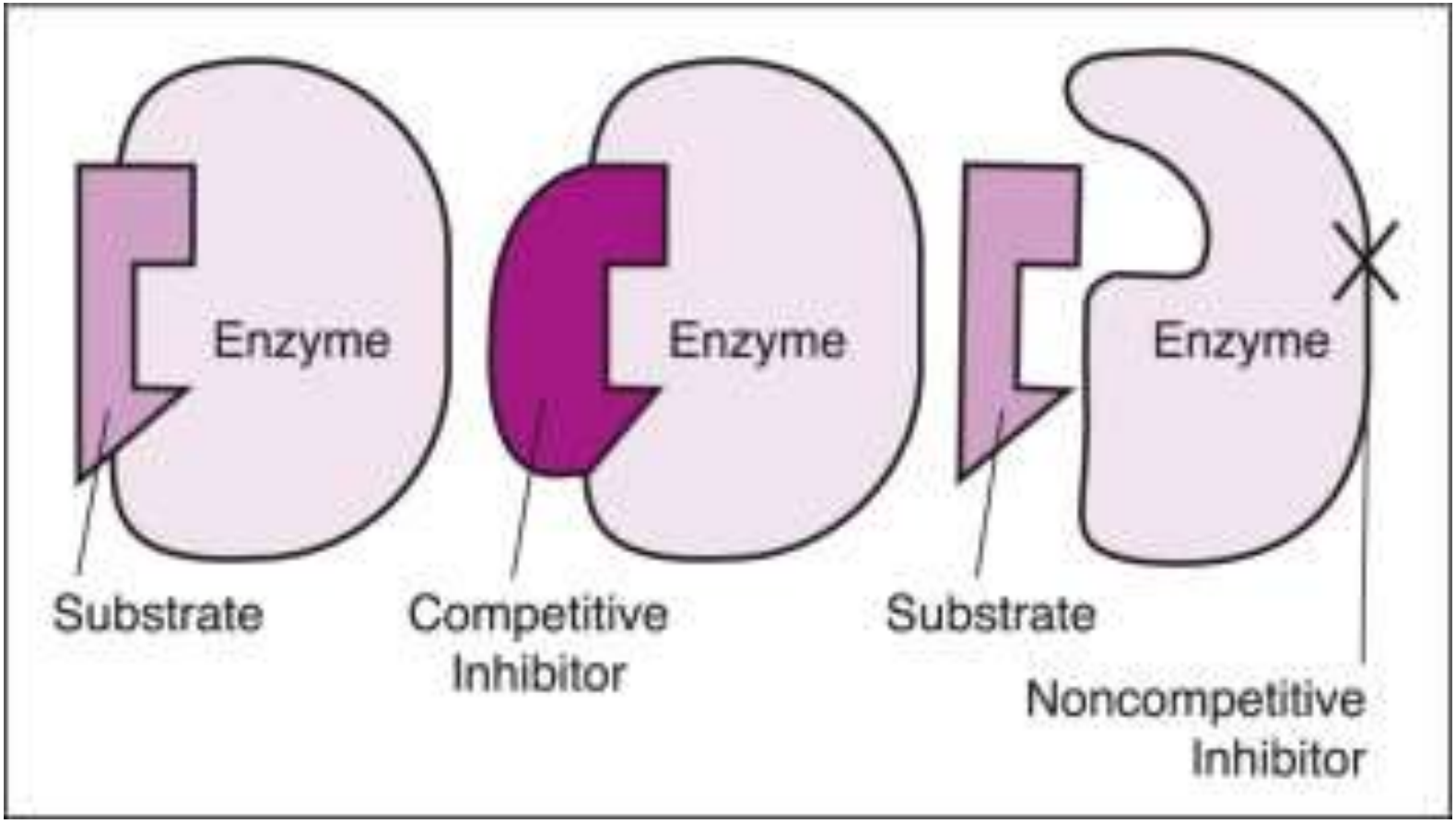
- Exposure of enzymes to ultraviolet, beta, gamma and X-rays inactivates certain enzymes due to the formation of peroxides. E.g. UV rays inhibit

ACTIVE SITE

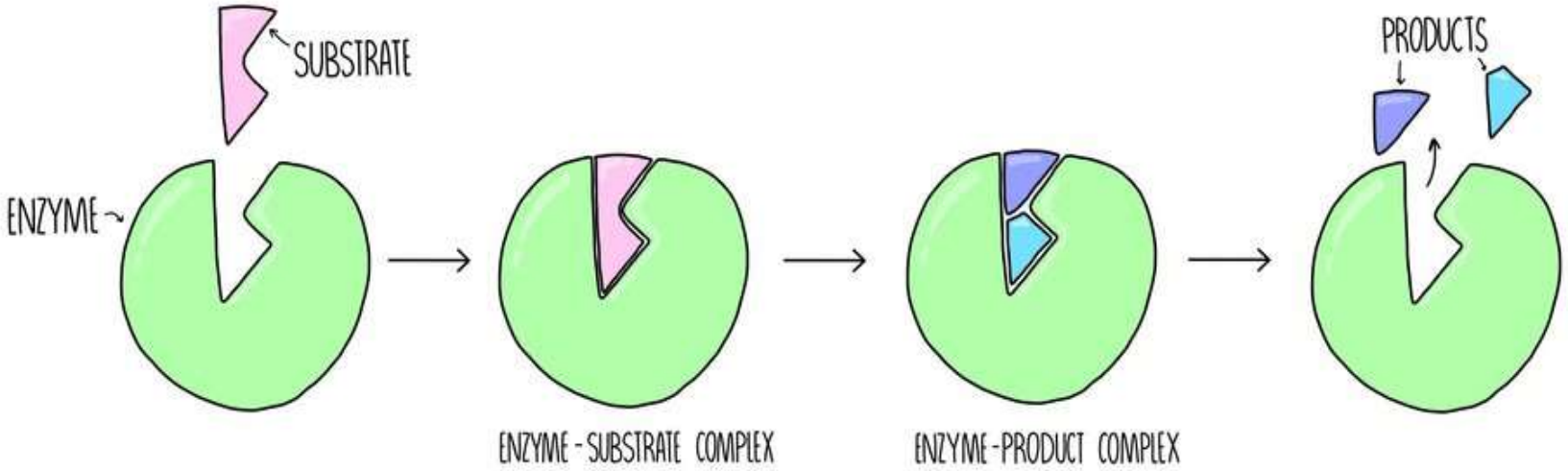
The active site (or active centre) of an enzyme represents as the small region at which the substrate(s) binds and participates in the catalysis.

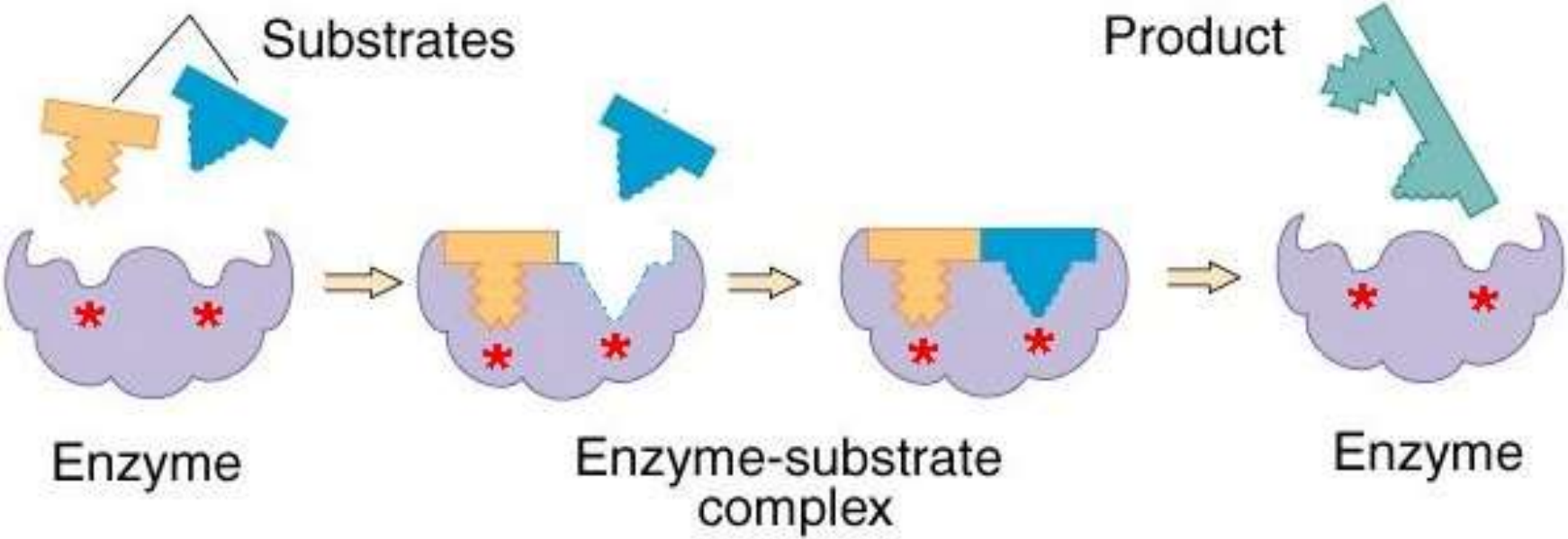





Enzyme
Enzyme
Enzyme
Substrate
Competitive Inhibitor
Substrate
Noncompetitive Inhibitor

LOCK AND KEY MODEL

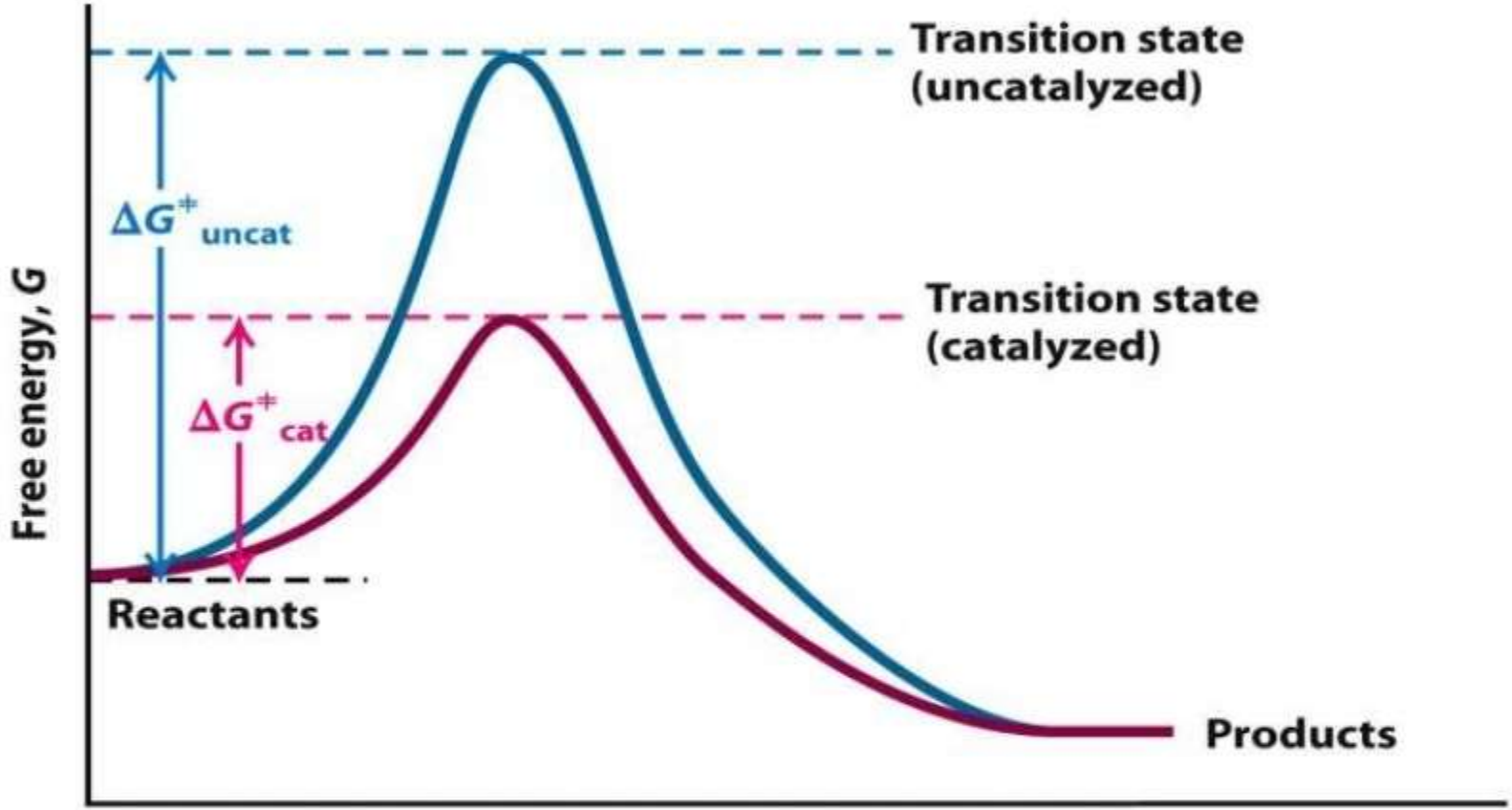



Substrates
Product
Enzyme
Enzyme-substrate complex
Enzyme

Salient features of active site

1. The existence of active site is due to the tertiary structure of protein resultin in three-dimensional native conformation.
2. The active site is made up of amino acids (known as catalytic residues) which are far from each other in the linear sequence of amino acids (primary structure of protein).
3. Active sites are regarded as clefts or crevices or pockets occupying a small region in a big molecule.
4. The active site is not rigid in structure and shape. It is rather flexible to promote the specific substrate binding.
5. Generally, the active site possesses a substrate binding site and a catalytic site.
6. The coenzymes or cofactors on which some enzymes depend are present as part of the catalytic site.
7. The substrate(s) binds at the active site by weak noncovalent bonds.

MECHANISM OF ENZYME ACTION



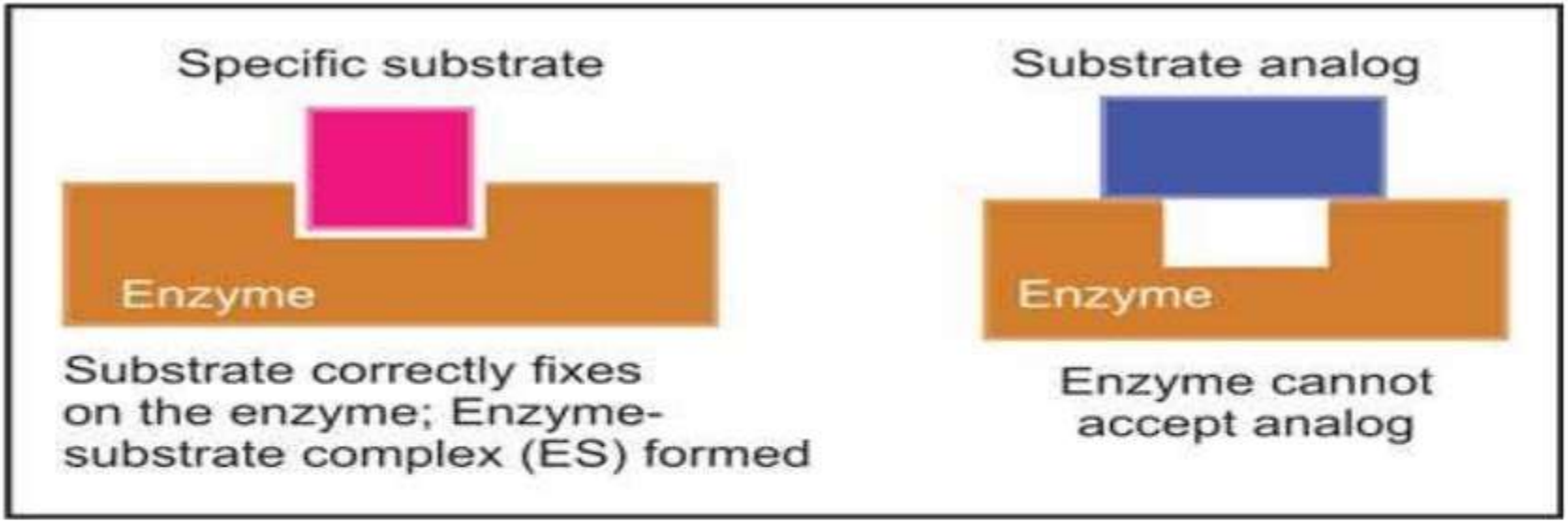
Enzyme-substrate complex formation

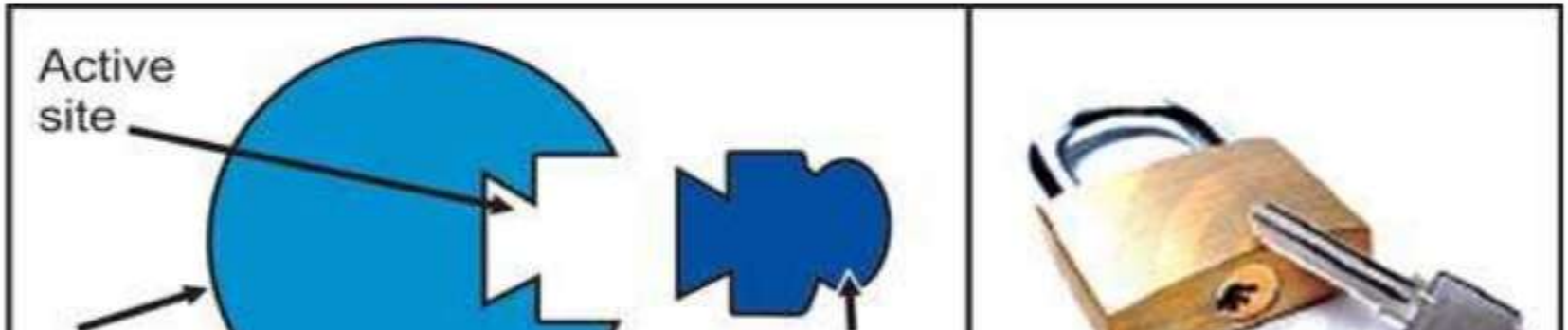
- Formation of enzyme-substrate (ES) complex is the first step in enzymatic catalysis which ultimately results in the product formation(p).
- $E + S \rightleftharpoons ES \longrightarrow E + P$
- Two models for substrate binding to the active site of the enzyme have been proposed to explain the specificity that an enzyme has for its substrate.

1. Lock and Key model or Rigid Template Model of Emil Fisher
2. Induced Fit Model or Hand-in-glove Model of Daniel E Koshland

❑ Lock and key Model

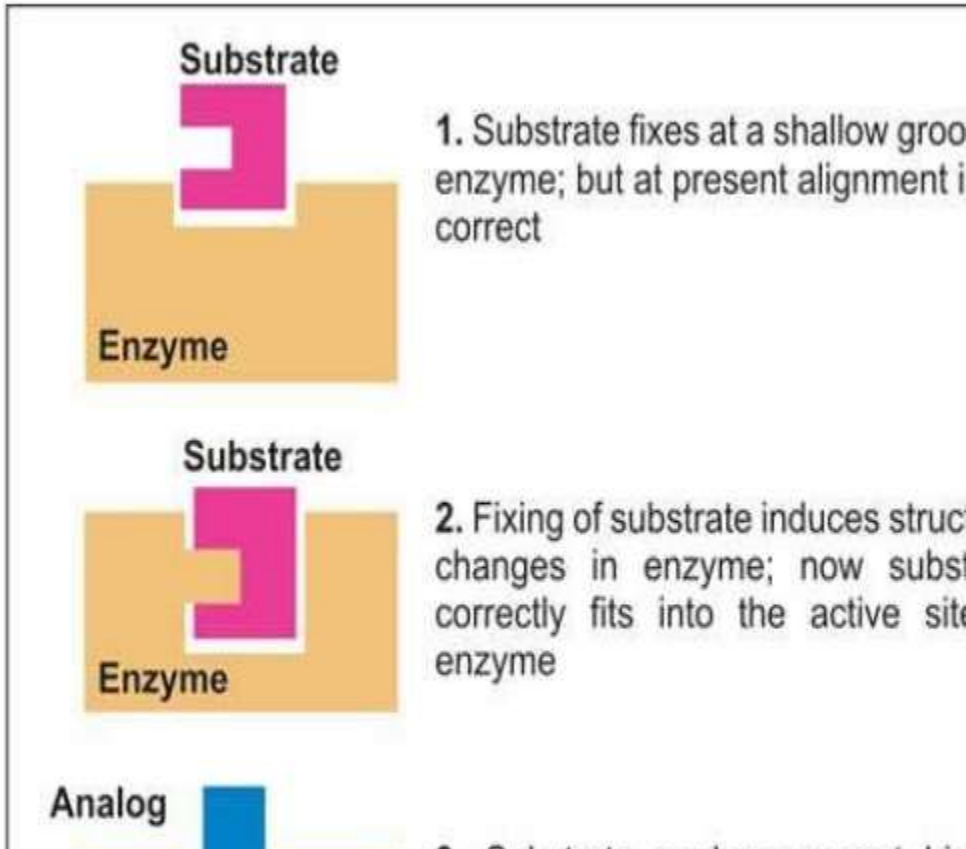
- First model purposed to explain an enzyme catalyzed reaction.
- According to this model, the structure of conformation of the enzyme is rigid The substrate fits to the binding site (now active site) just as a key fits into th proper lock


Specific substrate
Enzyme
Substrate correctly fixes on the enzyme; Enzyme-substrate complex (ES) formed
Substrate analog
Enzyme
Enzyme cannot accept analog


Active site

Induced Fit Model

- Daniel E Koshland in 1958 postulated that the enzymes are flexible and shapes of the active site can be modified by the binding of the substrate.
- In the induced fit model, the substrate induces a conformational change in the enzyme, in the same manner in which placing a hand (substrate) into a glove (enzyme) induces changes in the glove's shape. Therefore, this model is also known as hand-in-glove model.
- The functional groups of the



MECHANISM OF ENZYME CATALYSIS

- Enzymatic catalysis may take place by several processes:

1. Acid-Base catalysis
2. Covalent catalysis
3. Substrate strain
4. Proximity catalysis

Acid-Base catalysis

- The catalytic activity of an enzyme may be sensitive to pH, since pH can influence the state of protonation of the side chain at the active site.
- At physiological pH, the protonated form of histidine is the most important general acid while its conjugate base is an important general base.
- Various biochemical reactions may occur as a result of acid and/ or base catalysis.
- Ribonuclease A (RNase A), which hydrolyzes RNA to its component

Acid-Base catalysis

- Enzymes use amino acid residues as proton acceptors/do
  to aid in catalysis

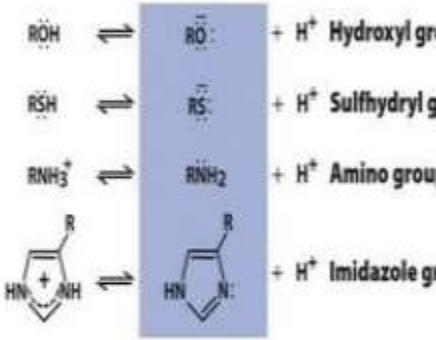


Figure 11-12
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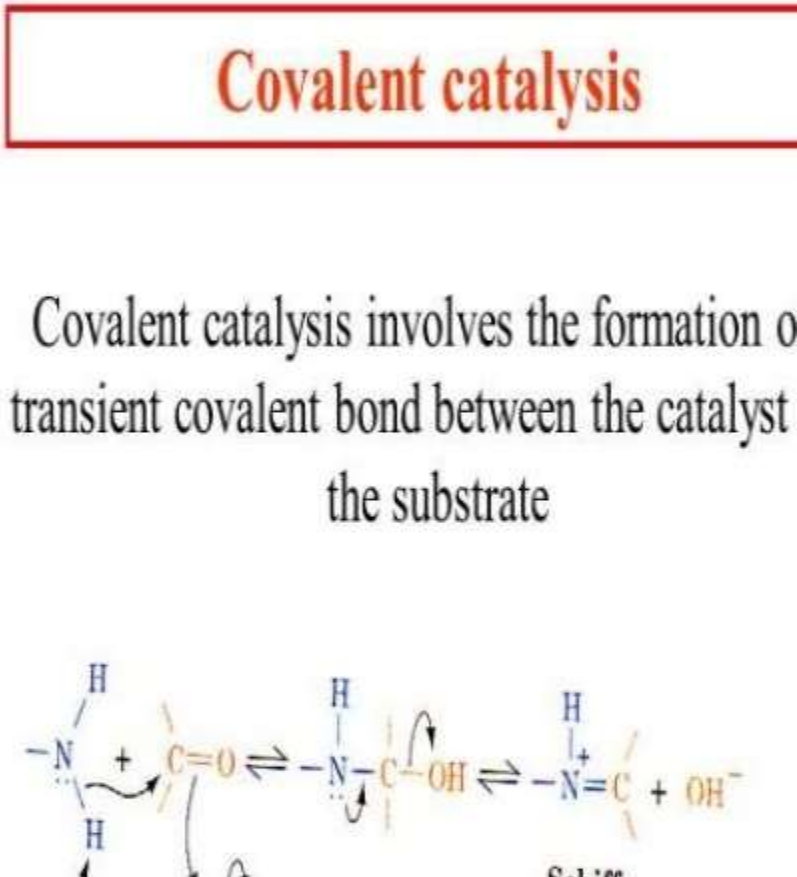
- Covalent catalysis: $A-B + :X \rightarrow A-X + B \rightarrow A + B + :X$
- Acid-Base catalysis:

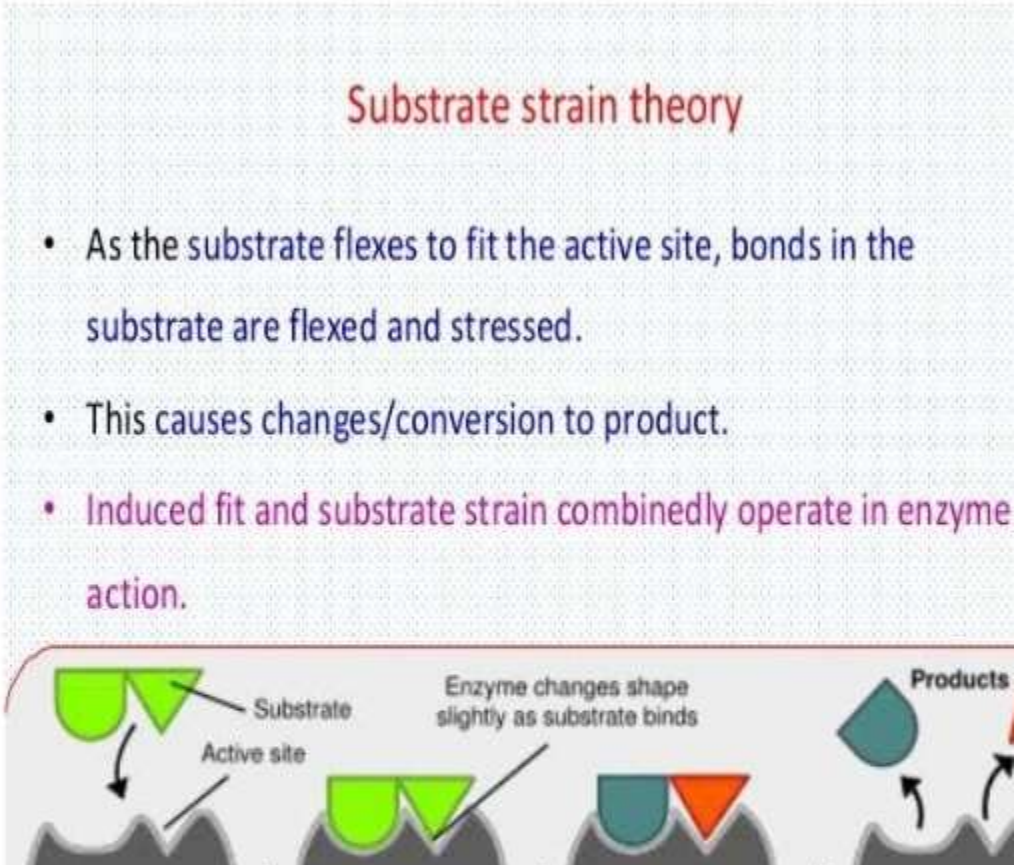
Covalent Catalysis

- In the covalent catalysis, the negatively charged (nucleophillic) or positively charged (electrophillic) group is present at the active site of the enzyme.
- This group attacks the substrate that results in the covalent binding of the substrate to the enzyme.
- Functional groups in proteins, such as the imidazole group of His, the –SH group of cysteine, the –COOH group of Asp and the –OH group of Ser, which have high polarity, are good covalent catalysts.



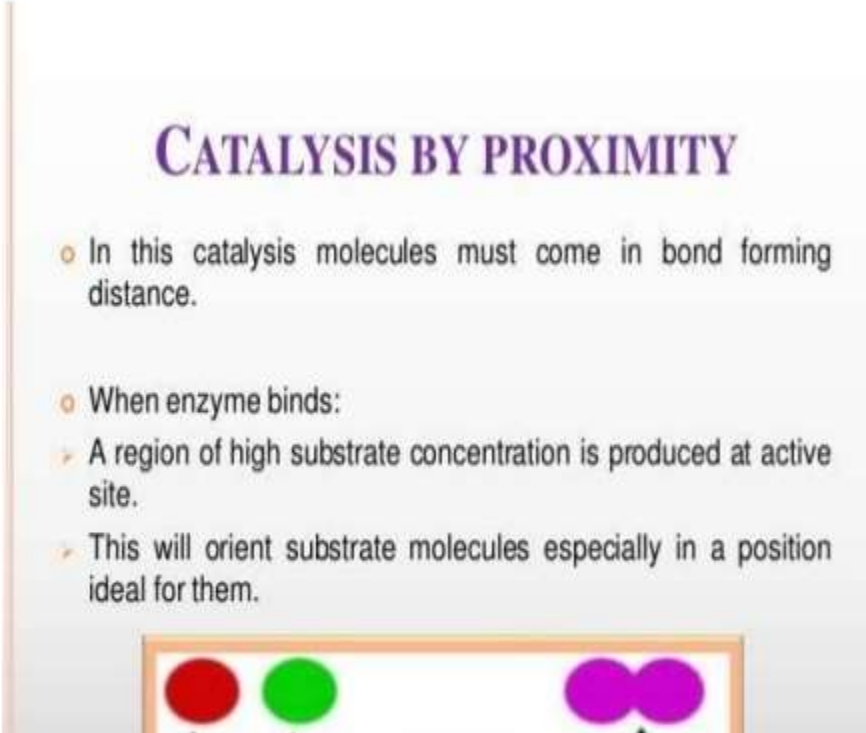
Substrate strain

- In this model, the substrate is strained due to the induced conformation change in the enzyme.
- It is also possible that when a substrate binds to the preformed active site, the enzyme induces a strain to the substrate. The strained substrate leads to the formation of product.
- The mechanism of lysozyme (an enzyme of tears, that cleaves



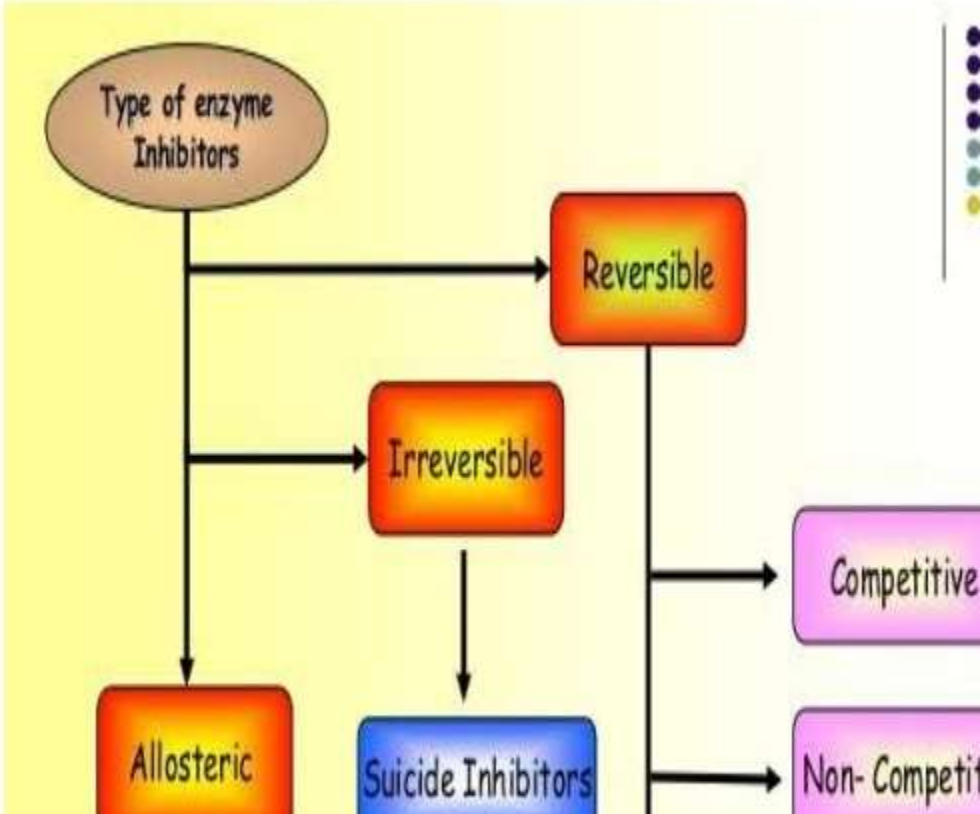
Proximity catalysis

- The reactants should come in close proximity to the enzyme for appropriate catalysis to occur.
- The higher the concentration of the substrate molecules, the greater will be the rate of reaction.



ENZYME INHIBITION

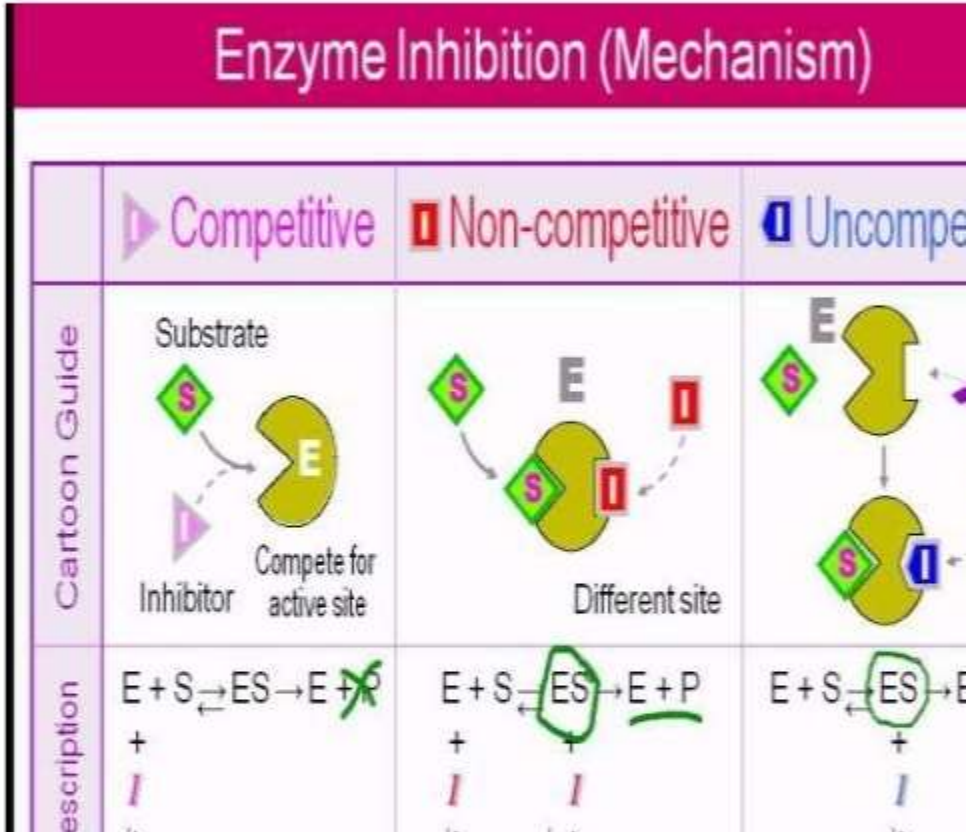
- Chemical substances which inhibit enzyme activity and reduce the velocity of an enzyme catalyzed reaction are called **inhibitors**, e.g. cyanide inhibits cytochrome oxidase.
- The phenomenon of a decrease in enzymatic reaction brought about by the addition of an inhibitor is called enzyme **inhibition**.
- There are 3 broad categories of enzyme inhibition.



REVERSIBLE INHIBITION

- The inhibitor binds non-covalently with enzyme.
- The enzyme inhibition can be reversed if the inhibitor is removed.
- The reversible inhibition is further sub-divided into

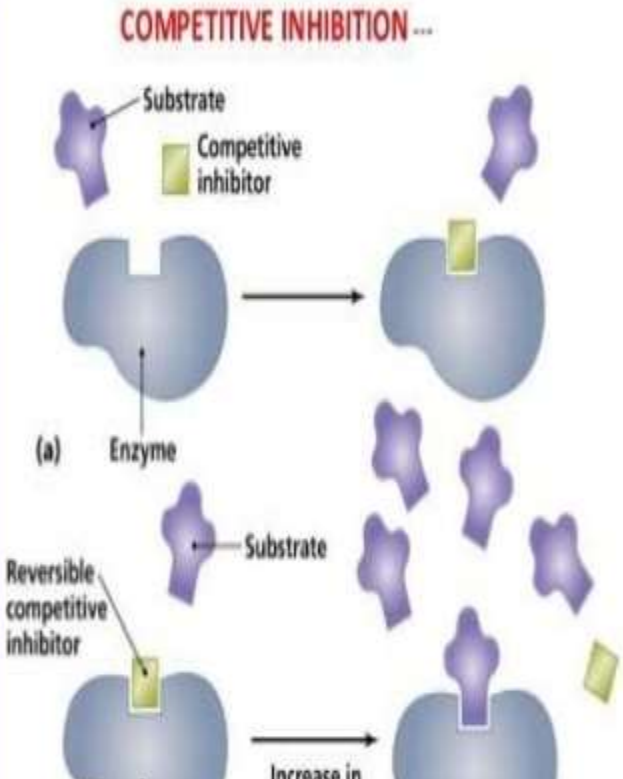
1. Competitive inhibition
2. Non-competitive inhibition
3. Uncompetitive inhibition



Competitive inhibition

- A substance that competes directly with a normal substrate for an enzyme's substrate-binding site is known as a competitive inhibitor.
- Chemical structure of the competitive inhibitor closely resembles with that of the substrate. They are called structural analogs.
- The inhibitor competes with the substrate for binding at the active site of the enzyme.
- The inhibitor forms a complex with the enzyme called as enzyme-inhibitor comple (EI), instead of the enzyme substrate complex (ES).
- A competitive inhibitor thus reduces concentration of free enzyme available for th substrate binding.
- The relative concentration of the substrate and inhibitor and their respective affini with the enzyme determines the degree of competitive inhibition.
- The degree of inhibition can be reduced by increasing the concentration of the substrate.

Competitive inhibitors
1 .competes with substrate for binding to active site but once bound substrate cannot be transformed into product by enzymes.
2.Inhibition by **Competitive inhibitors** can be reversed by simply increasing concentration of



Ethanol in the treatmen of methanol poisonini

- Methanol as such is only mildly toxic, in the liver.
- when acted upon by the enzyme alcohol dehydrogenase, methanc is converted into a highly toxic compound, i.e. formaldehyde.
- The toxicity of methanol can be overcome by giving ethanol.
- Ethanol competes with methano for binding to the active site of th enzyme and slows down the conversion of methanol to

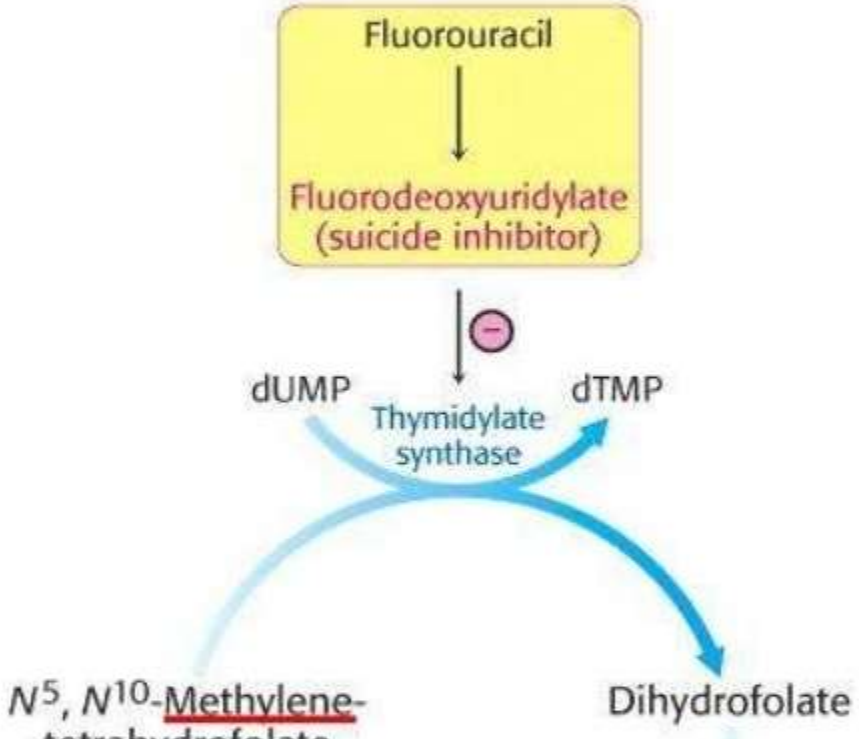
Clinical applications of competitive enzyme inhibition

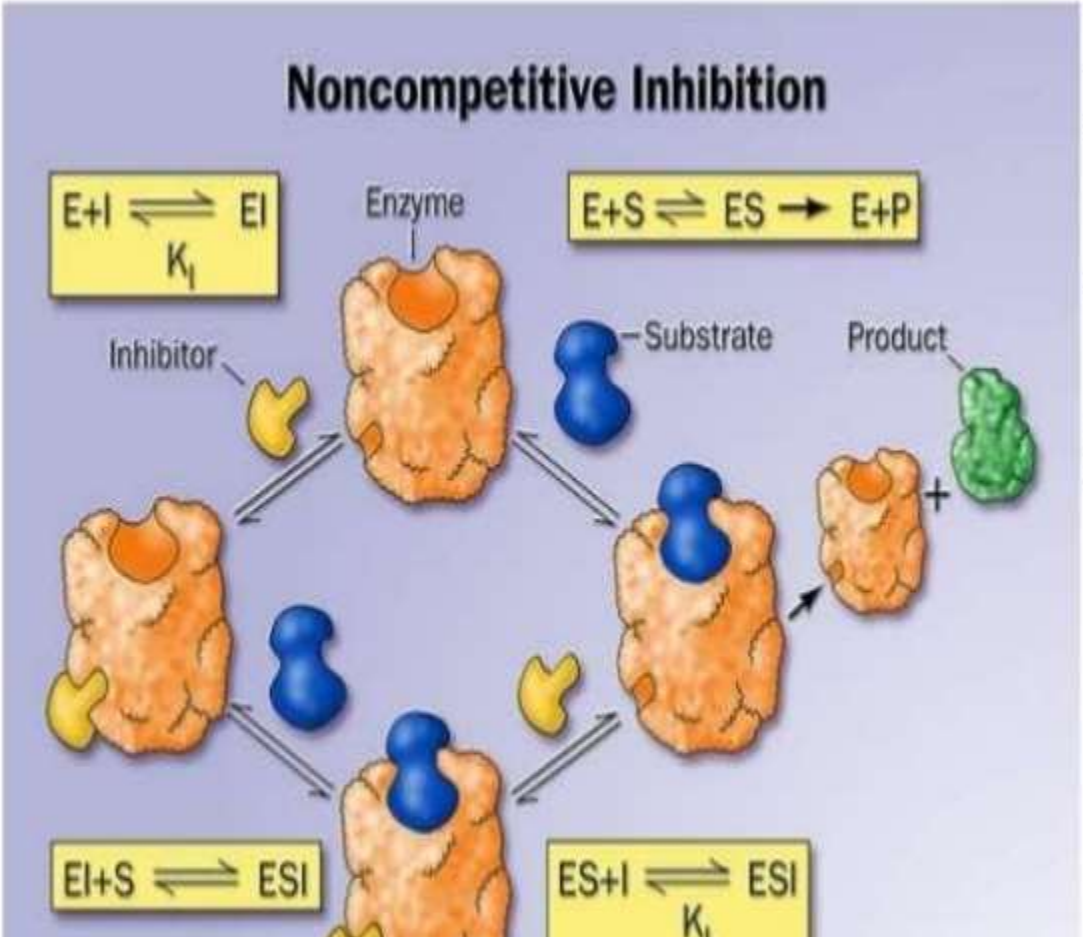
Drug	Enzyme	True substrate	Clinical application
Allopurinol	Xanthine oxidase	Hypoxanthine	Gout
Sulfonamides	Dihydropteroate synthase	Para-amino benzoic acid (PABA)	Bacterial infection
Methotrexate	Dihydrofolate reductase	Dihydrofolate	Cancer
Dicumarol	Epoxide reductase	Vitamin K epoxide	Thrombosis

Non-competitive inhibition (mixed inhibition)

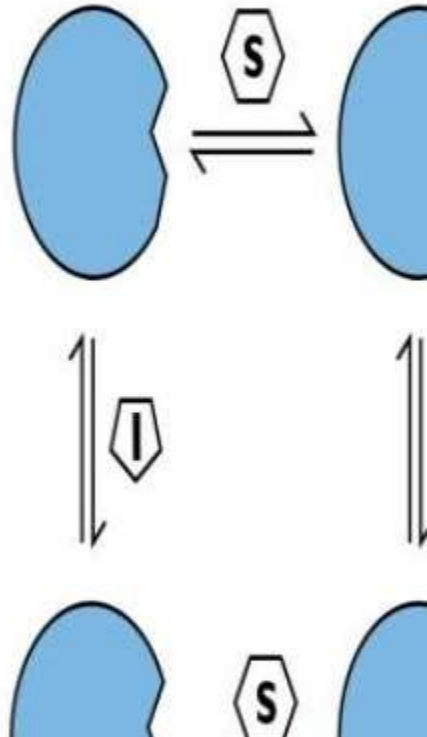
- The inhibitor (I) usually bears no structural similarity to the substrate(S) and thus there occurs no competition between I and S.
- A non-competitive inhibitor binds at a site other than the substrate binding sit hence the enzyme as well as the enzyme-substrate complex can bind to inhibitor. Therefore, both binary(EI) and ternary (ESI) complexes can be formed
- Since ESI may breakdown to form a product but at a slower rate, therefore, thi type of inhibition is also known as **mixed inhibition**.
- For non-competitive inhibition, the K_m value is unchanged whereas V_{max} is lowered.
- Certain analogs of purines and pyrimidines (called antimetabolites) are non-competitive inhibitors of some of the enzymes and are used as chemotherapeutic agents, e.g. 5-flurouracil. It is an analog of thymine and inhibits thymidylate synthetase noncompetitively

Thymidylate Synthase and DHFR: Targets of drug therapy



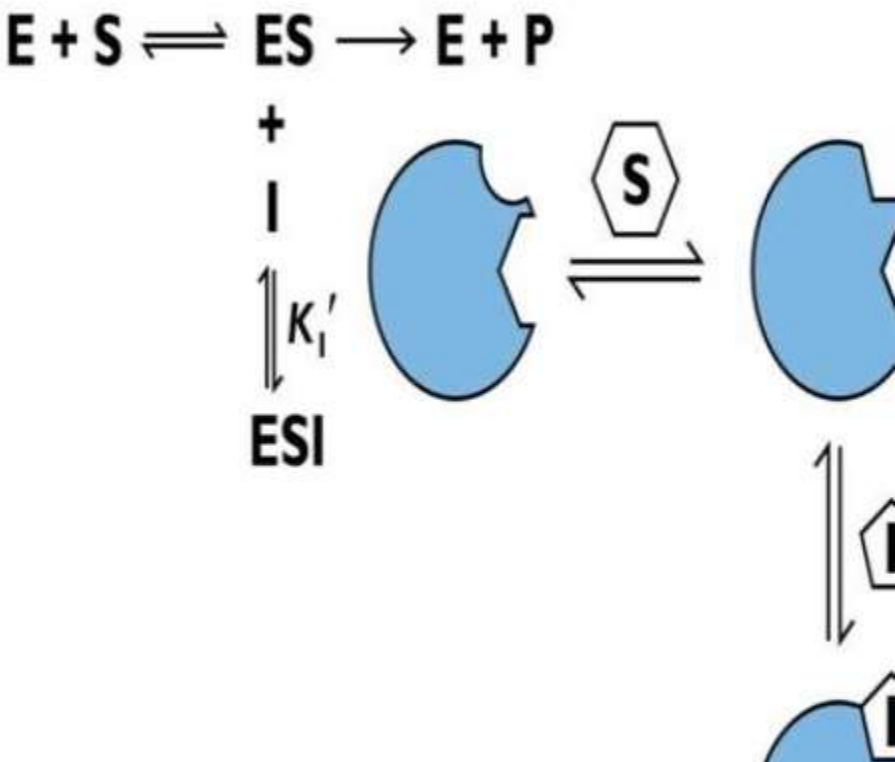


$$\begin{array}{ccc} E + S & \rightleftharpoons & ES \rightarrow E + P \\ + & & + \\ I & & I \\ \Big\| K_I & & \Big\| K_I' \\ EI + S & \rightleftharpoons & ESI \end{array}$$



Uncompetitive inhibition

- Uncompetitive inhibition occurs when the inhibitor binds after the substrate has bound to the enzyme, and then stops the reaction.
- Uncompetitive inhibitor can bind only to the enzyme-substrate(ES) complex and such an inhibitor may not resemble the substrate.
- In uncompetitive inhibition both K_m and V_{max} are decreased.
- An example is the inhibition of alkaline phosphatase by



IRREVERSIBLE INHIBITION

- An irreversible inhibitor bind covalently with the enzymes and inactivate the which is irreversible.
- Several oxidizing agents, enzyme poisons, and heavy metals cause irreversibl inhibition of enzyme activity.
- These inhibitors bear no structural similarity to the substrate, the inhibition cannot be reversed by increasing substrate concentration.
- Irreversible inhibitors can be divided in to three categories:

1. Group specific inhibitors
2. Reactive substrate analogs inhibitor or affinity labels
3. Suicide inhibitor resulting or mechanism based inactivation.

Group Specific Irreversible Inhibitor

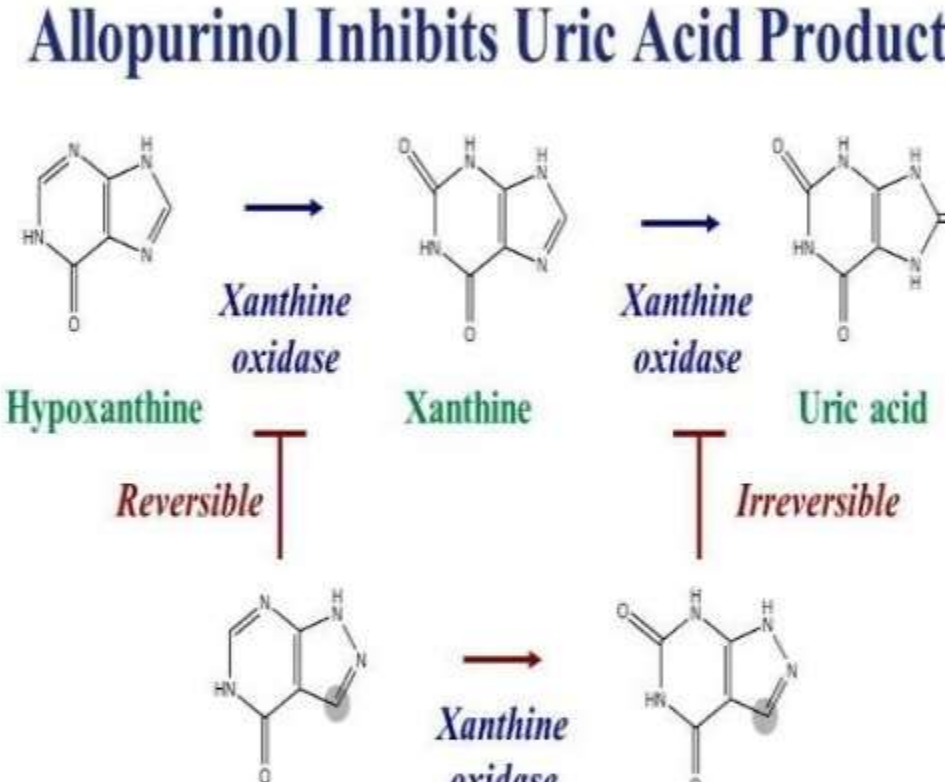
- These inhibitors react with specific R-groups of amino acid residues in the enzyme that plays essential roles in catalysis, substrate binding or maintenance of the enzyme's functional conformation.
- Diisopropylphosphofluoride (DIPF) can inhibit an enzyme acetylcholine esterase by covalently reacting, with hydroxyl group of an essential serine residue present at the active site of the enzyme. All DIPF inhibited enzyme have an essential serine residue in their active site.
- Iodoacetamide which can react with sulfhydryl (-SH) groups of essential cysteine residues or with the imidazole group of essential histidine residues o the enzyme.
- Heavy metals like Pb^{2+}, Ag^{+}, Hg^{2+} and etc. form tight covalent bond with

Reactive Substrate Analogs or Affinity Labels

- Reactive substrate analogs are molecules that are structurally similar to the substrate for an enzyme.
- These substrate analogs possess a highly reactive group which is not present in the natural substrate.
- The reactive group of affinity labels covalently reacts with amino acid residues of the active site and permanently blocks the active site of the enzyme.
- Tosyl-L-phenylalanine chloromethyl ketone (TPCK): It is a reactive substrate analog of the normal substrate for the enzyme chymotrypsin. TPCK binds at the active site of the enzyme and then reacts irreversibly with an essential histidine residues at that site and inhibits the enzyme.
- 3-Bromoacetol phosphate (BAP): It is a reactive substrate analog of the

Suicide inhibition

- Suicide inhibition is a specialized form of irreversible inhibition.
- In this case, the original inhibitor (the structural analogue/competitive inhibitor) is converted to a more potent form by the same enzyme that ought to be inhibited.
- The so formed inhibitor binds irreversibly with the enzyme in contrast to the original inhibitor which binds reversibly.
- A good example of suicide



ALLOSTERIC INHIBITION

- Some of the enzymes possesses additional sites, known as allosteric sites (Greek: allo- other), besides the active site. Such enzymes are known as allosteric enzymes or regulatory enzymes.
- The catalytic activity of some of the regulatory enzymes is modulated by certain low-molecular weight substances.
- Since these substances exhibit their effect after they bind at the site which is different from the catalytic site, i.e. they occupy another space, hence these substances are called as allosteric effectors (allosteric modulators).
- The catalytic site where the substrate binds, and the allosteric site which is occupied by the effector molecule, are physically distinct and often located far away from each other.

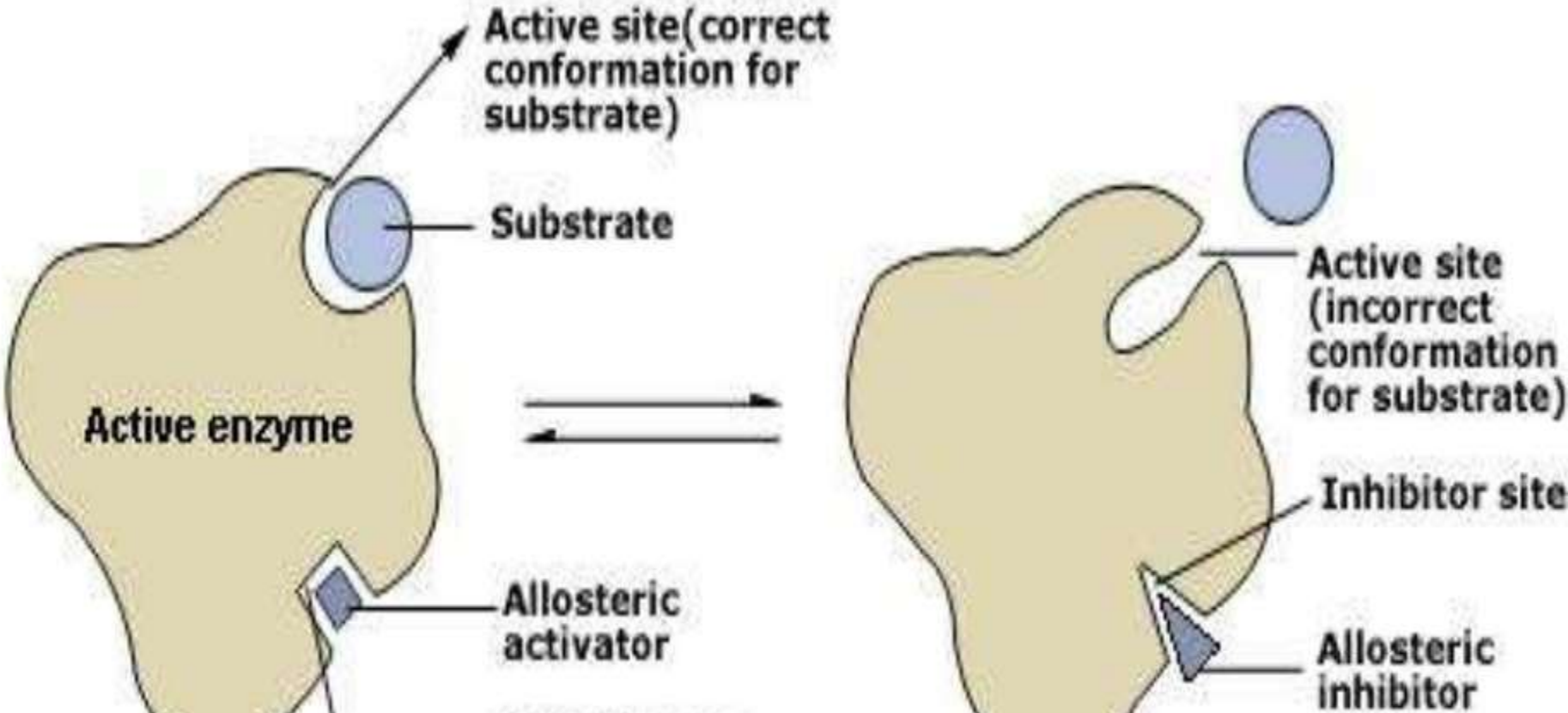
- Reversal of such an inhibition can be brought about by increasing the amoun of the substrate, relative to the amount of the inhibitor.
- If the effector substance is the substrate itself, it is called as the homotropic effect.
- On the other hand, if the effector molecule is a substance other than the substrate, then it is called as the heterotropic effect.
- Allosteric enzymes are divided into two classes based on the influence of allosteric effector on K_m and V_{max} .

1. K-class of allosteric enzymes

- The effector changes the K_m and not the V_{max} .
- E.g. phosphofructokinase

2. V-class of allosteric enzymes

- The effector alters the V_{max} and notnthe K_m .
- E.g. acetyl CoA carboxylase


Active site(correct conformation for substrate)
Substrate
Active enzyme
Allosteric activator
Active site (incorrect conformation for substrate)
Inhibitor site
Allosteric inhibitor

Allosteric modulation of some enzymes

Enzyme	Allosteric activator	Allosteric inhibitor
Hexokinase	ADP	Glucose-6-P, ATP
Isocitrate dehydrogenase	ADP	Glucose-6-P, ATP
Glutamate dehydrogenase	ADP	ATP, NADH

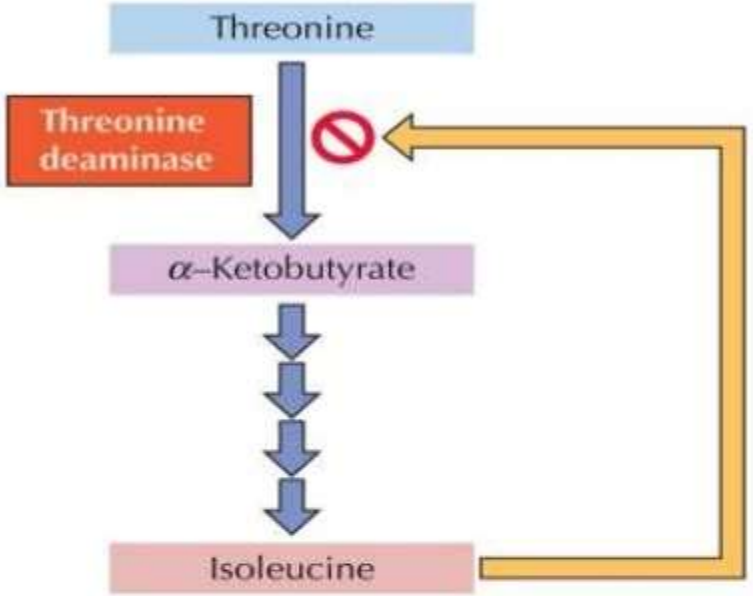
REGULATION OF ENZYME ACTIVITY IN THE LIVING SYSTEM

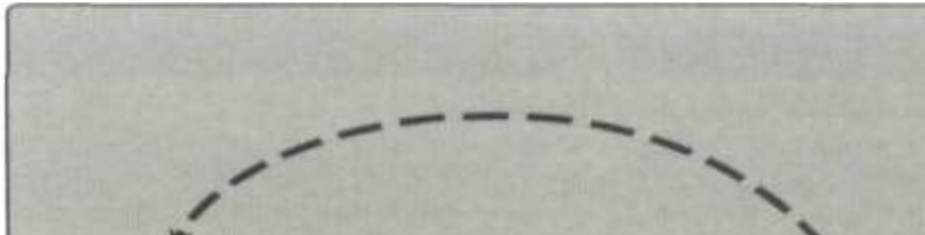
- In biological system, regulation of enzyme activities occurs at different stages in one or more of the following ways.

1. Allosteric regulation
2. Activation of latent enzymes
3. Compartmentation of metabolic pathways
4. Control of enzyme synthesis
5. Enzyme degradation

Feedback regulation

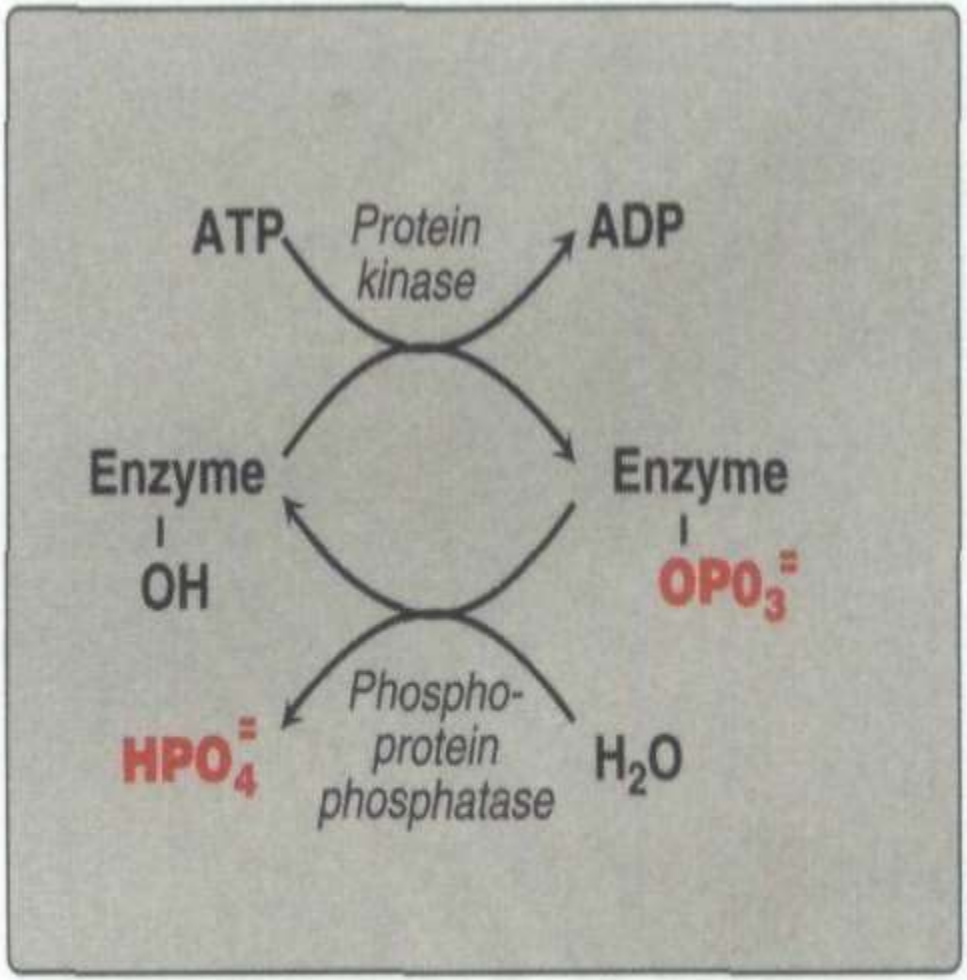
- The process of inhibiting the first step by the final product in a series of enzyme catalyzed reactions of a metabolic pathway is referred to as feedback regulation.
- When a substrate (A) is converted to an end product (P) through various intermediates, such as B,C,D, etc., the end product of the reaction inhibits the first enzyme of the pathway.
- In this type of inhibition, accumulation of the end product slows down the whole reaction

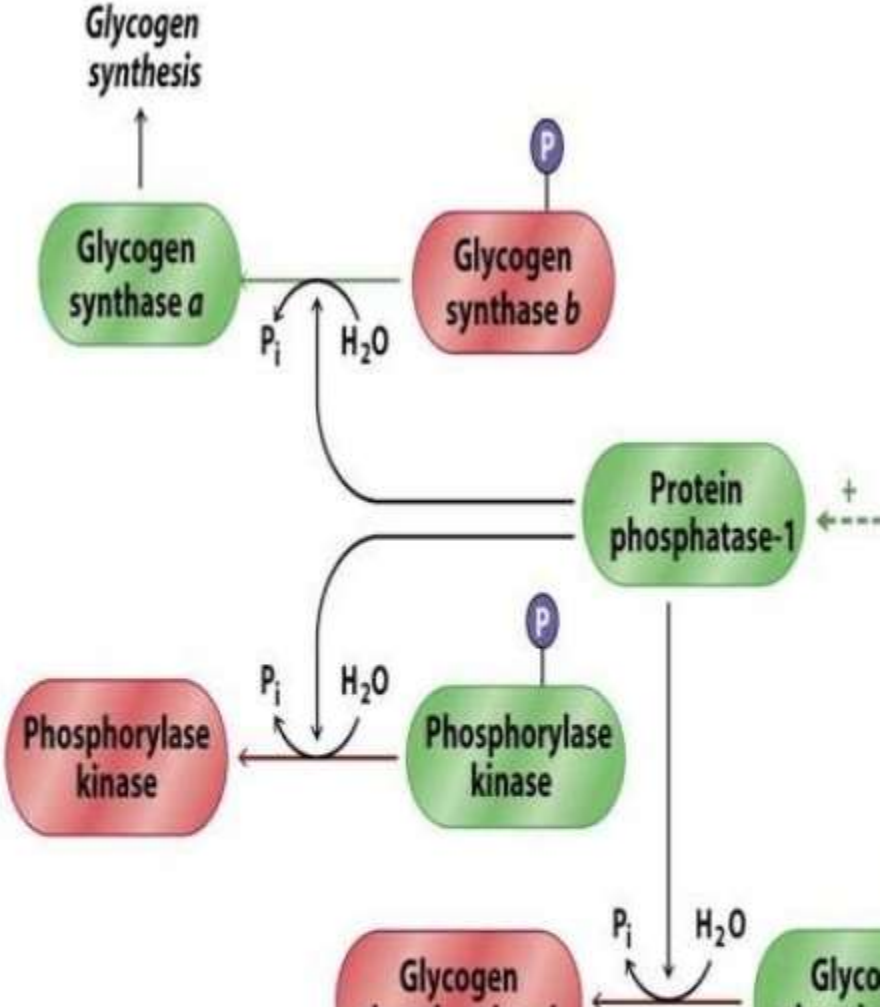




Activation of latent enzymes (covalent modification)

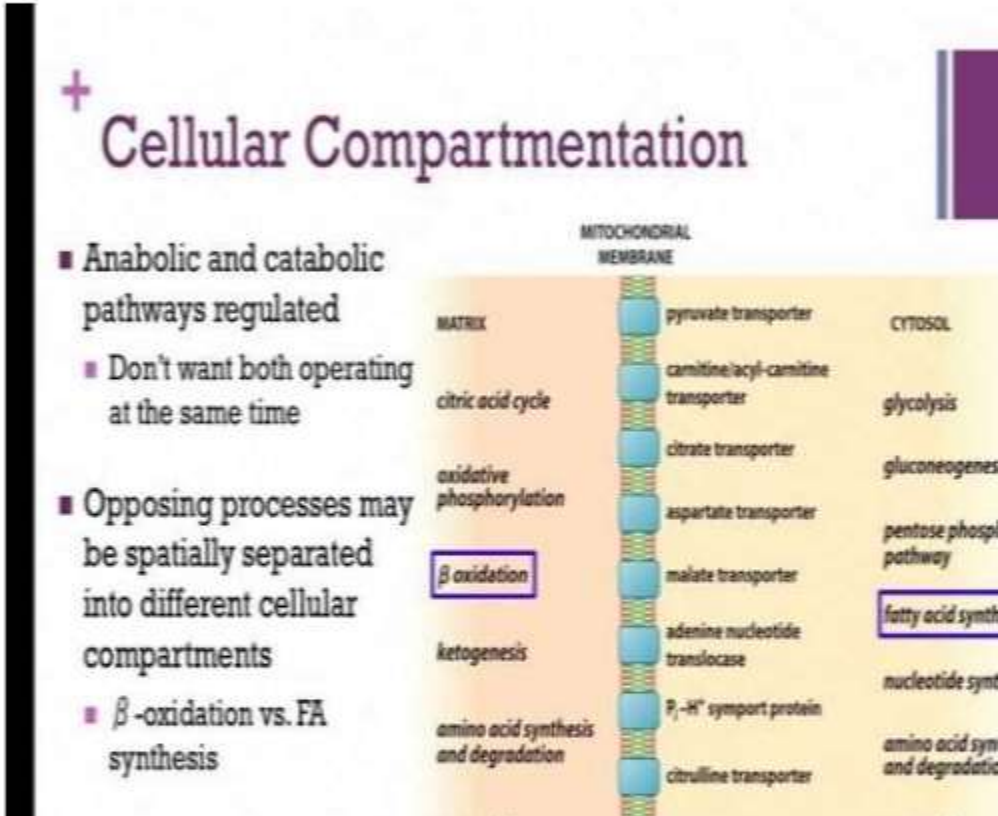
- Latent enzymes, as such are inactive.
- Some enzymes are synthesized as proenzymes or zymogens which undergo irreversible covalent activation by the breakdown of one or more peptide bonds.
- For instance, proenzymes-namely chymotrypsinogen, pepsinogen etc. are respectively converted to the active enzymes chymotrypsin, pepsin.
- Certain enzymes exist in the active and inactive forms which are interconvertible, depending on the needs of the body.
- The interconversion is brought about by the reversible covalent modifications, namely phosphorylation and dephosphorylation and oxidation and reduction of disulfide bonds.
- Glycogen phosphorylase is a muscle enzyme that breaks down glycogen to provide energy.
- This enzyme is a homodimer (two identical subunits) and exists in two interconvertible forms.
- This enzyme is active in phosphorylated state.


ATP
Protein kinase
ADP
Enzyme
OH
Enzyme
$OPO_3^=$
$HPO_4^=$
Phospho-protein phosphatase
H_2O


Glycogen synthesis
Glycogen synthase a
Glycogen synthase b
P
P_i
H_2O
Protein phosphatase-1
+
Phosphorylase kinase
Phosphorylase kinase
P
P_i
H_2O
P_i
H_2O
Glycogen

Compartmentation

- There are certain substances in the body (e.g., fatty acids, glycogen) which are synthesized and also degraded.
- Generally, the synthetic (anabolic) and breakdown (catabolic) pathways are operative in different cellular organelles to achieve maximum economy.
- For instance, enzymes for fatty acid synthesis are found in the



Control of enzyme synthesis

- Most of the enzymes, particularly the rate limiting ones, are present in very low concentration.
- The amount of the enzyme directly controls the velocity of the reaction, catalysed by the enzyme.
- Many rate limiting enzymes have short half-lives.
- This helps in the efficient regulation of the enzyme levels.
- There are two types of enzymes

1. Constitutive enzymes (house-keeping enzymes)

- Its levels are not controlled and remain fairly constant.

2. Adaptive enzymes

- Its concentrations increase or decrease as per body needs and are well

Induction and Repression

- The term induction is used to represent increased synthesis of enzyme while repression indicates its decreased synthesis.
- Induction or repression determines the enzyme concentration at the gene level through the mediation of hormones or other substances.
- Example of gene induction: The hormone insulin induces the synthesis of glycogen synthetase, glucokinase, phosphofructokinase and pyruvate kinase
- Examples of repression: In many instances, substrate can repress the synthesis of enzyme.
- Pyruvate carboxylase is a key enzyme in the synthesis of glucose from non-carbohydrate sources like pyruvate and amino acids.
- If there is sufficient glucose available, there is no necessity for its synthesis. This is achieved through repression of pyruvate carboxylase by glucose

Enzyme degradation

- Enzymes are not immortal, since it will create a series of problems.
- There is a lot of variability in the half-lives of individual enzymes.
- E.g. LDH_4 – 5 to 6 days; LDH_1 – 8 to 12 hours; amylase – 3 to 5 hours.
- Enzymes when not needed, they immediately disappear and, as when required they are quickly synthesized.

Isoenzymes

- Multiple forms of the same enzym will also help in the regulation of enzyme activity.
- Many of the isoenzymes are tissue specific.

APPLICATIONS OF ENZYMES

THERAPEUTIC IMPORTANCE

1. **Streptokinase :** is used in blood clot-dissolution during an acute myocardia infarction or in deep vein thrombosis.
2. **Asparaginase :** is used for some types of leukemias based on the rationale that the tumor cells are asparagine-dependent for their multiplication and survival.
3. **Deoxyribonuclease (DNAse)** : is administered by the respiratory route to clear up the viscid secretions in patients of cystic fibrosis.
4. **Serratiopeptidase :** used to minimize the edema that accompanies a physic trauma or an acute inflammation of the skin.
5. **Hyaluronidase :** Used for hypodermoclysis, to facilitate the subcutaneous

THERAPEUTIC APPLICATION

Enzyme	Reaction	use
αAmylase	Starch hydrolysis	Diagestive disorders
Collagenase	Collagen hydrolysis	Skin ulcers
Ficin, Papain, Proteinase	Protein hydrolysis	Deworming, digestive -disorders,narcotic tissue removal
Glutaminase	Gln→Glu	Leukemia
Aspargina	Asn→Asp	Leukemia

Enzyme	Reaction	Use
Rhodanase	Degradation of cyanide	Cyanide poisoing
RNase	RNA digestion	Antiviral RNA hydrolysis
β lactamase	Pencillin→Pencilloate	Pencillin allergy
Streptokinase/ urokinase	Plasminogen→ plasmin	Blood clot dissolving
uricase	UA→allatoin	Gout
Hyaluronidase	?HUA hydrolysis	Viral infection

DIAGNOSTIC IMPORTANCE

Measurement of various enzymes in serum has been found to be helpful in the diagnosis of various diseases.

66.4 Important enzymes in the diagnosis of diseases

Serum enzyme (elevated)	*Disease (most important)*
Amylase	Acute pancreatitis
Serum glutamate pyruvate transaminase (SGPT)	Liver diseases (hepatitis)
Serum glutamate oxaloacetate transaminase (SGOT)	Heart attacks (myocardial infarction)
Alkaline phosphatase	Rickets, obstructive jaundice
Acid phosphatase	Cancer of prostate gland
Lactate dehydrogenase (LDH)	Heart attacks, liver diseases

Table 11.10. Some serum enzymes of diagnostic significance

Enzyme	*Principal sources*	*Conditions in which altered (↑, increase; ↓, decrease)*
Acid phosphatase	Prostate, RBC	↑-Carcinoma prostate
Alkaline phosphatase	Liver, bone, placenta, kidney	↑-Obstructive jaundice, rickets, Paget's disease, hyperparathyroidism, growing children
Aldolase	Skeletal muscle, heart	↑-Muscular dystrophies, acute hepatitis
Amylase	Salivary glands	↑-Acute parotitis
Amylase	Pancreas	↑-Acute pancreatitis, intestinal obstruction
Ceruloplasmin	Liver	↑-Cirrhosis, ↓-Wilson's disease
Cholinesterase	Liver	↑-Nephrotic syndrome, organophosphorus poisoning
Creatine phosphokinase	Skeletal muscle, brain, heart	↑-Acute myocardial infarction, muscular dystrophies
Glutamate oxaloacetate transaminase (GOT)	Liver, skeletal muscle, heart, kidney, RBC	↑-Acute myocardial infarction, toxic liver cell necrosis, acute hepatitis, myositis
Glutamate pyruvate transaminase (GPT)	Liver, skeletal muscle, heart	↑-Viral hepatitis

Analytical application reagents (for estimation)

Enzymes	use
Glucose oxidase and peroxidase	Glucose
Urease	Urea
Cholesterol oxidase	Cholesterol
Uricase	Uric acid
Lipase	Triacylglycerols

ISOENZYMES

❑ Definition

- The multiple forms of an enzyme catalyzing the same reaction are isoenzyme or isozymes.
- Isoenzymes may be present in different tissues of the same organism, in different cell types or subcellular compartments.
- Besides the source, they also differ from each other with respect to their structure, electrophoretic mobility and immunological properties.

❑ Explanation for the existence of isoenzymes

- The most common mechanism for the formation of isoenzymes involves the arrangement of subunits, arising from different genetic loci, in different combinations to form the active polymeric enzyme.
- E.g. malate dehydrogenase of cytosol is different from that found in

Table 11.9. Diagnostic importance of some isoenzymes assayed in serum

Enzyme	*Isoenzymes*	*Predominant location*	*Diagnostic significance*
Creatine phosphokinase (CPK, a dimer with 2 polypeptide chains B [brain] and M [muscle])	CPK-1 (BB)	Brain	Head injury
	CPK-2 (MB)	Myocardium	Acute myocardial infarc
	CPK-3 (MM)	Skeletal muscle	Muscular dystrophies
Lactate dehydrogenase (LDH, a tetramer with 4 polypeptide chains, H [heart] and M [muscle])	LDH-1 (H_4)	Myocardium	Acute myocardial infarc
	LDH-2 (H_3M)	Erythrocytes	Hemolytic anemia
	LDH-3 (H_2M_2)	Brain	Cardiovascular accident
	LDH-4 (HM_3)	Liver	Hepatocellular damage
	LDH-5 (M_4)	Skeletal muscle	Muscular dystrophies
Alkaline phosphatase	α_1-ALP	Liver	Obstructive jaundice

ISOENZYMES OF LACTATE DEHYDROGENASE

- LDH systematic name is L-lactate-NAD^+ oxidoreductase (E.C. 1.1.1.27).
- It catalyzes the interconversion of lactate and pyruvate.

❑ Structure

- Lactate dehydrogenase (LDH) is a tetramer, i.e. it has four polypeptide subunits.
- Each subunit may be one of the two types, known as the H type (heart-type) and the M type (muscle-type) produced by different genes.
- M-subunit is basic while H subunit is acidic.
- The isoenzymes contain either one or both the subunits giving LDH_1 to LDH_5.

❑ Diagnostic Significance of LDH

- LDH_1 has 4H type of polypeptide chains (H_4) and is predominantly found in

- LDH_1 becomes greater than LDH_2 (known as flipped ratio) between 12 and 24 hours following an acute myocardial infarction.
- Rise in LDH starts 12 to 18 hours after the onset of acute myocardial infarction, with peak at 48 hours to 72 hours.
- The levels return to below the upper normal level after 6 to 10 days.
- Rise in total LDH parallels LDH_1 isozyme.
- Marked elevation of the total LDH activity, upto 50 times the upper normal value, may be observed in megaloblastic anemia.

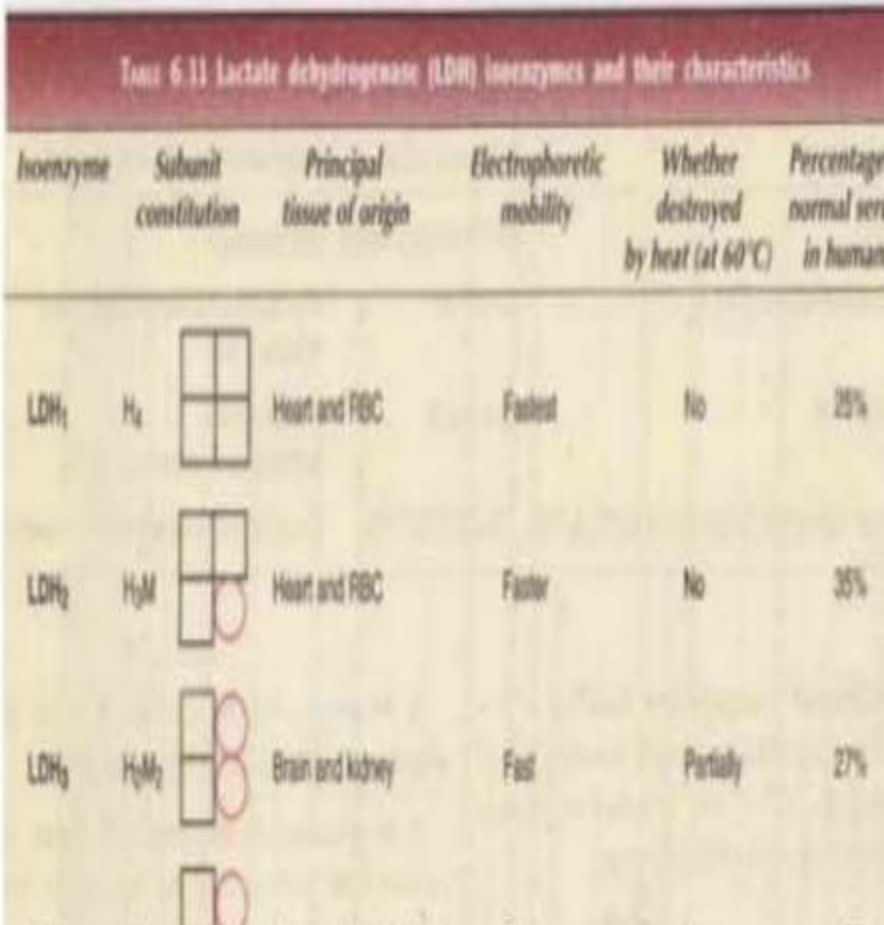


Table 6.11 Lactate dehydrogenase (LDH) isoenzymes and their characteristics

Isoenzyme	Subunit constitution	Principal tissue of origin	Electrophoretic mobility	Whether destroyed by heat (at 60°C)	Percentage normal ser... in human...
LDH_1	H_4	Heart and RBC	Fastest	No	25%
LDH_2	H_3M	Heart and RBC	Faster	No	35%
LDH_3	H_2M_2	Brain and kidney	Fast	Partially	27%

ISOENZYMES OF CREATINE PHOSPHOKINASE

- Creatine kinase (CK) or creatine phosphokinase (CPK) catalyses the inter-conversio of phosphocreatine (creatine phosphate) to creatine.
- It exists in three forms.
- Each isoenzyme is a dimer composed of two subunits, i.e. M (muscle) type and B (brain) type.
- The three isoenzymes are CPK_1 (BB) found in brain, CPK_2 (MB) in myocardium, and CPK_3 (MM) in skeletal muscle.
- CPK-MB isoenzyme is present in very small amount in serum i.e. almost undetectable in serum with less than 2% of total CPK.
- CK activity is greatly elevated in all types of muscular dystrophies. Usually only CK- is present in serum, in case of dystrophies and myopathies.
- CPK_2 isoenzyme is not elevated in skeletal muscle disorders . Therefore , estimatio of the enzyme CPK_2 (MB) is the earliest reliable indication of myocardial infarction.
- Its level rises within 4-6 hours in acute myocardial infarction and reaches to a maximum within 1 day of the infarction while return to normal occurs within 48-72 hours

ISOENZYMES OF ALKALINE PHOSPHATASE

- Different tissues contain different forms of alkaline phosphatase.
- A major portion of alkaline phosphatase in serum is derived from liver and its level rises in post-hepatic jaundice.
- As many as six isoenzymes of alkaline phosphatase (ALP) have been identified.
- ALP is a monomer, the isoenzymes are due to the difference in the carbohydrate content (sialic acid residues).
- The most important ALP isoenzymes are alpha1-ALP, alpha2-heat labile ALP, alpha2 heat stable-ALP, prebeta ALP, Gamma-ALP etc.
- In growing children, the major isoenzyme is from the bone which is related to its increased osteoblastic activity.
- During the last trimester of pregnancy, there is an increase in alkaline phosphatase which is of placental origin.
- Estimation of serum alkaline phosphatase (ALP) is of great significance in the diagnosis of hepatobiliary diseases and bone diseases associated with an increased osteoblastic activity.

ISOENZYMES OF ALCOHOL DEHYDROGENASE

- Alcohol dehydrogenase (ADH) has two heterodimer isoenzymes.
- Among the white Americans and Europeans, alpha-beta-1 isoenzyme is predominant whereas in Japanese and chinese (Orientals) alpha-beta-2 is mostly present.
- The alpha-beta-2 more rapidly converts alcohol to acetaldehyde.
- Accumulation of acetaldehyde is associated with tachycardia and facial flushing among Orientals which is not commonly seen in whites.
- It is believed that Japanese and Chinese have increased sensitivity to alcohol due to the presence of alpha-beta-2 isoenzyme of ADH.
- Normal value : 0.07 – 0.56 U/L

ENZYME PATTERN IN DISEASES

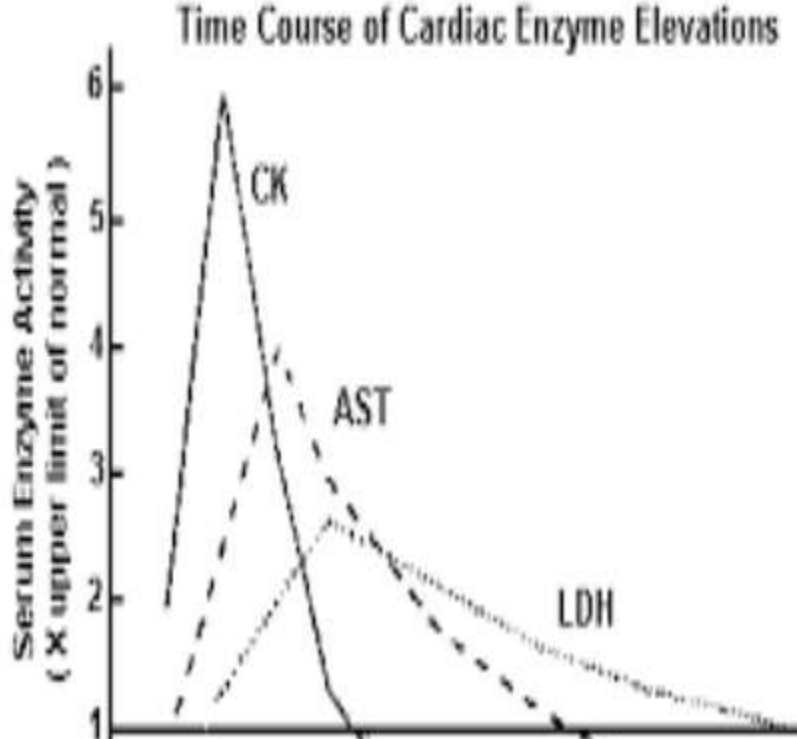
ENZYMES IN MYOCARDIAL INFARCTION

TABLE 6.12 Summary of diagnostic markers used for the evaluation of acute myocardial infarcti

Diagnostic marker	*Time of peak elevation*	*Time of return to normal level*	*Diagnostic importance*
Myoglobin	4-6 hrs	20–25 hrs	Earliest marker, however not cardiac specific.
Cardiac troponin I	12-24 hrs	5-9 days	Early marker and cardiac specific.
Cardiac troponin T	18-36 hrs	5-14 days	Relatively early marker and cardiac specific.

Time Course for Serum Markers in Acute MI

Test	Onset	Peak	Duration
Troponin	3 – 12 hours	18 – 24 hours	Up to 10 days
CK (total) & MB	3 – 12 hours	18 – 24 hours	36 – 48 hours
LDH	6 – 12 hours	24 – 48 hours	6 – 8 days (5 – 10 days)



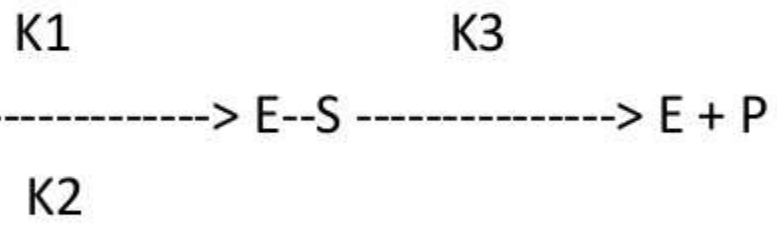
ENZYMES PATTERN IN OTHER DISEASES

Table 6.3. Enzyme patterns (Enzyme profiles) in diseases

Hepatic diseases	
1. Alanine amino transferase(ALT)	Marked increase in parenchymal liver diseases
2. Nucleotide phosphatase(NTP)	Elevated in liver dysfunction with cholestasis
3. Alkaline phsophatase(ALP)	Marked increase in obstructive liver disease
4. Gamma glutamyl transferase(GGT)	Increase in obstructive and alcoholic liver disease
Myocardial infarction	
1. Creatine kinase(CK-MB)	First enzyme to rise following infarction, CK-MB isoenzyme is specific
2. Aspartate amino transferase(AST)	Rises after the rise in CK and returns to normal in 4-5 days
3. Lactate dehydrogenase(LDH)	Last enzyme to rise. LDH-1 becomes more than 2 (Flipped pattern)
Muscle diseases	
1. Creatine kinase(CK-MM)	Marked increase in muscle diseases. CK-MM fraction is elevated
2. Aspartate amino transferase(AST)	Shows an increase in different types of muscle disease; not specific
3. Aldolase(ALD)	Earliest enzyme to rise, but not specific
Bone diseases	

ENZYME KINETICS AND K_m VALUE

- The study of reaction rates and how they change in response to changes in experimental parameters is known as kinetics.
- The relationship between substrate concentration and reaction velocity can be derived by Michaelis-Menten equation.
- Michaelis and Menten proposed that an enzyme (E) forms enzyme-substrate complex (ES), with a single substrate (S).
- The complex (ES) is broken down, relatively slowly, into free enzyme (E) and the product (P).



$$Km = \frac{K2 + K3}{K1}$$

Km is called as **Michaelis Constant.**

It is further shown that

$$\text{Velocity } (v) = \frac{Vmax\ [S]}{Km + [S]}$$

When concentration of substrate is made equal to Km
i.e.

When [S] = Km

MICHAELIS-MENTEN CONSTANT(Km)

- It is defined as the substrate concentration (expressed in moles/l) to produce half-maximum velocity in an enzyme catalyzed reaction.
- It indicates that half of the enzyme molecules (i.e. 50%) are bound with the substrate molecules when the substrate cocentration equals the Km value.

❑ Salient Features of Km

1. Km value is substrate concentration (expressed in moles/L) at half-maximal velocity.

2. It denotes that 50% of enzyme molecules are bound with substrate molecules at that particular substrate concentration.

3. Km is independent of enzyme concentration. If enzyme concentration is

4. Km is the Signature of the Enzyme. Km value is thus a constant for an enzyme. It is the characteristic feature of a particular enzyme for a specific substrate.

5. The affinity of an enzyme towards its substrate is inversely related to the dissociation constant, Kd for the enzyme–substrate complex.

$$E + S \underset{K2}{\overset{K1}{\rightleftharpoons}} E\text{–}S \text{ complex} \xrightarrow{K3} E + P$$

- $Kd = \frac{K1}{K2}$ and $Km = \frac{K2 + K3}{K1}$

Therefore, the smaller the tendency for the dissociation of the complex, the greater is the affinity of the enzyme for the substrate.

6. Km denotes the affinity of enzyme for substrate. The *lesser the numerical value of Km, the affinity of the enzyme for the substrate is more*

SIGNIFICANCE OF Km

1. Physiological significance

- Glucose can be phosphorylated to glucose 6-phosphate by glucokinase (the enzyme present in the liver, is specific for glucose) or hexokinase (nonspecific for hexoses) except galactose, present in all tissues). Glucokinase has a high Km (i.e. low affinity for glucose), hence it is importa during the fed state when glucose is in excess. On the other hand, under post-absorptive/fasting conditions, hexokinase having a low Km (i.e. high affinity for glucose) is important so that glycolysis continues to provide energy to the vital organs even at low blood glucose levels.

2. Laboratory significance

- During enzyme assay in the laboratory, the substrate concentration is kept at saturating amounts (at least 10 times the Km) so that the reaction proceeds

3. Clinical significance

- The Km value for a given enzyme may differ person to person and explains th varied response to drugs/chemicals.
- Aldehyde dehydrogenase enzyme oxidizes acetaldehyde (formed from alcoho into acetic acid. People having a low Km (i.e. high affinity) variant of the enzyme metabolize acetaldehyde rapidly and are less susceptible to its adverse effects such as headche and flushing. On the other hand, having the high Km variant are more prone to these side effects of alcohol.

LINEWEAVER-BURK DOUBLE RECIPROCAL PLOT

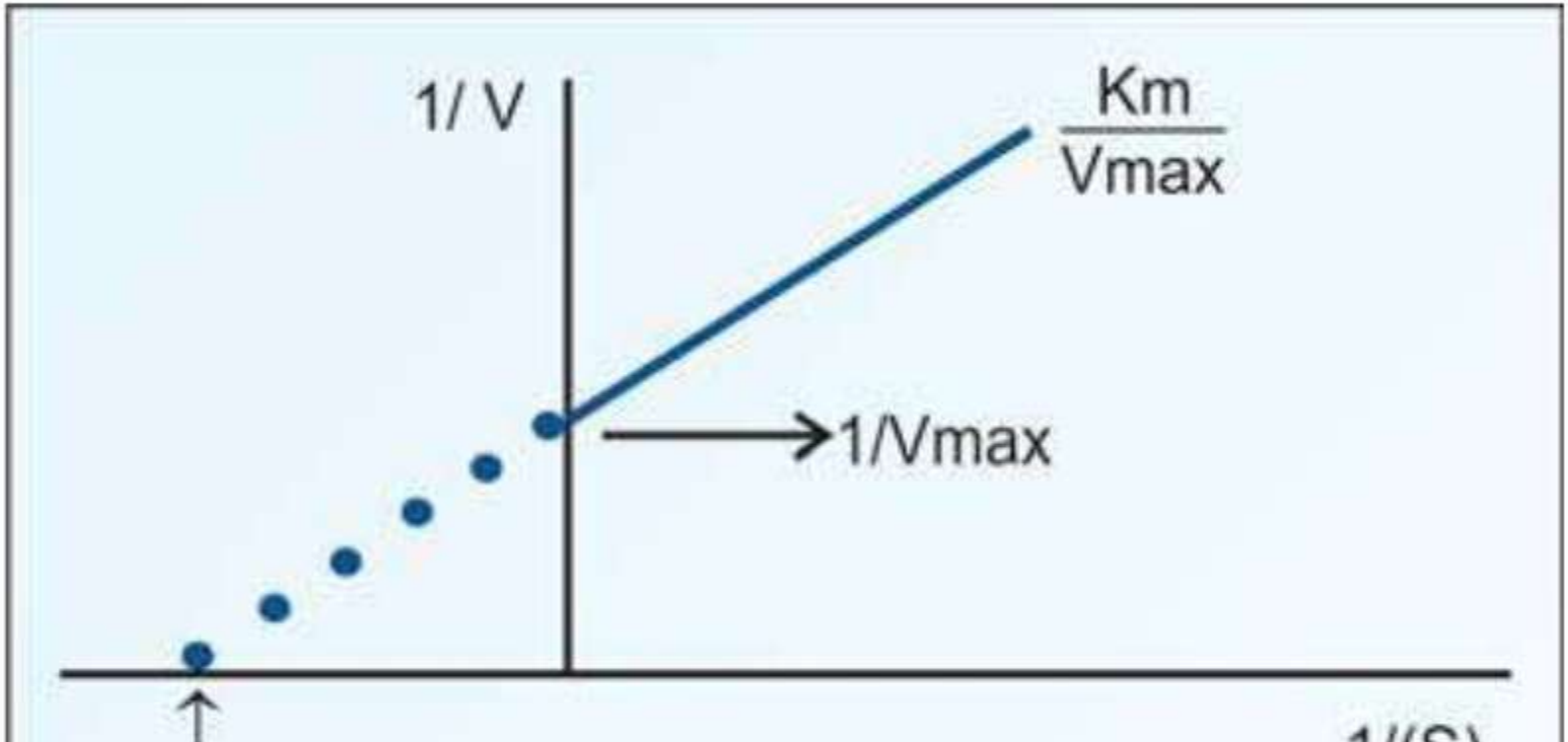
- Sometimes it is impractical to achieve high substrate concentrations to reach the maximal velocity conditions. So, ½ Vmax or Km may be difficult to determine.
- Then, the experimental data at lower concentrations is plotted as reciprocals.
- The straight line thus obtained is extra plotted to get the reciprocal of Km .Thi is called Lineweaver-Burk Plot or Double Reciprocal Plot which can be derived from the Michaelis-Menten equation.

$$v = \frac{Vmax\,[S]}{Km + [S]}$$

When inverted, the equation is:

$$\frac{1}{v} = \frac{Km + [S]}{Vmax\,[S]}$$

- If we plot 1/V against 1/[S], it will give a straight line graph. Intercepts in X-axis is minus 1/Km, from which Km can be calculated.



Evaluation of Herbal Drugs

Objectives: upon compilation of this module the student should be able to:

1. Understand WHO and ICH guidelines for assessment of herbal drugs
2. . Know the Stability testing of herbal drugs
3. Patenting aspects of traditional knowledge and natural products
4. Know about the various Regulatory issues in India

Learning outcomes: the student will be able to:

1. Learn the WHO guidelines for evaluation of herbal drugs.
2. Learn about the methods for stability testing of herbal drugs
3. Learn about the patent, IPR, Farmers Right, Bioprospecting and Biopiracy
4. Learn about the Regulation of manufacture of ASU drugs, Cosmetic Act and Schedule Z drugs.
5. Introduction: The safety and efficacy of

Introduction:

The safety and efficacy of herbal drugs remain major issues of concern especially in the developing world where the use is high. The evaluation of herbal drugs involves confirmation of its identity, quality, purity and detection of nature of adulteration. Thus, the evaluation parameters are based upon chemical, physical, microbiological, therapeutic and toxicological studies. It also serves as an important tool in stability studies.

WHO GUIDELINES:

The WHO guidelines present general consideration on potentially hazardous contaminants and residues in herbal medicines.

The objectives of these guidelines are to provide:

a. Quality control of crude drugs material, plant preparations and finished products.

b. Stability assessment and shelf life.

c. Safety assessment; documentation of safety based on experience or toxicological studies.

d. Assessment of efficacy by ethno-medical informations and biological activity evaluations.

The other WHO documents relating to the quality assurance of herbal medicines with regard to safety should include the following steps:

1. **Authentication (Stage of collection, parts of the plant collected, regional status, botanical identity like phytomorphology, Microscopical and histological analysis, taxonomical identity, etc.)**

2. **Foreign matter (herbs collected should be free from soil, insect parts or animal excreta, etc.)**

3. **Organoleptic evaluation (sensory characters – taste, appearance, odor, feel of the drug, etc.)**

4. **Tissues of diagnostic importance present in the drug powder.**

5. **Ash values and extractive values.**

6. **Volatile matter**

7. **Moisture content determination**

.

8 Chromatographic and spectroscopic evaluation. TLC, HPTLC, HPLC methods will provide qualitative and semi quantitative information about the main active constituents present in the crude drug as chemical markers in the TLC fingerprint evaluation of herbals (FEH). The quality of the drug can also be assessed on the basis of the chromatographic fingerprint.

9. Determination of heavy metals – e.g. cadmium, lead, arsenic, etc.

10. Pesticide residue – WHO and FAO (Food and Agricultural Organization) set limits of pesticides, which are usually present
in the herbs. These pesticides are mixed with the herbs during the time of cultivation. Mainly pesticides like DDT, BHC, toxaphene, aldrin cause serious side-effects in human beings if the crude drugs are mixed with these agents.

11**. Microbial contamination** – usually medicinal plants containing bacteria and molds are coming from soil and atmosphere. Analysis of the limits of E. coli and molds clearly throws light towards the harvesting and production practices. The substance known as aflatoxins will produce serious side-effects if consumed along with the crude drugs. Aflatoxins should be completely removed or should not be present.

12. Radioactive contamination – Microbial growth in herbals are usually avoided by irradiation. This process may sterilize the plant material but the radioactivity hazard should be taken into account. The radioactivity of the plant samples should be checked accordingly to the guidelines of International Atomic Energy (IAE) in Vienna and that of WHO.

The quality of the raw materials can be tested according to the following format:

- Name of the drug (English, Regional names, Exact botanical nomenclature)
- **Part of the plant used**
- **Area of collection • Distribution details • Season of Crop**
- **Time and year of collection**
- **Pesticide and insecticides**
- **Condition of the drug (fresh or dry)**
- **Form of the drug (powdered or intact or cuttings like etc.**

Standardization & Quality Evaluation of Herbal drugs

- Colour
- Odour
- Taste
- Texture
- Fracture

- Shape
- External
- Marking

Macroscopic

Microscopic

- Qualitative
- Quantitative
- SEM Studies
- Powder Studies

- Moist. Cont.
- Extrac. Values
- Ash Values
- Fluores. Analy.

Microbial Contamination
A) Total viable aerobic count
B) Determination of pathogens
C) Aflatoxins content

- Pharmacological
Bitterness value
Haemolytic activity
Foaming index
Swelling index
- Toxicological
Determination of pesticide residues
Determination of heavy metals

Antagonistic

- Bacterial
- Fungal

HPTLC
GLC
HPLC

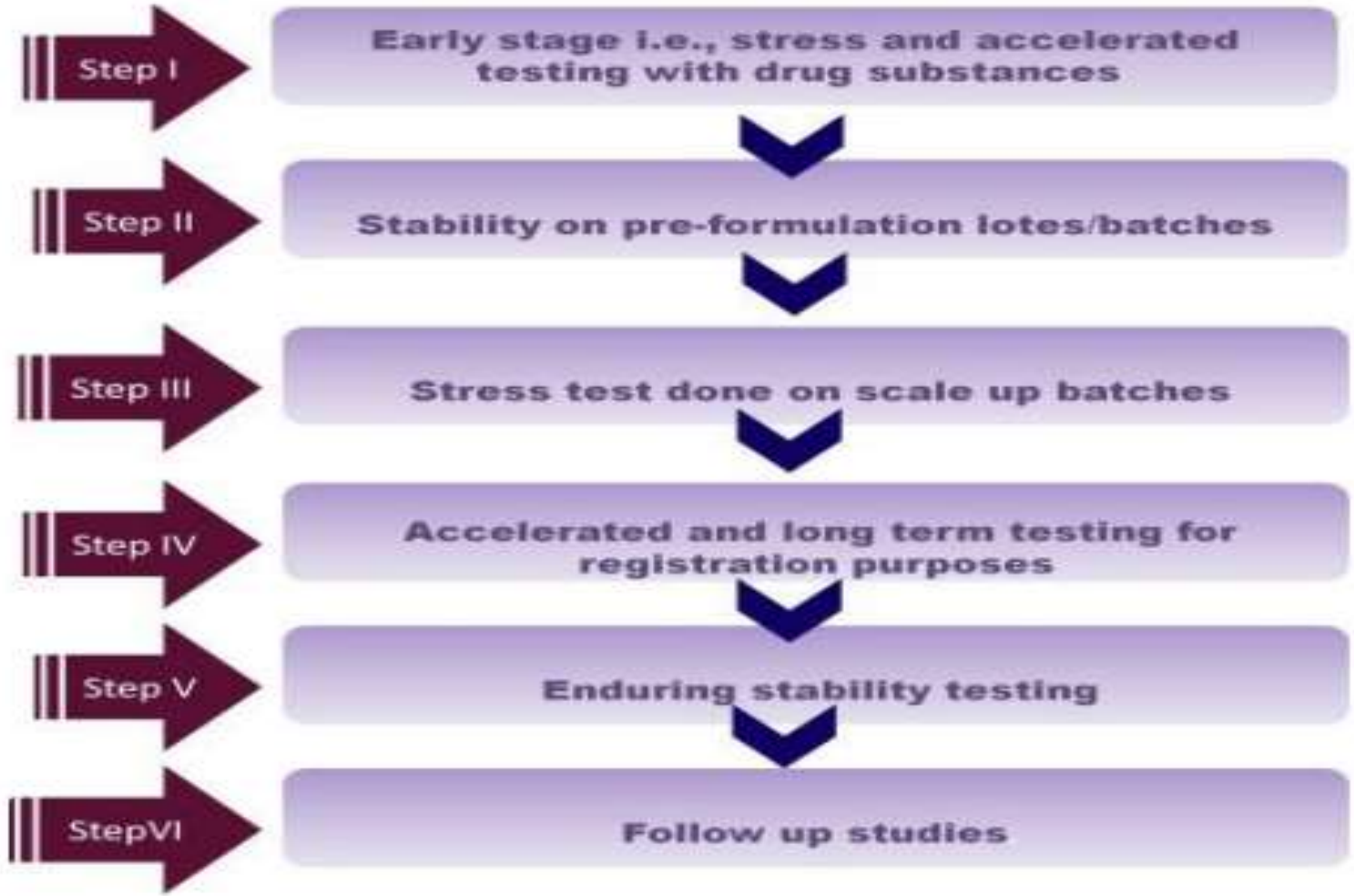
- Qualitative
- Quantitative
- Chromatography
- Radioactive contamination

HPTLC Finger printing
Sec. Metabolites
DNA Finger printing

Stability testing of herbal drugs: It is a challenging risk, because the entire herb or herbal product is regarded as the active matter, regardless of whether constituents with defined therapeutic activity are known. The most important aspect in the evaluation of the stability study of a product and its storage condition. The purpose of a stability testing is to provide proof on how the quality of the herbal products varies with the time under the influence of environmental factors such as temperature, light, oxygen, moisture, other ingredient or excipients in the dosage form, particle size of drug, microbial contamination, trace metal etc.

Shelf-life The determination of shelf life of herbal medicinal drug products is same as chemically defined APIs, but special nature of herbal product should be taken into consideration. It is recommended that in case of a herbal medicinal product containing a natural product or a herbal drug preparation with constituents of known therapeutic activity, the variation in component during the proposed shelf-life should not exceed ± 5% [5,6]of the initial assay value, unless justified to widen the range up to ±10 per cent or even higher.

Challenges in Stability testing of herbal medicinal product: 1. Active substances (herbal substances and/or herbal preparations) in HMPs consist of complex mixtures of constituents and in most cases the constituents responsible for the therapeutic effects are unknown. 2. The situation is further complicated when two or more herbal substances and/or herbal preparations are combined in a Herbal formulations. 3. In addition, many herbal substances/herbal preparations are known to be unstable.


Step I
Early stage i.e., stress and accelerated testing with drug substances
Step II
Stability on pre-formulation lotes/batches
Step III
Stress test done on scale up batches
Step IV
Accelerated and long term testing for registration purposes
Step V
Enduring stability testing
StepVI
Follow up studies

Importance of Stability testing: It evaluates the efficacy of a drug. Stability studies are used to develop suitable packaging information for quality, strength, purity & integrity of product during its shelf life. It is used for determination of the shelf life.

Stress testing: Stress testing help to identify the degradation product, which can help to establish the degradation pathway. Stress tests are usually considered unnecessary for herbal drug & its preparation.

Selection of batches: Long term testing is to be provided with on at least two batches of the drug substance and three batches of drug product. In some cases of combination products, it is hardly possible to provide the required two batches of each extract at the same time due to different harvesting times. This should be taken into consideration when planning the schedule for stability study.

Predictable changes in Herbal medicinal Product: Following predictable changes may occurs in herbal medicinal product during storage and in shelf life determination: Hydrolysis, Oxidation, Racemization, Geometric isomerization, Temperature, Moisture and Light

Hydrolysis: Reaction with water takes place results in degradation of product.

Oxidation: Due to addition of electro negative atom, Removal of electro positive atom, radicals formation results in decomposition of natural products.

Racemization: Racemization is the process in which one enantiomer of a compound, such as an L-amino acid, converts to the other enantiomer. The compound then alternates between each form while the ratio between the (+) and (–) groups approaches 1:1, at which point it becomes optically inactive.

Geometric isomerization: Products can be change in trans or cis form. One form may be more therapeutically active.

Polymerization: There is combination of two or more identical molecule to form much larger & more complex molecule.

Temperature: The rate of most chemical increase with increase in temperature. So that “Tropical” area must be taken in consideration during preparation of the formula of the herbal substance.

Moisture: Moisture absorbed on to the surface of solid drug will often increase the rate of decomposition, if it is susceptible to the hydrolysis.

Light: Many type of chemical reaction induced by exposure to light of high energy. Autoxidation of volatile oil / fixed oil takes place and substance becomes colored.

EXPECTORANTS AND EMETICS

By

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Expectorants and emetics

EXPECTORANT

These are the agents which enhance the secretion of sputum from trachea, bronchi or lungs and hence they are used in treatment of cough.

OR

They are also defined as agent that facilitates the removal of broncho-pulmonary mucus secretion membrane.

Classification of expectorants

Based on MOA they are categorized into two types:

1. Sedative expectorants
2. Stimulant expectorants

1. Sedative Expectorants

These are stomach irritants which are able to produce their effect through stimulation of gastric reflux. E.g. bitter drugs as Ipecac, senega, and compounds such as antimony potassium tartarate, ammonium chloride, potassium iodide.

2. Stimulant Expectorants

Expectorants which bring about stimulation of secretory cells of the respiratory tract directly or indirectly since these drugs stimulates secretion , more fluid gets produced in respiratory tract and hence sputum is diluted. E.g. Eucalyptus oil, Lemon, Anise and Terpine oil.

Potassium Iodide

- **Mol. Formula_** KI
- **Mol. Weight_** 166 gm
- **Synonyms_** Potide, Kalli Iodidum
- **Standard_** It contain not less than 99% KI with reference to a dried basis
- **MOP_**

a) Laboratory Method

b) Industrial Method

A)Laboratory Method

Prepared by treating slight excess of iodine with a hot aqueous solution of Potassium hydroxide. The pale yellow solution is evaporated to dryness and residue is heated with charcoal to reduce iodate to iodide.

$$6KOH + 3I_2 \longrightarrow KIO_3 + 5KI + 3H_2O$$

$$KIO_3 + 3C \longrightarrow KI + 3CO$$

B) Industrial Method

It can be prepared by using potassium carbonate and iron fillings. Iron filling are agitated in the iodine solution to form ferro ferric iodide which on further boiling with conc. solution of potassium carbonate gives potassium iodide.

$$4Fe + 5I_2 \longrightarrow 2FeI_2.FeI_3$$

$$FeI_2.FeI_3 + 4K_2CO_3 \longrightarrow 8KI + FeO.FeO_3 + 4CO_2$$

Physical properties

- White granular powder
- Slightly hygroscopic in nature
- Taste is saline and slight bitter
- Soluble in water, glycerin and alcohol
- On exposure to air, it become yellow

Chemical Properties

1. With silver nitrate it gives yellow ppt of silver iodide

   $KI + AgNO_3 \longrightarrow AgI\ (yellow) + KNO_3$

2. Iodine ion gets oxidised to iodine when treated with oxidising agents

   $2I \longrightarrow I + 2e^-$

Storage condition-

It should be stored in well closed container

Incompatibillity-

It is incompatible with salt of iron, bismuth, mercury, potassium chlorate and alkaloidal salts.

Uses-

1. As an expectorant
2. Act as source of iodine and potassium
3. In treatment of goiter
4. Use as saline diuretics
5. As anti-fungal agent in veterinary practices

Ammonium chloride

- **Molecular Formula**-NH_4Cl
- **Molecular weight-** 53.49g
- **Synonyms**- Salmiac, Amchlor
- **Standard**- contains NLT 99.5% of ammonium chloride calculated with reference to dried substance
- **Method of Preparation-**

1. By neutralizing hydrochloric acid with ammonia

$$NH_3 + HCl \longrightarrow NH_4Cl$$

2. By ammonium sulphate with sodium chloride

$$2NaCl + (NH_4)_2SO_4 \longrightarrow 2NH_3 + 2HCl + Na_2SO_4$$

$$2NH_3 + 2HCl \longrightarrow 2NH_4Cl$$

Physical Properties-

1. White, fine crystalline powder
2. Odourless and cooling saline taste
3. Hygroscopic in nature
4. Freely soluble in water but slightly soluble in alcohol

Chemical properties-

In its vapour form, it dissociate in ammonia and hydrochloric acid

$$NH_4Cl \longrightarrow NH_3 + HCl$$

Storage condition-

It should be stored in well closed container

Uses-

1. As Expectorant
2. As Diuretics
3. As systemic Acidifier

EMETICS

Emetics are the agents which when administered orally or by injection induce induce the vomiting

Mechanism of action-

1. By stimulation of chemoreceptor trigger zone
2. By refluxly producing irritation on g.i.t. tract

COPPER SULPHATE

- **Molecular formula-** $CuSO_4.H_2O$
- **Molecular weight-** 249.7 g
- **Synonym**- Blue vitriol, Cupric sulphate
- **Standard**- contains NLT 98.5% and NMT 100.5% calculated with reference to the dried substance
- **Physical properties-**

1. Deep blue crystals of pentahydratein granules or powder form
2. soluble in water insoluble in alcohol
3. Acidic in nature

Method of preparation

Two step reaction-

1. Copper granules are heated with sulphur, a mixture of copper sulphate and cupric oxide is obtained. Solution is filtered to separate copper sulphate crystals.
2. in second step residue CuO is again treated with dil. Sulphuric acid to convert into copper sulphate.

$$3Cu + S \longrightarrow CuSO_4 + 2CuO$$

$$\downarrow \text{ dil.HCl}$$

$$2CuSO_4.H_2O$$

STORAGE CONDITION-

It must be protected from air, heat and moisture

Incompatibility-

It has been incompatible with alkalis, phosphates, propylene glycol sulphathiaole

Uses-

- used as an emetic
- Use as chemical antidote in phosphorus poisoning
- Externally used as astringents and fungicidal
- As an ingredient in Benedicts and Fehling's reagents
- Use in preparation of absolute alcohol

THANK YOU

Definition:

Group of polyphenolic compounds which are found in fruits, flowers, seeds & vegetable.

They are more common in higher plants being abundant in families, Polygonaceae, Rutaceae, Leguminosae, Umbelliferae & Compositae.

Flavonoids (named from the Latin word **flavus** meaning **yellow**, their colour in nature) are a class of plant secondary metabolites.

Physiological role:

1- Role in plant defense mechanism:-

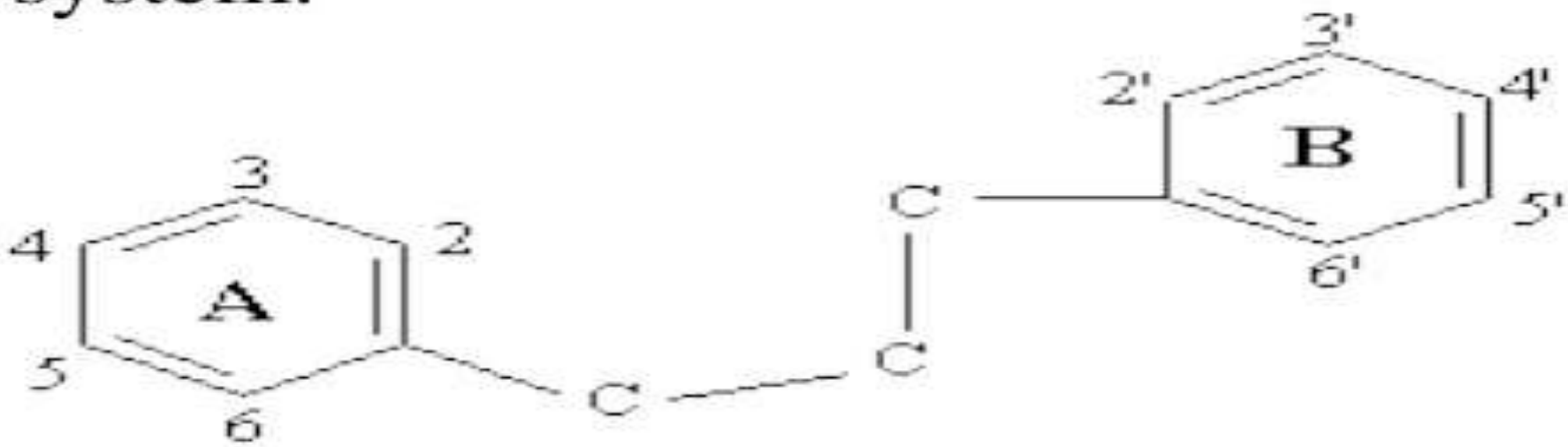
They have a very limited role in this respect due to their low toxicity when compared with other plant secondary metabolites such as alkaloids.

2- They are the pigments of flowers and attract pollinating insects.

3- They play a role in plant growth control by inhibiting & activating enzymes.

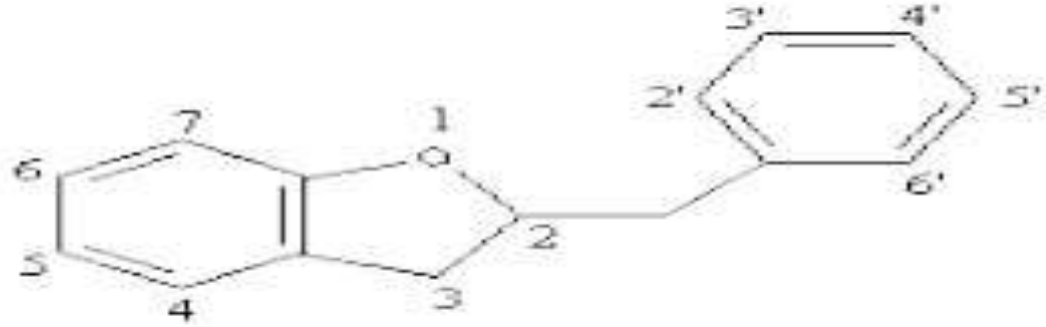
Structure of flavonoids

The flavonoids are possessing 15 carbon atoms; **two benzene rings** joined by a **linear three carbon chain** the skeleton can be represented as the C6 - C3 - C6 system.

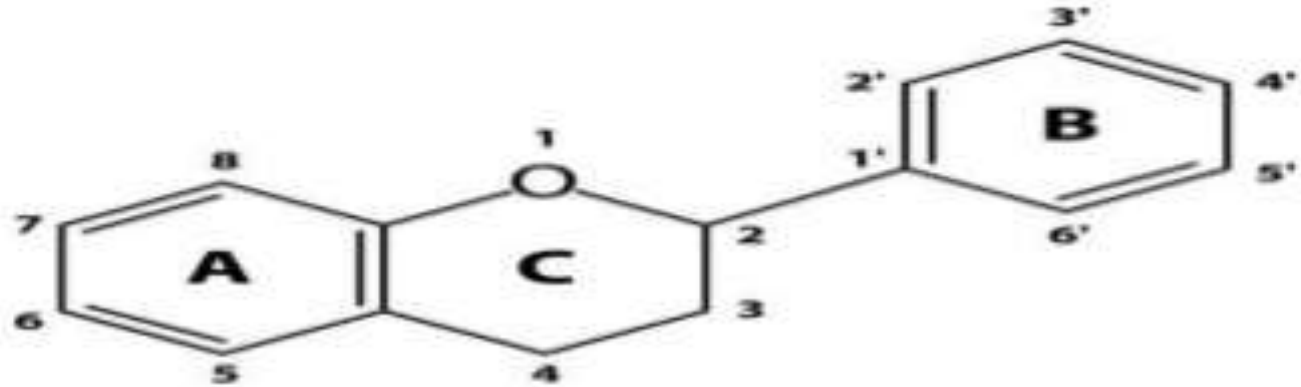


The three-carbon (-C3-) may be included through an oxygen bond between the two phenyl rings into:

1- A five-membered heterocyclic ring (furan) as in **aurones**.

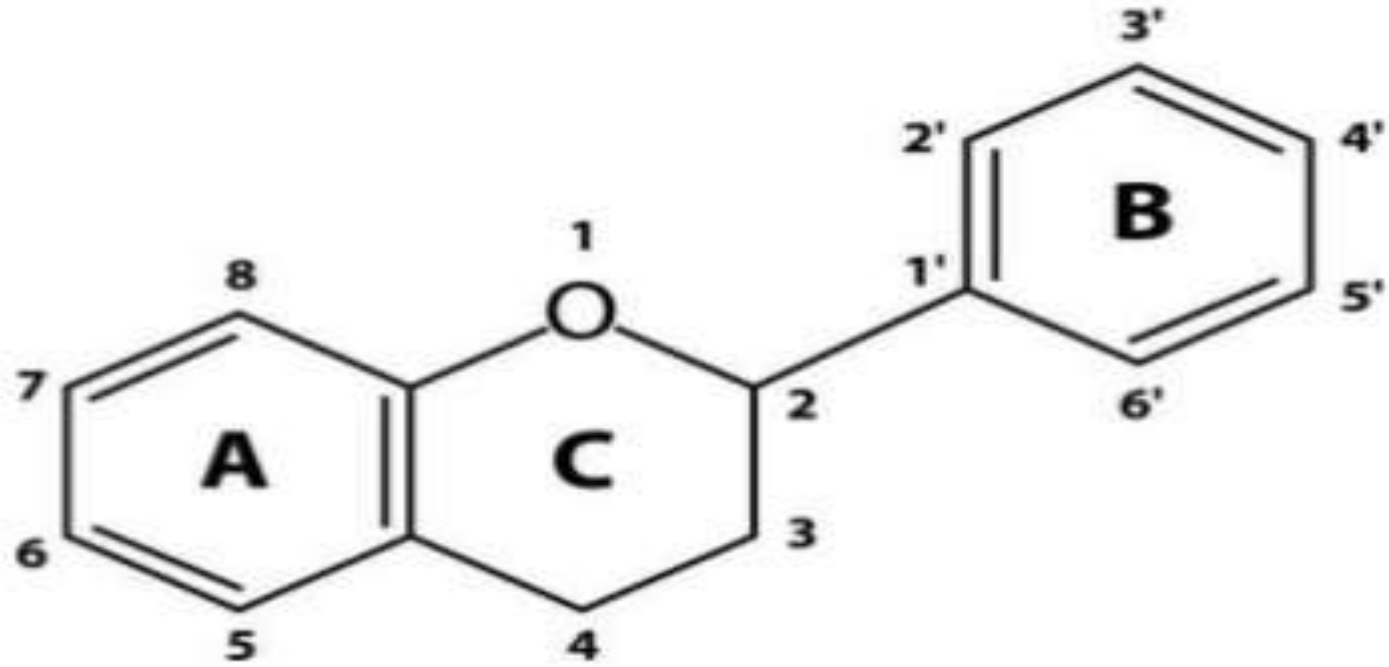


2- A six-membered heterocyclic ring (pyran) to give **flavonoids** which constitute the largest group.



Flavonoids occur as aglycones, glycosides and methylated derivatives.

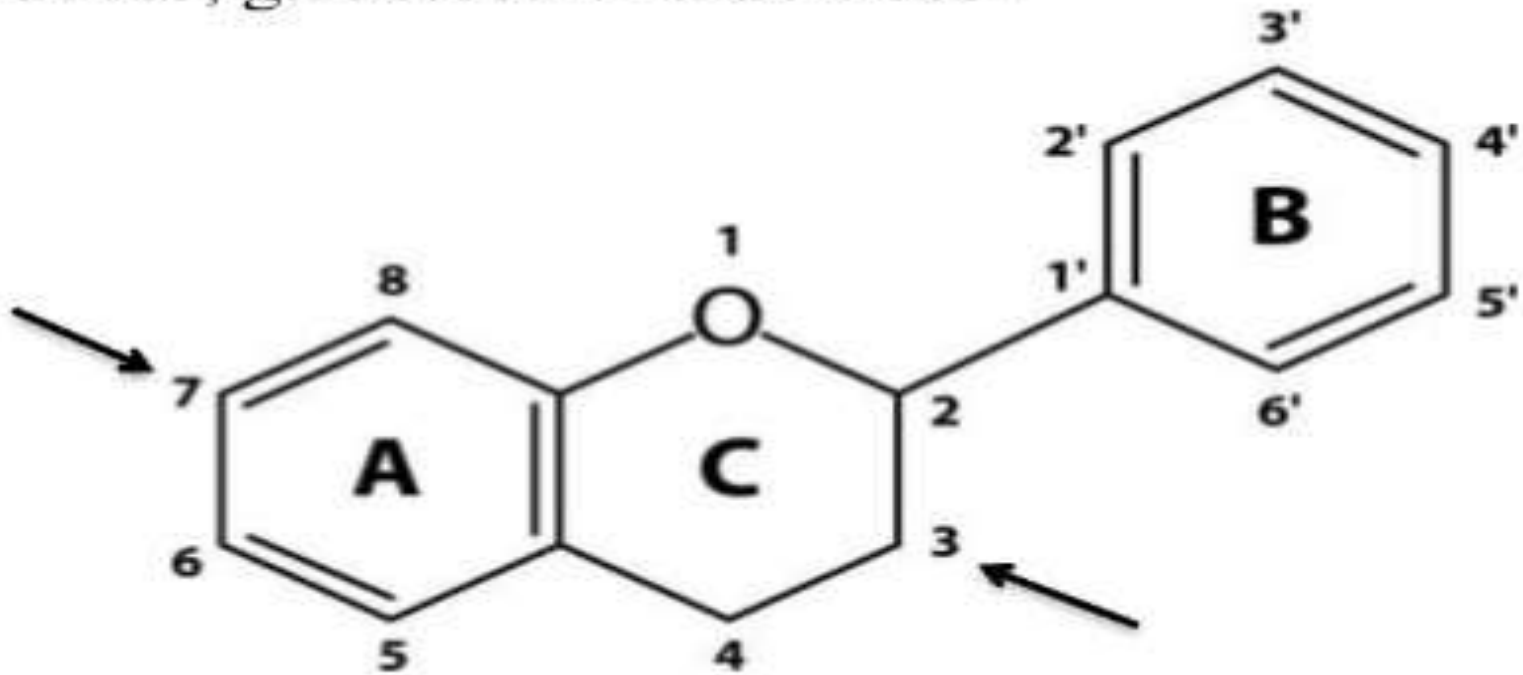
The flavonoid aglycone consists of a benzene ring (A) condensed with a six membered ring (C) pyran ring , which in the 2-position carries a phenyl ring (B) as a substituent.



The flavonoid glycosides:

Glycosides → aglycone (non sugar part)+glycone (sugar part)

When glycosides are formed, the glycosidic linkage can be located in positions 3 or 7 and may be L-rhamnose, D-glucose, galactose or arabinose .



Classification:

They are classified accorrding to chemical structure into:

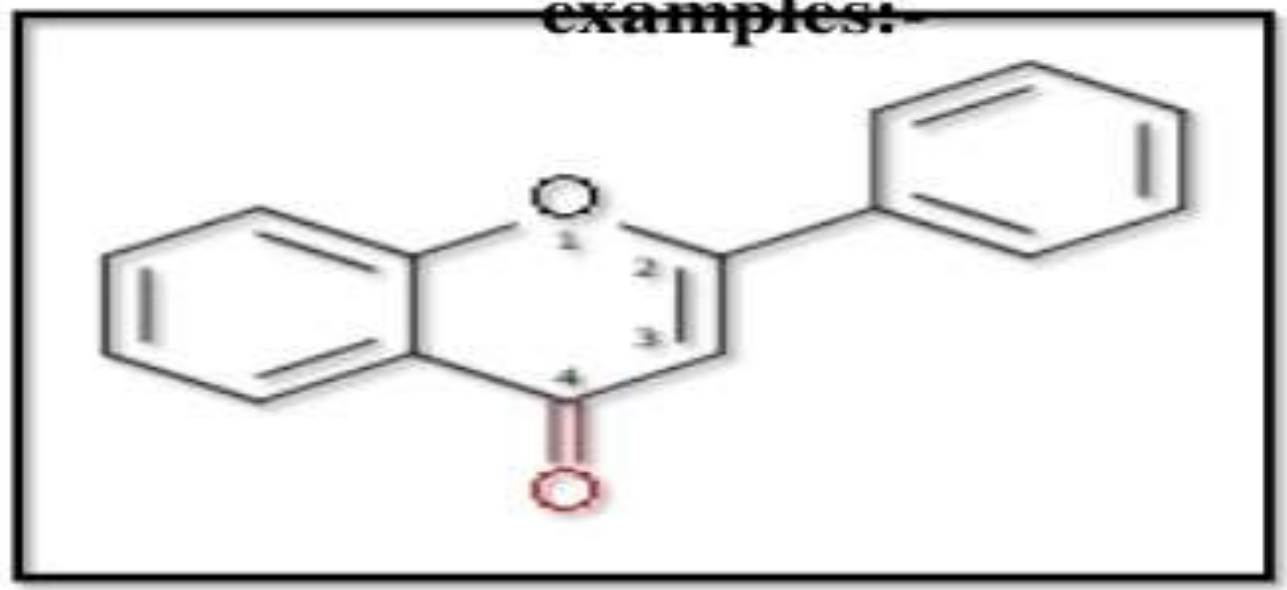
1-

Flavones:

2-phenylchromen-4-one

examples:-

1- Apigenin.
2- Luteolin.
3- Tangeritin.
4- Diosmetin.



2-Flavonol:

3-hydroxy-2-phenylchromen-4-one.

Examples:

1- Kaempferol.

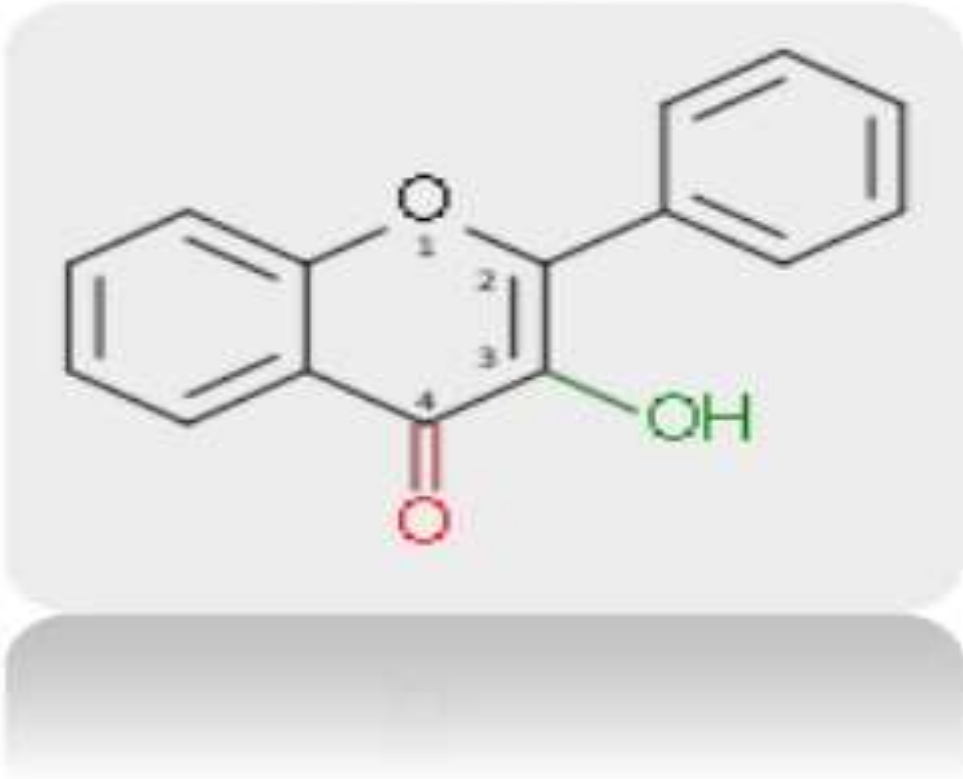
2- Rutin.

3-Myricetin.

4-Quercetin.

5- Quercetrin.

6- Fisetin.



3-Flavanone:

2,3-dihydro-2-phenylchromen-4-one

examples:-

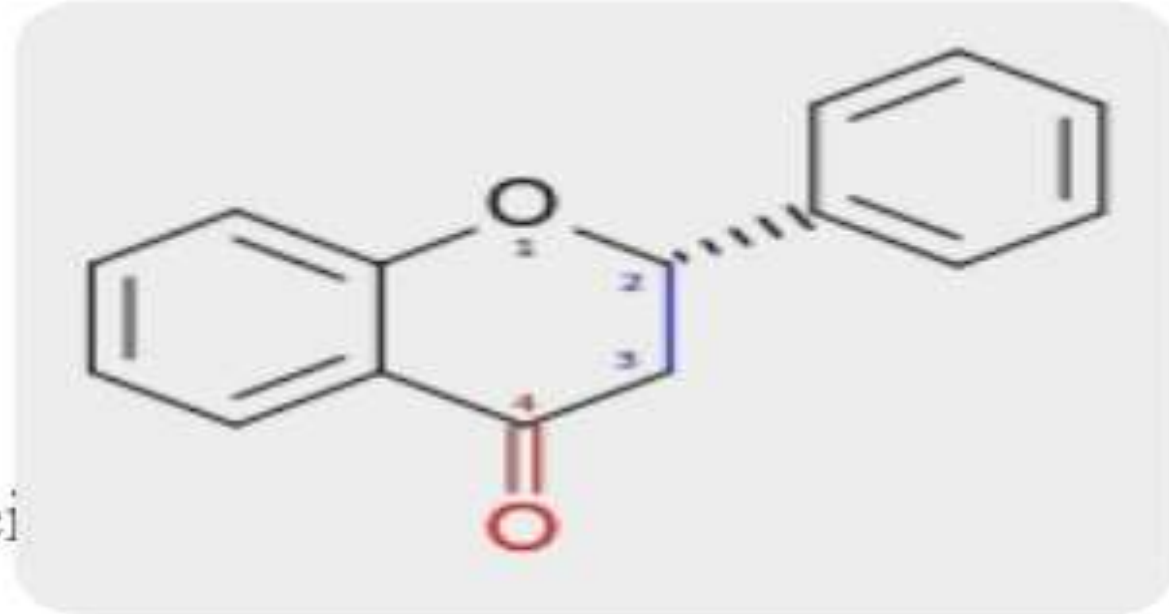
1-

Hesperetin.

2-

Hesperei

3-Naringenin.



4-Flavanonol:

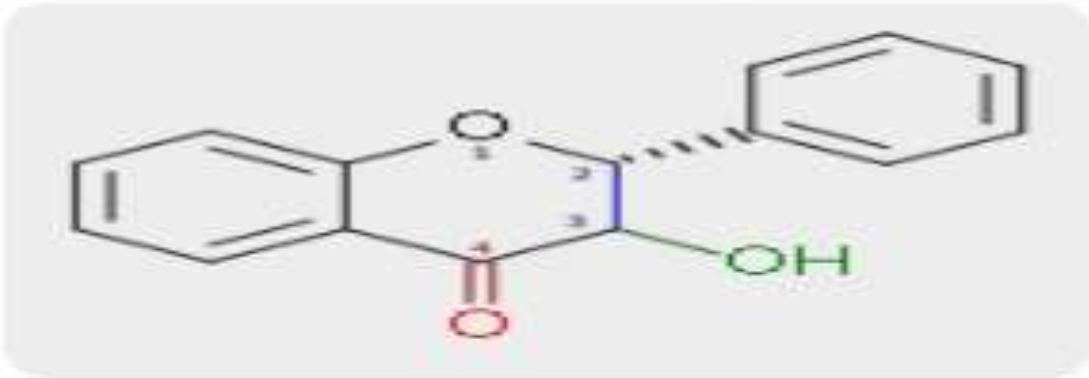
3-hydroxy-2,3-dihydro-2-phenylchromen-4-one

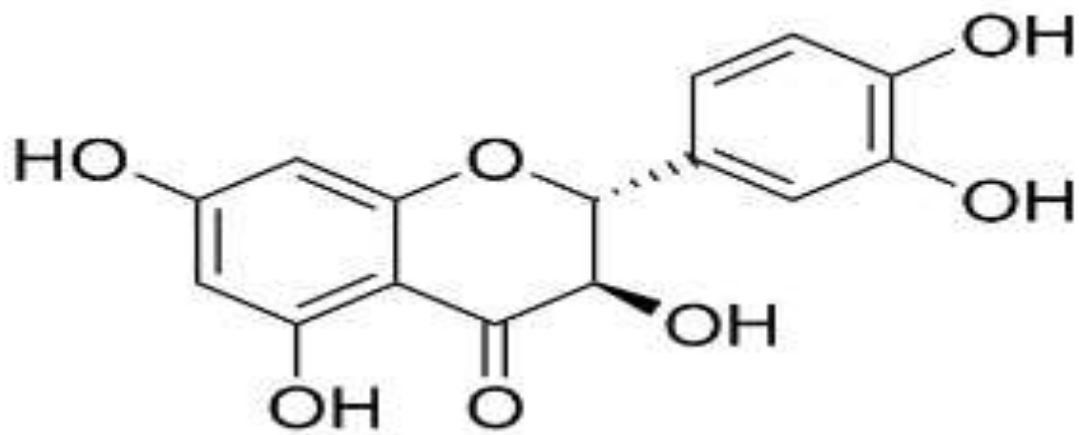
examples:-

1-Taxifolin.

2- Silymarin.

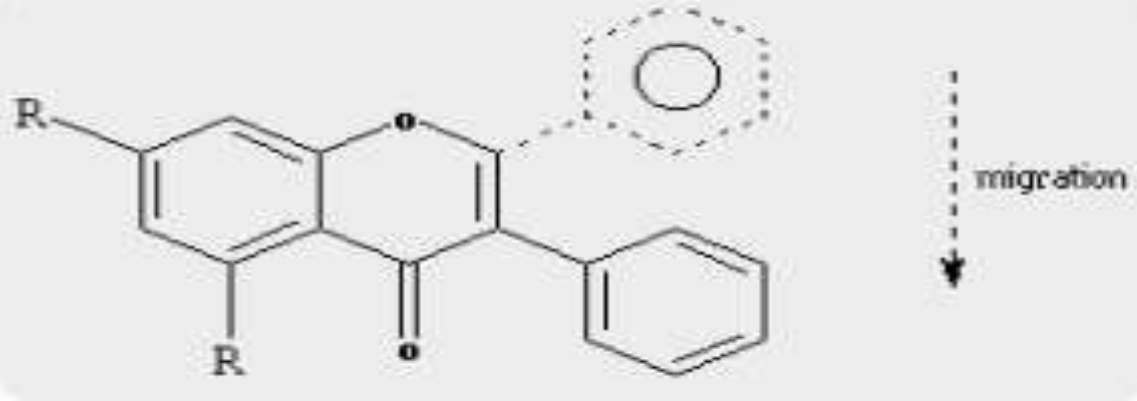
Taxifolin →

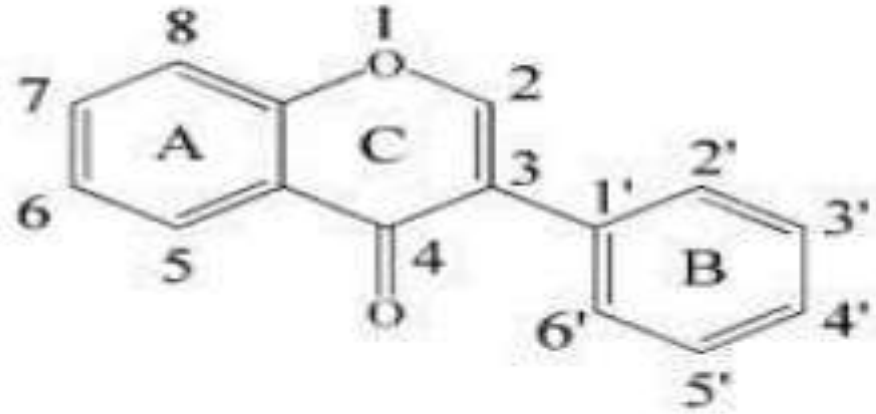




6- Isoflavones

3-Phenylchromen-4-one

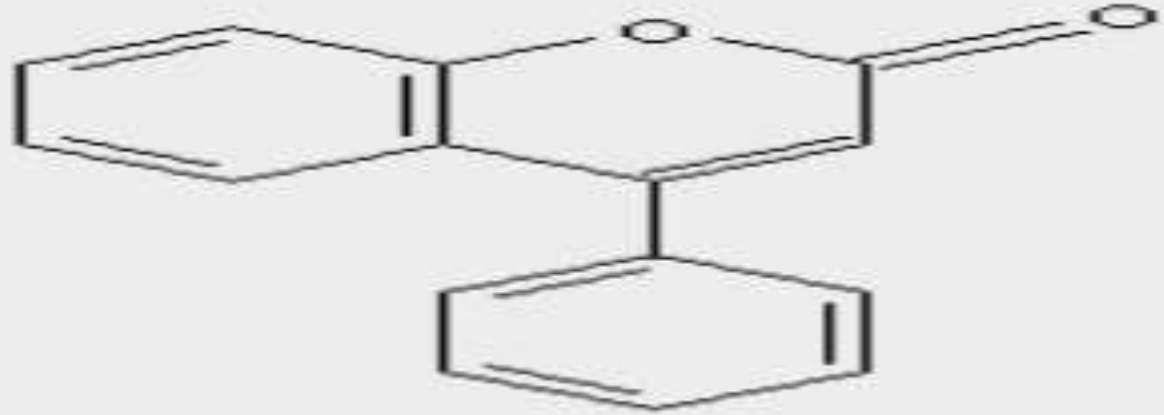




Isoflavone	3	5	7	4`
1- Genistein	-	OH	OH	OH
2- Daidzin	-	H	O-Glu	OH

7-Neoflavonoides

ring B in position 4 (4-phenyl-coumarins)

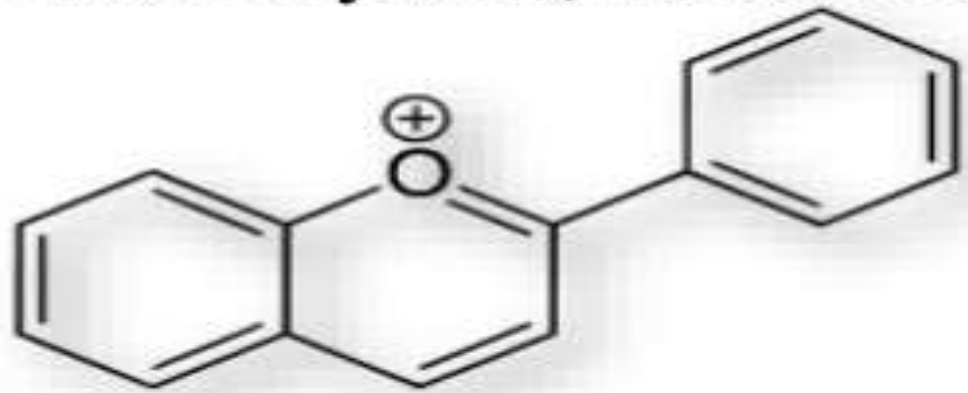


The Isoflavonoids and the Neoflavonoids can be regarded as abnormal flavonoids.

8- Anthocyanidins:

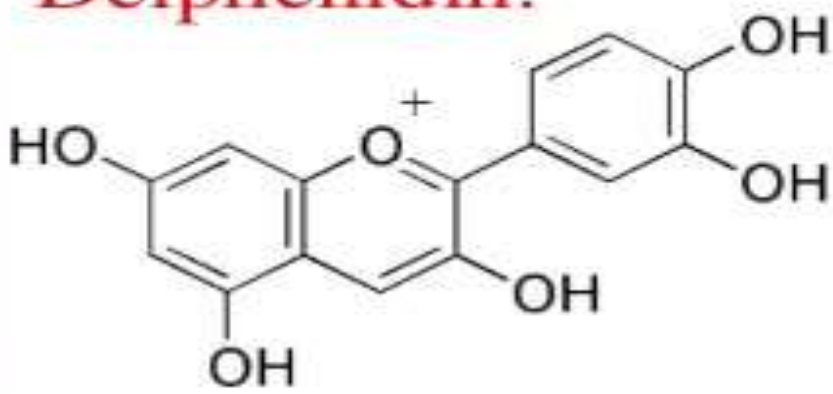
Flavylium (2-Phenylchromenylium) ion skeleton of anthocyanidins.

e.g



1-Cyanidin.

Delphenidin.



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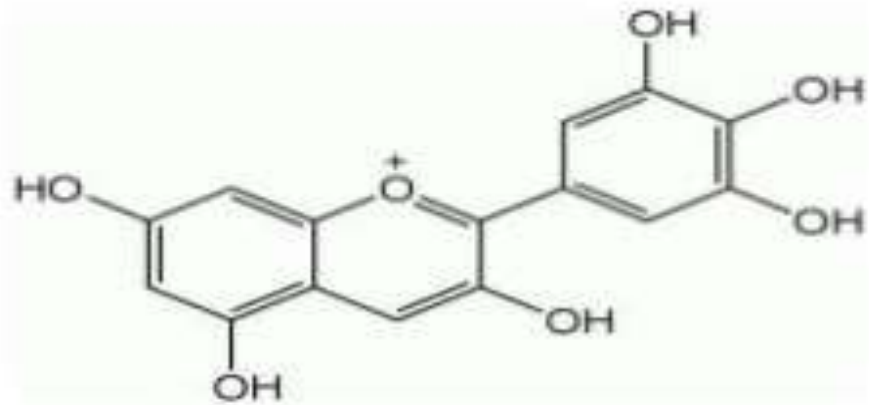


Actions of flavonoids

1-Flavonoids as antioxidants:

The **flavones** and **catechins** seem to be the most powerful flavonoids for protecting the body against reactive oxygen species (ROS). **Flavonoids are oxidized by radicals, resulting in a more stable, less-reactive radical.**

Because of the high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive. **Epicatechin** and **rutin** are also powerful radical scavengers.

2- Anti ulcer effect:

Majority of peptic ulcers are associated with helicobacter pylori, a spiral-shaped bacterium that lives in the acidic environment of the stomach. **Quercetin** seems to play a very important role in the prevention and treatment of peptic ulcer. It acts by promoting mucus secretion, thereby serves as gastroprotective agent, also quercetin has been shown to inhibit the growth of helicobacter pylori bacterium in-vitro studies.

3- Anti atherosclerotic effects:

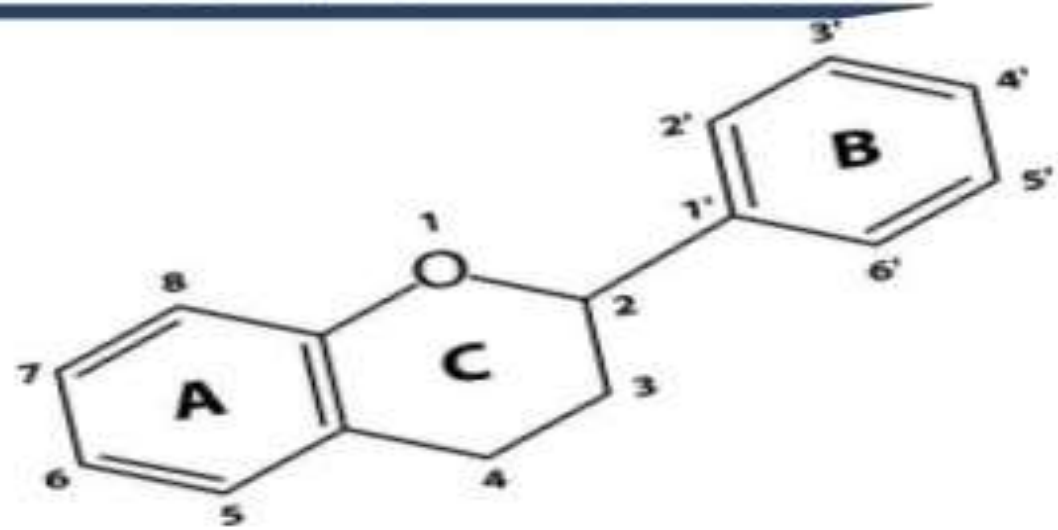
Atherosclerosis is a condition that results from the gradual build-up of fatty substances, including cholesterol, on the walls of the arteries. This build-up, called plaque, reduces the blood flow to the heart, brain and other tissues and can progress to cause a heart attack or stroke. This process is commonly referred to as hardening of the arteries. An elevated plasma low density lipoprotein (LDL) concentration is a primary risk factor for the development of atherosclerosis and coronary artery disease. Flavonoids seems to suppress LDL oxidation and inflammatory progression in the artery wall. A Japanese study reported an inverse correlation between flavonoid intake and total plasma cholesterol concentrations, other clinical studies, as mentioned earlier, stated that flavonoid intakes protect against coronary heart disease.

4- Anti-inflammatory effect:

Cyclooxygenase (COX) is an enzyme that plays an important role as inflammatory mediator and is involved in the release of arachidonic acid, which is a precursor for biosynthesis of eicosanoids like prostaglandins and prostacyclin. The release of arachidonic acid can be considered starting point for a general inflammatory response. Select flavonoids like **quercetin** are shown to inhibit the cyclooxygenase pathway. This inhibition reduces the release of arachidonic acid.

Isolation of flavonoids

A- Extraction:-



*Glycosides are polar due to sugar moiety and extractable with alcohol, water and hydroalcoholic solution.

*Aglycones are extracted with less polar solvents e.g ether, chloroform and ethyl acetate.

1-Plant preparation:

Flavonoids (particularly glycosides) can be degraded by enzyme action when collected plant material is fresh or non dried. It is advisable to use dry, lyophilized, or frozen samples. dry plant material is used, it is generally ground into a powder.

2-Solvent choosing:

For extraction, the solvent is chosen as a function of the type of flavonoid required. Less polar flavonoids (e.g., isoflavones, flavanones, methylated flavones, and flavonols) are extracted with chloroform, dichloromethane, diethyl ether, or ethyl acetate.

while flavonoid glycosides are more polar and are extracted with alcohols or alcohol–water mixtures.

The extractions of flavonoid-containing material are still performed by simple direct solvent extraction, can also be extracted in a Soxhlet apparatus, first with hexane,to remove lipids and then with ethyl acetate or ethanol to obtain phenolics. This approach is not suitable for heat-sensitive compounds.

A convenient and frequently used procedure is sequential solvent extraction. **A first step,with** dichloromethane, for example, will extract flavonoid aglycones and less polar material. **A subsequent step** with an alcohol will extract flavonoid glycosides and polar constituents.

B- Separation:-

Preliminary Purification

Preparative Methods

- ***HPLC***
  High-Performance Liquid Chromatography
- ***MPLC***
  Medium-Pressure Liquid Chromatography
- ***Centrifugal Partition Chromatography***

Identification and characterization

A- Test for identification:-

The different types of flavonoides can be characterized by their colored response to different tests (visible light) or colored fluorescence in UV light.

1- Reaction with aluminum chloride:

The different classes of flavonoids give yellow color with $AlCl_3$ but fluoresce differently in UV as shown in the following table .

light	Flavones	Flavonols	Flavanones
Visible	Yellow	Yellow	Yellow
UV	Green	Yellowish to Yellowish-green	Pale-brownish

2- Lead subacetate test:-

all flavonoids give yellowish precipitate with Pb subacetate.

3-Shinoda´s test for flavanones and flavonols:-

alcoholic solution + Mg metal / Hcl , an orange , red or violet color is produed.

4- Antimony pentachloride test for chalcones:

alcoholic solution + $SbCl_5$/ CCl_4 , red or violet color is produced.

LIGNAN

The lignans are bioactive, non-nutrient, non-caloric phenolic plant compounds that are found in highest concentration in flax and sesame seeds and in lower concentrations in grains, other seeds, fruits and vegetables. The enterolignans (sometimes referred to as mammalian lignans) are metabolites of food lignans produced by human intestinal bacteria. They have been identified in human urine and plasma , these are suggest potential for nutritional significance in the prevention of cardiovascular and other chronic diseases.

- Chemistry and occurrence of lignans

- Monolignols derived from hydroxycinnamic acids (*p*-coumaric, ferulic, and sinapic acids), are either dimerized to *lignans* in the cell or polymerized into larger lignin structures in the cell wall. These structurally diverse compounds are involved in plant defense (as antioxidants, biocides, phytoalexins, etc.),providing protection against diseases and pests, and possibly participating in plant growth control.

-

The plant *lignans* most commonly distributed in foods are lariciresinol, matairesinol, pinoresinol, Several other lignans are present in some foods, including medioresinol (in sesame seeds, rye, and lemons), syringaresinol (in grains), sesamin and the lignan precursor sesamolin (in sesame seeds). Other lignans found in foods but not often quantified include arctigenin, cyclolariciresinol (isolariciresinol), (Some cyclolariciresinol occurs naturally and some is formed from lariciresinol during extraction and analysis under acidic conditions.) The nutritional significance of lignans is unknown. Although *lignans* are not classified as dietary fibers, they share some of the chemical characteristics of lignin, which is an insoluble fiber.

Food sources of lignans

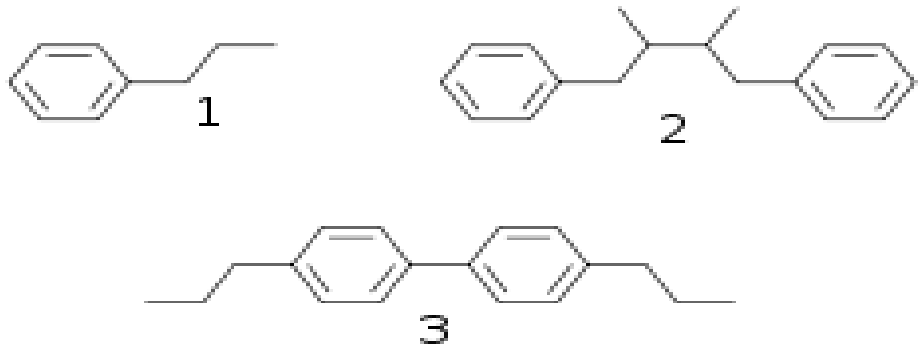
The lignan content of foods is generally low and usually does not exceed 2 mg/100 g. The exceptions are flaxseed(335 mg/100g) and sesame seeds (373 mg/100g), which have a lignan content a hundred times higher than other dietary sources.

They are present in many plant families, although the types and amounts vary from one family to another. Lignans are found in whole grains (especially in the bran layer) and seeds (in the seed coat). Barley, buckwheat, flax, millet, oats, rye, sesame seeds and wheat contain fairly high levels of lignans. Nuts and legumes are also reasonably good sources. Although in lesser amounts than in grains, lignans are also present in fruits and vegetables such as asparagus, grapes, kiwi fruit, lemons, oranges, pineapple, wine and even in coffee and tea.

The **lignans** are a large group of polyphenols found in plants. Some examples of lignans are enterolignans, enterodiol and enterolactone.

Definition

Plant lignans are polyphenolic substances derived from phenylalanine via dimerization of substituted cinnamic alcohols known as monolignols, to a dibenzylbutane skeleton **2**.



Many natural products, known as phenylpropanoids, are built up of C6C3 units (*n*-propylbenzene skeleton **1**) derived from cinnamyl units just as terpene chemistry builds on isoprene units. Structure **3** is a neolignan, a structure formed by joining the two propylbenzene residues

SOURCE

Flax seed and sesame seed contain higher levels of lignans than most other foods.

Other sources of lignans include cereals (rye, wheat, oat and barley - rye being the richest source),soybeans, cruciferous vegetables such as broccoli and cabbage, and some fruit, particularly apricots and strawberries. Secoisolariciresinol and matairesinol were the first plant lignans identified in foods. Typically, lariciresinol and pinoresinol contribute about 75% to the total lignan intake whereas secoisolariciresinol and matairesinol contribute only about 25%.Lignans are some of the secondary metabolites present in Cannabis sativa.

Source	Amount per 100 g
Flaxseed	300,000 µg (0.3 g)
Sesame seed	29,000 µg (29 mg)
Brassica vegetables	185 - 2321 µg
Grains	7 - 764 µg

- USES

- Anti-inflammatory and antioxidant activity neuroprotective It is also found to have anti cancer, anti viral and anti fungal properties.

RUTA

Ruta graveolens L., is a odoriferous herb belonging to the family Rutaceae. It is the source of Rue or Rue oil, called as Sadab or Satab in Hindi. It is distributed throughout the world and cultivated as a medicinal and ornamental herb. The ancient Greeks and Romans, held the plant in high esteem. It is used in Ayurveda, Homoeopathy and Unani. In 14 species of genus Ruta, R. graveolens and R. chalepensis are available in India and also cultivated in gardens.

Ruta graveolens (rue) contains acridone alkaloids, such as furacridone and gravacridone, quinoline alkaloids, such as graveoline and graveolinine, the furanoquinoline dictamnine, coumarins, such as gravelliferone, isorutarin, rutacultin, rutaretin, and suberenone, and the furanocoumarins 5-methoxypsoralen (bergapten) and 8-methoxypsoralen (xanthotoxine). It has been used as an abortifacient and emmenagogue .The essential oil of rue can cause contact dermatitis and phototoxic reactions and severe hepatic and renal toxicity. Therapeutic doses can lead to depression, sleep disorders, fatigue, dizziness, and cramps. The sap from fresh leaves can produce painful gastrointestinal irritation, fainting, sleepiness, a weak pulse, abortion, a swollen tongue, and a cool skin.

A series of furanoacridones and two acridone alkaloids (arborinine and evoxanthine) have been isolated from *R. graveolens*. It also contains coumarins and limonoids.Cell cultures produce the coumarins umbelliferone, scopoletin, psoralen, xanthotoxin, isopimpinellin, rutamarin and rutacultin, and the alkaloids skimmianine, kokusaginine, 6-methoxydictamnine and edulinine.[The ethyl acetate extract of *R. graveolens* leaves yields two furanocoumarins, one quinoline alkaloid and four quinolone alkaloids.The chloroform extracts of the root, stem and leaf shows the isolation of the furanocoumarin chalepensin.The essential oil of *R. graveolens* contains two main constituents, undecan-2-one (46.8%) and nonan-2-one (18.8%).

TEA

Biological Source

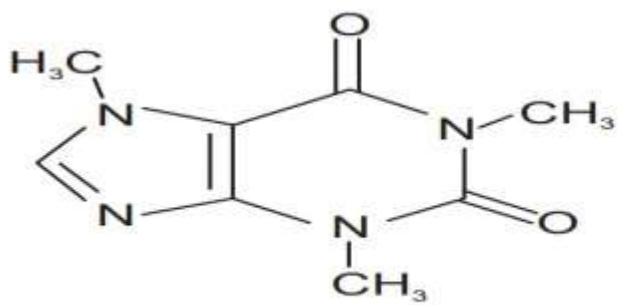
It contains the prepared leaves and leaf buds of *Thea sinensis* (Linne) kuntz., belonging to family Theaceae.

Geographical Source

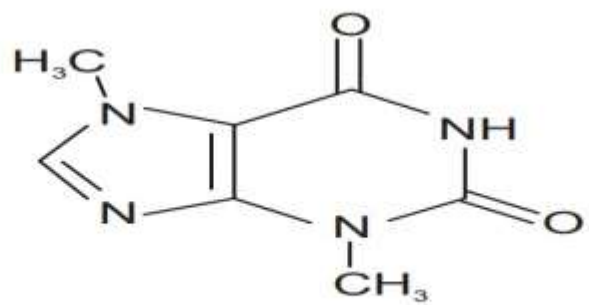
It is mainly cultivated in India (Assam), Ceylon, Japan and Java.

Cultivation and Collection

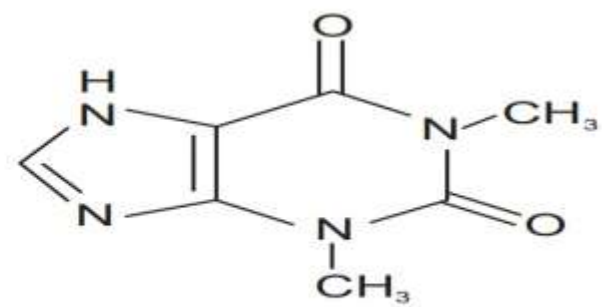
It is an evergreen shrub growing to 4 m by 2.5 m at a slow rate. The plant prefers light (sandy) and medium (loamy) soils and requires well-drained soil. The plant prefers acid and neutral soils and can grow in very acid soil. It can grow in semishade (light woodland). It requires moist soil and prefers a pH between 5 and 7. Prefers the partial shade of light woodland or a woodland clearing. It is reported to tolerate an annual rainfall of 70–310 cm, an average annual temperature range of 14–27°C and a pH in the range of 4.5–7.3. It prefers a wet summer and a cool but not very frosty dry winter. Seed can be sown as soon as it is ripe in a green house. Stored seed should be presoaked for 24 h in warm water and the hard covering around the micropyle should be filed down to leave a thin covering. It usually germinates in one to three months. Prick out the seedlings into individual pots when they are large enough to handle and grow them on in light shade in the green house for at least their first winter. Plant them out into their permanent positions when they are more than 15 cm tall and give them some protection from winter cold for their first year or three outdoors. Seedlings take 4–12 years before they start to produce seed.



Caffeine



Theobromine



Theophyline

Chemical Tests

1. Caffeine and other purine alkaloids, gives murexide colour reaction. Caffeine is taken in a petridish to which hydrochloric acid and potassium chlorate are added and heated to dryness. A purple colour is obtained by exposing the residue to vapours of dilute ammonia. In addition of fixed alkali the purple colour disappears.

2. Caffeine also produces white precipitate with tannic acid solution.

Uses

It is used as stimulant, astringent and also as diuretic.

- The **phenolic content in tea** refers to the phenols and polyphenols, natural plant compounds which are found in tea. These chemical compounds affect the flavor and mouthfeel of tea. Polyphenols in tea include catechins, theaflavins, tannins, and flavonoids.

- Polyphenols found in green tea include, epigallocatechin gallate (EGCG), epigallocatechin, epicatechin gallate, and epicatechin; flavanols such as kaempferol, quercetin, and myricitin are also found in green tea.

• Catechins

- Catechins include epigallocatechin-3-gallate (EGCG), epicatechin (EC), epicatechin-3-gallate (ECg), epigallocatechin (EGC), catechin, and gallocatechin (GC).The content of EGCG is higher in green tea.
- Catechins constitute about 25% of the dry mass of a fresh tea leaf,although total catechin content varies widely depending on species, clonal variation, growing location, season, light variation, and altitude. They are present in nearly all teas made from *Camellia sinensis*, including white tea, green tea, black tea
- 3,4-dihydroxybenzoic acid (protocatechuic acid), 3-methoxy-4-hydroxy-hippuric acid and 3-methoxy-4-hydroxybenzoic acid (vanillic acid) are the main catechins metabolites found in humans after consumption of green tea infusions.

- Catechin monomer structures are metabolized into dimers theaflavins and oligomers thearubigins with increasing degrees of oxidation of tea leaves. Theaflavins contribute to the bitterness and astringency of black tea. The mean amount of theaflavins in a cup of black tea (200 ml) is 12.18 mg.
- Three main types of theaflavins are found in black tea, namely theaflavin (TF-1), theaflavin-3-gallate (TF-2), and theaflavin-3,3-digallate (TF-3)**.**
- **Tannins**
- Tannins are astringent, bitter polyphenolic compounds that bind to and precipitate organic compounds. Gallic acid conjugates all of the catechins, such as EGCG (Epigallocatechin gallate), which are tannins with astringent qualities.

- **Theaflavin** (TF) and its derivatives, known collectively as *theaflavins*, are antioxidant polyphenols that are formed from the condensation of flavan-3-ols in tea leaves during the enzymatic oxidation (sometimes erroneously referred to as fermentation) of black tea. Theaflavin-3-gallate, theaflavin-3'-gallate, and theaflavin-3-3'-digallate are the main theaflavins. Theaflavins are types of thearubigins, and are therefore reddish in color. Those molecules contain a tropolone moiety.
- A 2020 review found low- to moderate-quality evidence that daily tea consumption might lower the risk for cardiovascular disease and death

- Tea has one of the highest contents of flavonoids among common food and beverage products. Catechins are the largest type of flavonoids in growing tea leaves.According to a report , in a 200-ml cup of tea, the mean total content of flavonoids is 266.68 mg for green tea, and 233.12 mg for black tea.
- In recent years tea has attracted more and more attention because of reported health benefits, in particularly as an antioxidant, but also as an anticarcinogenic and antiarteriosclerotic agent. It is generally believed that flavonoids are mainly responsible for these actions. Tea is now consumed throughout the world not just as a popular beverage, but, because its extracts have been prepared in a variety of physical forms, for example, strong infusions, soft extracts and powders, it is now widely available in a range of food, beverage, and toiletry and cosmetic products.
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-

- Tea polyphenols. Tea contains polyphenolic flavonoids. In green tea, catechins (catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate) represent 80–90% and flavonols (kaempferol, quercetin and myricetin glycosides) <10% of total flavonoids.[5]These polyphenols form complex condensation products (theaflavins, thearubigins) during the production of black tea. The catechin content of black tea is only 20–30%, whilst the theaflavins and thearubigins represent ~10 and 50–60% of total flavonoids.

FUNGI, YEASTS & MOULDS

Presented by

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Contents

- Definition
- Importance of fungi
- Characteristics of fungi
- Mycology & History
- Members of the kingdom fungi
- Growth & Physiology
- Structure of fungi
- Some important Fungi
  - Sacchromyces
  - Candida albicans
  - Penicillium
- Reproduction in yeasts & moulds

Definition of Fungi

- The kingdom Fungi includes a tremendous variety of organisms that are neither plant nor animal.
- These unique multicellular eukaryotes include edible examples like mushrooms and organisms such as yeast, which makes our bread rise and ferments our beer and wine.
- And their impact on our world as decomposers is absolutely crucial to every ecosystem.

Importance in Food Industry

- Fungi, especially the brewer's yeast Saccharomyces cerevisiae, provide us with numerous foods and beverages, including staples like bread and beer.
- The brewer's yeast is not only important for the production of delicious consumables but is nutritious, being especially rich in vitamin B12.
- Some moulds are important in the maturation of cheeses like blue cheeses (the colour comes

from the mould's spores) and for providing a meat-like flavor in the production of many rice, wheat, and soybean products (for example tempeh, miso, soy sauce) used extensively in Asian cuisine.

- Similarly, fungi are even used as a meat substitute in products mimicking meat, like Quorn.
- Edible mushrooms are also common 'vegetables' that provide an important source of dietary fiber and complete

Importance in Medicine

- Fungi provide extraordinarily powerful medicines that have revolutionised human health and have massive economic worth (eg antibiotics, immunosuppressants, etc.)
- The penicillins and cephalosporins, cyclosporine, and statin drugs are all based on natural chemicals produce by fungi.
- Mushrooms are also important ingredients in Traditional Chinese Medicine (TCM) and myriad therapeutic activities have been attributed to them, including

anti-inflammatory, anti-viral and even anti-tumour effects.

COMPARISON OF SELECTED FEATURES OF FUNGI AND BACTERIA:

CHARACTERISTICS	FUNGI	BACTERIA
• Cell type	Eucaryotic	Procaryotic
• Optimal pH	4 to 6	6.5 to 7.5
• Optimum temperature	25^0 to 30^0 C (saprophytes) 230 to 370 C (parasites)	32^0 to 37^0 C (mesophilic)
• Cell membranr	Sterols present	Sterols absent except mycoplasma
• Oxygen requirement	Strictly aerobic (moulds), Facultative anaerobic (some yeasts)	Aerobic to anaerobic

• Carbon source	Organic	Inorganic / Organic
• Concentration of sugar in laboratory media	4 to 5 %	0.5 to 1 %
• Cell wall components	Chitin, cellulose or hemicellulose	Peptidoglycan
• Susceptibility to antibiotics	Sensitive to grisefulvin, Resistant to penicillin's, Chloramphenicol etc.	Resistant to griseofulvin, sensitive to penicillin's, tetracycline, etc.

• Light requirement	None	Some photosynthetic groups occur

CHARACTERISTICS

- Like plants, fungi often grow in soil and, in the case of mushrooms, form conspicuous fruit bodies, which sometimes resemble plants, such as mosses.
- The fungi are now considered a separate kingdom, distinct from both plants and animals, from which they appear to have diverged around one billion years ago.
- Some morphological, biochemical, and genetic features are shared with other organisms, while others are unique to the fungi.

General characteristics of fungi are as follows :

- Fungi are eukaryotic organisms.
- They are non-vascular organisms.
- They reproduce by means of spores.
- Depending on the species and conditions both sexual and asexual spores may be produced.
- They are typically non-motile.
- Fungi exhibit the phenomenon of alteration of generation.
- The vegetative body of the fungi may be unicellular or composed of microscopic

threads called hyphae.

- The structure of cell wall is similar to plants but chemically the fungi cell wall are composed of chitin.
- Fungi are heterotrophic organisms.
- They fungi digest the food first and then ingest the food, to accomplish this the fungi produce exoenzymes.
- Fungi store their food as starch.
- Biosynthesis of chitin occurs in fungi.
- The nuclei of the fungi is very small.
- During mitosis the nuclear envelope is not dissolved.

Shared features

- With other eukaryotes : Fungi have membrane-bound cytoplasmic organelles such as mitochondria, sterile containing membranes, and ribosomes of the 80S types. They have a characteristic range of soluble carbohydrates and storage compounds, including sugar alcohols (eg. mannitol), disaccharides, (eg. trehalose), and polysaccharides (eg. glycogen)

- With animals : Fungi lack chloroplasts and are heterotropic organisms and so require preformed organic compounds as energy source.
- With plants : Fungi have a cell wall and vacuoles. They reproduce by both sexual and asexual means, like basal plant grows (ferns and mosses) produce spores. Similar to mosses and algae, fungi typically have haploid nuclei.
- With euglenoids and batteries : Higher fungi, euglenoids, and some bacteria produce the amino acid L-lysine in specific

Biosynthesis steps, called the a-aminoadipate pathway.

- The cells of most fungi grows as tubular, elongated, and thread-like structure called hyphae, which may contain multiple nuclei and extend by growing at there tips. Both fungi and oomycetes grow as filamentous hyphal cells. In contrast, similar-looking organisms, such as filamentous green algae, grow by repeated cell division within a chain of cells.

Unique features

- Some species grow as unicellular yeasts that reproduce by budding or binary fission. Dimorphic fungi can switch between a yeast phase and a hyphal phase in response to environmental conditions.
- The fungal cell wall is composed of glucans and chitin; while glucans are also found in plants and chitin in the exoskeleton of arthropods, fungi are the only organisms that combine these two structural molecules in their cell wall.

Mycology

- Mycology is the branch of biology concerned with the systematic study of fungi, including their genetic and biochemical properties, their taxonomy, and their use to humans as a source of medicine, food, and psychotropic substances consumed for religious purposes, as well as their dangers, such as poisoning or infection. The field of phytopathology, the study of plant diseases, is closely related because many plant pathogens are fungi.

- The use of fungi by humans dates back to prehistory; Ötzi the Iceman, a well-preserved mummy of a 5,300-year-old Neolithic man found frozen in the Austrian Alps, carried two species of polypore mushrooms that may have been used as tinder, or for medicinal purposes.
- Ancient peoples have used fungi as food sources–often unknowingly–for millennia, in the preparation of leavened bread and fermented juices. Some of the oldest written records contain references to the destruction of crops that were probably caused by pathologebic fungi.

HISTORY

- Mycology is a relatively new science that became systematic after the development of the microscope in the 16th century. Although fungal spores were first observed by Giambattista della Porta in 1588, the seminal work in the development of mycology is considered to be the publication of Pier Antonio Micheli's 1729 work Nova plantarum genera.
- Micheli not only observed spores but also

showed that, under the proper conditions, they could be induced into growing into the same species of fungi from which they originated.

- The 20th century has seen a modernization of mycology that has come from advances in biochemistry, genetics, molecular biology, and biotechnology. The use of DNA sequencing technologies and phylogenetic analysis has provided new insights into fungal relationships and biodiversity, and has challenged traditional morphology-based groupings in fungal taxonomy.

Members of the Kingdom Fungi

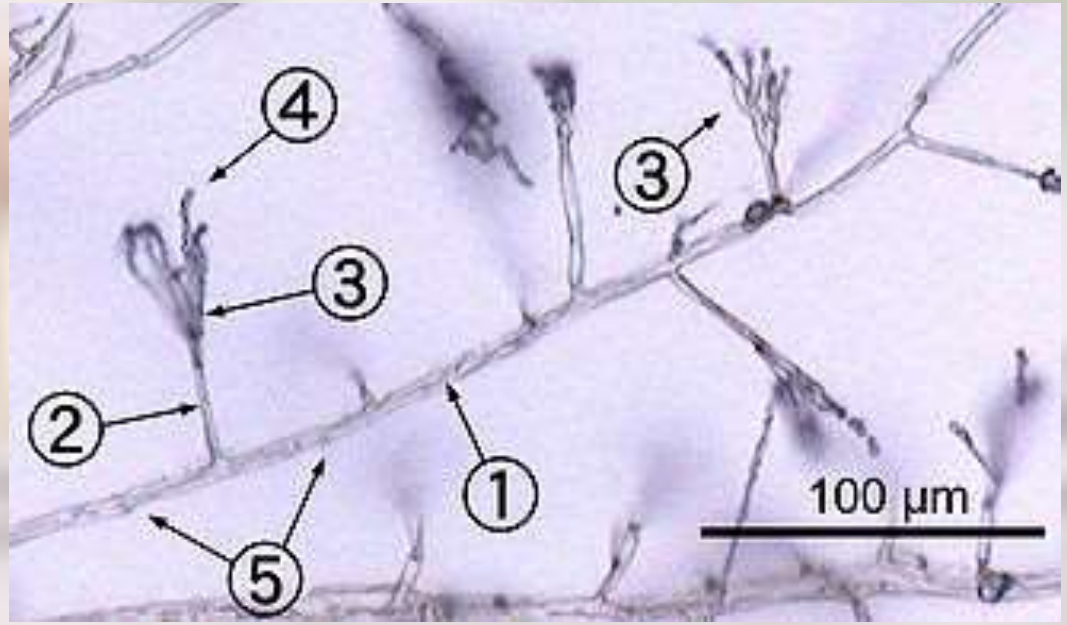
- Mycorrhizae - More than 90% of the plants are symbionts of mycorrhizae. Myco means fungus and rhiza means root. Mycorrhizae are of two types ectomycorrhizae and endomycorrhizae.
- Ectomycorrhizae - These are fungus forms sheath outside the root. Endomycorrhizae - They are also known as vesicular- arbuscular- mycorrhizae (VAM). Fungus does not form sheath around the roots.

- Lichens - They are symbionts. They have a symbiotic relationship between a fungus and a alga. Neither of the organisms can survive on their own.

MORPHOLOGY

- *Microscopic structures* : An environmental isolate of Penicillium

1. Hypha
2. Conidiophore
3. Phialide
4. Conidia
5. Septa



- Most fungi grow as hyphae, which are cylindrical, thread-like structures 2–10 µm in diameter and up to several centimeters in length.
- Hyphae grow at their tips (apices); new hyphae are typically formed by emergence of new tips along existing hyphae by a process called branching, or occasionally growing hyphal tips fork, giving rise to two parallel-growing hyphae.
- The combination of apical growth and

branching/forking leads to the development of a mycelium, an interconnected network of hyphae.

•Hyphae can be either septate or coenocytic.

- Septate hyphae are divided into compartments separated by cross walls (internal cell walls, called septa, that are formed at right angles to the cell wall giving the hypha its shape), with each compartment containing one or more nuclei; coenocytic hyphae are not compartmentalized.
- Septa have pores that allow cytoplasm, organelles, and sometimes nuclei to pass

Growth & Physiology

- Mold growth covering a decaying peach. The frames were taken approximately 12 hours apart over a period of six days.

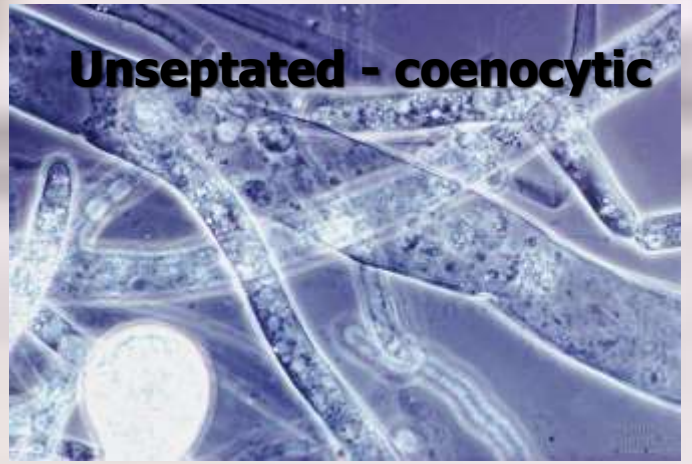
- The growth of fungi as hyphae on or in solid substrates or as single cells in aquatic environments is adapted for the efficient extraction of nutrients, because these growth forms have high surface area to volume ratios.
- Hyphae are specifically adapted for growth on solid surfaces, and to invade substrates and tissues.
- They can exert large penetrative mechanical forceseg., the plant pathogen Magnaporthe grisea forms a structure called an appress- orium that evolved to puncture plant tissues

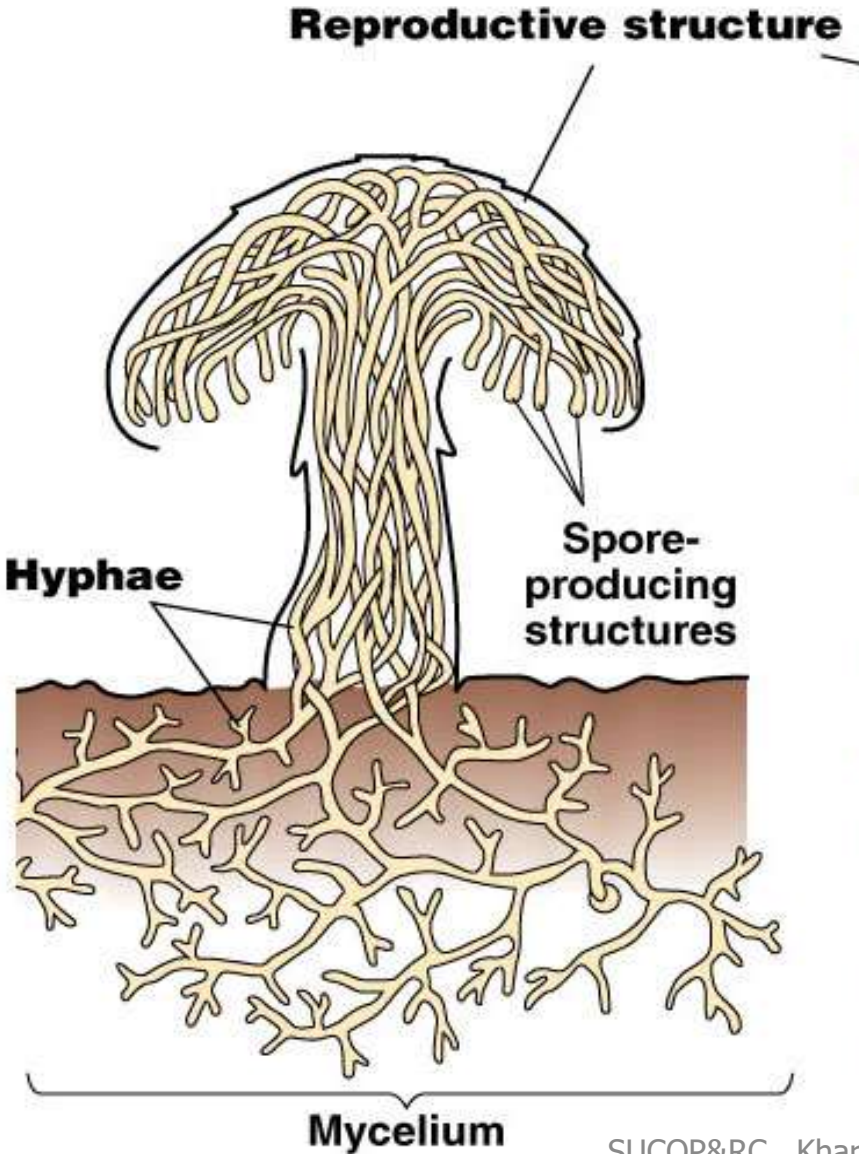
- The fungi are traditionally considered heterotrophs, organisms that rely solely on carbon fixed by other organisms for metabolism.
- Fungi have evolved a high degree of metabolic versatility that allows them to use a diverse range of organic substrates for growth, including simple compounds such as nitrate, ammonia, acetate, or ethanol.

- In some species the pigment melanin may play a role in extracting energy from ionizing radiation, such as gamma radiation.

Structure of Fungi

- Filaments of fungi are called hyphae.
- The cell walls contain chitin.
- The MYCELIUM is a mat of hyphae visible to the unaided eye
  (bread mold)
- Some hyphae may divided by cross sections called septa





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Some important Fungi

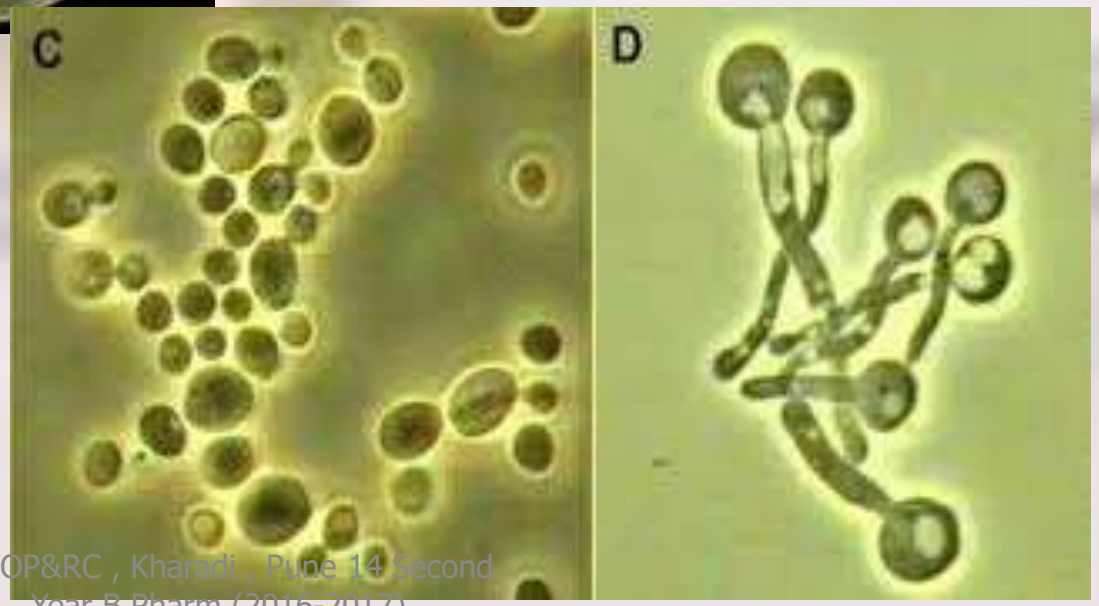
a) Saccharomyces
b) Candida albicans
a) Penicillium

Candida albicans

Scientific classification

- Kingdom : Fungi
- Division : Ascomycota
- Class : Saccharomycetes
- Order : Saccharomycetales
- Family : Debaryomycetaceae
- Genus : Candida
- Species : C. albicans
- Binomial name : Candida albicans

- Synonyms : Candida stellatoidea, Oidium albicans



- Candida albicans is a dimorphic fungus that grows both as yeast and filamentous cells and one of the few species of the Candida genus that cause the infection candidiasis in humans.
- C. albicans is responsible for 50–90% of all cases of candidiasis in humans.
- Systemic fungal infections (fungemias) including those by C. albicans have emerged as important causes of morbidity and mortality in immunocompromised

patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation).

- C. albicans is a common member of human gut flora and is detectable in the gastrointestinal tract in 40% of healthy adults.
- It is usually a commensal organism, but can become pathogenic in immunocompetent individuals under a variety of conditions.

Structure, Metabolism, and Life Cycle of Candida albicans

- C. albicans can take on either a unicellular (yeast) or multicellular (hyphae, pseudohyphae) form.
- The yeast form is 10-12 microns across, and is Gram-positive.
- A transcription repressor is needed to maintain the yeast state. While multicellular, the pseudohyphae are

formed by yeast buds that attach to one another.

- Spores form on the pseudohyphae called chlamydospores.
- C. albicans can create a biofilm as it becomes multicellular.
- The biofilm is made primarily of cellulose, but also contains polynucleotides, polypeptides, and fibrinogin.
- The form it takes depends on

environmental cues, switching to the hyphae phase based primarily on temperature and pH changes.

- The microbe is asexual, and does not perform meiosis.
- However, the diploids undergo parasex, meaning that diploid cells of opposite mating types (different from the characteristic male/female form) perform cell fusion to create a tetraploid.
- This unit then undergoes a split to return

to a diploid state.

- While splitting, chromosomes are randomly lost. Chromosomes are not typically exchanged, though some gene conjugation does occur to provide genetic diversity

Asexual and Sexual reproduction in Candida albicans.

ASEXUAL SPORES (cont.)

- **Blastospores**
  - A bud coming off the parent cell
  - *Candida albicans*

- Candida albicans cells are diploid (2N) and can divide asexually or can undergo heterothallic or homothallic mating.
- Mating type-like locus a (MTLa) and MTLα cells must switch from white to opaque to become mating-competent.
- Opaque cells secrete pheromones that result in the formation of conjugation tubes, and subsequently, cell and nuclear fusion occur to form tetraploid (4N) cells.

- Homothallic mating also occurs and can be driven by loss of Bar1 protease in MTLa cells.

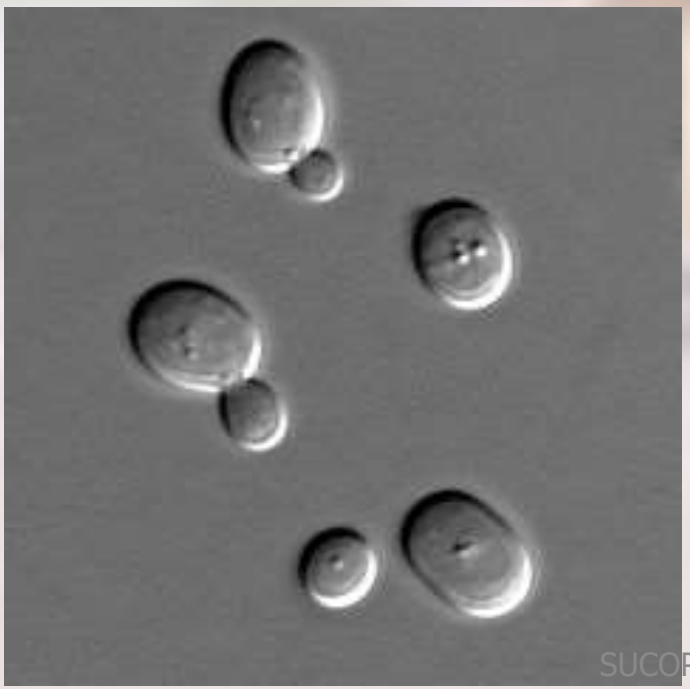
Saccharomyces

Scientific classification

- Kingdom : Fungi
- Division : Ascomycota
- Class : Saccharomycetes
- Order : Saccharomycetales
- Family : Saccharomycetaceae
- Genus : Saccharomyces

- Type species : Saccharomyces cerevisiae
- Species : Saccharomyces arboricolus Saccharomyces bayanus . Saccharomyces boulardii.

Etc.



- Saccharomyces is a genus of fungi that includes many species of yeasts.
- Saccharomyces is from Greek σάκχαρ (sugar) and μύκης (mushroom) and means sugar fungus.
- Many members of this genus are considered very important in food production.
- It is known as the brewer's yeast or baker's yeast.
- They are unicellular and saprophytic fungi.

- One example is Saccharomyces cerevisiae, which is used in making wine, bread, and beer.
- Other members of this genus include the wild yeast Saccharomyces paradoxus that is the closest relative to S. cerevisiae, Saccharomyces bayanus, used in making wine, and Saccharomyces boulardii, used in medicine.

Structure of Saccharomyces

Structure of Saccharomyces:

- In a culture of Saccharomyces 2 types of somatic cells can be seen

(a) 'Dwarf strain' yeast cells : They are small (3 x 2µm) haploid spherical yeast cells which exist in 2 opposite mating types (+ and – strains)

(b) 'Large strain' yeast cells : They are comparatively larger (15 x I0 µm) diploid,

ellipsoidal yeast cells.

- Structurally both strains of Saccharomyces are unicellular, uninucleate, hyaline, holocarpic, non-mycelial thallus.
- In a favourable sugary medium as many as 64 cells found temporarily connected to form a pseudomycelium.
- The cell surface has one concave birth scar and on e or many convex bud scars.
- Like a plant cell, it consists of protoplast surrounded by cell wall.

- Cell wall composed of glucan (30-40%), mannan (30%), proteins, lipids and chitin.
- The granular cytoplasm contains various organelles and reserve foods (glycogen and oil globules).
- The centril cytoplasm (endoplasm) contains a large vacuole with a nucleus at one end. From nucleus fine dark staining strands extend around the vacuole.
- The vacuole enclosed by tonoplast and fills with solution of volutin composed of RNA,.

lipoprotein and poly-phosphate granules

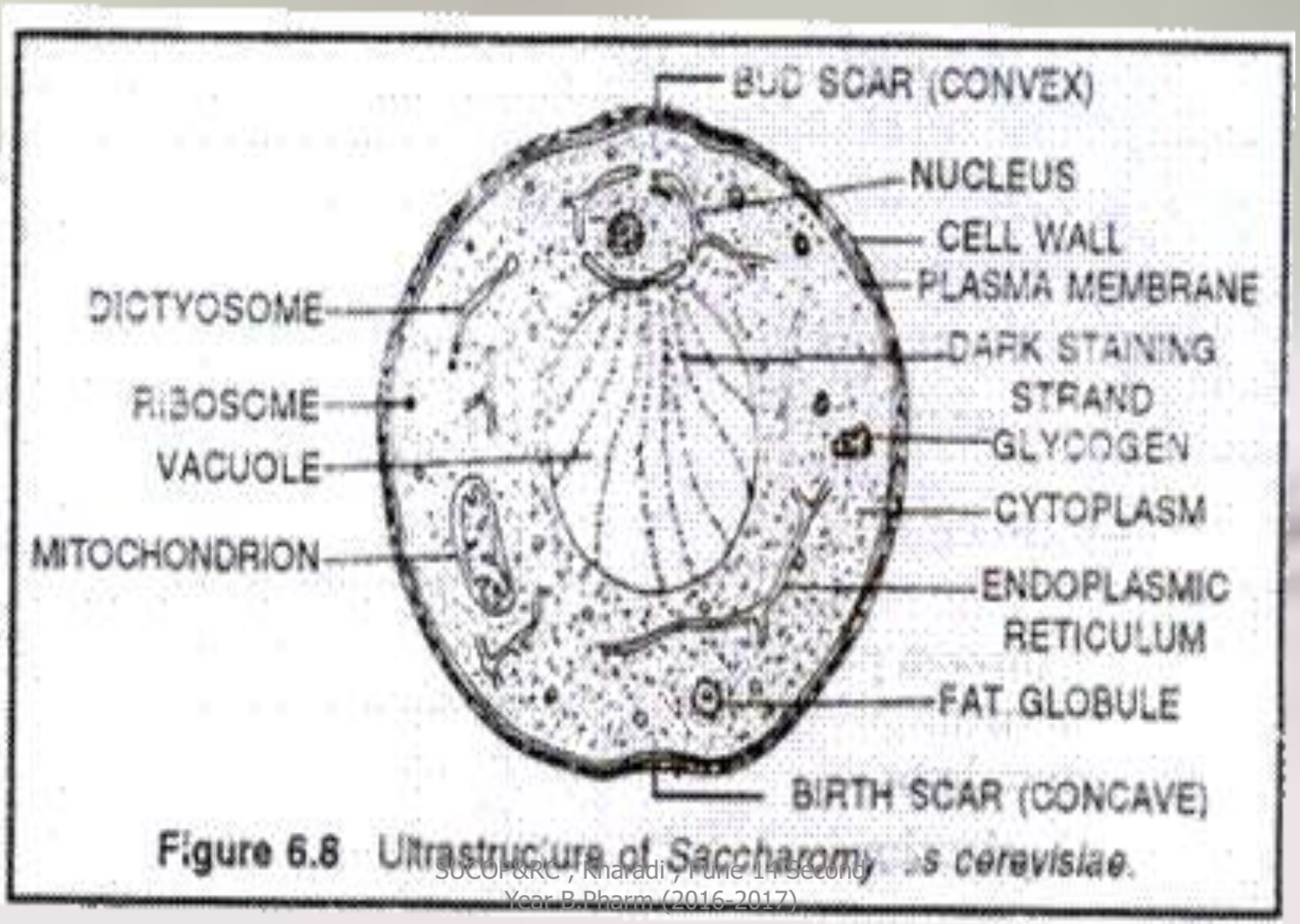


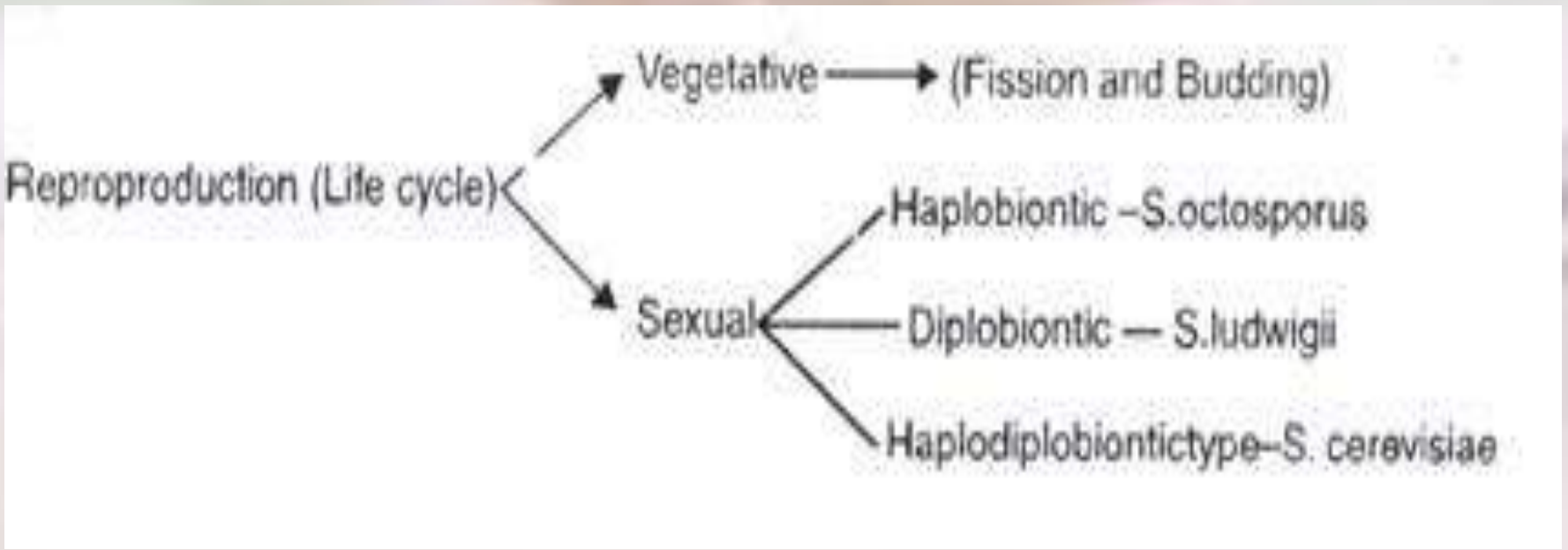
Figure 6.8 Ultrastructure of Saccharomyces cerevisiae.

Life Cycle of Saccharomyces

- In the life cycle of Saccharomyces cereviceae alternation of generations seen where 2 types of vegetative individuals alternate with each other's i.e. the 'dwarf strain' haploid yeasts ceils alternates with 'large strain' diploid yeast cells.
- The 'dwarf stain' cells represent haplophase or gametophyte phase while large strain' cells are diplophase or

sporophyte phase.

- Saccharomyces undergo asexual cycle in favourable period and sexual cycle under unfavourable period.



(A) ***Asexual cycle (budding)***:

- Under favourable conditions both ‘dwarf strain’ cells and ‘large strain’ cells multiply by asexual process called budding.
- During the process, near one pole cell wall softens by enzymatic action as a result an outgrowth appears.
- The nucleus divides mitotically and passes one daughter nucleus along with a part of vacuole into the outgrowth.
- The outgrowth now known as a bud that

- enlarges, and then constricts as a young yeast cell from the parent.
- Rapid budding may form pseudomycellium.

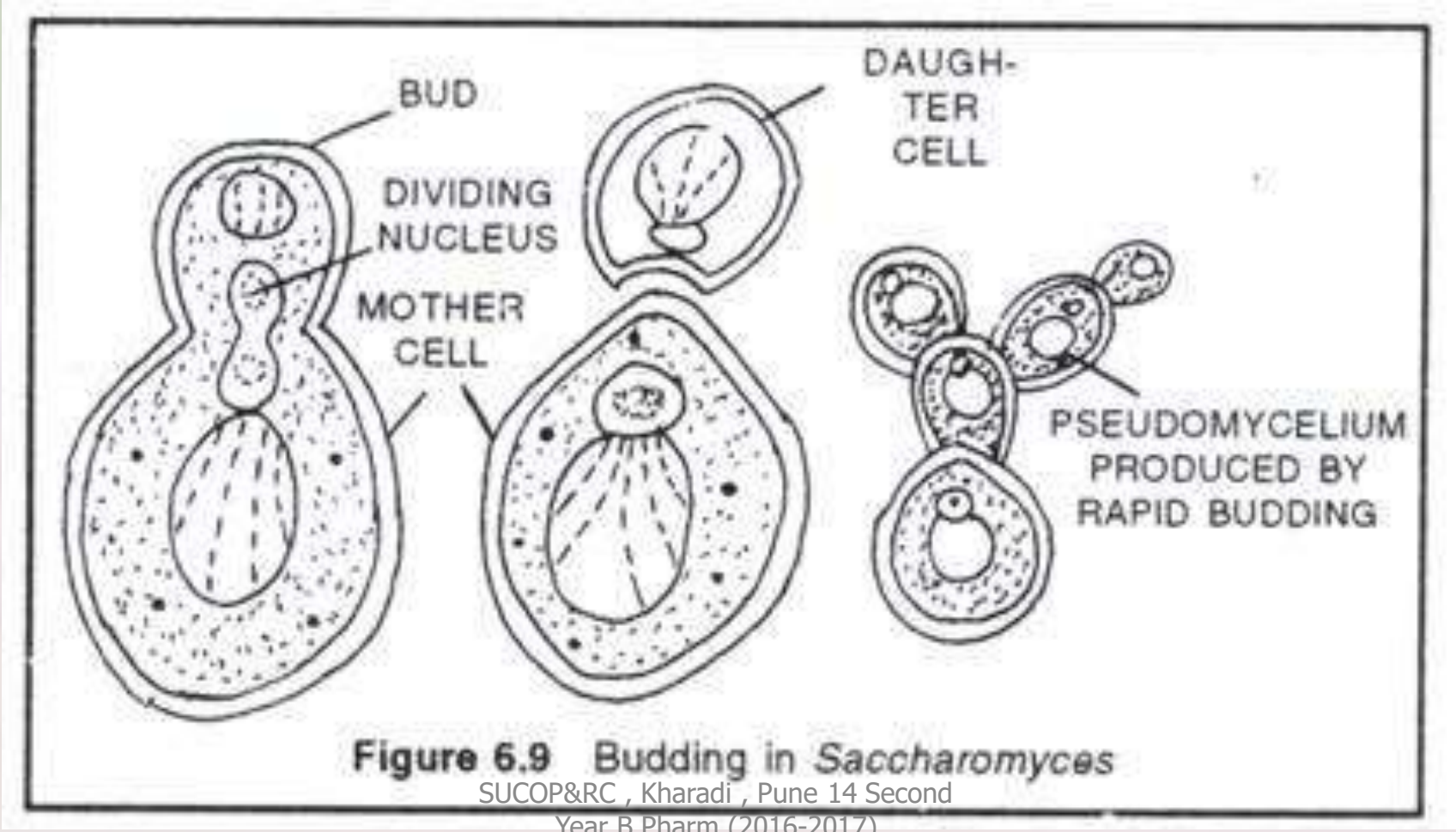


Figure 6.9 Budding in *Saccharomyces*

(B) ***Sexual cycle (Haplo- diplobiontic cycle)***:

- Under unfavourable condition Saccharo-myces cerevisiae undergo sexual cycle which is haplo-diplobiontic type.
- Here both haploid and diploid phases are equally represented.
- The haploid dwarf cells have two genetically distinct mating types or strains (+ and -).
- Herman (1971) and Hartwell (1974) have observed that when dwarf cells of opposite mating types (i.e. – and +) are not

in contact with each other, a sex hormone is secreted.

- This hormone induces enlargement and elongation in the yeast cells, and consequently they grow towards the opposite mating types, and conjugation follows.
- Such a fusion of cells or nuclei of opposite mating types of the same species is called legitimate copulation and the phenomenon is called heterothallism

- Rarely, cells of the same mating types may also fuse and depolarization takes place.
- Such a conjugation between the same mating types is called illegitimate copulation.

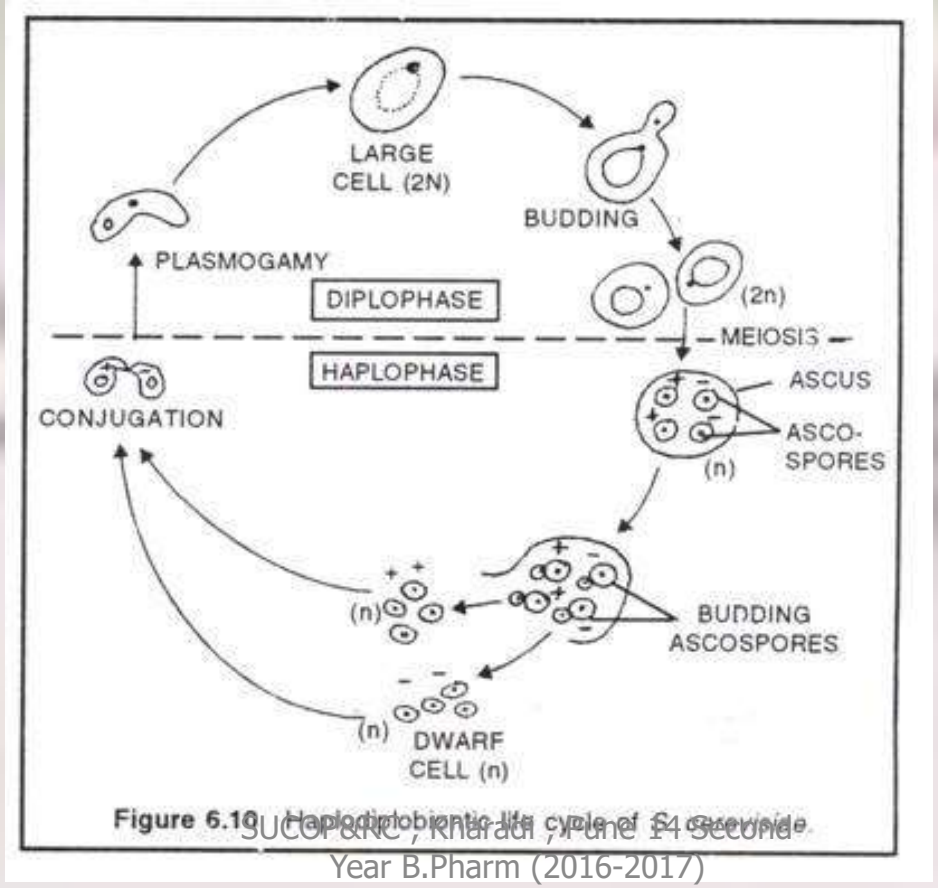


Figure 6.10 Haplodiplobiontic life cycle of *S. cerevisiae*

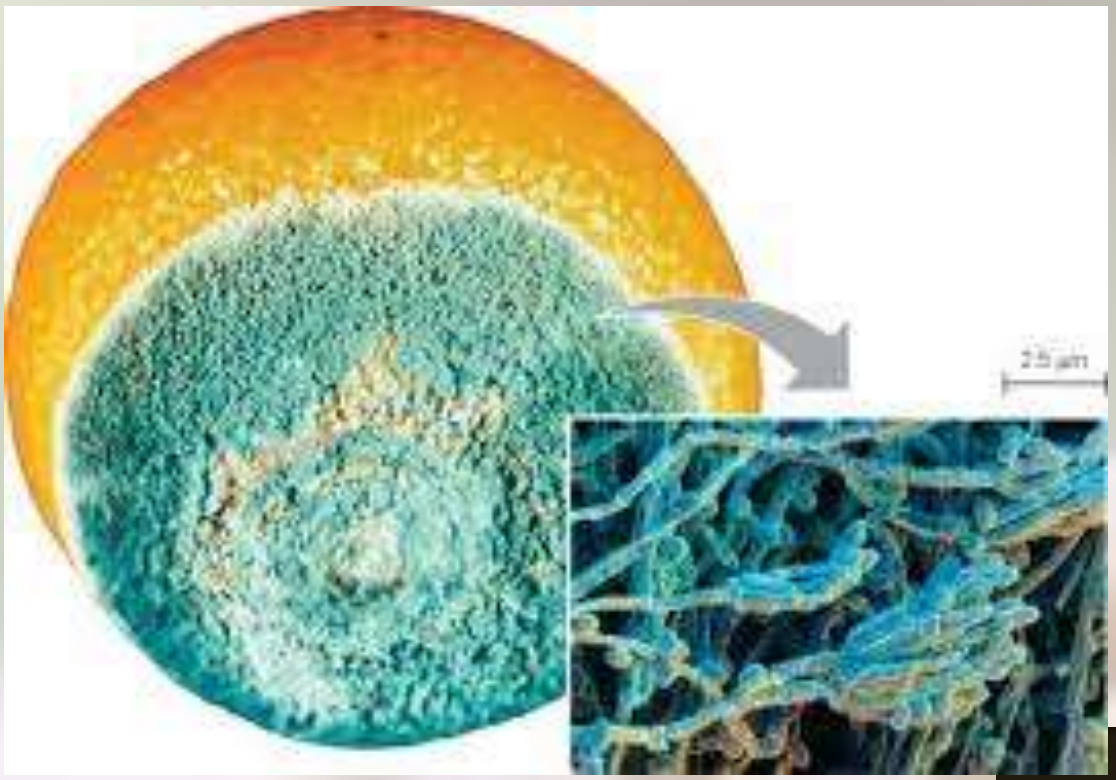
Penicillium

Scientific classification

- Kingdom : Fungi
- Phylum : Ascomycota
- Class : Eurotiomycetes
- Order : Eurotiales
- Family : Trichocomaceae
- Genus : PenicilliumLink

Type species

- Penicillium expansum
- Species : over 300List of Penicillium species


2.5 µm

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Structure of Penicillium

Plant body-Structure :

- The Mycelium: the mycelium is well developed and is profusely branched. It is composed of colorless, slender, tubular, branched, and septate hyphae.
- Some of the hyphae may invade the substratum to absorb nourishment from the substratum, whereas the other spread on the surface of the substratum.

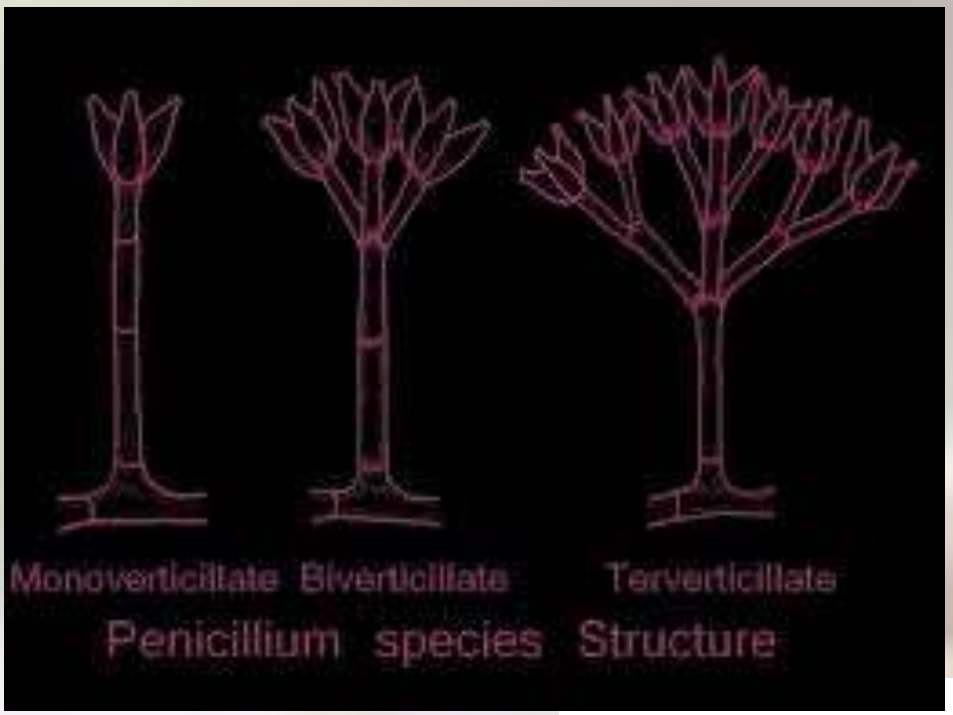
- Some of the hyphae may interwine to form rhizophores.
- In some species, mycelium may develop into a sclerotium.
- The mycelium becomes coloured when the fungus enters reproductive phase.

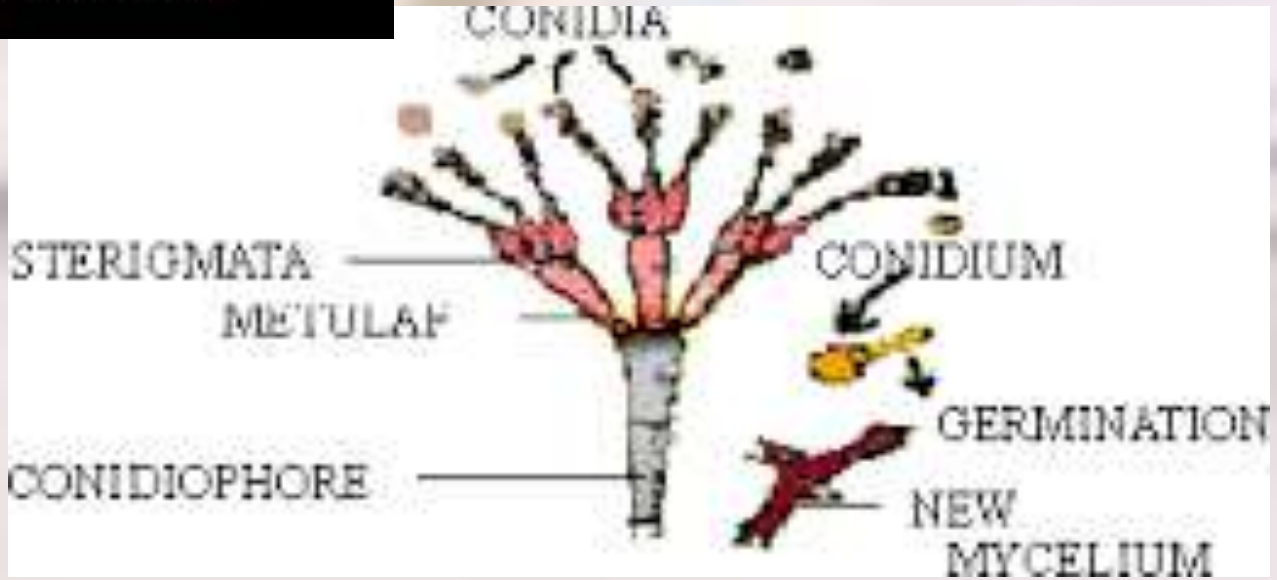
Cell structure :

The hyphae are septate and each cell is uninucleate.

The cell wall is microfibrillar and in

- Penicillium notatum it is reported to consist of three or four layers, the outer most layer is composed of glucans, the next of proteins, the third of chitin fibrils embedded in a granular matrix, and the inner most of pectic or hemicellulosic material.
- The plasma membrane surrounds the cytoplasm in which mitochondria, ribosomes and endoplasmic reticulum is embedded.


Monoverticillate Biverticillate Terverticillate
Penicillium species Structure

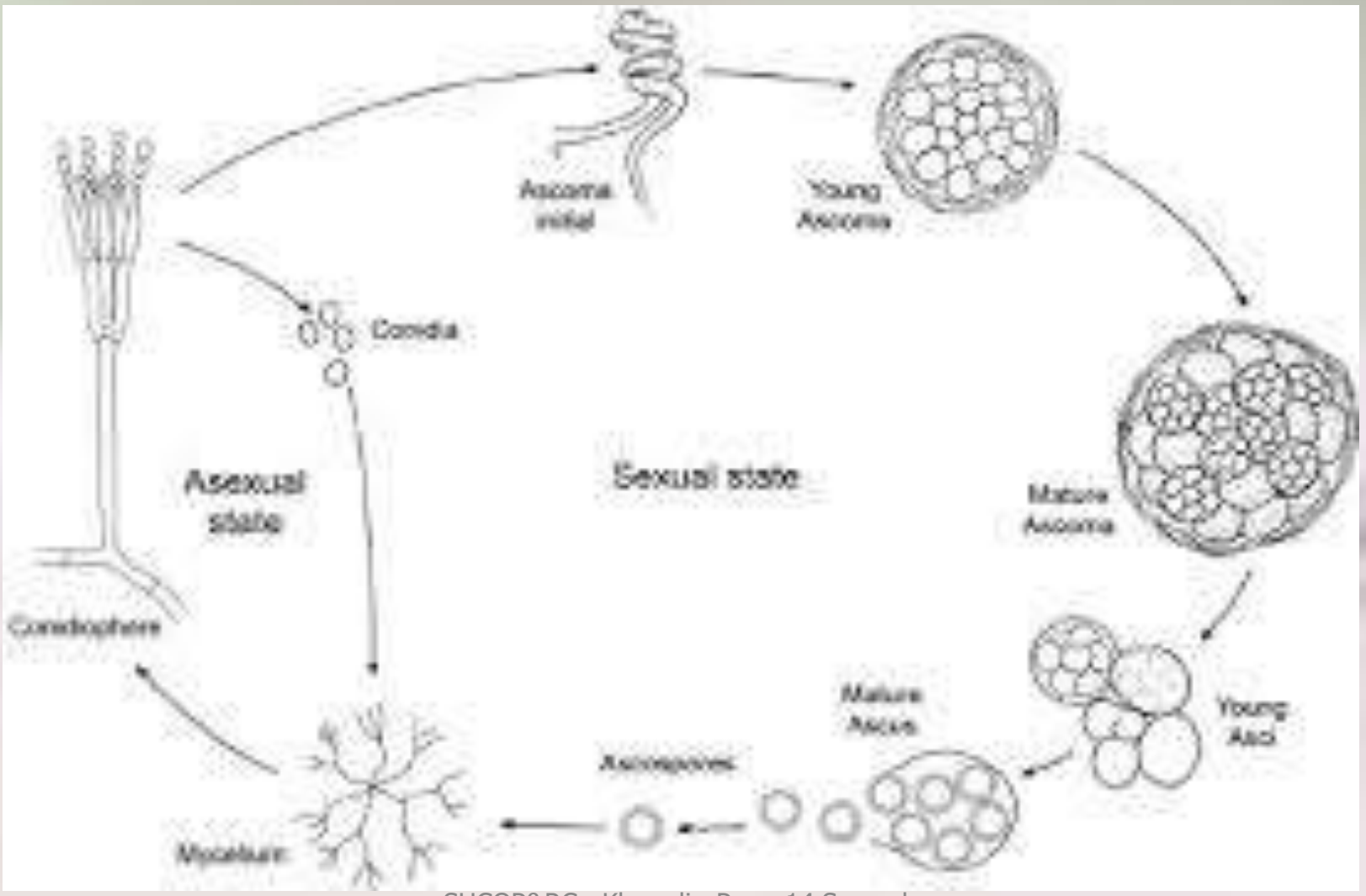

CONIDIA
STERIGMATA
METULAE
CONIDIUM
GERMINATION
CONIDIOPHORE
NEW MYCELIUM

Reproduction in Penicillium

LIFE CYCLE :

- The Penicillia reproduce vegetatively, asexually, and asexually.
- The perfect state of form –genus Penicillium is assigned to two different genera, the Eupenicillium and Talaromyces.
- Vegetative Propagation: The vegetative reproduction takes place by fragmentation during which the hyphae break up into short fragments, which grow by repeated

division into a new mycelium.



Vegetative Propagation :

- The vegetative reproduction takes place by fragmentation during which the hyphae break up into short fragments, which grow by repeated division into a new mycelium.

Asexual Reproduction :

- The asexual reproduction is by formation of non-motile, asexual spores, the conidia, and produce at the tip of special, erect, hyphae called conidiophores. Many crops

of conidia are produce during a growing season.

Conidiophores :

- The mycelium produces simple, long, erect, conidiophores that branch two third of the way to the tip, in characteristic broom like fashion.
- The branches of conidiophores end in a group of conidiogenous cells, the phialides that produce conidia at their tips in chain.

Structure and development of conidia :

- The conidia are tiny, uninucleate, spore like structures which may be globose to avoid in form.

- The spore wall is pigmented and is differentiated into two layers, an outer thick, ornamented layer, the exine; and inner smooth and thin layer, the intine.
- The conidia are detached from the conidiophores and are carried by wind to a suitable substratum where they germinate by forming a germ tube.
- The germ tube elongates, becomes septate to form a new hyphae.

Sexual Reproduction :

- The perfect state of Penicillium is assigned to two different genera, the Eupenicillium and Talaromyces.
- All the species are homothallic.
- In Penicilllium vermiculatum the sexual reproduction is oogamous.
- The male sex organs are antheridia and female sex organ are ascogonia.

Ascogonium :

- A mature ascogonium is a long erect, multinucleate, tubular structure with curved upper end.
- It arises from uninucleate, septate hyphae as a finger like, lateral outgrowth which elongates into an ascogonium.
- The nucleus of the ascogonium divides many times mitotically to produce 32 or 64 nuclei.

Anteridium :

- While the ascogonium is developing a uninucleate branchoriginates from a cell of the same hyphae adjacent to the developing ascogonium, or from neighbouring hyphae.
- This is the antheridial branch.
- It grows up band coils around the The tip of the antheridial branch swells up and is cut off from rest of the branches to form a uninucleate antheridium.

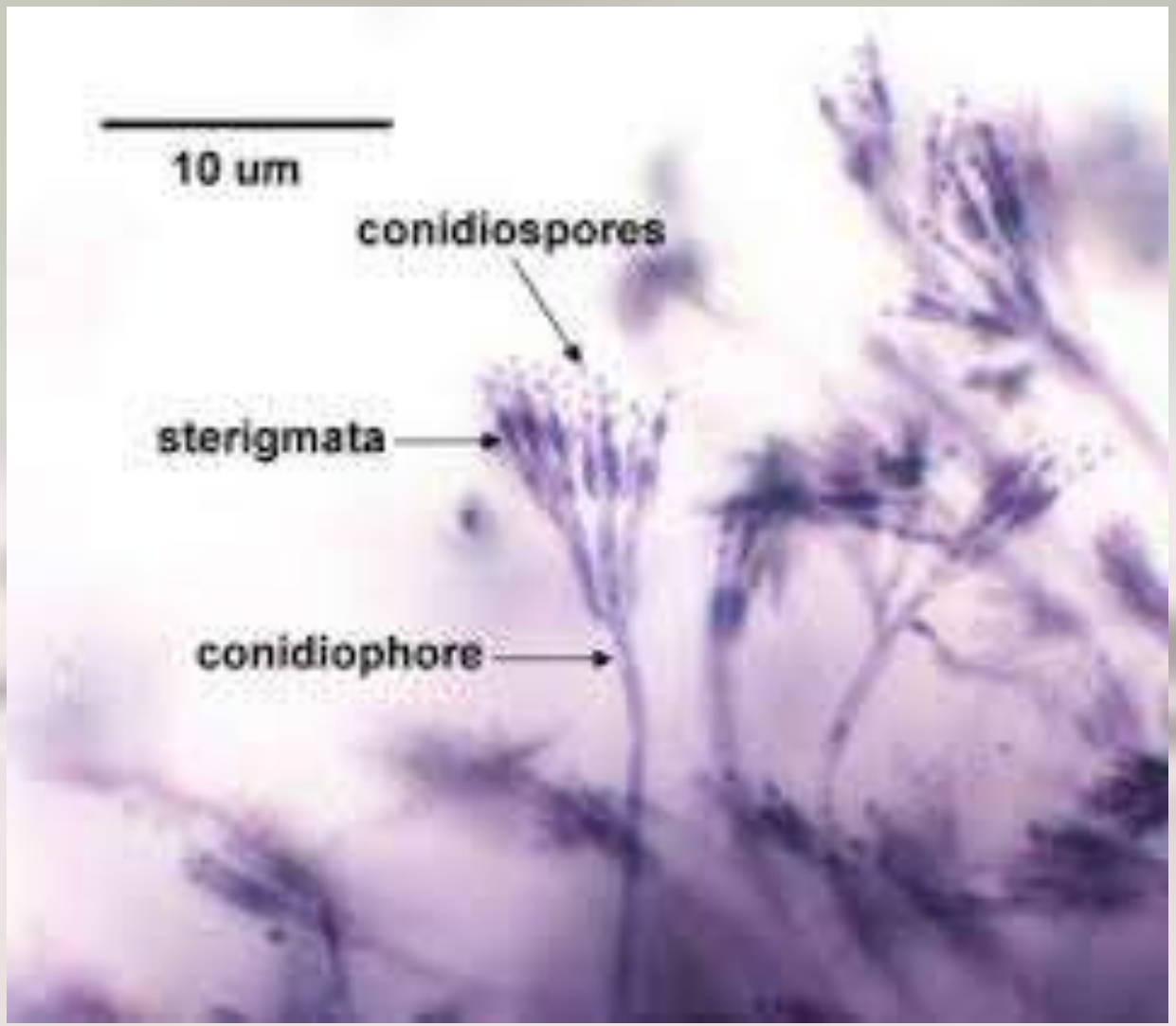
Development of Ascus and Ascospores:

- The stimulus of plasmogamy results in septation of ascogonium into binucleate cells.
- Some of these segments usually those present in the middle, produce outgrowth called ascogenoum which develop into ascogenous hyphae composed of binucleate cells.
- The tip cells of these hyphae act as ascus mother cells which develop either simply

by elongation, or by crozier formation into ascus.

- Karyogamy takes place in the ascus mother cell and this diploid uncleus undergoes meiosis to produce four haploid nuclei.
- A mitotic division results in the formation of eight haploid nuclei.
- These are transformed into ascospores by free cell formation.
- The ascus are globose or pear-shaped and the unicellular, uninucleate and lens

shaped with a groove around the edge.



Reproduction in Fungi

- Fungal reproduction is complex, reflecting the differences in lifestyles and genetic makeup within this diverse kingdom of organisms.
- It is estimated that a third of all fungi reproduce using more than one method of propagation; for example, reproduction may occur in two well-differentiated stages within the life cycle of a species, the teleomorph and the anamorph.

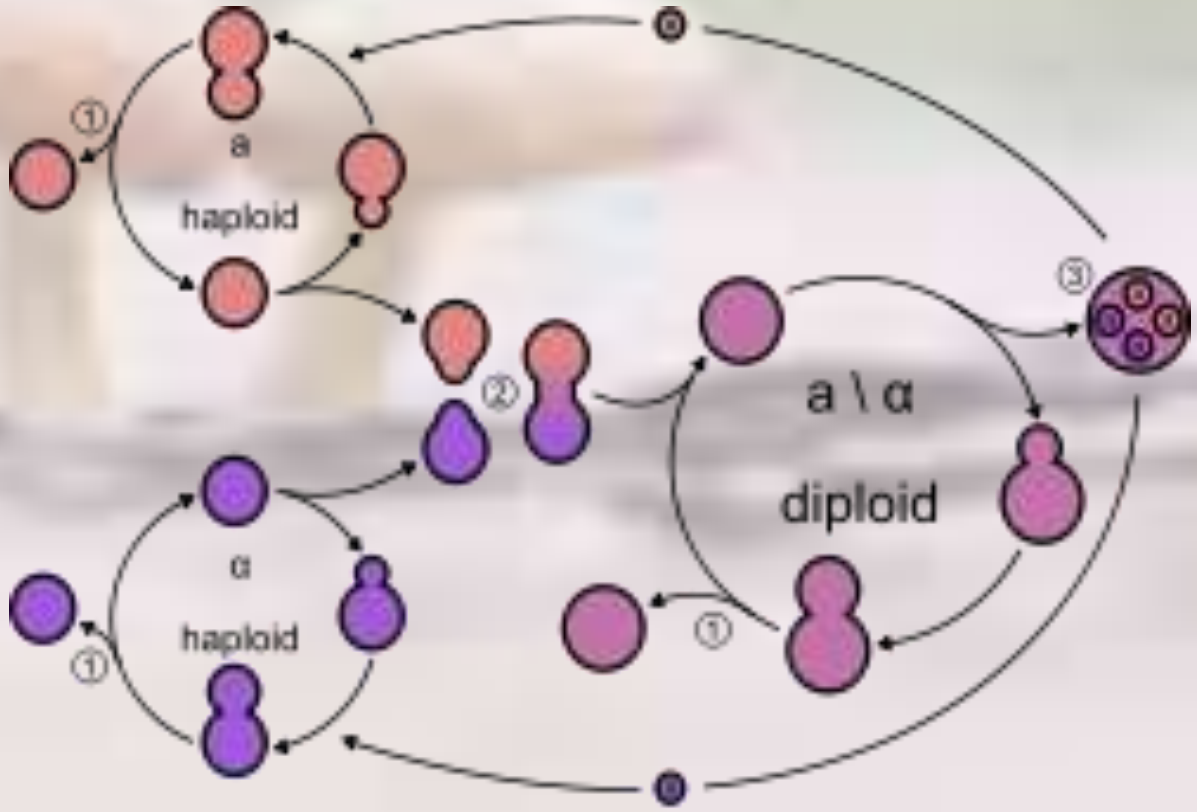
•Environmental conditions trigger genetically determined developmental to the creation of specialized structures for sexual or asexual reproduction.

- These structures aid reproduction by efficiently dispersing spores or spore-containing propagules.

Reproduction in Yeasts

- The yeast cell's life cycle:

1. Budding
2. Conjugation
3. Spore



- Yeasts, like all fungi, may have asexual and sexual reproductive cycles.
- The most common mode of vegetative growth in yeast is asexual reproduction by budding.
- Here, a small bud (also known as a bleb), or daughter cell, is formed on the parent cell.
- The nucleus of the parent cell splits into a daughter nucleus and migrates into the daughter cell.

- The bud continues to grow until it separates from the parent cell, forming a new cell.
- The daughter cell produced during the budding process is generally smaller than the mother cell.
- Some yeasts, including Schizosaccharomyces pombe, reproduce by fission instead of budding, thereby creating two identically sized daughter cells.

- The budding yeast Saccharomyces cerevisiae reproduces by mitosis as diploid cells when nutrients are abundant, but when starved, this yeast undergoes meiosis to form haploid spores.
- In nature, mating of haploid cells to form diploid cells is most often between members of the same clonal population and out-crossing is uncommon.
- Analysis of the ancestry of natural S. cerevisiae strains led to the conclusion that

- out-crossing occurs only about once every 50,000 cell divisions.
- These observations suggest that the possible long-term benefits of outcrossing (e.g. generation of diversity) are likely to be insufficient for generally maintaining sex from one generation to the next.

Reproduction in Mould/Mold

- A mold or mould is a fungus that grows in the form of multicellular filaments called hyphae. In contrast, fungi that can adopt a single-celled growth habit are called yeasts.

- Molds are a large and taxonomically diverse number of fungal species where the growth of hyphae results in discoloration and a fuzzy appearance, especially on food.
- The network of these tubular branching hyphae, called a mycelium, is considered a single organism.
- The hyphae are generally transparent, so the mycelium appears like very fine, fluffy white threads over the surface.

- Cross-walls may delimit connected compart- ments along the hyphae, each containing one or multiple, genetically identical nuclei.
- Molds are considered to be microbes and do not form a specific taxonomic or phylogenetic grouping, but can be found in the divisions Zygomycota and Ascomycota.
- In the past, most molds were classified within the Deuteromycota.

- Molds cause biodegradation of natural materials, which can be unwanted when it becomes food spoilage or damage to property.
- They also play important roles in biotechnology and food science in the production of various foods, beverages, antibiotics, pharmaceuticals and enzymes.
- Some diseases of animals and humans can be caused by certain molds: disease may result from allergic sensitivity to mold

- spores, from growth of pathogenic molds within the body, or from the effects of ingested or inhaled toxic compounds (mycotoxins) produced by molds.

Economic Importance of Fungi

Fungi are important in a variety of ways

- Recycling - Together with bacteria the fungi form a major role in recycling the dead and decayed matter.
- Food - Many mushrooms are used as food by humans. Mushrooms species are edible and are cultured in many parts of the world for sale.

- Medicines - Penicillin antibiotic is derived from a common fungi Penicillium. Many other fungi also produces antibiotics, which are used to control diseases in humans and animals.
- Bio-control Agents - Fungi are used to parasitise insects which help control pests. Spores of fungi are sprayed on crops, this method is cheaper and environmentally friendly.
- Plant and Animal Diseases - Many fungi live on and in plants and animals causing

diseases.

- They also co-exist harmoniously with plants and animals.
- Food spoilage - Fungi play a major role in recycling organic material. Fungal damage is responsible for large losses of stored food usually when the food contains moisture.

SOME IMPORTANT QUESTIONS

Q1.] State the importance of fungi or significance of fungi. {2014,2012}

Q2.] Differentiate between : {2012,2014,2013}

a.] Bacteria and fungi.

b.] Yeast and molds.

Q3.] write short notes on : {2013,2014}

a.] Saccharomyces cerevisiae.

b.] Candida albicans.

c.] Penicillium

d.] aspergillus.

e.] Dimorphic fungi.

Thank you

Pune District Education Association's Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre.

GAS CHROMATOGRAPHY

Presented by : Dr. Vijaya U. Barge

(Vice Principal & Professor)

Learning Objectives :

After completing this sub-unit the students should be able:

- To study the principle and types of Gas Chromatography.
- To study the instrumentation involved in Gas Chromatography.
- To study the derivatization in Gas Chromatography.
- To study the applications of Gas Chromatography.
- To study system suitability parameters used in GC/HPLC.
- To study Rate and Plate theory.

CONTENTS

- Introduction
- Principle
- Instrumentation
- Applications
- Conclusion
- References

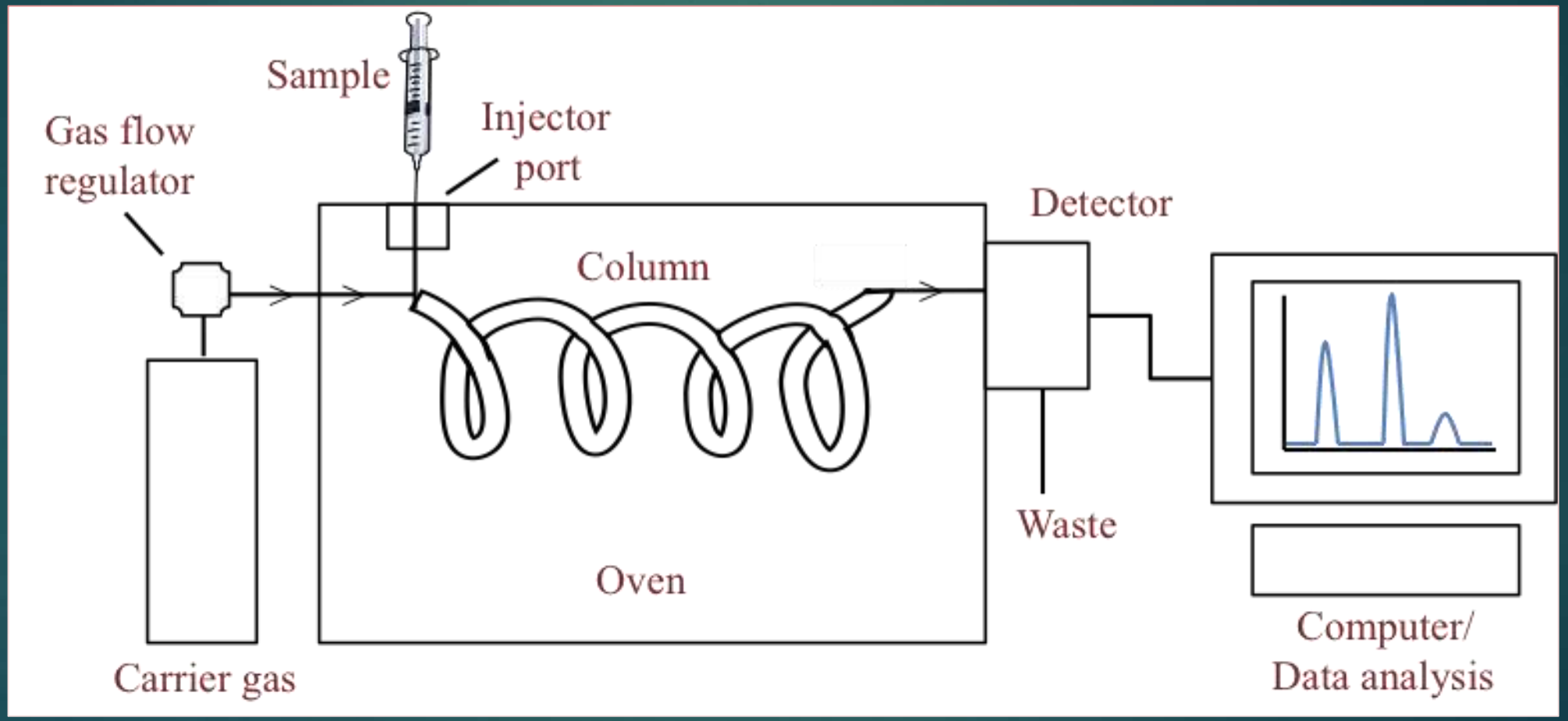
INTRODUCTION

- Chromatography dates to 1903 in the work of Russian scientist *Mikhail Tswett.*
- German graduate student *Fritz Prior* developed solid state gas chromatography in 1947.
- *Archer John Porter Martin* was awarded the **Nobel Prize** for his work in developing gas chromatography and he later produced liquid-gas chromatography (1950).
- Gas chromatography (GC) is an analytical technique used to separate the chemical components of a sample mixture and then detect them to determine their presence or absence and/or how much is present. These chemical components are usually organic molecules or gases.

PRINCIPLE

- Gas Chromatography is a chromatographic technique that can be used to separate volatile organic compounds.
- The sample solution injected into the instrument enters a gas stream which transports the sample into a separation tube known as the "column." (Helium or nitrogen is used as the so-called carrier gas.) The various components are separated inside the column. The detector measures the quantity of the components that exit the column. To measure a sample with an unknown concentration, a standard sample with known concentration is injected into the instrument. The standard sample peak retention time (appearance time) and area are compared to the test sample to calculate the concentration.

INSTRUMENTATION



CARRIER GAS

- It is the mobile phase that runs in column.
- Hydrogen, helium, argon or nitrogen is mostly used as carrier gas.
- The helium gas is mostly preferred because of its efficiency and safety.
- The carrier gas is filled in reservoir tank and regulator controls the flow of gas.
- The carrier gas must be pure.
- It is equipped with molecular sieve for filtering and removal of moisture and other impurities if present.

SAMPLE INJECTOR

- The sample that needs to be analyzed is injected in gas chromatography through sample injector.
- To inject the sample, calibrated syringe is used.
- As the sample needs to travel with mobile phase they both should be in same physical state. In gas chromatography, the mobile phase is in gaseous state hence sample also need to be gaseous state.
- The sample injector is equipped with heater that allows the vaporization of liquid sample.
- If sample is in solid state, it is crushed and grinded and converted to liquid state.

COLUMN

- The column consists of stationary phase (liquid adsorbed on inert solid support).
- The column is mostly made from stainless steel.
- The column can be packed with stationary phase called as packed column or thin layer of stationary phase is bonded on the inner walls of column forming hollow tube called open tubular column
- The length of column used in gas chromatography is in range from 1.5 to 10m with diameter of 2 -4 mm. It is present in the form of coil.
- The column is present inside the oven. The temperature of oven is controlled and monitored by the software.
- The temperature of oven increases gradually.

COLUMN

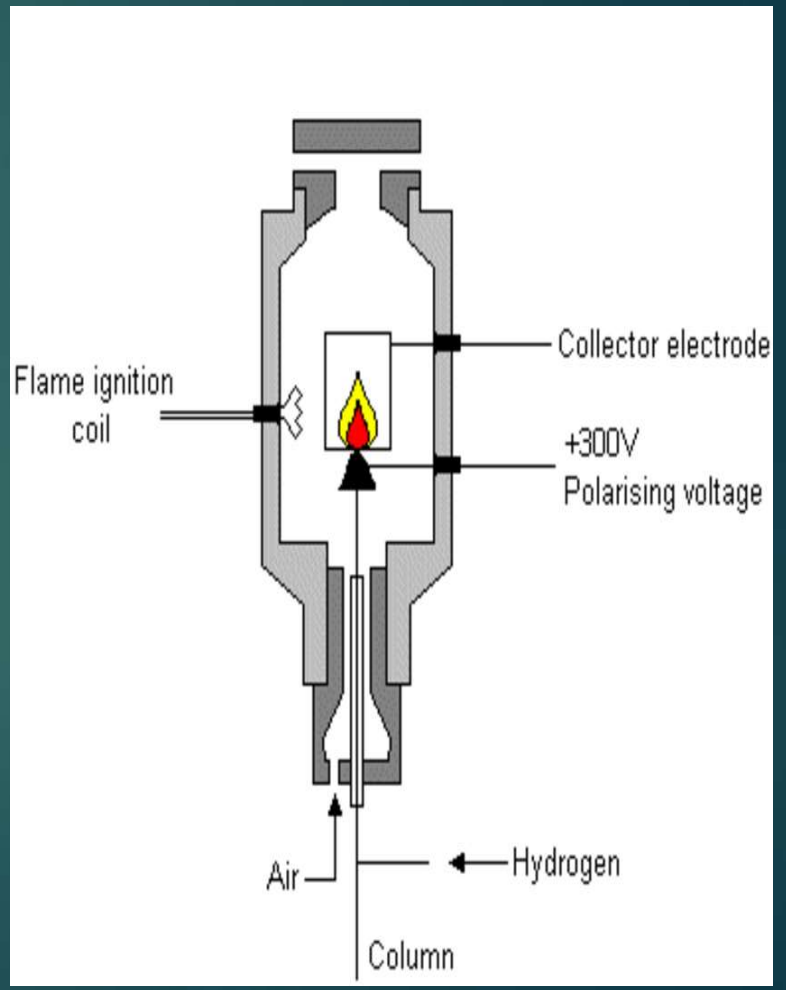
- The high temperature of oven ensures the sample to stay in gaseous state.
- The optimum temperature of oven is depends on sample's boiling point and hence it is varies from sample to sample.
- When the sample is injected via injector, it travels with carrier gas (mobile phase) in column filled with stationary phase. The components of mixture (sample) interact with both immiscible phases in differently.
- Due to difference in the interaction with stationary and mobile phase, the time taken by individual components to travel the column is different.
- The time spend by individual component in the column is called as retention time. This characteristic is used for identification of sample.

DETECTOR

- The detector measures the quantity of the components or ingredients of sample.
- There are different types of detectors available. The working mechanism of different detectors varies. They also differ in selectivity i.e. ability to quantify based on molecule's physical and chemical property.
- The most commonly used detectors are Flame Ionization and Thermal conductivity detector.

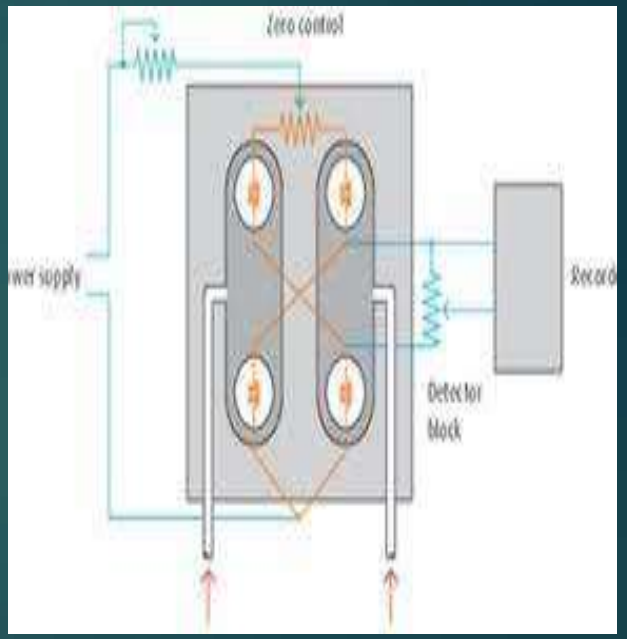
FLAME IONIZATION DETECTOR

- When the carrier gas that carries the sample in column and enters the detectors and passes through hydrogen air flame. It causes chemical decomposition and ionization of sample.
- The collector collects the produced ions. It causes increase in the current. The current is directly proportional to quantity of sample that is burned.
- Hence, the number of ions (produced during ionization) depicts the concentration or quantity of sample. The produced current is converted into digital form and present in the output device.
- As the works on ionization caused by burning of sample in hydrogen flame, the detector is called as Flame Ionization detector.



THERMAL CONDUCTIVITY DETECTOR

- This detector works by measuring the difference in thermal conductivity caused by flow on carrier gas with sample with reference to flow of only carrier gas.
- TCD is located at the end of the column consisting a paired tube like thermistors. When carrier gas and sample travels through tube, it causes change in the temperature. This change is temperature is sensed by Wheatstone bridge.
- When pure carrier gas is passed through both the tubes, there is no difference in the temperature of the tubes and hence bridge is balanced. When one tube carries pure carrier gas while carries carrier gas with sample, the bridge become unbalanced. It is because of difference in temperature of both the tubes.
- The extent of difference of temperature is directly proportional to the concentration of sample. The difference in the temperature is measured and recorded and converted to digital signal and presented in output device.



APPLICATIONS

- Gas chromatography is exclusively used for isolation, identification and quantification of volatile compounds or mixtures. Such analytical studies need to be conducted in pharmaceutical and cosmetic industries, medical fields and environmental studies.
- Qualitative:
  - The supplement identification of unknown by retention data.
  - To derivatives the unknown & see if the retention time of derivative compares with that of known derivatives.
  - It is useful for analysis of compound in a mixture of known composition.
- Quantitative:
  - External standardization
  - Internal standardization

APPLICATIONS

- In pharmaceutical analysis:
  - Used in quality control, analysis of new product and in monitoring metabolites in biological fluids. E.g.: antibiotics – penicillin & derivatives, gentamicin, kanamicin.
- Food analysis.
- Quality control.
- Research.
- Forensics.
- Measuring air pollution.
- Blood alcohol analysis.

CONCLUSION

- This technique is having very high resolution power so complex mixture can be resolved into it's components by this method.
- Sensitivity in detection is very high with thermal conductivity detectors.
- The speed of analysis is high and fast.
- It gives relatively good precision and accuracy.
- Qualitative and Quantitative analysis at a time is possible.

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Thank You!

GIT AGENTS

By

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GASTROINTESTINAL AGENT

- (GIT) consist of series of organs for the digestion of food; ingestion of food to the expulsion of waste as faeces.
- It starts from esophagus, oral or buccal cavity, stomach, the small intestines (consisting of duodenum, jejunum and ileum)
- The large intestines (consisting of caecum, ascending, transverse and descending colons)
- lastly rectum with exit anus.

Each organ has definite functional role.

- **Gastro Intestinal (GI)Agents** :- Drugs used in the treatment of GI disorders are known as GI Agents

Classifications

1. Acidifying Agents – Drugs which increase the gastric acidity are known as Acidifying Agents

   Eg:- Dil HCl

2. Antacids – Drugs which neutralizes the excess of HCL secreted in the stomach.

   Eg:- Sodium Bicarbonate, Alumnium hydroxide gel, Calcium carbonate etc.

3. Protective & Adsorbents :- used in treatment of dirrhoae

   Eg:- Bismuth sub carbonate, Kaolin

4. Saline Cathartics:- are used to relieve constipation Sodium Potassium Tartrate, Magnesium Sulphate etc.

List Of GI disorders

In event of impaired functions, the gastrointestinal (GI) disturbances follows eg:

- Absence or insufficient secretion of hydrochloric acid in stomach -leading to achlorhydria or hypochlorhydria.
- Excessive secretion of hydrochloric acid in stomach leading to hyperacidity, which causes ulcers inflammation Acute pain.
- Accumulation of toxins, gases, toxic substances etc; or insufficient absorption of fluids or electrolytes in the intestine stimulates intestine motility leading to diarrhea.
- Insufficient intestine motility leads to peristalsis leading to constipation.

Various inorganic GIT agents employed are:-

1. Acidifiers or Acidifying Agents for acidity
2. Antacids for hyperacidity
3. Protectives for intestinal inflammation
4. Adsorbents for intestinal toxins
5. Cathartics for constipation.

Gastric acidifiers:

Drugs uses to increase the acidity are known as Gastric acidifiers or acidifying agents.

- Some drugs are used to increase the Gastric HCl or some drugs to increase the metabolic acidosis.

Eg :-HCl

- Normal HCL is secreted which helps in digestion of food & due to secretion of HCl the PH of stomach is acidic
- Stomach PH varies from 1 when stomach is empty & when food is ingested
- The gastric mucosa is made up with epithelial cells which secrets HCL & enzymes which are required for digestion.
- The HCL softens the fibrous food promotes formation of proteolytic enzyme pepsin from pepsinogen
- Kills the bacteria by food or drink.

- Proteolytic activity of pepsin is based at PH 3-5.
- Due to some reason there is no secretion of HCl & it is called as Achlorhydria.

•Symptoms varies from patients to patients.

1)frequent bowel movement (mild diarrhea)

2) Epigastric pain.(upper middle portion of abdomen)

3) sensitivity to spicy food.

two types of patients of Achlorhydria.

Treatment :- counter effect the effect by using HCl.

Explain the term achlorhydria ?

absence or insufficient secretion of hydrochloric acid in stomach -leading to achlorhydria

Achlorhydria patients are of two kind

1 those who do not respond to histamine phosphate stimulation & remain free of gastric HCl .

.

2.Those who respond to histamine phosphate stimulation for the release of HCl in the stomach.

.

Monograph

HCl

- DIL HCl used to treat achlorhydria

Properties of HCl :-

1] It occurs as a colorless fuming liquid with pungent odour.

2] It is miscible with water, alcohol & has a specific gravity of 1.18.

Storage of HCl:-It is stored in glass-stoppered containers at a temperature not exceeding 30°C.

Uses of HCl:-

- Hydrochloric acid as such cannot be used as medicine.
- The dilute hydrochloric acid is used as acidifying agent.
- It is also used as a solvent in numerous industries.
- Also used as laboratory reagent.

2) ANTACID

- These are the agents that decrease gastric acidity by neutralizing HCL.
- Many used in the condition called hyperchlorhydria they give relief of pain due Hyperchlorhydria.(HCH)
- Effectiveness of antacids is measured in neutralizing capacity.
- In normal HCl is secreted by epithelial cell of gastric mucosa depending upon intake of food,
- The PH of stomach varies from 1-6.
- Due to inflammation of gastric mucosa as in gastritis ,there results hypersecretionof HCL called as H.C.H .
- Prolong excessive secretion of HCl leads to peptic ulcer.
- Erosion of mucosal layer & various portion results in Esophageal ulcer, gastric ulcer, & duodenal ulcer.

Treatment:- Anticholergenic drugs, use of certain type of food, antacids

1) As antacids are alkaline in bases they neutralize the acidity occurred in H.C.H.
2) Production is continuous therefore use of antacid is required but antacid should act gradually without producing rebound acidity.
3) As antacids are used for longer periods they may produce side effects,
4) To produce good effects with lesser side effects should have following properties.

Ideal Characteristic of Antacid

1. It should not be absorbable and cause systemic alkalosis
2. It should not be laxative or cause constipation
3. It should exert effect rapidly & over a long period of time
4. It should buffer in pH 4-6.
5. It should not produce large volume of gas
6. It should be palatable & inexpensive
7. It should probably inhibit pepsin

Antacids are of two types.

l) Systemic(absorbable) antacids: which are soluble readily absorbable and capable of producing systemic electrolytic alterations & alkalosis.

E.g. sodium bicarbonate.

2) Non-systemic (non.absorbable) antacids:- which a not absorbed to a significant extent and thus do not exert an appreciable systemic effect.

This group is further sub-divided into following.

a) Aluminum containing antacids:- e.g. aluminum hydroxide aluminum phosphate, dihydroxyaluminium, aminoacetate dihydroxyaluminium sodium carbonate, basic aluminium carbonate (gel).

b) Calcium containing antacids :- Eg. Calcium Carbonate tribasic calcium phosphate

c) Magnesium containing antacids:-Eg. magnesium carbonate magnesium citrate, magnesium hydroxide, magnesium oxide magnesium phosphate, magnesium trisilicate

d) Combination antacid preparations:- e g Aluminum Hydroxide gel & Magnesium Hydroxide, Aluminum Hydroxide gel, Magnesium trisilicate, Magaldrate Simethicone (deforming agent) containing Antacids, Calcium carbonate containing antacid mixtures

Drug Facts/ Información del Medicamento

Active Ingredients/ Ingredientes Activos **Purpose / Propósito**
In each pack / En cada empaque

Aluminum Hydroxide / Hidróxido de Aluminio gel seco 0.18g.....	
Bismuth Subcarbonate / Subcarbonato de Bismuto 0.21g	
Calcium Carbonate / Carbonato de Calcio 0.25g.....	.....Antacid
Magnesium Carbonate / Carbonato de Magnesio 0.59g.....	
Sodium Bicarbonate / Bicarbonato de Sodio 2.27g.....	

Uses / Uso

■For the relief of heartburn, sour stomach, and acid indigestion / Para el alivio de reflujo, acidez estomacal, y indigestión ácida.

Directions / Instrucciones

Add packet (3.5g) to a cup of water after each meal taken, maximum of 6 doses in 24 hours or as directed by physician. / Mezclar un paquete (3.5g) con un vaso de agua tres veces al día después de comer. No usar mas de 6 dosis dentro de 24 horas, o como le aconseje su médico.

Other information/Otra información

■In each pack/en cada empaque:
Calcium/Calcio 95mg, Magnesium/Magnesio 145mg, and Sodium/Sodio 605mg.

Inactive Ingredients/Ingredientes Inactivos

Colloidal silicon dioxide, glycerine anyhydrous,
perppermint oil/Dioxido de silicio coloidal, glicerina anhidra, aceite escencial de menta piperita.

Antacid Powder Antiacido en polvo
LIMSA

Estomarol®

This Unit Not labeled For Retail Sale. This label does not contain complete drug facts information. For complete information, please see outer carton. / esta unidad no es para la venta individual. Esta etiqueta no contiene toda la información sobre el producto. Para mayor información por favor referirse a la caja.

NetWeight 0.12oz(3.5g)/
Contenido Neto 0.12 Onzas

1. Antacids :- SB, ALHG, ALP, CC, MG, MT, MO, COAP.

A)SODIUM BICARBONATE.

Chemical formula :-$NaHCO_3$

Mol wt :-84.01

Common name :-Baking Soda, Mitha Soda

Synonym :-Sodium Hydrogen Carbonate.

Preparation:-

A) Industrial Scale:-

prepared by Solvay process.

- Brine Sol (NaCl) is saturated with ammonia to rem
- Sol is filtered & passed through carbonating towe o enhance ppt.
- $NaHCO_3$ is less soluble in water below 15°C the ppt is filtered & dried

$H_2O + CO_2 \longrightarrow H_2C03$

Water Carbonic acid

$NH3 + H_2C03 \longrightarrow NH_4C03$

Carbonic acid ammonium Carbonate

$NaCl + NH_4C03 \longrightarrow NaHCO3 + NH_4Cl$

ammonium Carbonate Sodium biCarbonate

B) LAB SCALE :-prepared by passing through CO_2 gas through sol of NaOH .

Sol is conc to obtain the product.

$$2NAOH + CO_2 \longrightarrow Na_2C03 + H_2O$$

$$2Na_2C03 + H_20 + CO_2 \longrightarrow 2NaHCO_3$$

Properties:-

Colour:-White

Nature:-Amorphus

Taste:-Saline

Solubility:-Soluble in water insoluble in alcohol.

Incompatibility:- It inhibits the absorption of tetracycline from the gastrointestinal tract

Storage: it should be stored in well-closed containers.

USES:-

1) Sodium bicarbonate **was** used as an antacid to reduce excessive acidity of gastric juice. sharp Increase in gastric pH 7 Neutralizes HCL but results in the production of CO_2 causing belching and flatulence, is not desirable.

2) treatment of systemic acidosis.

3) It is used as electrolyte replenisher.

4) 5% solution of sodium bicarbonate is used as ear drops to an remove ear wax.

5) 3.5% solution warm water is used as eye lotion.

6) Ingredients in Compound Sodium Bicarbonate .

7) It is also an ingredient in many effervescent antacid preparation.

NOTE:-Cardio vascular patients or those on sodium restricted diet . advised to **avoid this antacid**.

Usual dose :-300 mg to 2 g four times a day.

2) ALUMINIUM Hydroxide Gel:-$Al(OH)_3$

Synonym:- Aluminium Hydroxide/Hydrate Powder

Colour:-White

Nature:-Amorphus

Taste:- Tasteless

Solubility:-Soluble in mineral acids i

Chemical:-

When Heated To Redness (Ignited) it

Aluminium Oxide.

$Al(OH)_3 \longrightarrow Al_2O_3 + H_2C$

It reacts readily with acids, such as H minium salts.

$Al(OH)_3 + 3HCl \longrightarrow AlCl_3$

Uses:-

1. As an Antacid(Above Reaction)

Imp Note:-Acts as antacid not by neutralization

but by physical adsorption of acid. When gel passes into the intestine the acid is released.

2. In treatment of Phosphate Urinary Calculi $Al(OH)_3$ finally forms Aluminium phosphate in intestine, thereby reducing phosphate absorption.

3. Externally As mild astringent & desiccant.

4. Infrequently as protective in treatment of diarrhea & cholera.

5. Externally as dusting powder for desiccating foul wounds.

6. In Silicosis therapy, Given by inhalation.

Storage:- In Airtight container at temp not exceeding 25.°C but not to be freezed.

Aqueous Suspension of Hydrated Aluminium containing

(1) aluminium carbonate

(2) and contains not less than 3.5% w/v and not more than 4,4% w/v of aluminium oxide ($AI_2 0_3$).

(3) sodium benzoate as preservative (antimicrobial agent),

(4) oil of Mentha as a flavoring agent

(5) and glycerin or sucrose as sweetening agent.

Properties: White Viscous Suspension . Clear Liquids gets Separated when it is Kept Standing For some time.

Advantages

1)Non absorbale.no evolution of CO_2

2) This allows the gastric PH at just about the desires level.

Disadvantage

1)Loss of Antacid property on aging- Particularly with Dried Aluminium Hydroxide.

2) It also have adsorbent properties:-Caution should be exercised in co-administration with other drug simultaneously.

Preparation:-

- A hot sol of potash alum is added slowly with constant stirring to a hot sol of sodium carbonate after complete removal of C02,
- The precipitated aluminium hydroxide is filtered, washed thoroughly with hot water until it becomes free from sulphate ions.
- The precipitate may be suspended in the purified water to required strength of aluminium hydroxide gel.

Assay:-performed by gravimetric method as per IP.

Procedure:-

1)Weigh about 5gm + add 10ml of HCL + 100ml water .

2)methyl orange is used as an indicator & sufficient amount of dil NH_3 sol is added to it.

3) End point:- distinct yellow color.

4) Heat to boil & filter wash with 2.5% w/w solution of ammonium nitrate until the ppt is free from Cl dry the ppt at 120°C & weigh it.

Aluminium Phosphate

Synonym:- Dried Aluminium Phosphate

Chemical formula :-$AlPO_4$

Physical properties

Colour :-White

Nature:- powder containing suitable freiable aggregate.

Solubility:- Practically insoluble in water ethanol&

Soluble in dil mineral acids.

It also contain preservatives, flavoring & sweetening agent.

Uses:- As an Antacid: on absorbable, useful where loss of phosphate may create problem to patient.

(Endogenous phosphates spared)

MEDI-FIRST
Antacid Calcium Carbonate
Antiácido
Carbonato de Calcio
Calcium Rich Chewable Tablets
Tabletas Masticables, Ricas en Calcio
Tamper Evident Unit Dose Packets
Empaquetado con Sellado Evidente en Dosis Unitarias
250 Tablets (125 x 2)
MEDI-FIRST®
Antacid Calcium Carbonate
Antiácido
Carbonato de Calcio
Pull to Open
Calcium Rich Chewable Tablets
Tabletas Masticables, Ricas en Calcio
Compare active ingredient to:
Tums®
Tamper Evident Unit Dose Packets
Empaquetado con Sellado Evidente en Dosis Unitarias
Información del Medicamento
ANTACID

Chemical Properties

Water Solubility increase in presence of CO_2& ammonium salts due to formation of bicarbonate & free calcium ion respectively.

$$CaCO_3 + H_2CO_3 \longrightarrow Ca(HCO_3)_2$$

$$CaCO_3 + 2NH_4 \longrightarrow Ca + 2NH_3 + H_2O + CO_2$$

Calcium Carbonate Neutralizes acid by effervescence.

Physical Properties

Colour:-White microcrystalline powder.

- Odour :- odourless,
- Nature:-Amorphus
- Taste:-tasteless
- Solubility:-insoluble in water alcohol .

USES:-

- As an Antacid. It neutralizes gastric acid& forms CaCl2
- Which gets converted in calcium salt which is insoluble & tends to be constipating
- It is used as an dentifrice because of its mild abrasive action.

Disadvantage:-

1. The Ca^{2+} ions can be either absorbed systemically or precipitated as calcium phosphate salt in the intestine or as insoluble calcium soaps (formed from the glycerides resulting from the hydrolysis of digested food containing oils and fats).
2. Burnett syndrome (commonly known as milk-alkali syndrome), characterised by an acute alkalosis, hypercalcaemia, hyperphosphatemia, renal failure and azotemia.
3. Though insoluble can cause systemic alkalosis.

- This syndrome is often is misunderstood as hyperparathyroidism.
- On account of calcium's constipating effect, calcium antacids are preferred in combination with magnesium antacid which have cathartic action.
- However, they should not be used in patients with hypertension, renal insufficiency, a history of urinary calculi, gastrointestinal hemorrhage or dehydration an electrolyte imbalance due to excessive loss of fluids and electrolytes consequent to diarrhea, vomiting or aspiration of gastric contents

    - Usual dose 1 g, 4 to 6 times a day.
    - Usual dose range 1 to 10 g

MAGNESIUM CONTAINING ANTACIDS

- magnesium salts e.g. magnesium carbonate magnesium citrate, magnesium oxide, magnesium phosphate and magnesium trisilicate are common antacids.
- Magnesium antacids are practically insoluble in water, but dissolve slowly in acidic media due to neutralization reaction Except magnesium trisilicate, they all act in the same manner.
- Mg^{2+} ion is not readily absorbed from GIT because its absorption is retarded by alkaline media, thus, magnesium salts absorption takes place only in the acid medium of the duodenum.

Due to the slow absorption of Mg+ ions, a saline laxative action occurs

- Most of the magnesium is excreted in the faeces as Insoluble magnesium salt.

- A small amount of Mg^{2+} ions are also absorbed which are excreted by the kidneys.
- On account of this, magnesium salts are not to be used in patients with renal insufficiency because magnesium retention leading to **magnesium poisoning** may occur.
- Magnesium antacids are practically insoluble in water, but dissolve slowly in acidic media due to neutralization reaction.

Magnesium carbonate

- It is a hydrated basic magnesium carbonate of varying composition.
- It exists in two forms viz.
- Heavy Magnesium Carbonate,
- and Light Magnesium Carbonate.
- Physical Properties

Colour:-Light, White, friable mass or as a bulky white

Odour :- Odourless,

Taste:-Tasteless

Solubility:-Insoluble in water alcohol but dissolves in dilute acids with effervescence

Usual dose range:-500 mg to 2 g four times daily.

USES:-

- As an antacid
- Abrasive in tooth powder
- Magnesium carbonate also finds its use as saline cathartic when given in adequate dosage (8 g).
- also used as an abrasive in some tooth powders, as a cosmetic and as an ingredient in silver polishes.

Storage:- In air tight container.

MAGNESIUM OXIDE

Chemical formula :-MgO

Synonym:- Magnesia

Mol Wt:- 40.30

Properties :

- Magnesium oxide Occur in two different varieties commonly known as "light" magnesium oxide and "heavy" magnesium oxide.
- On exposure to air, it absorb carbon dioxide and forms basic carbonates, also absorbs moisture.
- Light magnesium oxide hydrates more easily than heavy Mg(OH)2 oxide and will dissolve readily in water & is clearly alkaline & phenolpthalein.carbon dioxide or acid.
- $MgO + 2H_20 \longrightarrow Mg(OH)_2 + H_20$

Magnesium hydroxide so formed goes into the sol & reacts gastric HCl

$Mg(OH)_2 \longrightarrow Mg^{2+} + 2OH^-$

$\downarrow 2H_3O^+$

$\downarrow 4H_2O$

$Mg(OH)_2 + 2HCl \longrightarrow MgCl_2 + H_2O$

Storage:- It should be stored in airtight containers.

Uses:- low dose works as an antacid and higher dose laxative.

Usual dose an Antacid 250 mg 4 times daily; Laxative: 4 g.

Usual dose range 250 mg to 4g daily.

A mixture of magnesium oxide, tannic acid & charcoal is well know as universal antidote.

MAGNESIUM TRISILICATE

Chemical Formula:-(2Mg0.3Si0 2.xH_2O) or $(MgSiO_3)_2SiO_2.xH_2O$

Mol wt : Anhydrous 260.86

Physical Properties:-

Colour:- White

Odour:- Odourless

Taste :- Tasteless

Solubility:-Insoluble in water alcohol but readily decomposed by mineral acids with HCL forms Magnesium Chloride & gelatinous trisilic acid

Storage:- It should be stored in airtight containers.

Magnesium trisilicate when treated with acid

E.g.. HCl 'decomposes to form and gelatinous trisilicic acid.

$(MgSIO_3)_2SiO2 \cdot XH_2O + 4HCl \longrightarrow 2MgCl_2 + 2H_2Si0_3 + SiO_2 + {}_XH2O$

Colloidal mixture

(gelatinous)

1. In the stomach, a similar reaction takes place with HCl & gelatinous mass is formed with gastric content which also works as protective & adsorbent
2. The colloidal silicate protects the ulcer from further acid & peptic attack and possibly adsorbs the pepsin.

Usual dose 1 g 4 times a day.

Usual dose range 1 to 16 g a day.

- Magnesium trisilicate powder is an emulsifying agent for mineral, vegetable and animal oils when used in proper proportions.

Importance of combination preparations of antacid-

No single antacid meets all requirements for ideal antacid hence Antacids are used in combination.

Antacids are used in combination for following reasons-

1. To balance constipating effect of Aluminum and Calcium containing antacids with laxative effect of Magnesium containing antacids.

2.Some antacids gives rapid action but for short duration while some antacids gives delayed action but for longer duration. So to get quick and longer duration of action antacids are used in combinations.

3. Few combination antacids contain Simethicone, Dimethicone as they are Antiflatulents or antifoaming agents and reflux suppressants like alginic acid.

Examples of Combined Antacids-

1. Combination of Aluminium hydroxide gel and Magnesium hydroxide
2. Combination of Aluminium hydroxide gel and Magnesium trisilicate
3. Combination of Aluminium hydroxide gel and Calcium carbonate
4. Combination of Aluminium hydroxide gel, Calcium carbonate and Magnesium hydroxide
5. Sodium bicarbonate and alginic acid
6. Simethicone containing antacids
7. Magaldrate- $Mg(OH)_2$ & $Al(OH)_3$
8. Aluminum hydroxide gel & kaolin combination etc.

Protective & adsorbents

- Def:- Inert sub Treat mild Diarrhea dysentery, GI disturbance associated with indigestion. Adsorbs gases toxins, bacteria.
- Protective layers on painful ulcers.
- Dysentery:- Frequent elimination of watery fluid with or without mucus/blood
- Causes:-Infection (Amoeba)
- Diarrhea:- Frequent elimination of intestinal contents in form of watery fluids .Electrolyte imbalance
- Causes. Improper digestion bacterial infection improper digestion of food bacterial toxins chemical or poisonous drug.
- Action:-
- Bacteria, bacterial toxins stimulate flow of electrolyte into intestine causing watery stools.

Protective & Adsorbents- Adsorb bacteria & Bacterial toxins .

They are adsorbents insoluble salts. therefore forms protective coating on mucosal membrane & provide mechanical protection .

Note:- Antibacterial drug should be taken along with P & A if diarrhea is due to bacterial infection.

P & A do not have antibacterial activity.

Three different classes namely.

1) Bismuth salts or Bismuth Containing Product:-. Bismuth Subnitrate, Bismuth Sub carbonate Milk of Bismuth, Bismuth subgallate, Bismuth Subsalicylate, Bismuth Ammonium citrate.
2) Activated Clays and other Adsorbents e.g. Light Kaolin
3) Activated Charcoal.

• Bismuth Containing Products

- Oral Administration of bismuth-containing' results in **black stool** due to the action of intestinal hydrogen sulphide to bismuth sulphide.
- Bismuth compounds to their toxicity coupled with discovery & availability of newer & more effective drugs the been gradually withdrawn from the medicinal practice .

BISMUTH SUBCARBONATE

Chemical formula Approx. $(BiO)_2 CO_3)_2. H_2O$

Synonym :-Basic Bismuth Carbonate

Properties

Colour:- white or pale-yellow

Odour :- odourless

Taste:- tasteless powder

Storage:-light or well-closed light resistant containers.

Solubility:-It is practically insoluble in water and in alcohol but dissolves completely in HCl and nitric acid with effervescence and forms corresponding salts.

Uses:-

- as mild antacid,
- protective, mild astringent, dusting powder and antiseptic.
- it was earlier used internally in gastric disorders, ulcers, diarrhea, dysentery, ulcerative colitis etc. as protective.

•It is still used as topical protective in lotion and ointments.

Note:-

On account of **systemic toxicity & availability of safer drugs** it is deleted from pharmacopoeia.

ACTIVATED CLAYS AND OTHER ADSORBENTS

- This group is mostly composed of clays which have excellent adsorbent properties and most of them are used for that purpose industrially as well. They appear to have a definite clinical use, at least in mild diarrhea of short duration.

Light Kaolin

Chemical formula Approx:. $Al_20_3.2SiO_2 \cdot 2H_2O$

Properties

colour:-white yellowish-white powder

Nature:-unctous to touch

Odour:- Clay like odour

Taste:- clay like earthy

Solubility:-insoluble in water, Dil acids, or in alkali hydrooxide.

Chemically it is inert, since it is insoluble in all the common solvents.

Uses:-

- Kaolin is used internally for its adsorptive properties as well as its ability to coat irritated intestinal mucosa.
- Used in diarrhea, enteritis, colitis & dysentery caused by food or alkaloidal poisoning
- It helps in by absorbing gases, toxins & bacteria .
- Usual dose:-15 to 17 g in three divided doses
- Infants:-1g one to three times daily
- Children:-(1-5years) 2g one to three times daily.

Externally as dusting powder, adsorbent in ulcers .

Cathartics

- Def:_Drug that brings about defecation & relieves a person from individual from constipation.

Constipation:- Inability to defecate intestinal contents for a period

Causes:- Weakness of intestine, intestinal spasm, injury, use of certain drugs, dietary habits, emotional factors, less water/ fluid intake

Purgatives:- Also cathartics, act similarly but mild in action.

Laxatives:- Mild type of Purgative

Define Cathartics.(1Marks) Classify with examples.(3Marks)

Ans.**Cathartics are the agents that quicken & increase evacuation from bowels & also drugs used to** relieve constipation.

Classification:

The cathartics can be considered under the following class,

1.**Mild purgatives or laxatives** : It helps to promote defaecation causing minimum adverse effects.

Drugs included in this group are:

a)Bulk –producing drugs: Which promote evacuation by increasing the stools bulk-volume & water contents e.g. Isapgol, agar-agar, methycellulose, bran, psyllum seed, sodium carboxymethylcellulose & karaya gum.

b)Stool softners (Emollient): Which penetrate ,lubricate & soften the stool e.g. D-octyl sodium sulphosuccinate, liquid paraffin.

2.Strong Purgatives: It cause complete evacuation of the bowel & the bowel become inactive.

There are two kinds of strong purgatives:

a)Irritant or stimulant purgatives e.g. Phenolphthalein, senna glycosides ,aloe, cascara sagrada, Rhubarb extract, Podophyllin, castor oil, bisacodyl.

b)Saline cathartics/Purgatives (osmatic laxatives) these are further sub classified as under:

i)Sodium-containing products e.g. Sodium biphosphate, sodium phosphate, potassium sodiumTartrate

ii)Magnesium-containing product e.g. magnesium hydroxide, magnesium citrate, Magnesium sulphate

iii) Sulphur as cathartic

iv)Non official cathartics e.g. sodium sulphate , potassium phosphate, potassium bitartrate,calomel.

Properties & uses of magnesium sulphate:-

Chemical Formula:-$MgSO_4.7H_2O$

Synonyms:-Epsom Salt, Mag Sulph

- **Properties :**
- It occurs as colorless crystals, with cool, saline and bitter taste.
- It is freely soluble in water, sparingly soluble in alcohol and dissolves slowly in glycerin.
- **Uses :**
- Magnesium sulphate is given orally in dilute solutions as a cathartic & parenterals as an anticonvulsant.
- Given as enema
- Used in cholecystitis
- Ingredient of Mineral water

SODIUM POTASSIUM TARTRATE

Synonym:- Potassium Sodium tartrate, Rochelle Salt,

Seignette's Salt.

Properties:- white colorless

nature:-crystalline powder,

odour :-odourless,

Taste:-cooling saline taste and

Solubility:-. It is freely soluble in water and practically insoluble in alcohol.

and sodium carbonates.

Uses:-

1. It is a saline purgative; depending upon dose, its action ranges from mild laxative to active hydragogue.
2. Ingredient of compound Effervescent powder.
3. The tartrate anion has very poor intestinal permeability and causes a watery evacuation of bowel after 1 to 2 hours without producing irritation.
4. Also used as food additive.

GLYCOSIDES

- Glycosides, defined as the organic compounds from plant or animal sources, which on enzymatic or acid hydrolysis give one or more sugar moieties alongwith non sugar moiety.
- The former is called as glycone and the later is aglycone or genin.
- The glycone can consist of a single sugar group (monosaccharide) or several sugar groups (oligosaccharide).
- The sugars found in glycosides may be glucose and rhamnose (monosaccharides) or, more rarely, deoxysugars such as the cymarose found in cardiac glycosides.

Properties

- In plants glycosides are both synthesized and hydrolysed under the influence of more or less specific enzymes.
- They are crystalline or amorphous substances that are soluble in water or alcohols and insoluble in organic solvents like benzene and ether.
- The aglycone part is soluble in organic solvents like benzene or ether.
- They are hydrolysed by water, enzymes and mineral acids. They are optically active.
- Glycosides do not themselves reduce Fehling's solution, the simple sugars which they produce on hydrolysis will do so with precipitation of red cuprous oxide.
- The sugars present in glycoside are of two isomeric forms, that is, α form and β form, but all the natural glycosides contain β-type of sugar.

Classification

- The glycosides can be classified by the glycone, by the type of glycosidal linkage, and by the aglycone.
- **On the Basis of Glycone**
- If the glycone group of a glycoside is glucose, then the molecule is a glucoside; if it is fructose, then the molecule is a fructoside; if it is glucuronic acid, then the molecule is a glucuronide, etc.
- **On the Basis of Glycosidic Linkage**
- **1. O-glycosides:** Sugar molecule is combined with phenol or –OH group of aglycone, for example, anthraquinone glycosides like sennosides etc

- **N-glycosides:** Sugar molecule is combined with N of the –NH (amino group) of aglycon, for example, nucleosides
- **S-glycosides:** Sugar molecule is combined with the S or SH (thiol group) of aglycon, for example, Sinigrin.
- **C-glycosides:** Sugar molecule is directly attached with C—atom of aglycon, for example, Anthraquinone glycosides like Aloin, Barbaloin, Cascaroside and Flavan glycosides, etc.

On the Basis of Aglycone

S. No.	Class	Examples
1.	Anthraquinone glycosides	Senna, Aloe, Rhubarb, etc.
2.	Sterol or Cardiac glycosides	Digitalis, Thevetia, Squill, etc.
3.	Saponin glycosides	Dioscorea, Liquorice, Ginseng, etc.
4.	Cyanogenetic and Cyanophoric glycosides	Bitter almond, Wild cherry bark, etc.
5.	Thiocynate and Isothiocynate glycosides	Black mustard
6.	Flavone glycosides	Ginkgo
7.	Aldehyde glycosides	Vanilla
8.	Phenol glycosides	Bearberry
9.	Steroidal glycosides	Solanum
10.	Bitter and Miscellaneous glycosides	Gentian, Picrrohiza, Chirata, etc.

DISTRIBUTION

- Glycosides are the class of compounds abundant in nature. Some plant families containing important glycosides are listed bellow:
- 1. Scrophulareaceae (*Digitalis purpurea*).
- 2. Apocyanaceae (*Nerium oliander* and *Thevetia peruviana*).
- 3. Liliacea (*Urgenea indica* and *U. maritima*, *Aloe vera*)
- 4. Leguminocae (*Cassia angustefolia*, *Gly-cyrrhiza glabra,*)
- 5. Dioscoreaceae (*Dioscorea floribunda*)
- 6. Rosaceae (*Prunus amygdalus, Carategus oxycantha*)
- 7. Cruciferae (*Brassica* sp.)
- 8. Gentianaceae (Gentian and Chirata)
- 9. Acanthaceae (Kalmegh)
- 10. Simarubaceae (Quassia)
- 11. Umbelliferae (*Ammi majus, Ammi visnaga*)
- 12. Rutaceae: Citrus sp. (*Ruta graveolens*)
- 13. Polygonaceae (Fagopyrum sp.)
- 14. Myrtaceae (Eucalyptus sp.)

CHEMICAL TESTS

- Generally, aqueous or alcoholic extracts of crude drugs are tested with specific reagents for presence of various types of glycosides.
- **Chemical Tests for Anthraquinone Glycosides**
- ***Borntrager's test***
- To 1 gm of drug add 5–10 ml of dilute HCl boil on water bath for 10 min and filter. Filtrate was extracted with CCl_4/ benzene and add equal amount of ammonia solution to filtrate and shake. Formation of pink or red colour in ammonical layer due to presence of anthraquinone moiety.
- ***Modified borntrager's test***
- To 1 gm of drug, add 5 ml dilute HCl followed by 5 ml ferric Chloride (5% w/v). Boil for 10 min on water bath, cool and filter, filtrate was extracted with carbon tetra-chloride or benzene and add equal volume of ammonia solution, formation of pink to red colour due to presence of anthraquinone moiety. This is used C-type of anthraquinone glycosides.

- **Chemical Tests for Saponin Glycosides**
- ***Haemolysis test***
- A drop blood on slide was mixed with few drops of aq. Saponin solution, RBC's becomes ruptured in presence of saponins.
- ***Foam test***
- To 1 gm of drug add 10–20 ml of water, shake for few minutes, formation frothing which persists for 60–120 s in presence of saponins.
- **Chemical Tests for Steroid and Triterpenoid Glycosides**
- ***Libermann burchard test***
- Alcoholic extract of drug was evaporated to dryness and extracted with $CHCl_3$, add few drops of acetic anhydride followed by conc. H_2SO_4 from side wall of test tube to the $CHCl_3$ extract. Formation of violet to blue coloured ring at the junction of two liquid, indicate the presence of steroid moiety.
- ***Salkowaski test***
- Alcoholic extract of drug was evaporated to dryness and extracted with $CHCl_3$, add conc. H_2SO_4 from sidewall of test tube to the $CHCl_3$ extract. Formation of yellow coloured ring at the junction of two liquid, which turns red after 2 min, indicate the presence of steroid moiety.

- **Chemical Tests for Cardiac Glycosides**
- ***Keller-kiliani test***
- To the alcoholic extract add equal volume of water and 0.5 ml of strong lead acetate solution, shake and filter. Filtrate was extracted with equal volume of chloroform. Chloroform extract was evaporated to dryness and residue was dissolved in 3 ml of glacial acetic acid followed by addition of few drops of $FeCl_3$ solution. The resultant solution was transferred to a test tube containing 2 ml of conc. H_2SO_4. Reddish brown layer is formed, which turns bluish green after standing due to presence of digitoxose.
- ***Legal test***
- To the alcoholic extract of drug add equal volume of water and 0.5 ml of strong lead acetate solution, shake and filter. Extract filtrate with equal volume of chloroform and the chloroform extract was evaporated to dryness. Dissolve the residue in 2 ml of pyridine and sodium nitropruside 2 ml was added followed by addition of NaOH solution to make alkaline. Formation of pink colour in presence of glycosides or aglycone moiety.

- ***Baljet test***
- Thick section of leaf of digitalis or the part of drug containing cardiac glycoside, when dipped in sodium picrate solution, it forms yellow to orange colour in presence of aglycones or glycosides.

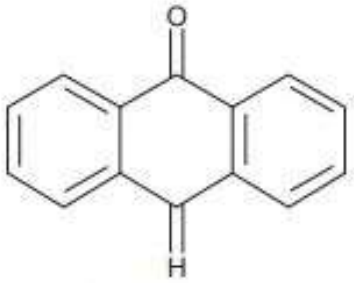
ISOLATION

- **Stas-Otto Method**
- The drug containing glycoside is finely powdered and the powder is extracted by continuous hot percolation using soxhlet apparatus with alcohol as solvent.
- During this process, various enzymes present in plant parts are also deactivated due to heating.
- The thermolabile glycosides, however, should be extracted at temperature preferably below 45°C.
- The extract is treated with lead acetate to precipitate tannins and thus eliminate nonglycosidal impurities.
- The excess of lead acetate is precipitated as lead sulphide by passing hydrogen sulphide gas through solution.

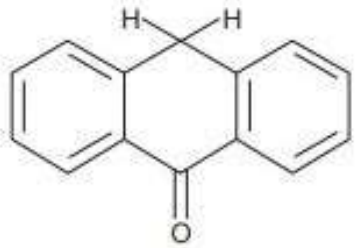
- The extract is filtered, concentrated to get crude glycosides. From the crude extract, the glycosides are obtained in pure form by making use of processes like fractional solubility, fractional crystallization and chromatographic techniques such as preparative thin layer and column chromatography.
- The characterization of isolated purified compounds is done by IR, UV, visible, NMR and mass spectrometry and elemental analysis.

ANTHRACENE GLYCOSIDES

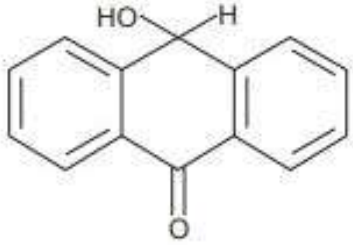
- Anthracene glycosides are chiefly found in dicot plants but to some extent it is also found in monocot and lower plants.
- It consists of glycosides formed from aglycone moieties like anthraquinones, anthranols, anthrones or dimers of anthrones or their derivatives.
- Anthrones are insoluble in alkali and do not show strong fluorescence with them, while anthronols which are soluble in alkali show strong fluorescence.
- The reduced anthraquinones are biologically more active.
- Anthroquinones that are present in fresh drugs are in reduced form, which on long storage get oxidized and hydrolysed .
- Glycosides of reduced derivatives are more active than oxidized aglycones. This is due to the fact that sugars take the glycosides to the site of action and thus are more active.



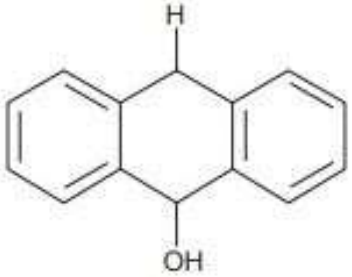
Anthraquinone



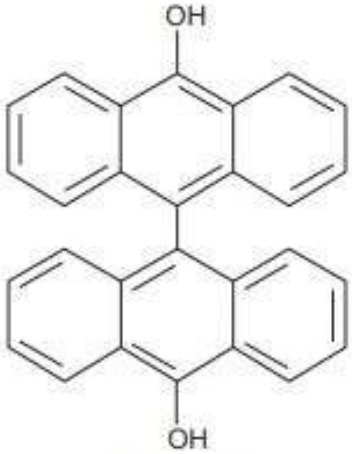
Anthrone



Oxanthrone



Anthranol



Dianthranol

- Anthraquinone is an aromatic organic compound and a derivative of anthracene.
- It has the appearance of yellow or light grey to grey-green solid crystalline powder.
- Its chemical formula is $C_{14}H_{8}O_{2}$.
- It melts at 286°C, boils at 379.8°C.
- It is insoluble in water or alcohol, but dissolves in nitrobenzene and aniline.
- It is chemically fairly stable under normal conditions.
- Anthraquinone naturally occurs in some plants (e.g. aloe, senna, rhubarb and cascara), fungi, lichens and insects, where it serves as a basic skeleton for their pigments.
- Natural anthraquinone derivates tend to have laxative effects.
- These glycosides are characterized by a chemical test, known as Borntrager test
- Most of the glycosides are O-glycosides.

SENNA

- **Synonyms**

  Alexandrian senna, Tinnevelly senna, Folia senna, Sonmukhi.

- **Biological Source**

  Senna leaf consists of the dried leaflets of *Cassia acutifolia* known as Alexandrian senna and *C. angustifolia* which is commercially known as Tinnevelly senna.

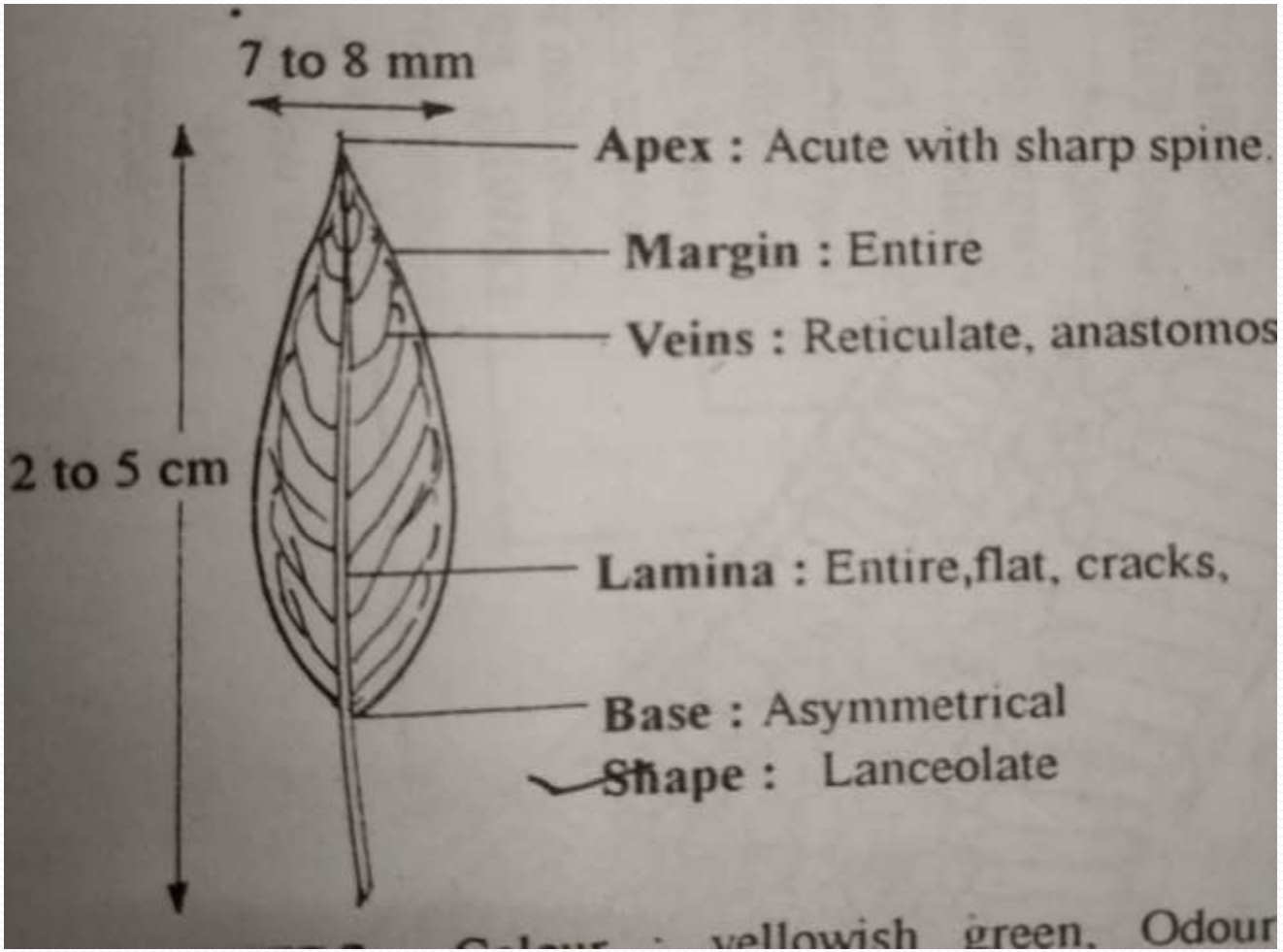
  It belong family Leguminosae.

- **Geographical Source**

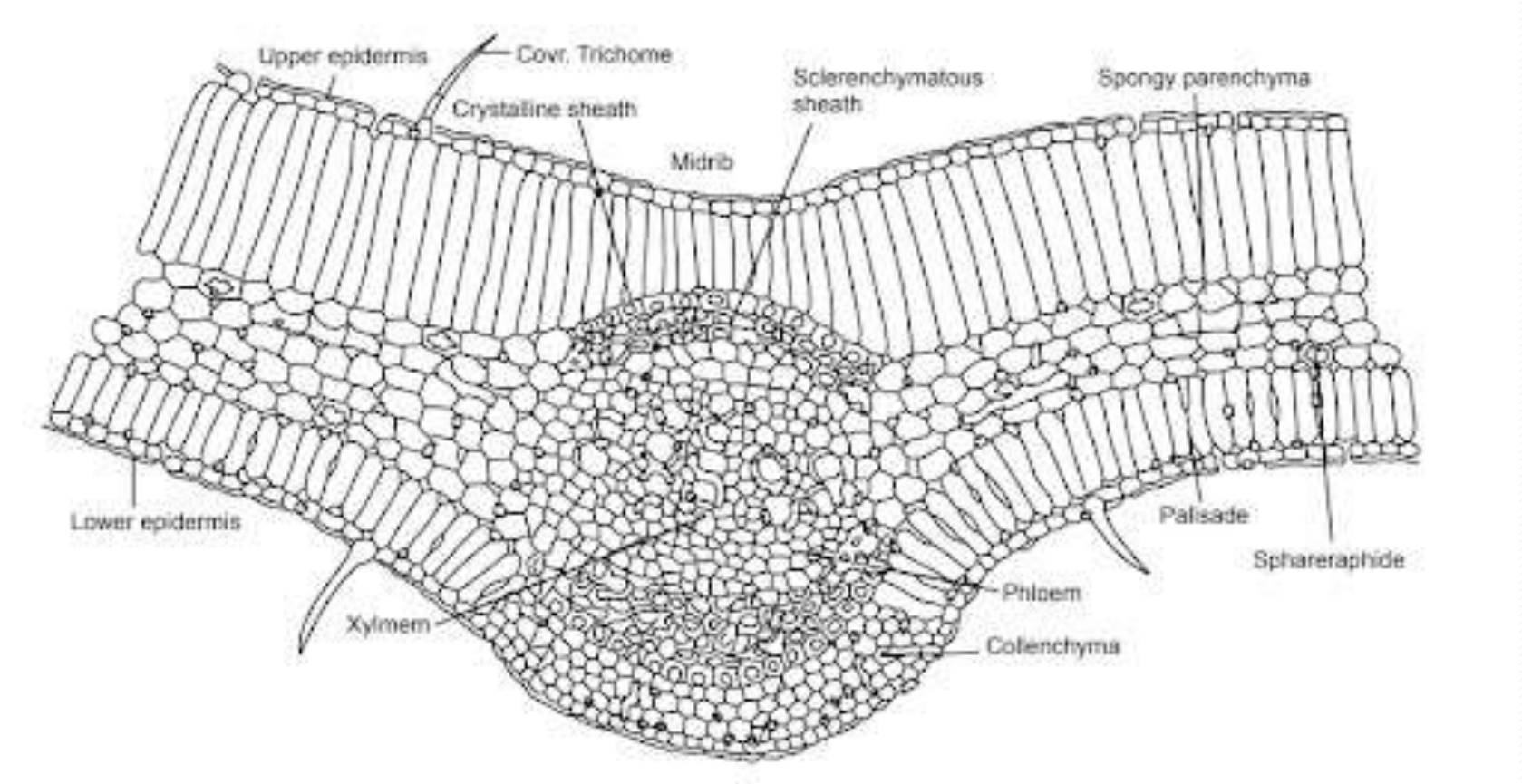
  Alexandrian senna is indigenous to South Africa.

  Indian or Tinnevelly senna is cultivated largely in Tinnevelly and Ramnathpuram districts of Tamilnadu. It also grows in Sindh and Punjab region.

Morphology

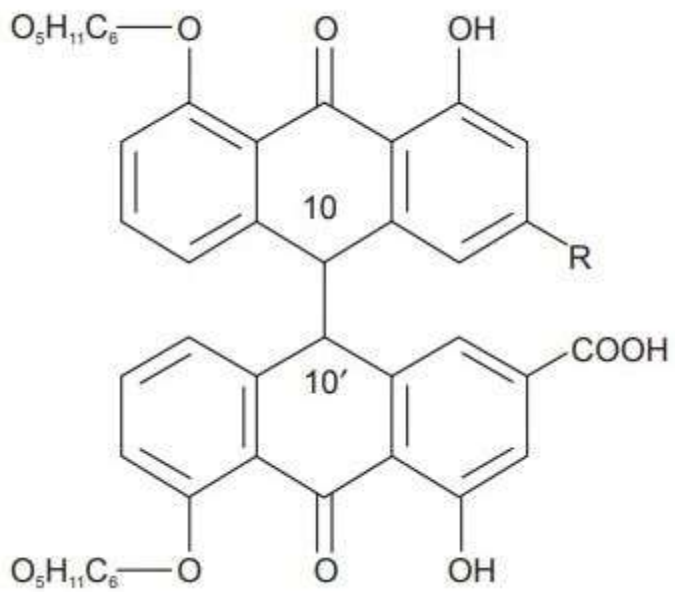

7 to 8 mm
2 to 5 cm
Apex : Acute with sharp spine.
Margin : Entire
Veins : Reticulate, anastomos
Lamina : Entire,flat, cracks,
Base : Asymmetrical
Shape : Lanceolate
yellowish green, Odour

Microscopy


Upper epidermis
Covr. Trichome
Crystalline sheath
Midrib
Sclerenchymatous sheath
Spongy parenchyma
Lower epidermis
Palisade
Sphareraphide
Phloem
Xylmem
Collenchyma

Chemical constituents

- Senna contains sennosides A and B (2.5%) based on the aglycones sennidin A and B, sennosides C and D which are glycosides of heterodianthrones of aloe-emodin and rhein
- Others include palmidin A, rhein anthrone and aloe-emodin glycosides.
- Mucilage is present in the epidermis of the leaf and gives red colour with ruthenium red.



Glycoside	10 - 10′	R
Sennoside A	trans	COOH
Sennoside B	meso	COOH
Sennoside C	trans	CH_2OH
Sennoside D	meso	CH_2OH

USES

- Senna leaves are used as laxative.
- It causes irritation of large intestine and have some griping effect. They are prescribed along with carminatives.
- Senna is stimulant cathartic and exerts its action by increasing the tone of the smooth muscles in large intestine.

Adulterants

- *Cassia obovata* (Dog Senna):
- They occur as small pieces with Alexandrian senna but can be easily identified by its obovate shape and obtuse and tapering apex. It has only 1% anthraquinone derivatives.
- *Cassia auriculata* (Palthe senna) Characterized by absence of anthraquinone glycoside, can be identified by treating with 80% sulphuric acid. It gives red colour.
- Bombay or Mecca or Arabian senna a variety of Indian senna have the morphology similar to that of Tinnevelly senna but the leaflets are narrow, more elongated and brownish green in colour

ALOE

- **Biological Source**

  Aloe is the dried juice collected by incision, from the bases of the leaves of various species of Aloe. *Aloe vera* or *Aloe barbadensis or Aloe perryi* and *Aloe ferox* , belonging to family Liliaceae.

- **Geographical Source**

  Aloes are indigenous to East and South Africa, but have been introduced into the West Indies and into tropical countries, and will even flourish in the countries bordering on the Mediterranean.

- ***Curacao aloe***

- It is usually opaque and varies in colour from bright yellowish or rich reddish brown to black.
- Curacoa Aloes possesses the nauseous and bitter taste that is characteristic of all Aloes and a disagreeable, penetrating odour.
- It is almost entirely soluble in 60% alcohol and contains not more than 30% of substances insoluble in water and 12% of moisture.
- It should not yield more than 3% of ash.

- ***Socotrine aloe***

- It may be distinguished principally from Curacao aloes by its different odour., it is yellow-brown to dark-brown in colour and opaque

- ***Zanziber aloe***

➢ Zanziber aloes often very closely resembles Curacao in appearance, and it is brown coloured.

➢ It has a pleasant odour and bitter taste.

- ***Cape aloes***

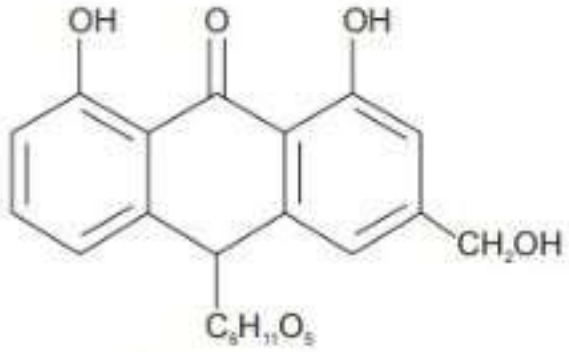
➢ Its translucent and glossy appearance is very characteristic sufficiently distinguish it from all other varieties of Aloes.

Cultivation and Collection

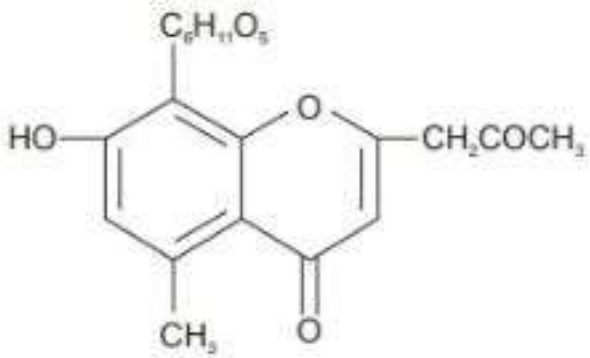
- It is an evergreen perennial growing to 0.8 m to 1 meter.
- The plant prefers light (sandy) and medium (loamy) soils, requires well-drained soil and can grow in nutritionally poor soil.
- It can tolerate drought.
- It is xerophytic plant.
- It can be propagated by seeds. Seeds are sown in the spring.
- The seed usually germinates in 1–6 months.
- The seedlings are transferred to the pots containing well-drained soil.
- They are allowed to grow in sunny part.
- In the second year leaves are collected.
- The leaves are cut near the base, kept inside of kerosene tins and taken them to a central place for the preparation of aloe.
- Juice of aloe is present in parenchymatous cells of pericycle that are mucilage cells.
- In a single incision mucilage cells exert pressure on pericycle cells and the entire juice from the leaves is drained out.

Chemical Constituents

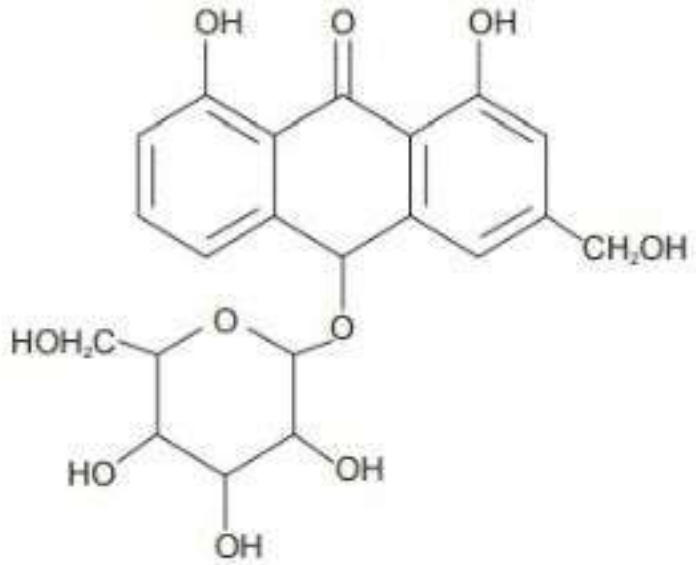
- The most important constituents of Aloes are the three isomers of Aloins, Barbaloin, β-barboloin and Isobarbaloin, which constitute the so-called 'crystalline' Aloin, present in the drug at from 10 to 30%.
- Other constituents are amorphous Aloin, resin, emodin and Aloe-emodin.
- Barbaloin is present in all the varieties; it is slightly yellow coloured, bitter, water soluble, crystalline glycoside.
- Isobarbaloin is a crystalline substance, present in Curacao aloe and in trace amount in Cape aloe and absent in Socotrine and Zanzibar aloe.
- The chief constituents of Socotrine and Zanzibar aloe are Barbaloin and β-Barbaloin.



Barbaloin



Aloesin



Aloin

Chemical Tests

- Boil 1 gm of drug with 100 ml water, allow it to cool; add 1 gm kieselguhr, stir it well and filter through filter paper
- 1. *Borax Test:* Take 10 ml of aloe solution and to it add 0.5 gm of borax and heat; a green coloured fluorescence is produced indicating the presence of aloe-emodin anthranol.
- 2. *Modified Anthraquinone Test:* To 0.1 gm of drug, 5 ml of 5% solution of ferric chloride is added followed by the addition of 5 ml dilute hydrochloric acid. The mixture is heated on water bath for 5–6 min and cooled. An organic solvent (benzene or chloroform) is added and shaken. Separate the organic solvent layer and add an equal volume of dilute ammonia. The ammoniacal layer produces pinkish red colour.
- 3. *Bromine Test:* To 5 ml of aloe solution, add equal volume of bromine solution; bulky yellow precipitate is formed due to the presence of tetrabromaloin.

Uses

- Stimulating purgatives, its action is exerted mainly on the large intestine.
- It is useful as a vermifuge.
- It is emollient, stimulant, stomachic.
- Extracts of the plant have antibacterial activity.
- The clear gel of the leaf makes an excellent treatment for wounds, burns and other skin disorders, placing a protective coat over the affected area, speeding up the rate of healing and reducing the risk of infection.

ALMOND

- **Biological Source**

  Almonds are obtained from the dried ripe seeds of *Prunus amygdalus* var. dulcis (sweet almonds), or *P. amygdalus* var. amara (bitter almonds).

  family- Rosaceae

- **Geographical Source**

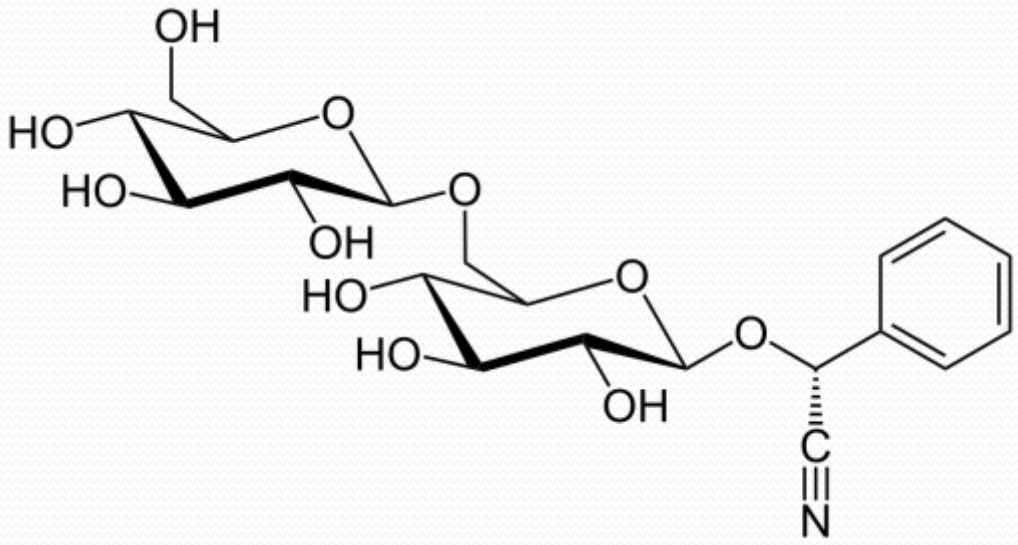
  It is mainly produced from almonds grown in the countries like Italy, France, Syria, Spain and North Africa) and Iran.

Ref. Annals of Plant Sciences 7(6):2363

Chemical constituents

Both varieties of almond contain 40–55% of fixed oil, about 20% of proteins, mucilage and emulsin.

The bitter almonds contain in addition 2.5–4.0% of the colourless, crystalline, cyanogenelic glycoside amygdalin



- Almond oil consists of a mixture of glycerides of oleic (62–86%), linoleic (17%), palmitic (5%), myristic (1%), palmitoleic, margaric, stearic, linolenic, arachidic, gadoleic, behenic and erucic acid.
- Bitter almond oil contains benzaldehyde and 2–4% of hydrocyanic acid.
- Purified volatile oil of bitter almonds has all its hydrocyanic acid removed and, therefore, consists mainly of benzaldehyde.

Uses

- Used as sedative because of its hydrocyanic acid content
- Almond is often eaten on its own, raw or toasted, it is also a component of various dishes.
- Almonds are available in many forms, such as whole, slivered, and ground into flour.
- Almonds are included as a good source of protein among recommended healthy foods.
- A regular consumption of almonds may reduce the risk of heart disease by lowering blood levels of LDL cholesterol

Herbal Cosmetics

Prof. R.Y Patil
PDEAS Shankarrao Ursal College of Pharmaceutical Sciences and Research centre

Hair Care Cosmetics

Medicinal Plants used as Hair Tonic and Hair care

Amla: It consists of fresh or dried fruits of *Emblica officinalis* of family Euphorbiaceae. It is commonly observed in India and cultivated at commercial level in Uttar Pradesh, Gujarat, Rajasthan and Maharashtra. Active principle of amla is 6% of tannins such as gallic acid, ellagic acid, phyllembelin, vitamin C, and minerals such as phosphorus, iron and calcium. It is commonly used as oil or aqueous extract in the hair care formulations like hair oil or shampoo. It acts as hair tonic, stimulates hair growth and prevents premature graying of hairs.

Brahmi: It consists of herb of *Centella asiatica* belonging to family Umbelliferae. It grows well in wet damp and marshy places of north India. Brahmi shows presence of essential oils, sterols, flavonol, glycoside, triterpenoid, saponins etc. Brahmi is used as oil and extract in hair care formulations. It is rasayana drug hence maintain proper body health. It avoids premature graying of hairs and helps to relieve mental fatigue and stress.

Bhringraj: It is obtained from plant *Eclipta alba* Linn of Family Asteraceae. It is an annual or perennial plant grows in moist places throughout India. Plant shows presence of coumestans like wedelolactone and dimethyl wedelolactone; ecliptine alkaloid; β-amyrin glycosides, triterpenic acid and ecalbasaponins steroids etc. Brahmi oil is very good hair tonic and used for getting healthy, black and long hairs.

Henna: It is natural colourant obtained from leaves of *Lawsonia inermis* of Family Lythraceae. Henna leaves are used as natural colour dye in hair formulations. It contains lawsone which give dark intense orange color at basic pH.

Neem: It consists of dried or fresh leaves of plant *Azadirachta indica* belonging to family Melliaceae. The plant is indigenous to all plains in Indian subcontinent. Neem leaves shows constituents such as flavonoids, steroids, terpenoids, sterols and nimbolide. Neem seed and seed oil consists of nimbin, nimbibin, salanin etc. It is used in treatment of ticks, fleas and lice. Neem oil is used in herbal hair oils, hair tonics, shampoos and conditioners.

Hibiscus: It consists of flowers of *Hibiscus rosa sinensis* of family Malvaceae. Hibiscus has constituents such as taraxeryl acetate, β-sitosterol, campesterol, stigmasterol, cholesterol, erogosterol, flavonoids and flavonoid, glycosides, lipids, citric and oxalic acids. It acts as hair tonic, stimulates hair growth and prevents premature graying, hair loss and scalp disorders. It is used as hair conditioner and in hair washes.

Fenugreek: It consists of herb of *Trigonella foenum graecum* of family Leguminoseae. In India it is cultivated in Rajasthan, Gujarat, Uttar Pradesh, Maharashtra and Tamilnadu. Seeds are used in hair care formulations. Fenugreek seed contain alkaloids like Neurin, Trigonelline, Choline, Gentianine; amino acids like Isoleucine, 4-Hydroxyisoleucine, Histidine, Leucine, Lysine; Saponins like Graecunins, fenugrin B, fenugreekine, trigofoenosides A-G, lipids, vitamins and fibers. Fresh fenugreek leaves are hair growth promoter, preserves natural color, keeps hair silky and used in dandruff treatment.

Shikakai: It consists of pods of plant *Acacia concinna* of family Mimosacea. It is observed in plains of central and south India. Shikekai consists of lupeol, spinasterol, acacic acid, lactone, natural sugars, hexacosanol, spinasterone, oxalic acid, tartaric acid, citric acid, succinic acid, ascorbic acid, alkaloids calyctomine and nicotine. Shikakai acts as natural hair cleanser, astringent and detangle hairs. It is used to make hair formulations like soaps and shampoos for hair wash. It promotes hair growth; strengthen hairs and acts as antidandruff agent.

Ginko: It consists of dried leaves of plant *Ginko biloba* belonging to family Ginkgoaceae. It is mainly found in North India. It consists of terpenes, ginkolides, flavanol glycosides and kaemferol. Leaf extract is used in formulations like shampoos and hair lotions.

Volatile oil and fixed oil used in Hair care products

Coconut oil: It consists of fixed oil obtained from kernels of plant *Cocos nucifera Linn*, of family Palmae. Phytoconstituents of coconut oil are lauric, caproic, caprylic, capric, myristic, palmitic, and stearic acid. It has good saponification value hence it is used in shampoos and hair oil.

Eucalyptus oil: It consists of volatile oil obtained from leaves of *Eucalyptus globules* of family Myrtaceae. The volatile oil is composed of cineole, aldehyde, terpenes, alcohol and phenol. It is colourless or pale yellow liquid with characteristic aromatic odour and a pungent, spicy and cooling taste. It acts as anti-dandruff agent.

Rose oil: It is volatile oil obtained from petals of *Rosa damascena* and *Rosa centifolia* of family Rosaceae. The steam-distilled product is called as rose otto while the solvent extracted product is rose absolute. β-damascenone, β-damascone, β-ionone and oxide are main constituents of Rose oil. It is used as perfume.

Basil Oil (Tulsi oil): It is essential oil obtained by steam distillation of leaves and flowering tops of *Ocimum Sanctum* belonging to family Labiatae. The basil oil consists of 1, 8 cineol, linalool, citral, methyl chavicol (estragole), eugenol and methyl cinnamate etc. It is good hair growth promoter.

Lemon oil: It is essential oil obtained from cold expression of peels (fruit) of *Citrus limonum* belonging to family Rutaceae. It is cultivated in north-west region of India. The constituents of lemon oil are α-pinene, camphene, β-pinene, sabinene, myrcene, α-terpinene, linalool, β-bisabolene, limonene, trans-α-bergamotene, nerol and neral. It is potent hair cleaning agent.

Rosemary oil: It is obtained from plant *Rosmarinus officinale* Linn of family Labiatae. It consists of volatile oil, resin, ursolic acid, α-pinene, borneol, β-pinene, camphor, bornyl acetate, camphene, 1,8-cineole and limonene etc. It is good hair growth promoter and used in hair lotions and gels.

Sandal wood oil: It is essential oil obtained from steam distillation of heartwood of *Santalum album*, of family Santalaceae. It grows well in South India, western ghat. The the main constituents of sandalwood oil are 90-97% of sesquiterpenes alcohol (Santalol), hydrocarbons, acids, aldehydes and ketones. It is used as perfume in hair care products.

Sage oil: It is essential oil obtained by steam distillation of dried leaves of *Salvia officinalis Linn* of family Labiatae. The sage oil is composed of α-pinene, cineole, linalyl acetate, thujone, borneol, bornyl acetate, farnesol, and camphor. It is potent anti-dandruff agent.

Jojoba oil: It is fixed oil obtained from cold expression of seeds of *Simmondsia chinensis* of family Simmondiaceae. In India it is cultivated in Rajasthan, Gujarat, Maharashtra, and Tamilnadu. It consists of eicosenoic acid, docosenoic acid and oleic acid. It is potent hair revitalizing agent.

Almond oil: It is fixed oil obtained from cold expression of ripe seeds of plant *Prunus dulcis* of family Rosaceae. It has nourishing, softening, cleansing and hair strengthening property. It contains 40-55% fixed oil, 20% proteins, mucilage etc. The fixed oil composed of oleic acid, linoleic acid, and palmitic acid. It is used in formulations like hair lotion and hair tonic.

Sesame oil: It is fixed oil expressed from ripe seeds of *Sesamum indicum* of family Pedaliaceae. It is cultivated in Gujarat, West Bengal, Rajasthan, Tamilnadu, Orissa, Madhya Pradesh, Andhra Pradesh, Maharashtra, Uttar Pradesh, Punjab and Karnataka. Sesame oil contains palmitic acid, oleic acid, linoleic acid, linolenic acid, stearic acid, arachidic acid, behenic acid and gandoleic acid as major phytoconstituents. It is potent hair tonic and avoids premature graying of hairs.

Olive oil: It is a fixed oil extracted from the fruits of *olea europaea* belonging to family Oleaceae. It is mainly produced at Spain, Italy and Greece. Olive oil contains phytoconstituents like triolein, tripalmitin, trilinolein, tristearate, monosterate, triarachidin, squalene, β-sitosterol and tocopherol. It is skin and hair conditioner and fatty acid penetration enhancer.

Neem oil: It is fixed oil expressed from seeds, kernels or leaves of *Azadirachta indica* belonging to family Meliaceae. It is native of India. Neem oil contains linoleic acid, palmitic acid, stearic acid and oleic acid. It acts as hair tonic and anti-dandruff agent.

Natural Colorants used in Hair care products

Henna: Henna leaves consist of active principle lawsone which is responsible for red orange colour. It is used as non-oxidizing hair dye. Tannins and flavonoids present in henna acts as mordant. Carbohydrates present in Henna helps to bind it on hairs. It also balances pH of hairs hence avoids graying of hairs and avoid hair fall.

Madder: It consists of roots of Rubia tinctorum belonging to family Rubiaceae. It is red coloured dye. The colouring property is due to presence of anthraquinones such as alizarin and purpurin. Madder has anticancer, antimicrobial, antifungal, hypotensive, analgesic, antiseptic, blood purifier and antirheumatic properties.

Chamomile: These are obtained from leaves and flowers of *Matricaria recutita* (German chamomile) and *Chamaemelum nobile* (Roman chamomile) of family Asteraceae. It is responsible for producing yellow coloured shades. Chamomile flower contains active principle apigenin (1,3,4- trihydroxyflavone). The plant is used as softening agent, moisturizing agent and has skin soothing properties. It is also used for indigestion, diarrhea, sleeplessness and sciatica etc.

Fats and Waxes used in hair care products

Light and heavy liquid paraffin: It is colourless or odourless oily liquid consisting of a mixture of hydrocarbons. Due to good spreadibility it is used to formulate bath oils, hair oils, lotions and creams etc.

Waxes: They are the esters formed from condensation of high molecular straight chain fatty acids and high molecular straight chain monohydric alcohol. Waxes forms base of cosmetics. The different waxes used in cosmetics are:

1. **Beeswax:** It is most commonly used purified wax obtained from the honeycomb of bees, *Apis mellifera* of family Apidae. Beeswax contains 70% ester myricyl palmitate. It is solid in nature, yellowish brown in colour, sweet in taste and has characteristic odour. Its melting point is 60 to 65°C.
2. **Carnauba wax:** It is obtained from leaves of plant *Copernica cerifera* belonging to family Palmae. It is light brown to pale yellow in colour, occurs as coarse powder or flakes with characteristic odour and taste. Its melting range is 81-86°C.
3. **Paraffin wax:** It is distillation product of petroleum. It mainly consists of paraffins and their isomers. It is white, transluscent wax, colourless, odourless and greasy to touch. Its melting range is 50 to 57°C.
4. **Spermaceti:** It is wax obtained from *Physester colodon* belonging to family Physeteridae. Spermaceti contains cetyl palmitate and cetyl myristate. It is transluscent, crystalline, pearly white with characteristic odour and taste.

Raw materials used in Oral Hygiene products

Oral hygiene is important part of human health but it is neglected. Oral health problems like dental carries and periodontal diseases are increasing day by day. Dental treatments are usually expensive and utilize mainly analgesics and antimicrobial agents. These agents have several side effects such as alteration in oral microflora, diarrhoea, vomiting, burning sensation and tooth staining etc. It is well documented that many of the phytochemicals have anti-microbial activity and are safe, effective and economic option to treat oral diseases. Herbs used for oral hygiene are classified as:

- **Antimicrobial agents:** e.g. Aloe, black pepper, eucalyptus, garlic, turmeric etc.
- **Antioxidant agents:** e.g. Spinach, pepper, black tea, broccoli, green tea, carrot, potato tomato, blackberry, grape etc.
- **Analgesic agents:** e.g. Capsicum, clove, ginger, peppermint etc.
- **Anti-inflammatory agents:** e.g. Aloe, garlic, ginger, onions, neem, periwinkle, turmeric etc.

Herbs used in Oral hygiene products

Neem: It consists of leaflets of *Azadirachta indica* of family Meliaceae. Neem contains chemical constituents such as genin, sodium nimbinate, salannin, nimbin, azadirachtin, nimbidiol, quercetin, nimbidin, fiber, carbohydrates, amino acid, proteins, calcium, carotenoids etc.

It acts as antiviral, antifungal, antimicrobial, antibacterial, antipyretic, anti-inflammatory, antitumor, analgesic, antihelminthic, anticariogenic, antioxidant agent. Neem is used in dental problems such as dental caries, gingivitis and periodontitis. It has anti-plaque activity by removing bacteria from teeth surface, inhibiting bacterial growth, adhesing to hydroxyapatite on tooth surfaces, and producing insoluble glucan. It avoids sore and bleeding gums. Neem oil is used to treat mouth ulcers.

Miswak: It is dried stem bark of *Salvadora persica* of family Salvadoraceae. It contains salvadorin, trimethylamine, tannins, flavonoids, saponins, silica, chloride, and high amount of fluoride, sulphur, vitamin C. Miswak keeps the gum healthy and prevents inflammation. Calcium salts and fluorides prevent dental decay. Trimethylamine decreases plaque formation. Tannins and sulphated compound, benzylisothiocyanate has antimicrobial effect.

Turmeric: It consists of rhizomes of *Curcuma longa Linn.* of family Zingiberaceae. It possesses wide range of therapeutic activities such as analgesic, antibacterial, anti-inflammatory, anti-tumor, anti-allergic, antioxidant, antiseptic, antispasmodic, appetizer, astringent, cardiovascular, carminative, cholagogue, digestive and diuretic etc.

Turmeric consists of 6% volatile oil composed of a number of monoterpenes and sesquiterpenes, including zingiberene, curcumin, α- and β-turmerone etc. The main colouring principles are curcuminoids, 50-60% containing main constituents such as curcumin, mono des methoxy curcumin and bis des methoxy curcumin.

It is used in dental caries, oral lichen planus and halitosis. It also acts as colorant in pit, fissure sealant and used in dental-plaque detection system. Turmeric can be utilized in the treatment of premalignant lesions in oral cavity. Roasted, turmeric may eliminate pain and swelling of teeth. Turmeric water boiled with clove and guava leaves gives instant relief from dental problems. Burnt turmeric powder along with bishops seed powder not only cleanses teeth but also make them strong.

Volatile oils used in Oral Care Products

Volatile oils are antibacterial, antifungal and antioxidant in nature. In dentistry, volatile oils has following applications:

- Volatile oils inhibit plaque hence acts as oral hygiene adjunct.
- They reduce anxiety in dental patients and reduce needle insertion pain.
- They possesses wound healing activity after dental procedures
- Volatile oils have antibiofilm activity hence used in dental implant.
- They also act as preservatives.

1. **Clove oil:** It is volatile oil obtained from *Syzygium aromaticum* of family Myrtaceae. Clove oil contains eugenol, eugenol acetate and β-caryophyllene. It has analgesic, antibacterial, antiviral, anti-inflammatory, antioxidant property. It acts as mild anesthetic and an anti-bacterial agent. Clove oil is used in root canal therapy, in temporary fillings, and to relieve dental and gum pain.

2. **Eucalyptus oil:** It is volatile oil obtained from leaves of *Eucalyptus globules* of family Myrtaceae. The eucalyptus oil mainly consists of 1,8-cineole, cryptone, α-pinene, p-cymene, α-terpineol, trans-pinocarveol, phellandral, cuminal, globulol, limonene, aromadendrene, spathulenol and terpinene-4-ol. It exhibits antimicrobial property against gram-positive and gram-negative bacteria. Eucalyptus oil significantly affects plague accumulation, gingival index, bleeding on probing, periodontal depth probing. It reduces gingivitis and gingival bleeding.
3. **Lavender oil:** It is volatile oil obtained by steam distillation from the flowers of *Lavandula angustifolia* of family Lamiaceae. The main chemical constituents in Lavender oil are linalyl acetate, linalool, lavandulol, 1,8-cineole, lavandulyl acetate, and camphor etc. The oil has good antimicrobial activities against most of the bacteria, fungi, and yeast. It helps to reduce stress, anxiety and improve mood of patients.

4. **Tea Tree oil:** It is volatile oil obtained from distillation of leaves of plant *Melaleuca alternifolia* Cheel of family Myrtaceae. It is antiseptic, immunomodulator, an antifungal agent, non-irritant and a mild solvent. The major constituent is terpinen-4-ol (30%–40%). When used orally, it may cause serious side effects such as confusion, loss of muscle control or coma. In dentistry, tea tree oil is used as antimicrobial agent in the mouth before dental surgery, it removes smear layer when used in root canal and to alleviate mouth soreness.
5. **Rosemary:** It consists of volatile oil obtained from *Rosmarinus officinalis* of family Lamiaceae. Rosemary consists of volatile oil, carnosol, ursolic, linalyl acetate (25-46%), linalool (20-45%), lavendulyl acetate (>1.0%), 1,8-cineole, eucalyptol (<2.5%), 3-octanone (<2.5%), camphor (<1.2%), limonene (<1.0%), and α-terpineol (<2.0%) etc. It is used as antibacterial, antifungal, antioxidant, anti-inflammatory property. It helps to relieve toothache and disinfects GP cones.

Herbal Cosmetics

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Cosmetics

- Derived from Greek word ‘Kosmtikos’
- Meaning skilled in Decorating
- BEAUTY -Skin and Hair -Health, habit, routine job, Climate
- Concept of Beauty- Women?

- According to Drugs and Cosmetics Act (1940), “cosmetic” means any article intended to be rubbed, poured, sprinkled or sprayed on, or introduced into, or otherwise applied to, the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance.

- Why?

✓ Give sense of well being
✓ Avoid premature ageing of skin
✓ Improove overall look & personality

- Basically six different types of products are used during Herbal cosmetic preparation

1. Fresh material
2. Dried material
3. Acellular products (Oils, gums, resins)
4. Galenical preparations (Infusions, decoction, Extracts)
5. Processed extracts
6. Pure isolated compounds

Advantages

- Herbal cosmetics are made up from natural products free from all the harmful synthetic chemicals. Herbal cosmetics utilize plant parts, plant extracts or nutrients which are easily available in nature such as aloe vera gel, neem, coconut oil, vitamin E.
- Natural cosmetics are safe to utilize as proved and tested by dermatologists. As they contain natural ingredients there are less chances of getting skin rashes, skin itchiness or allergies.
- Herbal cosmetics are compatible with all skin types as they contain natural ingredients and avoid skin degradation.
- Wide varieties of herbal cosmetics are now available.
- They are available at affordable prices.
- Herbal cosmetics are tested in laboratories using state of the art equipment and there is no need of their animal testing.
- They are mostly free from side effects.
- Herbal cosmetics have long history of use and better patient acceptance.
- Herbal ingredients are widely available at cheaper rates.

Market overview

- The global herbal beauty products market size was valued at USD 78.5 billion in 2019
- It is anticipated to grow at a compound annual growth rate (CAGR) of 5.2% from 2020 to 2027.
- Rising focus on appearance and looks coupled with increased acceptance of herbal products among consumers are some of the factors that are expected to help the expansion of the market worldwide.
- The increased demand for chemical-free beauty products along with growing awareness about cruelty-free cosmetics is supporting market growth.

Classification of Herbal Cosmetics

- Classified in main Two ways

a. Based on dosage forms

b. Based on application on part of the body

a. Based on dosage forms

i. Cream/ emulsion- Cold cream, Vanishing cream.

ii. Powder- Face powder, talcum powder.

iii. Oil- hair oil

iv. Solution- after shave lotion

- b. Based on application on part of the body
- i. Cosmetics for Skin care- creams, lotions, sunscreen products, deodorants.
- ii. Cosmetics for Hair care- Shampoo, hair oil, hair removal products, shaving products
- iii. Cosmetics for nail care- Nail polish, manicure
- iv. Cosmetics for dental and oral care- Dentifrices, mouthwashes

Raw materials used in Skin care Herbal cosmetics

- Skin is the largest organ of human body
- It protects against external environment, microbes, sunlight, chemicals etc.
- Consist of
  1. epidermis
  2. Dermis
  3. Subcutaneous fatty layer
- Herbal cosmetics are prepared with the purpose to protect the skin against exogenous and endogenous harmful factors

Basic requirements for skin care products are

- **Cleansing agent:** These agents should remove the dust, dead cells and dirt that blocks skin pores. E.g. Vegetable oils like coconut, sesame and palm oil.
- **Toners:** They help to tighten the skin and avoid the contact with toxins present in air or other environmental pollutants. E.g. hazel, geranium, sage, lemon, ivy burdock and essential oils.
- **Moisturizing agents:** Moisturizers keep the skin soft and supple, maintains healthy glow and have antiaging effects. E.g. Vegetable glycerin, sorbitol, rose water, jojoba oil, aloe vera etc.

Fixed oil used for dry skin treatment

1. **Coconut oil:** It is also called as copra oil. It is edible oil obtained from fruit or seed of the coconut palm tree *Cocos nucifera*, family Arecaceae. Coconut oil is extracted by dry pressing of dried copra or by wet processing from coconut milk. It can be used in both liquid and solid forms as its melting point is 24 to 25°C. Coconut oil contains 99% of saturated fat. Coconut oil mainly consists of lauric acid (41.8%), myristic acid (16.7%), palmitic acid (8.6 %), and caprylic acid (6.8 %). It contains 6% Monounsaturated fats, 2% polyunsaturated fats and phytosterols. It is potent skin moisturizer and softener. It helps to prevent protein loss from the wet combing of hair.
2. **Sunflower Oil:** It is the fixed oil expressed from sunflower seeds of plant *Helianthus annuus*, family Asteraceae. The largest producers of sunflower oil are Ukraine and Russia as. Sunflower oil is extracted using chemical solvents like hexane, or by crushing seeds or by cold-pressing, means crushing the seeds under low-temperature conditions. It is light amber color with a mild flavour. Sunflower oil consists of Palmitic acid (5%), Stearic acid (6%), Oleic acid (30%), Linoleic acid (59%), polyunsaturated fat, monounsaturated fat, lecithin, tocopherols, carotenoids and waxes. It has soothing properties. It is widely used in face and body care products.

3. **Jojoba oil:** Jojoba oil is extracted from the seeds of shrub *Simmondsia chinenesis*, family Simmondsiaceae. The plant is native to southern Arizona, southern California, and northwestern Mexico. At room temperature unrefined jojoba oil is clear golden liquid with a slightly nutty odour while refined jojoba oil is colourless and odourless. The melting point of jojoba oil is about 10 °C. It consists of fatty acids such as stearic acid, palmitic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, Behenic acid, Erucic acid, Nervonic acid, Lignoceric acid, 11-Eicosenoic acid and arachidonic acid, and triglycerides. It is used in cosmetics as a moisturizer and as solvent for exotic fragrances. It functions like Human sebum. Jojoba oil acts as humecant, create a protective film over the skin, thus it protects and moisturizes the skin and hair, replenishes skin and hair loss and restores their natural pH balance. Jojoba oil is used in lotions, moisturizers, hair shampoos and conditioners. The pure oil can be directly applied on skin, hair, or cuticles. It also has analgesic, antipyretic, antiinflammatory, antioxidant, antibacterial and antiparasitic properties.
4. **Olive oil:** Olive oil is a fixed oil extracted from the fruits of *Olea europaea*, family Oleaceae. Olive plant mainly grows in Mediterranean basin. Spain, Italy and Greece are largest producers of olive oil. Olive oil contains oleic acid (up to 83%), linoleic acid (up to 21%) and palmitic acid (up to 20%). It also contains Myristic acid, heptadecanoic acid and eicosanoic acid in traces. It also consists of phyconstituents such as triolein, tripalmitin, trilinolein, tristearate, monosterate, triarachidin, squalene, β-sitosterol and

5. **Castor oil:** This is obtained from the Castor seeds *Ricinus communis* of family Euphorbiaceae. Castor plant is commonly observed in southeastern Mediterranean Basin, Eastern Africa and India. Castor oil is viscous, colourless to very pale yellow liquid with a distinct taste and odour with boiling point 313 °C. The main chemical constituent is ricinoleic acid. Other constituents include oleic acid, palmitic acid, stearic acid, Linoleic acid; α-Linolenic acid, Dihydroxystearic acid. Water-soluble toxin ricin is also present in oil. The oil protects skin from against harsh climate, and has skin soothing effect. It is moisturizer and use to treat rough skin and acne. Hydrogenated castor oil or its esters, act as vehicles or carriers, emollients or solvent for toiletry, cosmetic, hair and skin care formulations. It is potent skin cleanser and conditioner.
6. **Cocoa butter:** It is also known as theobroma oil which is yellow coloured fat obtained from *Theobroma cacao* of family Sterculiceae. It is extracted from dried; fermented and roasted seeds separated from their hulls. The cocoa nibs are ground to form chocolate liquor. Then to separate the cocoa butter chocolate liquor is pressed. It melts at temperature 34–38 °C. It is rich in saturated fats and monounsaturated oleic acid (34.5%). Other constituents are Arachidic acid 1.0%, Linoleic acid 3.2%, Palmitic acid 26.0%, Palmitoleic acid 0.3%, Stearic acid 34.5%, and Other Fatty Acids 0.5%. It has soothing effect on windburn or sunburn. It acts as vehicle in suppositories and pessaries. Cocoa butter consists of oleic, stearic and palmitic acids. It is good emollient and a natural antioxidant.

Skin protecting and Anti ageing agents

1. **Aloe:** It is obtained from leaves of *Aloe vera, Aloe barbadensis* Miller family Liliaceae. Plant is fleshy, spiny-toothed leaves with red or yellow flowers. The mucilage obtained from inner part of leaf act as potent moisturizer, skin softener, antioxidant and use to heal cuts, wounds and burns. Aloe contains various constituents such as amino acids like leucine, isoleucine, saponin 12 anthraquinone glycosides, proteins, carbohydrates, minerals, vitamins A,C,E,B, choline, B12 and folic acid which can soothe, heal, moisturize and regenerate the skin. Aloe gel consists of 99% water, glucomannans, amino acids, lipids, sterols and vitamins etc. Aloe gel is useful to treat burns, sunburn and mild abrasion, and inflammation of skin. It can act as antibacterial, antifungal, antiviral, antioxidant and antihistaminic agent. Aloe gel also act as a cleanser, anaesthetic, antiseptic, antipyretic, antipruritic, nutrient and moisturizer and promote cell proliferation. Aloe sticks the epidermal cells together and softens the skin. It stimulates collagen and elastin fibres synthesis hence exhibit antiageing properties. Aloe possesses wound healing effect by keeping the wound moist and allowing oxygen to penetrate the wound, leading to regeneration of skin. Mucopolysacharides present in aloe causes remodeling of the wound. Aloe also protects the skin against harmful effects of UV rays.

Aloe Plant

2. **Oats:** It is obtained from *Avena sativa* L of family Graminae. Main producers of oats are European union, Russia Canada, Poland, Australia and Finland. Oats are rich in protein, and other constituents include minerals, lipids, β-glucan, polysaccharide, fibers, avenanthramides, an indole alkaloid-gramine, flavonoids, flavonolignans, triterpenoid saponins, sterols and tocols etc. Oats protect and repair the skin and hair from damaging effects of UVA or UVB irradiation, pollution, smoke, bacteria, chemicals and free radicals etc. It is used in treatment of geriatric dermatosis, eczema and sunburn, irritation and inflammation of the skin. Because of its soothing, moisturizing and an anti-irritating properties it is used in preparation of facial masks, facial scrubs and soap-free skin cleansers. Hydrolysed oat protein form protective film on skin and is used in formulation of baby cleansing products. Its constituent Polysaccharide β-glucan reduces signs of aging, protect skin against UV damage, stimulate metabolic activity, collagen synthesis, and improve the tensile strength of hair. Oat protein and β-glucan moisturizes, soothe the skin and reduce redness due to abrasion.

Carrot: It is obtained from the plant *Daucus carota* belonging to family Apiaceae. It is rich source of β-carotene, Vitamin A, B, C, E and K, and lesser amount of α-carotene, γ-carotene, lutein, and zeaxanthin. Polyacetylenes such as Falcarinol and falcarindiol are observed in carrot. Other constituents such as pyrrolidine, 6-hydroxymellein, 6-methoxymellein, eugenin, 2,4,5-trimethoxybenzaldehyde (gazarin) or falcarindiol 3-acetate are also present in carrot. Carrot seed oil possesses anti-aging, revitalizing, rejuvenating properties and is natural toner. Carrot keeps the skin healthy, removes scars and blemishes, prevents wrinkles, and protects the skin against sun rays. As carrot is rich in potassium, it keeps the skin hydrated. It helps to treat pimples, acne, rashes, dermatitis and other skin problem. It can be applied on wounds, cuts, and inflammation for quick healing.

Turmeric: It is obtained from the underground stems of herb *Curcuma longa* of the family Zingiberaceae. Turmeric contains curcumin, demethoxycurcumin, bisdemethoxy curcumin, zingiberene, curcumol, curcumenol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones and turmeronols. It is used as antiseptic, analgesic, anti-inflammatory, antioxidant, antimalarial, insect-repellant, natural colourant etc. It inhibits activity of phosphorylase kinase, hence is used for prevention, treatment or control of psoriasis, acne, wounds, burns, ulcers, eczema, sun damage to the skin and premature aging.

Volatile oils used in skin care products

1. **Calendula:** It is obtained from *Calendula officinalis* of family *Asteraceae*. The plant is mainly obtained from southwestern Asia, Western Europe, Macaronesia, and the Mediterranean region. The Calendula essential oil consists of constituents such as α-thujene, α-pinene, 1, 8-Cineole, dihydrotagetone and T-muurolol. It acts as antioxidant, anti- inflammatory and wound healing agent.
2. **Rose oil:** It is obtained from four species of roses mainly *Rosa damascena* Mill., R. *gallica* L., R. *moschata* Herrm. And R. *centifolia* L. belonging to family Rosaceae. It is used to treat depression, grief, nervous stress and tension. It also helps to heal wound and maintain good skin health. It contains constituents such as citronellol, geraniol, nerol, linalool, phenyl ethyl alcohol, farnesol, stearoptene, α-pinene, β-pinene, α-terpinene, limonene, p-cymene, camphene, β-caryophyllene, neral, citronellyl acetate, geranyl acetate, neryl acetate, eugenol, methyl eugenol, rose oxide, α-damascenone, β-damascenone, benzaldehyde, benzyl alcohol, rhodinyl acetate and phenyl ethyl formate etc.

Calendula flower

Rose flower

3. **Eucalyptus oil:** It is obtained by steam distillation from the leaves of various *Eucalyptus* species mainly *E. cinerea F. Muell., E. baueriana F. Muell., E. smithii R. T. Baker, E. bridgesiana R. T. Baker, E. microtheca F. Muell., E. foecunda Schau., E. pulverulenta Sims, E. propinqua Deane and Maiden, E. erythrocorys F. Muell.* of family Myrtaceae. They are widely used in the preparation of liniments, inhalants, cough syrups, ointments, toothpaste and also as pharmaceutical flavours. It consists of 1,8-cineole (eucalyptol; not less than 70%), limonene (4-12%), α-pinene (2-8%), α-phellandrene (less than 1.5%), β-pinene (less than 0.5%), camphor (less than 0.1%). The essential oil has soothing, cooling properties hence useful for massage. It is anti-inflammatory and analgesic agent.

4. **Chamomile:** It is obtained from German chamomile *Matricaria recutita L.* and Roman chamomile *Anthemis nobilis* Linn belonging to family Compositae. Chamomile oil is used to treat inflammation of skin and mucous membranes, eczema and other skin disorders. It helps to reduce itching and improve damaged skin. It possesses skin repairing, regenerating, and strengthening properties. Roman chamomile oils consist of α-pinene, camphene, β-pinene, sabinene, myrcene, 1,8-cineole, γ-terpinene, caryophyllene, and propyl angelate and butyl angelate. While German chamomile oil contains chamazulene, α-bisabolol, bisabolol oxide A, bisabolol oxide B and bisabolone oxide A. α-bisabolol (levomenol) and chamazulene present in chamomile inhibit leukotriene synthesis and thus act as anti-inflammatory and analgesic agent. It is also helpful in treatment of age spots, boil, minor wound, burn, or an insect bite, dry skin, windburn, sunburn, and chronic skin conditions such as acne and psoriasis.

5. **Citrus oil:** It is essential oil extracted from rinds of *Citrus sinensis* [L.] Pers and other citrus species belonging to family Rutaceae. Citrus oil is mixture of acids, alcohol, aldehydes, esters, ketones and hydrocarbons. It is composed of limonene, sabinene, α-pinene, β-pinene, myrcene, octanal, linalool, decanal etc. It is strong antioxidant, antibacterial, immunomodulator, flavouring agent, anti-stress agent, cleanser and helps to get rid of skin problems such as warts, bunions, coms or calluses etc.
6. **Geranium oil:** It is obtained from water and steam distillation of shoots of plant *Pelargonium graveolens* (L.) L Her. ex Ait of family Geraniaceae. Oil consists of α-pinene, myrcene, limonene, menthone, linalool, geranyl acetate, citronellol, geraniol and geranyl butyrate. Geranium oil is acts as cleansing, toning and sharpening agent. It is helpful in treatment of greasy, over-oily skin, acne, congested skin and eczema. It helps in skin tightening and has antiageing effects. It helps to avoid scars and after marks of fat-cracks, surgeries, boils, acne or pox. It has wide applications in fragrance industry and in aromatherapy. It helps to balance hormones, acts as tonic antiaxiety, anti-inflammatory, antihypertensive, anthelmintic agent. It eliminates problems of menopause, improve circulation and dental health and boost kidney health etc.

7. **Lavender oil:** It is obtained from plants *Lavendula angustifolia, L. latifolia, L. stoechas* and *L. x intermedia* of family Lamiaceae. The essential oil consists of linalool, α and γ-terpeniol, borneol, perillyl alcohol, linalyl acetate, geranyl acetate, neryl aceatae, α-pinene, β-pinene, camphor, limonene, tannins, triterpenes, coumarins, β-caryophyllene and cineole. It stimulates cell growth and used widely in skin disorders like bites, stings, boils, burns, stretch marks, rashes, spots, cold sores and sunburns etc. It is antibacterial agent hence helps to avoid acne. It is used in treatment of psoriasis and eczema. It helps to avoid wrinkles and hyperpigmentation.

Protecting agent against Acne, spots and Pimples

The skin condition which affects sweat glands and hair follicles, causing inflammation, black heads, white heads and pustules is termed as acne. They can leave scars and pitting.

1. **Artemisia:** It is obtained from *Artemisia vulgaris, Artemisia absinthum, A. campestris* of family Compositae. The plant is mainly found at Europe, Asia, northern Africa, Alaska and North America. The volatile oil mainly contain camphor, camphene, 5-thujone, germacrene D, 1,8-cineole, and C-caryophyllene etc. The plant decoction is used to treat skin diseases like eczema, herpes and prurulent scabies, ulcerative sores and wounds.
2. **Tulsi:** It is obtained from *Ocimum sanctum* of family Lamiaceae. Tulsi consists of oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, β-caryophyllene. Tulsi acts as anti-inflammatory agent by inhibiting both the cyclo-oxygenase and lipoxygenase pathways of arachidonate metabolism. It also has antiseptic properties. It has anti acne, skin brightening, tightening properties. It helps to remove the scars and spots from the face.

3. **Pumpkin:** It is obtained from *Cucurbita pepo* of family Cucurbitaceae. Pumpkin seed oil has linoleic, oleic, palmitic, stearic acid, vitamin A, vitamin C, β-carotene, carbohydrates, fats and proteins. It acts as anti-inflammatory agent. It is used in treatment of herpes lesions, venereal sores, acne, pimples, blackheads, chronic leg ulcers, as a poultice on sprains and pulled ligaments etc. It is suitable for all skin types. It avoids skin damage, soothes and moisturizes skin.

Antioxidants used in skin care

Most of the plants contain polyphenolic compounds hence they have free radicals scavenging effect. Various antioxidants used in skin care products are as follows:

1. **Tea:** It is obtained from *Camellia sinensis* of family Theaceae. It has black and green variety. Black tea is prepared by fermentation process, while green tea is produced by steaming the leaves immediately after harvesting. Tea consists of tannins, flavonoids, amino acids, vitamins, caffeine and polysaccharides. It has antiinflammatory, antioxidant, antiallergic, antibacterial and antiviral effects. Green tea mainly contains Catechins, flavandiols, and flavonols to repair UV photo-damage and phototoxicity. Green tea stimulates the formation of ceramides and sphingolipids in the skin and thus stimulates the lipid barrier. Black tea also acts as antioxidant and has skin protective properties.
2. **Grape seed:** The seeds are obtained from *Vitis vinifera L.* of family Vitaceae. Procyanidins present in grape seed are potent antioxidants. They inhibit lipid peroxidation, collagen and elastin degradation, acts as tyrosinase inhibitor and enhance wound healing process. Hence, it is applied in various skin disorders.

3. **Red clover:** It is obtained from *Trifolium pretense* L. of family Fabaceae. It is rich in isoflavones. It is useful in treatment of various skin disorders such as psoriasis, eczema, acne and rash. It also acts as anti-inflammatory agent and sunburn protective agent.
4. **Fenugreek:** It consists of herb of *Trigonella foenum-graeceum* L. of family Fabaceae. Diosgenin, coumarin, hydroxy-isoluceine, luceine, tyrosine, protein, B-Complex Vitamins, vitamin C and β-carotene are the constituents of fenugreek. Its seeds have antioxidant potential and mucilage has emollient properties. Fenugreek is used to treat severe skin inflammations, chapped lips and mouth ulcers.

Natural colorants used in skin care

Avocado: The oil is obtained from *Persea gratissima* of family Lauraceae. Plant contains constituents such as carotenoids and chlorophylls mainly lutein, α-carotene, β-carotene, neoxanthin, violaxanthin, zeaxanthin, antheraxanthin, chlorophylls a and b, and pheophytins a and b. Lutein is main colourant responsible for producing green or yellow colour and is lipophilic in nature.

Beet root: It is obtained from *Beta vulgaris* belonging to family Amaranthaceae. It contains natural colourant betanin. It is hydrophilic compound but degrades when subjected to light, heat and oxygen. It produces red or pink coloured shades.

Butterfly Pea: It is obtained from flowers of *Clitoria ternatea* of family Fabaceae. The flower shows presence of anthocyanins, p-coumaric acid and ferulic acid. Flowers are used to produce purple or blue coloured shades. It is due to presence of hydrophilic constituent Delphinidin.

Calendula: It is obtained from flowers of plant *Calendula officinalis* of family Asteraceae. Flower extract produces yellow, orange to red coloured shades because of presence of carotenoids. Flavoxanthin is the main colourant present in calendula. Flavoxanthin is water soluble colourant. It also shows presence of lycopene, lutein and about nineteen different carotenoids.

Carrot: It is obtained from roots of *Daucus carotasativa* belonging to family Apiaceae. It contains lipophilic colourant β-carotene and produces orange coloured shades. It also shows presence of α-carotene, γ-carotene, lutein and zeaxanthin.

Chamomile: It is obtained from essential oil of *Matricaria recutita*, family Asteraceae. The oil consists of Lipophilic pigment Chamazulene responsible for giving blur colour.

Henna: It is obtained from leaves of plant *Lawsonia inermis* of family Lythraceae. Leaves contain hydrophobic compound called Lawsone (2-hydroxy-1,4-naphthoquinone) or hennotannic acid. It yields red-orange coloured shades. Lawsone is also present in flower of water hyacinth *Eichhornia crassipes*.

Hibiscus: It is obtained from flowers of plant *Hibiscus rosa-sinensis* belonging to family Malvaceae. It consists of red hydrophilic pigments Cyanidin-3-sophoroside.

Indigo: It is obtained from leaves of *Indigofera tinctoria* of family Leguminosae. Indigo dye is a derivative of indican, a glucoside and this is converted to blue indigotin by an enzyme process. It produces blue to mauve coloured shades. The dye is obtained from fermented leaves of plant which is processed into cakes. Then cake is finely powdered. When powder is exposed to air, blue colour develops. The dye shows good fastness property.

Iris: It is obtained from roots of plant Iris germanica of family Iridaceae. It consists of main constituents Mangiferin, Delphinid which produces Purple or Blue or Green coloured shades.

B.Pharmacy VI Semester Herbal Drug Technology Herbs as raw materials Definition of herbs, herbal medicine, herbal medicinal product, herbal drug preparation Source of herbs, Selection, identification and authentication of herbal materials, Processing of herbal raw material . CONTENTS Herbs as raw materials: Definition of Herb Herbal medicine Herbal medicinal product Herbal drug preparation Source of Herbs Selection, identification and authentication of herbal materials. Processing of herbal raw material

INTRODUCTION: Herbs have used in the traditional system of medicine since time immemorial to alleviate human illness and for the maintenance of general health.

 As per WHO, 80% of world population of the developing countries still rely on the crude drugs and folklore medicines for their primary health care needs.

 The interest of people in herbal medicines has increased significantly in both developed and developing countries.

 There is a great demand for herbs, hence there is a need to adopt systematic scientific methods for their selection, cultivation, collection, processing and to ensure the quality, purity, safety, potency and develop modern methods for their quality control so that maximum benefit is obtained from these herbal medicines

1.HERBS: It consists of entire plant or any part of the plant.

2. The word herb is derived from the Latin word herba, which means grass, green stalk or blades. Depending upon the perspectives, the word herb has different meanings.

3. In commercial terms: Herbs gently refers the plants used for culinary purposes.

4. In horticulture: Herbs refers to herbaceous, describes the exterior of plant In botany: Herbs refers to entire or aerial parts of small, annual or biennial, non-woody plant.

5. In taxonomy: Herbs refer to above ground/aerial parts such as leaf, flower and stem. In herbal medicines: Herbs refer to all types of plants (herbs, shrubs or trees) used in various forms or preparations valued for their therapeutic benefits

6.5. HERBAL MEDICINE: It is also known as herbalism or phytomedicine.

7.These consist of medicinal plants or any parts of medicinal plants, usually in unprocessed or crude forms which have medicinal value.

8. They include different parts of plants like entire aerial part, roots, rhizomes, stem, leaves, flower, fruits, seeds, bark etc.

9.It includes modern standards of testing and evaluation of herbs.

10.The constituents and their therapeutic activity may be known or unknown.

1.WHO GUIDELINES FOR HERBAL MEDICINES: 1. Herbal medicines are finished labeled medicinal product that contain active ingredients; aerial or underground parts of plants or combination of plants, either in crude or processed formulations.

2. Herbal medicines may include juices, gums, volatile oils, fixed oils or other substances derived from plants.

3. Herbal medicines might possess excipients

4. Medicines possessing crude material mixed with chemically distinct substances including isolated phytoconstituents are not termed as herbal medicines.

5. The main objectives are: a. Provisions for recommended general test methods. b. General limits for contamination for herbal drugs.

HERBAL MEDICINAL PRODUCTS: Products produced exclusively from herbs and used in medicinal purposes are called herbal medicinal products.

 These are defined as any medicinal product, exclusively containing one or more active ingredients of herbal origin. In addition they may contain excipients.

 In India there are variety of herbal medicinal products that are produced as well as exported to other countries, which play an important contribution in country’s GDP

 It includes various herbal formulations like syrups, mixtures and tablets and novel dosage forms like

1.HERBAL DRUG PREPARATIONS

2. These are preparations derived from herbal drugs which are prepared by various techniques like extraction, infusion, decoction, maceration, distillation, expression, fractionation, purification, concentration and fermentation. Herbal drug preparation are available in market in the form of powders, extracts, tinctures, fixed oils, volatile oils, resins, gums, etc.

3. They contain a mixture of various constituents. However pure isolated compounds do not come under this category.

4. Sometimes phytoconstituents are isolated and formulated in appropriate dosage form in place of entire herb or parts.

SOURCE OF HERBS: Herbs or medicinal plants can be obtained from three sources

viz: A. Wild source B. Cultivated source C. By using modern scientific techniques like tissue culture, polyploidy, mutation, hybridization, genetic engineering, germplasm etc. on cultivated plants

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A. WILD SOURCE: The plants are obtained from wild source and grow themselves without any type of care at unutilized land such as forests, plains, river banks, etc. The wild plants also have reasonable active constituents and sometimes new variety is produced. These plants grow under favorable conditions in natural habitat. Advantages:

B. Economical; No cost of land, caring, fertilizers, irrigation etc. Less time consuming.

Disadvantages of wild plants:

 The quality of the plants cannot be predicted due to various environmental changes.

 The plants will not be uniform in their growth and yielding characteristics.

 The collection is uneconomical as these are widely distributed in different locations. They are sparsely distributed, so chances of adulteration and substitution are more.

 Modern scientific techniques cannot be applied to increase the yield as well as quality.

 If the plants are obtained continuously from wild source for prolonged period, may lead to depletion of raw material. They cannot fulfill the demand when required.

 Sometimes genetically different plants are developed.

B. CULTIVATED SOURCE

The plants are obtained from cultivated source. These plants are grown with proper care by human. Care is taken toward soil, climate, rainfall, irrigation, time of sowing and collection, altitude, temperature, fertilizers, manures, pesticides, weeds etc. Advantages of cultivated plants:

- The quality and purity of medicinal plants can be ensured.
- Better yield and therapeutic quality, and give more profit.
- It ensures regular supply of raw materials due to planned cultivation.
- Application of modern scientific techniques like tissue culture, genetic engineering, hybridization, germplasm, mutation, polyploidy etc. are possible.
- Cultivation of medicinal and aromatic plants leads to industrialization and has given rise to several cottage and small scale industries.
- Rapid growth of phytopharmaceuticals, perfumery and allied industries is possible due to cultivation of medicinal and aromatic

By using modern scientific techniques like tissue culture, polyploidy, mutation, hybridization, genetic engineering, germplasm etc. on cultivated plants a. Plant tissue culture techniques as source of drugs: Plant tissue culture is in vitro cultivation of plant cell or tissue under aseptic and controlled environmental conditions, in liquid or on semisolid well-defined nutrient medium for the production of primary and secondary metabolites or to regenerate plant. This technique affords alternative solution to problems arising due to current rate of extinction and decimation of medicinal plants. The whole process requires a well-equipped culture laboratory and nutrient medium. This process involves various steps, viz. preparation of nutrient medium containing inorganic and organic salts, supplemented with vitamins, plant growth hormone(s) and amino acids as well as sterilization of explant (source of plant tissue), glassware and other accessories inoculation and incubation.

1.STEPS INVOLVED IN PROCESSING OF HERBAL DRUGS

2. Selection of Herb 2. Identification and Authentication 3. Cultivation of herbs 4. Collection of herbs 5. Processing of herbal raw material

3. SELECTION OF HERBS:

The species or botanical variety selected for cultivation should be the same as specified in the official Pharmacopoeia or official document. In case of newly introduced medicinal plants, the variety selected for cultivation should be identified and documented.

4. IDENTIFICATION & AUTHENTICATION

Botanical Identity: The species, subspecies, genus, variety etc of the plant for cultivation should be verified from a qualified botanist/Institute and recorded. Specimens: In case of a new plant with medicinal properties whose identity is not known, a specimen of the plant should be submitted to a national herbarium for identification and documented. Seeds and other propagation materials: The suppliers of seeds and other propagation materials should specify all the necessary information relating to the identity, quality as well as their breeding history. The seeds and propagation material should be free from contamination and disease in order to promote healthy plan

1.CULTIVATION OF MEDICINAL PLANTS:

Cultivation of medicinal plants requires intensive care and management as various factors like environment, soil, irrigation, pests etc. play a vital role. These factors vary from one plant to another.

Scientific documented methods should be followed. If no data available, traditional methods should be adopted and a systematic method should be developed through research.

Good agricultural practices (GAP) and conservation agriculture (CA) improve, conserve and make more efficient use of natural resources.

COLLECTION OF HERBS: The proper time of collection is important because the nature and quantities of constituents vary greatly in some species according to the season.

The two basic factors which should be considered during collection are:

The plant parts should be collected when the level of active constituents are maximum and when the material will dry to give the maximum quality and appearance.

The environmental conditions in which the plant give maximum active constituents should be considered. The different environmental factors to be considered are light, humidity, altitude, rainfall, soil type etc.

1.General scheme for collection of different parts of the plants is as follows:

Roots and Rhizomes: Roots are collected in the beginning of the spring season just before the flowering. Rhizomes are collected during the reproductive phase ie when they contain maximum amount of the reserve food stores and active constituents. Eg. Liquoroice and Rauwolfia.

Leaves: leaves of the plants are collected before plant reaches the flowering stage. Eg. Senna, Belladonna.

Flowers: flowers are collected before pollination takes place. They are collected when the weather is dry and especially during morning hours. Eg. Clove, Saffron.

Bark: the time period of collection differs from different plant barks. Bark is collected during spring, early summer, winter or in autumn. The age of the tree at the time of collection is also important. There are three methods to collect bark as follows:

Felling method: Trees are cut from the base and bark is peeled out.

Uprooting method: entire tree is uprooted pulled out along with its roots. And the bark is removed.

Coppicing method: the plant is cut at a certain distance from soil leaving behind a stump known as stool. The stool give out new shoots which are cut off to collect their barks. This method is highly economical and rapid. Eg. Cinnamon and Cascara.

1.PROCESSING OF HERBAL RAW MATERIALS Processing of herbal raw materials involves various stages for which the crude drugs undergo after harvesting. Processing is classified as primary and secondary processing which are further subcategorized as follows:… Primary processing Secondary processing Garbling Cutting/sectioning Washing Ageing/sweating Boiling Banking/Roasting Leaching Boiling/streaming Drying Stir/frying Fumigation

Drying: Slight excessive moisture in the crude drug deteriorate the quality of the drug and also increases its weight. Before marketing a crude drug it is necessary to dry it properly because:

Drying removes sufficient moisture to ensure good keeping qualities.

Drying prevents molding, the action of enzymes, the action of bacteria and chemical or other possible changes in the crude drugs.

Drying preserve the drug for a longer time and give better pharmaceutical elegance.

1.Drying fixes the constituents and facilitates grinding and milling.

Drying converts the drug into a more convenient form for commercial handling.

Drying improves the quality of the drug and make it resistant to the growth of micro organisms.

Proper and successful drying involves two principles; control of temperature and regulation of air flow

Methods of drying:

 Depending upon the type of chemical constituents, suitable drying method can be chosen. There are two methods of drying: Natural drying and Artificial drying. Natural drying (Sun drying): when the contents of the drugs are quite stable to the temperature and sunlight, the drugs can be dried directly in sunshine eg. Gum acacia, seeds, small sized fruits. Shade drying: When the natural color and volatile principles of the drugs are to be retained, drugs are dried in shade. Eg. Digitalis, clove, Senna, peppermint, leafy and flower drugs.

Artificial Drying: 3 methods a.Tray Dryer (oven drying): this method is used for the drugs which do not contain volatile oils and are quite stable to heat or which need deactivation of enzymes. In this process hot air of required temperature is circulated in the oven. Eg. Roots and bark drugs.

b.Vacuum dryer; the drugs which are sensitive to high temperature are dried by this method. Eg. Tannins, digitalis leaves.

c.Spray dryer: few drugs which are sensitive to atmospheric conditions and also to temperature of vacuum drying are dried by this method. Eg. Papaya latex, pectin etc.

Special methods of drying:

 In certain drugs, some special methods are required to attain specific standards. Eg. Fermentation is done in case of cinnamon bark and gentian root.

 For fleshy and thick plant parts; slicing and cutting into smaller pieces is done to enhance drying.

 The flowers are dried in shade to retain their color and volatile oil contents.

Garbling (Dressing):

 Garbling consists of the removal of extraneous matter such as other parts of same plant, dirt, sand, foreign organic matter and added adulterants.

 This step is done to some extent during collection and harvesting, but should also be carried out after the drug is dried and before packaging. Garbling may be done by hand picking or by mechanical means. Eg. Clove stalks are removed from the clove.

 Rhizomes are separated from roots, rootlets and stem bases.

 Small pieces of bark are removed the gum.

Packaging: The packaging of drugs depend on the final disposition. While packing of crude drugs the main points to be considered are: oThe morphological and chemical nature of drugs. oIts ultimate use. oEffect of climatic conditions during transportation and storage. oThe type of packaging should be choosen that provide protection to the drug and gives economy of space.

Examples of packaging:

 Most of the leafy drugs are baled with power balers into a solid compact mass and then packed into burlap or gunny cover. Aloe is packed in goat skin.

 Resins like colophony and balsams of tolu are packed in kerosene tin.

 Volatile oil containing drugs are packed in well closed containers, protected from sunlight to prevent loss of volatile oil and oxidation.

 The drugs which are very sensitive to moisture and also costly need special attention. Eg. Squill, Ergot and Digitalis.

 Squill becomes flexible, ergot becomes susceptible to microbial growth and digitalis looses its potency if come in contact with slight excess of moisture. So desiccating agents such as CaO/silica

1.Storage and Preservation:

 Proper storage and preservation are important factors in maintaining high degree of quality.

 Compactly and hard packed bales, barks and resinous drug reabsorb a little moisture but drugs that are not compactly packed can absorb moisture upto 10% to 30%.

 Moisture in the drug: reduce the percentage of active constituents.

Increase the weight of the drug.

Facilitate enzymatic activity and fungal growth.

Light adversely affect the colored drugs, make them unattractive and cause undesirable changes in the constituents.

1.The oxygen of the air increases oxidation of the constituents of the drugs especially when oxidases are present. Therefore the warehouses should be cool, dark and well ventilated.

 The insects also infest vegetative drugs. The simplest method used for destruction of insects and prevention of their attack is to expose the crude drugs at 650 C.

 Large lots of drugs are stored in ware houses and are fumigated with methyl bromide.

 If drugs in small quantities are stored in tight containers, insects attack can be controlled by adding few drops of chloroform or carbon tetra chloride from time to time

 Small lots of drugs may be stored in tight and light resistant containers. The tin cans, covered metal bins or amber glass containers are most satisfactory.

 Crude drugs should not be stored in open, wooden boxes or drawers and never in paper bags. It not only hasten the deterioration but their odors are communicated from one drug to another, attacks by insects are facilitated and destruction by mice and rats may occur.

 In the case of digitalis and ergot where low moisture content must be maintained at all times, a suitable non-liquefying, inert, dehydrating agent may be introduced into the tight container.

High temperature accelerate all chemical reactions. So drugs must be stored at possible low temperature. The ideal temperature is just above freezing, but since this is impractical in most cases, the ware house or other storage places should be kept as cool as possible.

History of Microbiology

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History

Contribution of Anatomy Van Leeuwenhoek: (1632- 1720)

- He was a first to observe & accurately to describe the shape of human RBCs as well as little agent of disease i.e. animalcule's.
- He observed the motility of bacteria.
- In 1683 he described different shapes or morphological forms of bacteria.
- Antonie van Leeuwenhoek (1632–1723) was one of the first people to observe microorganisms, using a microscope of his own design, and made one of the most important contributions to biology.
- He had hobby of glass grinding and preparation of lenses and this led him to assemble 250 single microscope.
- He describe the inhibitory effect of acetic acid on M.O.

Edwerd Jenner

- Observed that the milk maids who had milder form of cowpox were not prone to Smallpox.
- After observing cases of cowpox and smallpox for few years, In 1796 he removed the fluid of a cowpox from milkmaid and inoculated James Phipps, an eight-year-old boy, who soon came down with cowpox.
- Six weeks later, he inoculated the boy with smallpox. The boy remained healthy.
- Jenner had proved his theory that the pus in the blisters which milkmaids received from cowpox protected milkmaids from smallpox.

Contribution of Louis Pasteur

[1822 - 1895]

- **Father of Microbiology**

1) Louis pasture first demonstrated that air contains micro- organism.

He pass large quantity of air through a tube which contain a plug of guncotton to serve as filter.

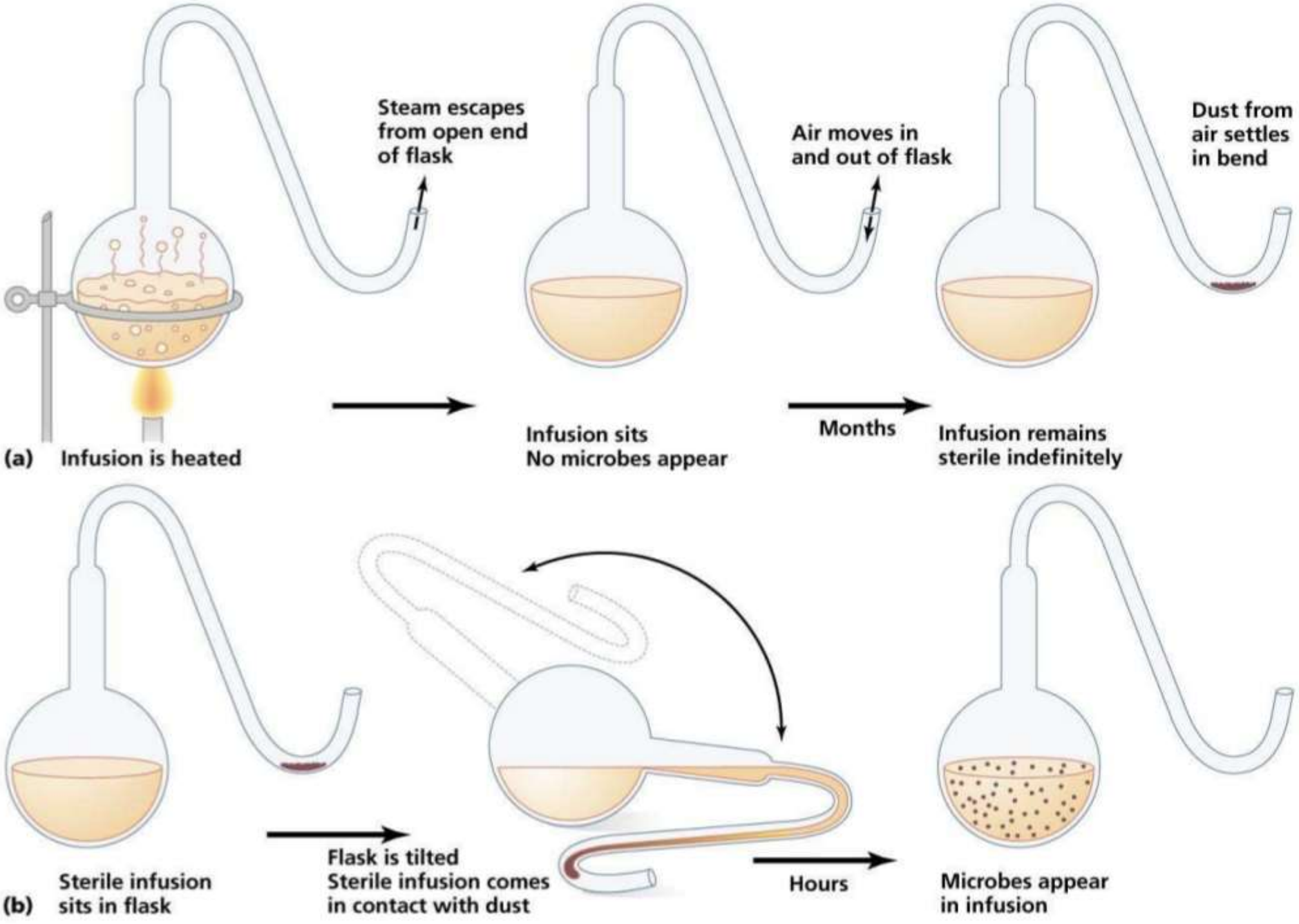
The guncotton was then removed & dissolved in mixture of alcohol-ether sediment was examine microscopically.

He found that this sediment contains not only organic matter but also a large number of small micro-organism.

2) Pasture Resolved the Controversy of Spontaneous Generation vs. Biogenesis.

- He performed a series of experiment to prove that although micro-organism were present in the air they were not spontaneously produce.
- Pasture took boiled meat infusion in several swan neck flack. The flack opening were freely open to the air but curve so that gravity would cause any air born dust particles to deposited in the lower part of the neck.
- The flask were heated to sterilize the broth & then incubated. No growth occurred even through the contents of the flack were exposed to air.

- The flask were heated to sterilize the broth & then incubated. No growth occurred even through the contents of the flack were exposed to air.
- Pasture pointed out that no growth took place because dust & germs had been trapped on the wall of the curved necks but if the necks were broken off so that dust fell directly down into the flack, microbial growth commenced immediately.
- In 1861, pasture finally resolved the controversy of spontaneous generation vs. biogenesis & proved that micro-organism are spontaneously generated from in animated matter.


Steam escapes from open end of flask
Air moves in and out of flask
Dust from air settles in bend
(a) Infusion is heated
Infusion sits
No microbes appear
Months
Infusion remains sterile indefinitely
Sterile infusion sits in flask
Flask is tilted
Sterile infusion comes in contact with dust
Hours
(b) Microbes appear in infusion

3) Pasture also showed that micro-organism are not evenly distributed in the atmosphere & that there number varies from place to place for this experiment .

- He took a large number of sealed flack containing boiled & cooled infusions & opened a few at a time for a short period at various places & resealed.
- Out of the 20 flacks which he opened & resealed on a dusty road & all showed spoilage, while out of the 20 that he opened on the top of a mountain only 5 showed spoilage & out of 20 that he opened near a glacier, only one showed spoilage.
- From these experiment, pasture concluded that the air contained micro-organism & there number of varied from place to place.

4) **Pasteurization:**

- Pasture made an intensive study of the bear & wine fermentation. He found that wine spoilage was caused by the growth of undesirable contaminating micro- organism.
- After some experiment, he showed that wine did not undergo spoilage if it was held for a few min. at 50 to 60^0c. This gave rise to the new process of preserving wine, fruit juice, milk etc. & was called **pasteurization.**

5) Aerobic, Anaerobic & Facultative Anaerobic:

- Pasture also found that many other micro-organism including yeast could grow either in presence or absence of food, oxygen.
- He designated life in the presence of oxygen as 'aerobic' in the absence of oxygen as 'anaerobic' & in presence or absence of oxygen as 'facultative anaerobic'

6) Pasture opened the field of sterilization by stating the boiling rendered the fluid sterile.

- Pasture established the practice of heating fluid material to 120^0c under pressure of sterilization (autoclaved).
- He also introduced the particle of sterilizing glassware by dry heat at 170^0c.

7) Chicken Cholera Vaccine:

- In 1880, pasture isolated the bacterium responsible for chicken cholera & grow it in pure culture. But on the day of public demonstration he failed to repeat his experimental results. The chicken get survived after injecting the isolated bacterial culture & this result surprised him.
- On subsequent experiment his mead conclusion that on long preservation or sub culturing the virulence power of the pathogenic bacteria was destroyed.
- Such microbial culture with decreased virulence was referred as attenuated culture. Such attenuated culture cannot caused infection but induced immunity in host.

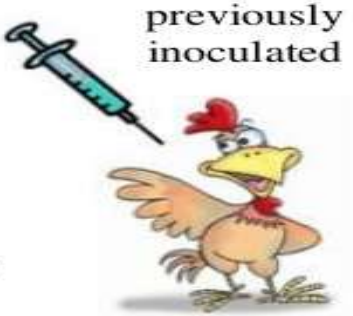
Pure culture of chicken Cholera bacteria 8 weeks old

Chicken inoculated

Remains Healthy

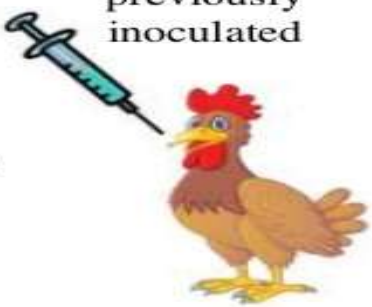
Fresh virulent culture of chicken Cholera bacteria

Chicken previously inoculated



Remained Healthy

Chicken which is not previously inoculated



Died

8) Pasture further introduced the vaccine for Anthrax & Rabies.

- He know that the causative agent of rabies attacked the brain & spinal cord.
- He took spinal cord from rabbits which died from the disease & suspended these cord in dry sterile air.
- By these process rabies micro-organism in the nervous tissue lost their virulence & emulsion of the spinal cord was used as vaccine.
- It was found to be effective in dose.

Joseph Lister

[1827 - 1912]

- **Father of Antiseptic surgery**
- Professor of surgery.
- Applied Pasteur's work and introduced Antiseptic techniques in Surgery .
- Use of Carbolic acid in Antiseptic surgery.
- Resulted in drop in morbidity and mortality due to surgical sepsis.

Robert Koch

[1843 - 1910]

- **Father of Bacteriology**
- He was a German physician, first isolated bacillus anthracis.
- Anthrax:- The first direct detection of the role of bacteria in causing was provided by the Robert Koch
- His method of preparing, fixing & staining, film preparation of bacteria using staining film.
- Preparation of bacteria using aniline dyes, opened a new area in the bacteriological techniques.

- In 1881, he described his method of preparing culture on solid media & it made possible for the isolation of pure strains of bacteria from single colonies.

- In 1881, solid media using potato & gelatin were in practice for isolation of fungi

- In 1882, he discovered Mycobacterium Tuberculosis & in 1883 the Vibrio Cholera.

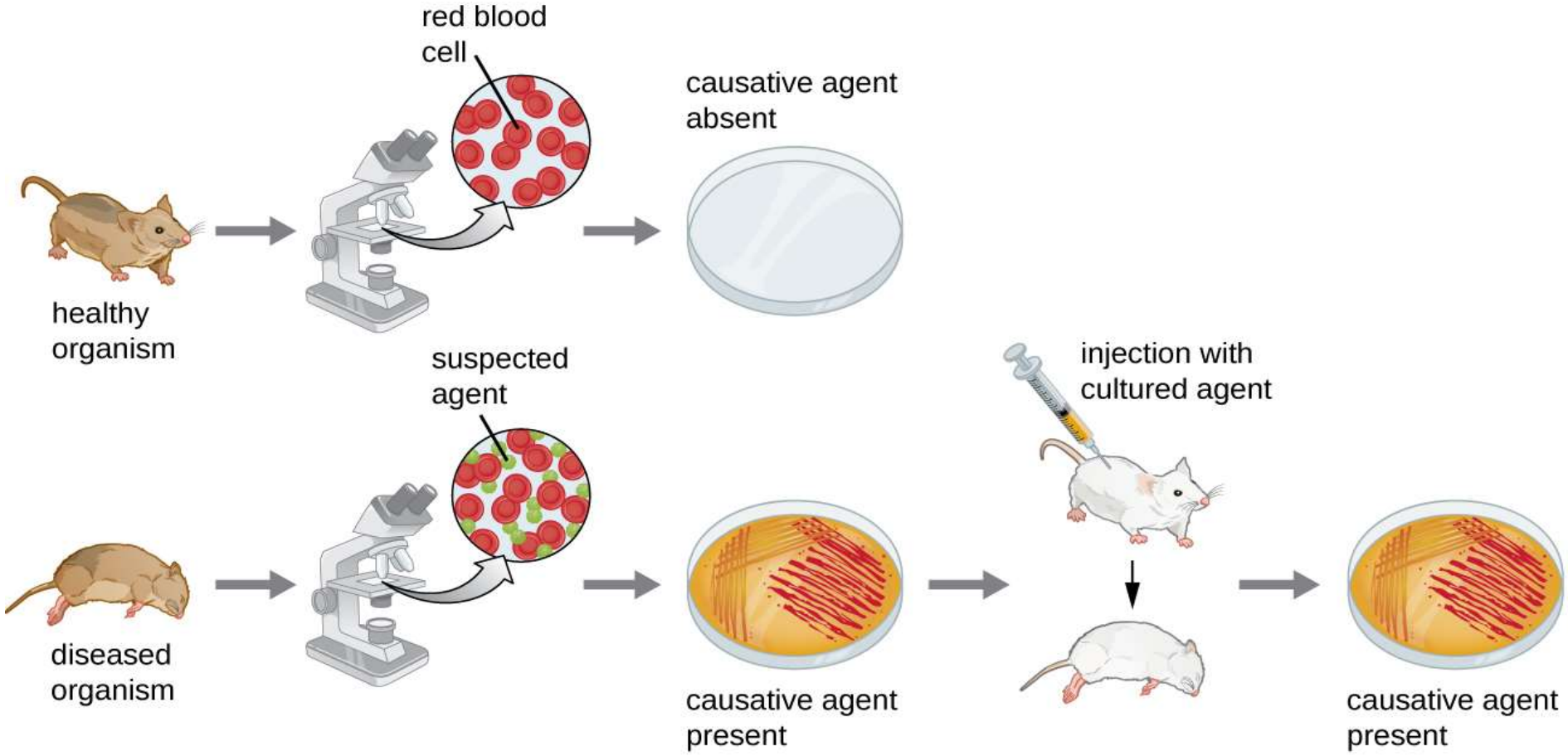
Koch's Postulates

The most notable constitution of Koch was the establishment of the causal relationship between a micro-organism & a specific disease by applying a set of criteria referred to as a

The postulate are:-

1) The micro-organism must be presently in every case of the disease
2) The micro-organism must be isolated from the disease animal & grown in pure culture.
3) The disease must be reproduced when a pure culture of the micro-organism is inoculated into a susceptible host
4) The same micro-organism must be isolated from the experimentally infected host.

Koch's Postulates



1. The suspected causative agent must be absent from all healthy organisms but present in all diseased organisms.
2. The causative agent must be isolated from the diseased organism and grown in pure culture.
3. The cultured agent must cause the same disease when inoculated into a healthy, susceptible organism.
4. The same causative agent must then be reisolated from the inoculated, diseased organism.

Paul Ehrlich

[1854 - 1915]

- **Father of Chemotherapy**
- In 1904 found that the dye trypan red was active against the trypanosome that causes African sleeping sickness.
- This dye with antimicrobial activity was referred to as 'magic bullet'.
- In 1910 Ehrlich in collaboration with sakahiro hata, Japanese physician the drug salvarsan as a treatment for syphilis caused by treponema pallidum.
- In 1912, he discovered neosalvarsan. This gave him the title "the father of chemotherapy"

Alexander Fleming

- Discovery of 1st Antibiotic [1928]
- Accidentally discovered Penicillin produced by a fungus Penicillium
- Left his Staphylococcus culture on an agar plate for 2 weeks → went on vacation → came back & found mold on his plate which prevented bacterial growth

Thank you

Pune District Education Association ,
Shankarrao Ursal College of
Pharmaceutical Sciences & Research
Centre, Kharadi, Pune

High performance liquid chromatography (HPLC)

Presented by :- Dr. Vijaya Barge
(Vice Principal & Professor)

LEARNING OBJECTIVES :

After completing this sub-unit the students should be able:

- To study principle, advantages and disadvantages of HPLC.
- To learn different types of HPLC.
- To study instrumentation and different components used in HPLC.
- To study derivatization techniques under HPLC.
- To study different applications of HPLC.

Contents

1. Background
2. Different types of chromatography
3. Introduction
4. Latest instrument
5. Principle
6. Types of HPLC
7. Application of HPLC
8. Reference

Background

- **Chromatography :-**

Chromatography is technique which is used to separate component mixture due to the different time take for each component travel through stationary phase carry to mobile phase.

Basically, All chromatography systems consists of two phases.

- **Mobile phase :-** liquid or gaseous flows over through the stationary phase .
- **Stationary phase :-** solid , liquid mixture which is immobilized.

- **Analyte :-** Substance that is separate during chromatography.
- **Effluent :-** Mobile phase leaving the columns.

Different types of chromatography:-

1. Paper chromatography
2. Liquid chromatography
3. Gas chromatography
4. High performance liquid chromatography

Introduction

- HPLC stands for “High-performance liquid chromatography” (sometimes referred to as High-pressure liquid chromatography).
- HPLC was developed in 1960 .
- HPLC is a form of liquid chromatography used to Separate compounds that are dissolved in solution.
- HPLC instruments consist of a reservoir of mobile phase, pump, an injector, a separation column, and a detector.
- Compounds are separated by injecting a sample mixture into the column.

- The mobile phase must be degassed to eliminate the formation of air bubbles.
- This technique base on the same mode of separation as column chromatography.
- HPLC is most powerful tool of Analytical techniques.
- In HPLC coloumns are tightly packed and the eluent is forced through the column under high pressure up to 5,000 psi by a pump.
- HPLC allows to used a very smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing through it.
- HPLC allows a much better separation of the components of the mixture.

Latest instrument

Principle

- To understand the principle of HPLC, we must first look at the principle behind liquid chromatography.
- Liquid chromatography is a separation technique that involves:-

1. The injection of a small volume of liquid sample
2. Into a tube packed with porous particles (stationary phase)
3. Where individual components of the sample are transported along the packed tube (column) by a liquid moved by gravity.

- The main principle of separation is adsorption.

- They travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent, travels slower.
- The component which has less affinity towards the stationary phase travels faster.
- Since no two components have the same affinity towards the stationary phase, the components are separated.

- HPLC is a separation technique that involves:-
- The injection of a small volume of liquid sample into a tube packed with tiny particles (3 to 5 micron in diameter called the stationary phase).
- Where individual comments of the sample are moved down the packed tube (column) with a liquid (mobile phase) force through the column by **high pressure delivered by a pump.**
- These components are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles.

- These separated components are detected at the exit of this tube (column) by a flow-through device (detector) that measures their amount. The output from the detector is called a liquid chromatogram.
- In principle, LC and HPLC work the same way except the speed, efficiency, sensitivity and ease of operation of HPLC is vastly superior.

Types of HPLC

- Based on modes of chromatography

1. Normal phase mode
2. Reverse phase mode

- Based on principle of chromatography

1. Absorption chromatography
2. Ion exchanging chromatography

- Based on elution technique

1. Isocratic Separation
2. Gradient separation

- Based on the type of Analysis

1. Qualitative analysis
2. Quantitative Analysis

Based on modes of chromatography

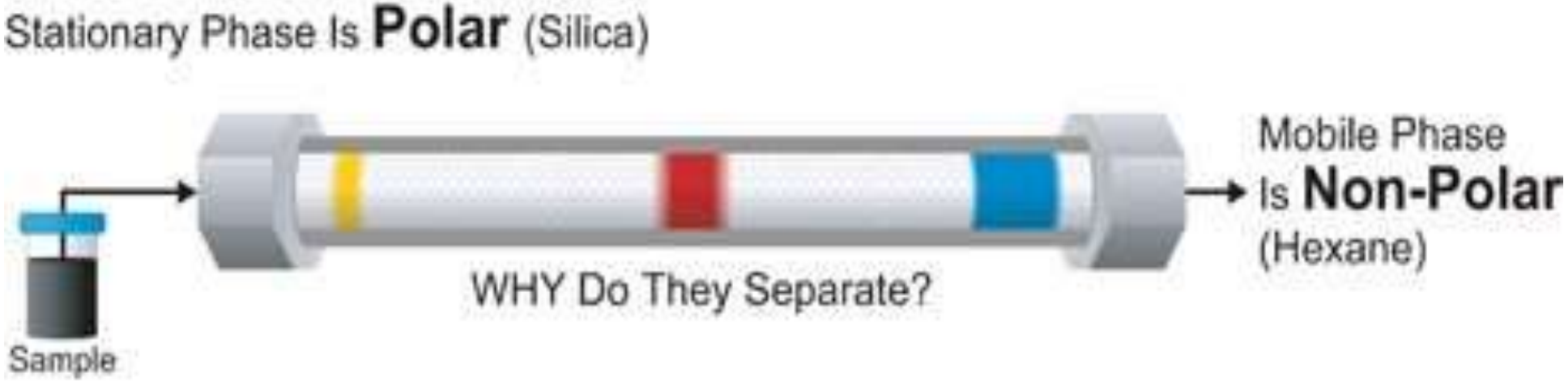
- Based on modes of chromatography there are two modes such as Normal phase mode and Reverse phase mode.
- Before explaining the modes, It is important to know the interaction which occurs between solute, stationary and mobile phase.
- Polar-polar :- Interaction or affinity is more .
- Non-polar – Non-polar :- Interaction or affinity is more .
- Polar - Non-polar :- interaction or affinity is less

Normal phase mode

In Normal phase mode , the Stationary phase (e.g. silica gel) is polar in nature and mobile phase is Non-polar.

In this technique Non-polar compound travels faster and are eluted first.

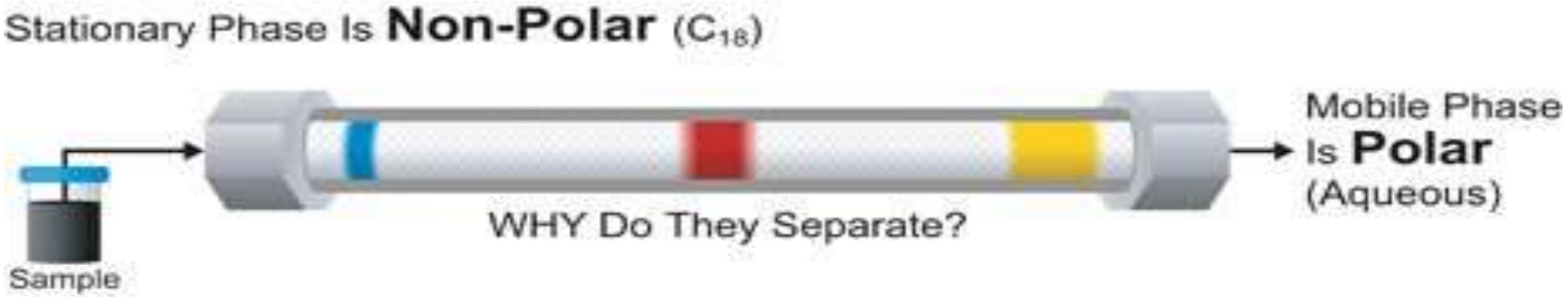
This is because of less affinity between solute and stationary phase.



Reverse phase mode

In reverse phase technique , a Non-polar Stationary phase is used. The Mobile phase is polar in nature.

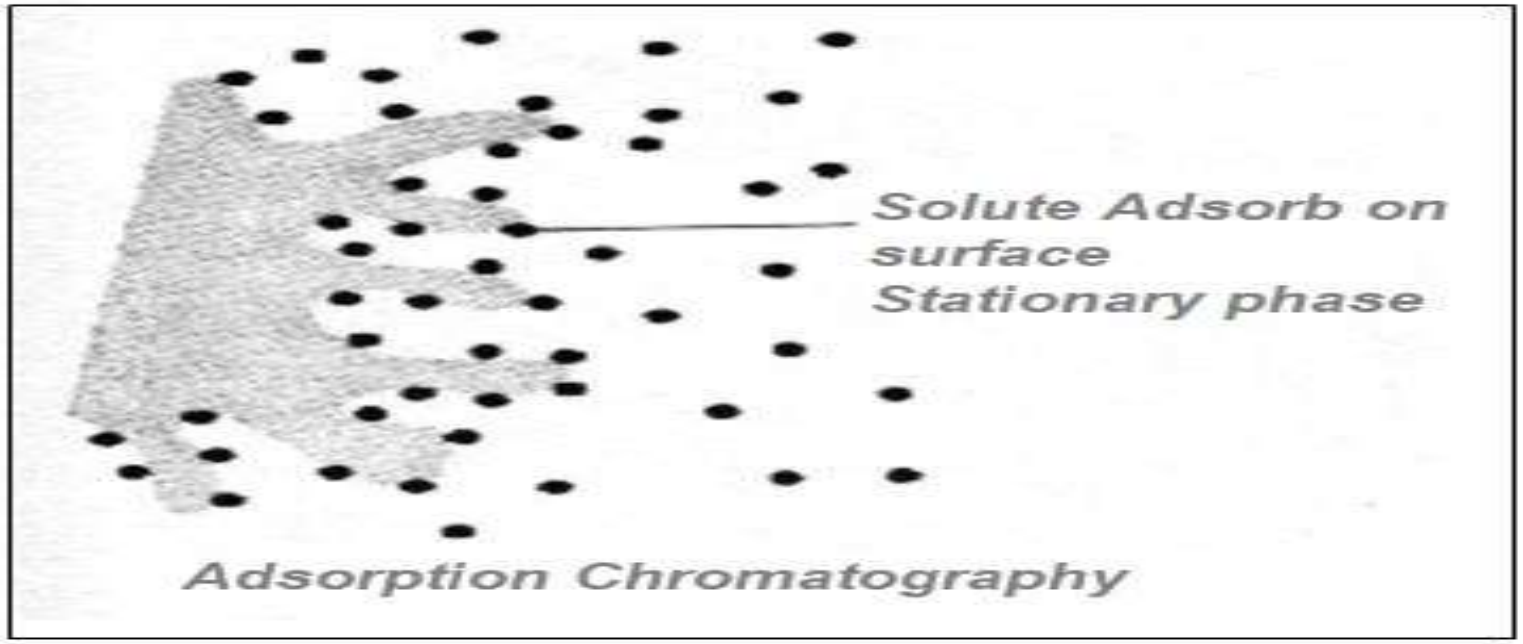
Hence polar components get eluted first and Non-polar compound are retained for longer time.



Based on principle of separation

Absorption Chromatography :-

The principle of absorption Chromatography separation of components takes place because of the difference in affinity of compound towards stationary phase.

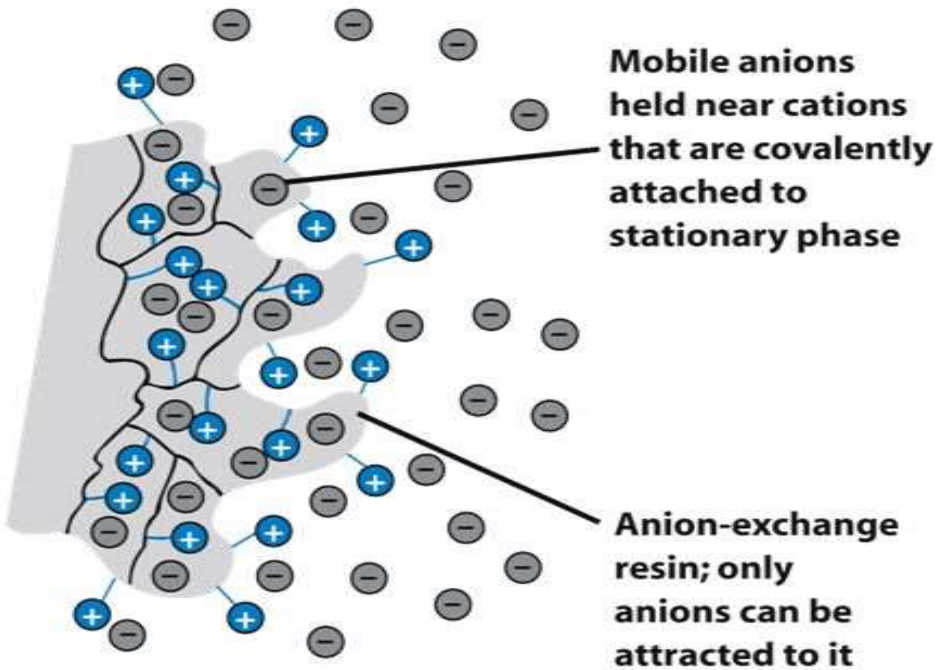


Ion exchange chromatography

The principle of separation is ion exchange, which is reversible exchange of functional groups.

In ion exchange chromatography, an ion exchange resin is used to separate a mixture of similar charged ions.

1.1. Ion-Exchange Chromatography



What is exchanged?

Anion for anion and cation for cation

How are the ions exchanged?

Ions with higher affinity displace ions of lower affinity to the stat. phase

Affinity increases as:

1. Charge increases
2. Size of (solvatized) ions decreases
3. Polarizability increases

Based on elution Technique

Isocratic separation :-

In this technique the same mobile phase combination is used throughout the process of separation.

The same polarity or elution strength is maintained throughout the process .

Gradient separation :-

In this technique a mobile phase combination of lower polarity or elution strength is used followed by gradually increasing the polarity or elution strength.

Based on the type of analysis

Qualitative Analysis :-

Which is used to identify compounds, detect the presence of impurities, to find out the number of compound etc. This is done by using retention time values.

Quantitative Analysis :-

This is done to determine the quantity of the individual or several components in a mixture. This is done by comparing the peak area of the standard and sample.

Application of HPLC

Chemical

polystyrenes
dyes
phthalates

Bioscience

proteins
peptides
nucleotides

Pharmaceuticals

tetracyclines
corticosteroids
antidepressants
barbiturates

Consumer Products

lipids
antioxidants
sugars

Environmental

polyaromatic hydrocarbons
Inorganic ions
herbicides

Clinical

amino acids
vitamins
homocysteine

1. HPLC which is used in Chemistry and Biochemistry research analysing complex mixtures.
2. HPLC which is used to purifying chemical compounds.
3. HPLC which is used quality control to ensure the purity of raw material.
4. HPLC which is used to analysing air and water pollutants.
5. HPLC which is used to purification of some compounds of natural or synthetic origin on preparative scale.
6. HPLC which used in stability studies.
7. HPLC which used in Biopharmaceutical and pharmacokinetic studies.
8. HPLC which is used in isolation and identification of drugs or metabolites in urine , plasma, serum etc. Can be carried out.
9. HPLC which is used in isolation and identification of mixtures of components of natural or synthetic origin.

References

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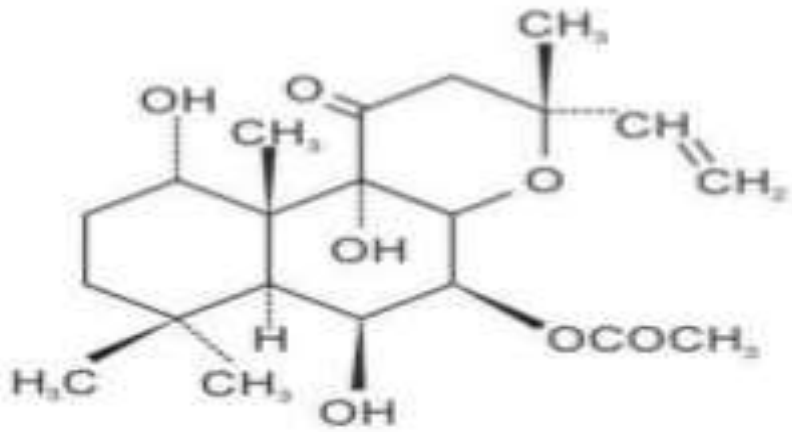
2. http ://scimedia.com
3. http ://www.forumsci.co.il/HPLC.
4. Dr.Walode S.G, Dr.Chandan R.S., Instrumental method of Analysis, Nirali Prakashan, first edition sep2020, page no 13.1, 13.36

Thank You!

FORSKOLIN

Biological Source: Labdane diterpenoid extracted from roots of *Coleus forskohlii*, family- Lamiaceae.



Chemical Structure of Forskolin

Industrial Production:

1. Roots & bark powder extracted with toluene at 60°C for 2 hours.
2. Filtrate collected & concentrated at temperature not exceeding 40°C.
3. Concentrated extract mixed with n-hexane, yields crude forskolin in the form of brown ppt.
4. Purified using column chromatography.

- **Estimation:**

TLC & HPTLC

Mobile phase – Toluene: ethyl acetate (8.5: 1.5 v/v)

Stationary phase- Silica gel F254

Visualizing agent- 5% vanillin in glacial acetic acid and 10% sulphuric acid in water.

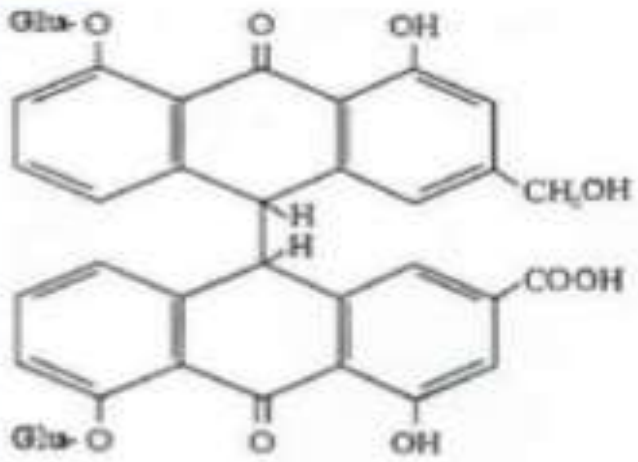
- **Utilization:**

1. Antidepressant
2. Vasodilating
3. Antiobesity
4. In glaucoma
5. Antiasthmatic

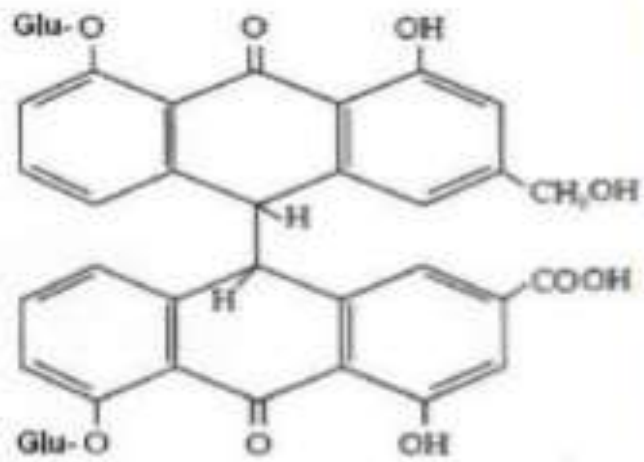
SENNOSIDES

- **Source:** Dianthrone glycosides, leaflets of *Cassia angustifolia* (Indian senna) & *C. acutifolia* (Alexandrian senna).

  Family- Leguminosae.



Sennoside A



Sennoside B

Industrial production:

1. Dried senna leaves powder extracted with benzene for 2-3 hrs.
2. Marc is dried and extracted with methanol for 4-6 hrs.
3. Mix both the extracts and concentrated .
4. pH of extract adjusted to 3.2 by HCl.
5. Extract is mixed with hydrous calcium chloride in 25 ml denatured spirit.
6. pH adjusted to 8 using ammonia & set aside for 2hrs, results into ppt of sennosides.

- **Estimation:**

Column- C18

Mobile phase- 1% acetic acid in water: Acetonitrile (82:18)

Flow rate- 1ml/min

Detection- 350 nm

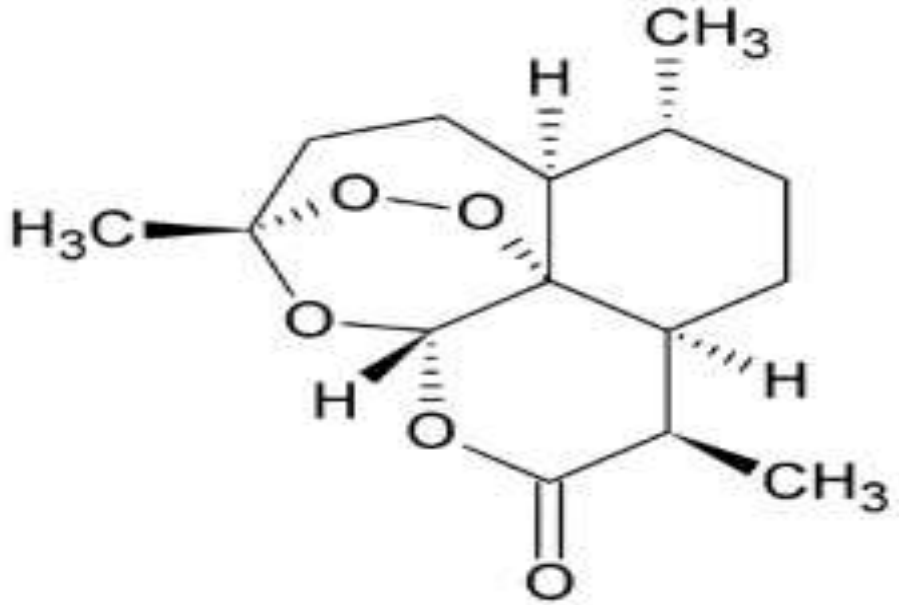
- **Utilization:**

1. Treatment of constipation
2. In skin diseases
3. As an anthelmintic
4. Useful in loss of appetite, dysentry, indigestion, malaria, jaundice, gout, rheumatism & anaemia.

ARTEMISININ

- **Source:** sesquiterpene lactone obtained from the leaves & unexpanded flower heads of *Artemisia annua*.

  Family- Asteraceae.



Industrial production:

1. Fresh leaves are dried below 60°C, powder is extracted with methanol by maceration.
2. Methanol extract partitioned with hexane
3. The hydro alcoholic extract partitioned with ethyl acetate until the colourless.
4. Contentrated at controlled temperature at 40°C under vacuum.
5. Artemisinin obtained as fine white crystals after recrystallization with cyclohexane.

- **Estimation:**

HPLC & HPTLC method

Mobile phase- n-hexane : ethyl acetate (7.5: 2.5 v/v)

Stationary phase- silica gel F254

Visulazing agent- anisaldehyde sulphuric acid reagent followed by heating to 110°C.

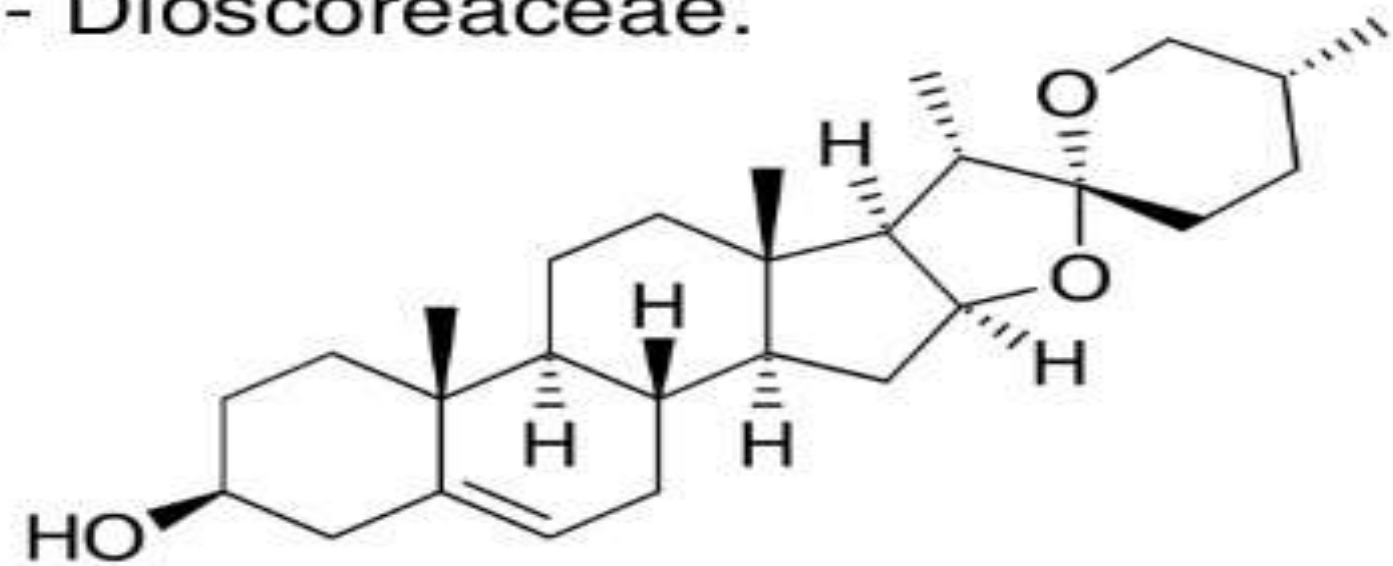
- **Utilization:**

1. Antimalarial
2. In gastric infections
3. Suppress inflamatory immune reactions
4. Anticancer

DIOSGENIN

- **Source:** Aglycone obtained after the hydrolysis of steroidal saponin glycoside dioscin present in *Dioscorea deltoidea, D. composite.*

  Family- Dioscoreaceae.



Industrial production:

1. Dried powder hydrolyzed with 2.5N H_2SO_4 by reflux or autoclave.
2. Marc washed with 10% sod. Bicarbonate to neutralize acid.
3. Hydrolyzed powder extracted with benzene for 6-8 hrs.
4. Benzene extract is filtered, residue dissolve in chloroform and concentrated by recystallization.

- **Estimation:**

HPTLC method

Mob. Phase- toluene: ethyl acetate: formic acid (5:4:1)

St. phase- Silica gel F 254

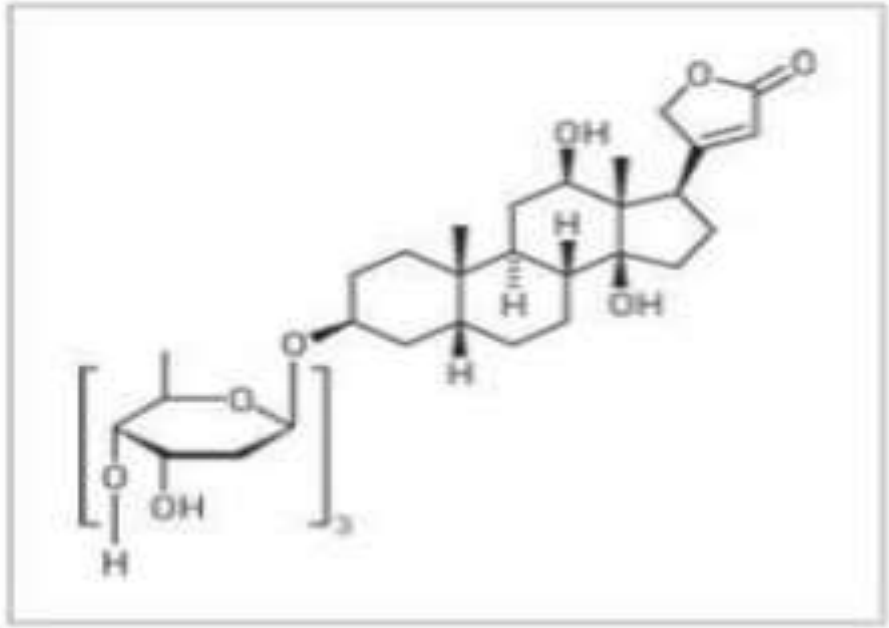
- **Utilization:**

1. As a precursor for steroidal synthesis
2. In preparation of oral contraceptives
3. In treatment of rheumatism.

DIGOXIN

- **Source:** Cardiac glycoside obtained from leaves of *Digitalis lanata.*

  Family- Scrophulariaceae



Industrial production:

1. Fresh leaves made into paste & treated with neutral salt.
2. Paste is defatted with benzene & followed by extraction with ethyl acetate
3. Extract contain lanatoside C, which after hydrolysis yields digoxin.

- **Estimation:**

Assay- 40 mg test & std solution of digoxin dissolve in sufficient ethanol.

5 ml of resulting solution, add 3ml picric acid solution.

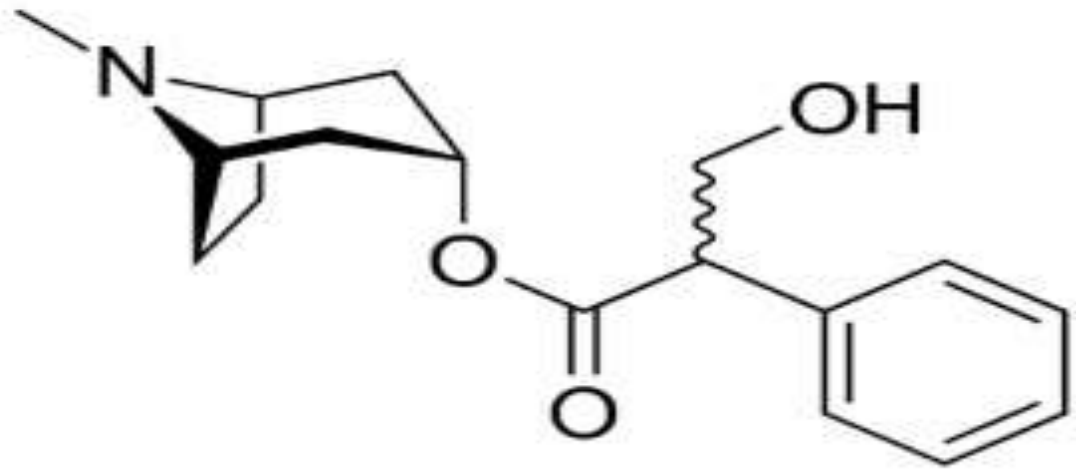
Measure absorbance at 495 nm.

- **Utilization:**

treatment of cardiac disorders.

ATROPINE

- **Source:** tropane alkaloid, flowering tops of *Atropa belladonna, Datura stramonium* & *Hyoscyamus niger.*
- Family- Solanaceae.



- **Industrial production:**

1. Powdered drug extracted with ether or benzene
2. Concentrate the non-polar extract & partitioned with acetic acid.
3. Add sodium bicarbonate leading to ppt alkaloid
4. Dry the ppt & crystallized by dissolving in solvent ether

- **Estimation:**

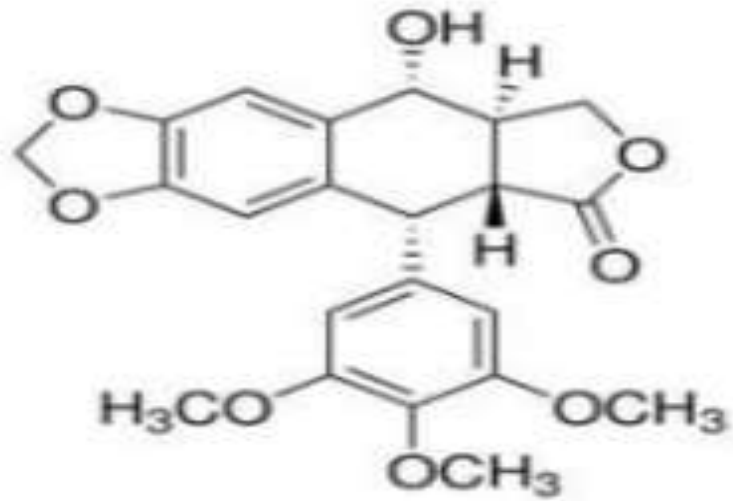
Assay- sulphate salt of atropine titrated against 0.1 N perchloric acid.

- **Utilization:**

1. As preanesthetic medication
2. Antispasmodic

PODOPHYLLOTOXIN

- **Source:** resin, roots & rhizomes of *Podophyllum hexandrum, P. emodi & P. peltatum.*
- Family- Berberidaceae.



- **Industrial production:**

1. Dried roots & rhizomes extracted with methanol
2. Evaporate the filtrate to semisolid mass
3. Dissolve in acidic water results into pptn of podophyllotoxin

- **Estimation:**

HPLC

Mob. Phase- methanol: water (62: 38 v/v)

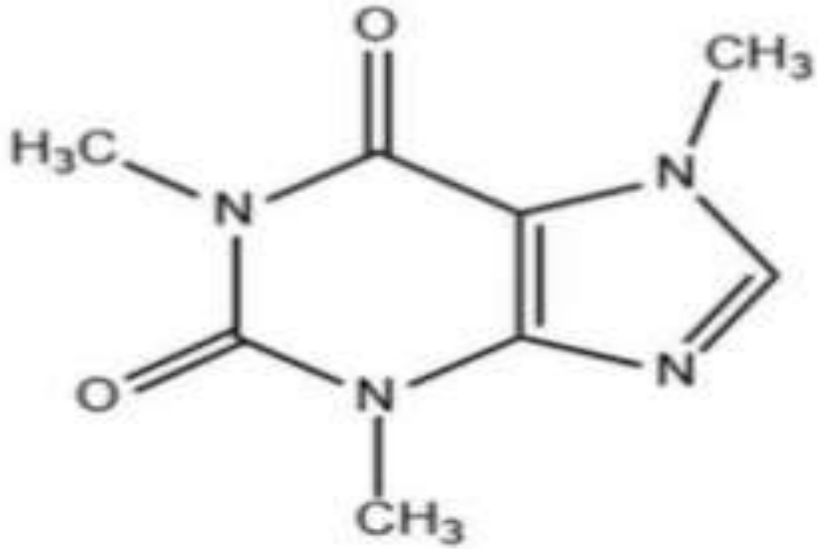
Detector wavelength- 280nm.

- **Utilization:**

1. Antitumour
2. Purgative
3. Emetic
4. Treatment of warts

CAFFEINE

- **Source:** xanthine alkaloid, leaves of *Camellia sinesis* (Theaceae), seeds of *Coffea arabica* (Rubiaceae).



- **Production:**

1. Leaflet powder boiled with 2% sodium carbonate water for 10 min & filtered.
2. Evaporate & partitioned with dichloromethane
3. Evaporate to get crystals of caffeine.
4. Purified by recystallization from hot ethanol.

- **Estimation:**

HPLC method

Mob. Phase- methanol: acetonitrile (65: 35 v/v)

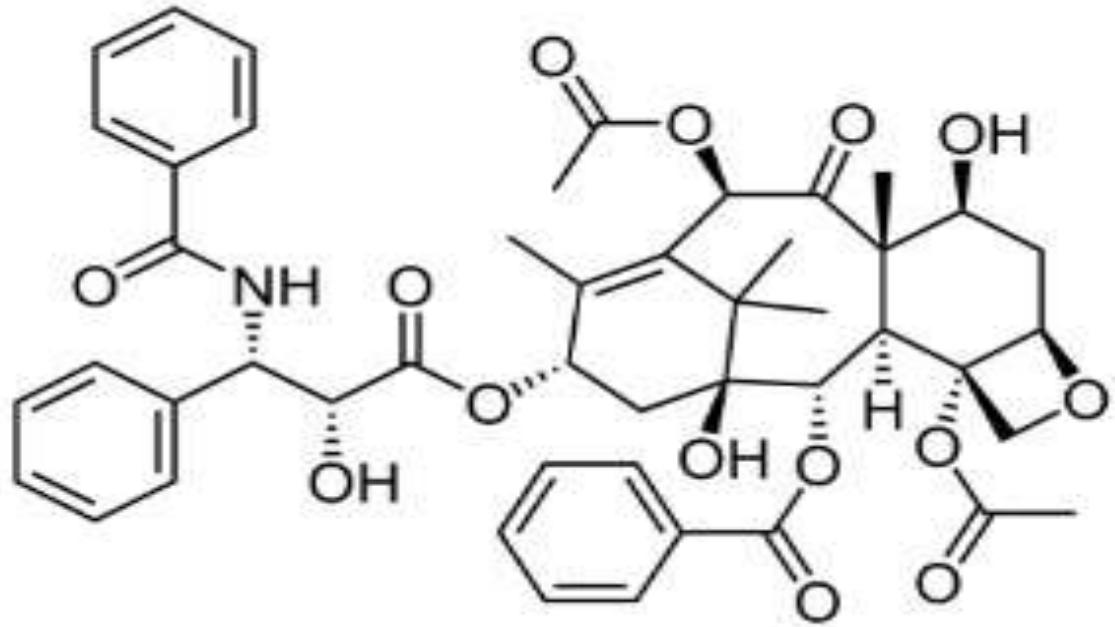
Column- C18

- **Utilization:**

Stimulant

TAXOL

- **Source:** nitrogen containing subs, bark of *Taxus brevifolia*, fam- taxaceae.



- **Production:**

1. Powdered bark extracted with methanol, filtered & evaporated to dryness.
2. Partition with the mixture of carbon tetrachloride & water, filter & evaporated.
3. Dried CCl_4 fraction again extracted with CCl_4 : methanol, evaporate to obtain crude taxol.

- **Estimation:**

HPTLC method

Mob phase- chloroform:methanol (7:1v/v)

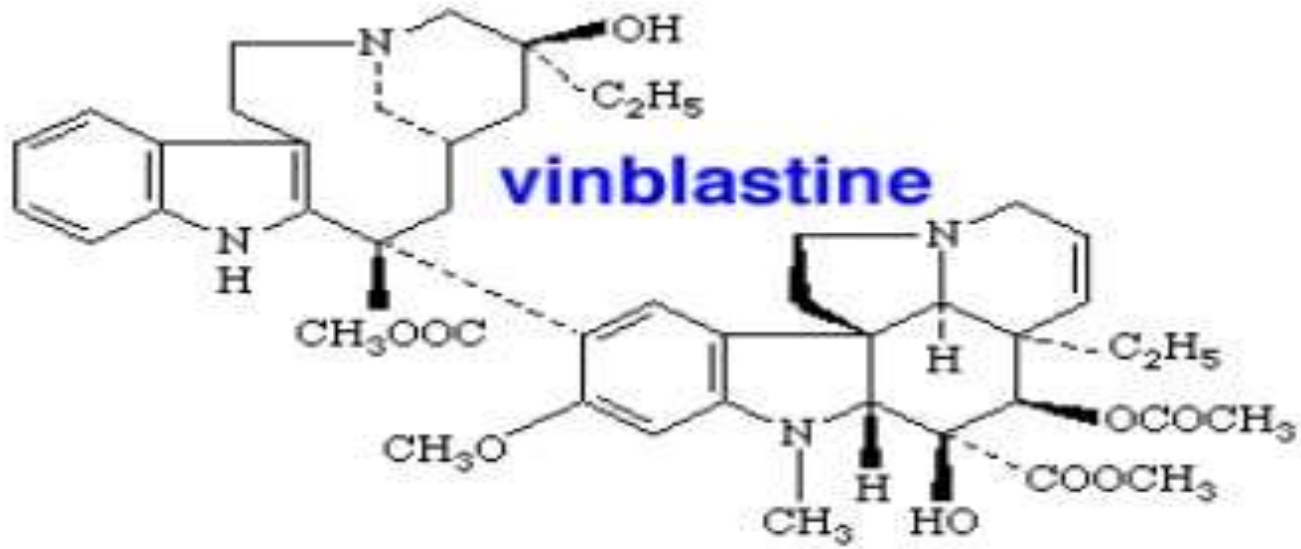
Visualizing agent- vanillin sulphuric acid.

- **Utilization:**

1. Treatment of ovarian, lung, bladder, esophageal & other types of cancers.
2. Antiproliferative agent.

VINCRISTINE & VINBLASTINE

- **Source:** Indole alkaloid, *Vica rosea*, family- Apocynaceae.



VINCRISTINE & VINBLASTINE

- **Production:** Plant tissue culture technique.

- **Estimation:** HPLC method

Mob phase- acetonitrile: 0.1 M phosphate buffer.

Wavelength- 254nm.

- **Utilization:**

1. In chemotherapy regimens
2. Childhood leukemia
3. immunosuppressant

Introduction of Audits and Regulatory Compliance

By

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Department of Pharmaceutical Chemistry
Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune

Contents

- Introduction.
- Definition
- Scope of Auditing
- Qualities of an Auditor
- Techniques of Auditing
- Objectives of Audits.
- Management of Audit.
- Responsibilities.
- References

Definition Of Audits

- Auditing in simple terms could be defined as inspection of a process or a system to make sure that it complies with the requirements of its intended use.
- Auditing is a systematic and independent examination of data, statements, records, operations and performances (financial or otherwise) of an enterprise for a stated purpose. In any auditing situation, the Auditor perceives and recognizes the propositions before him for examination, collects evidence, evaluates the same and on this basis, formulates his judgment which is communicated through his audit report."

Scope of Auditing

- In comparison with earlier days, where the main objective of auditing was to detect fraud, we now have enhanced ways to determine a true and fair view of financial statements. In recent times, almost every country of the world has introduced various legislations and framed rules and regulation of auditing.
- In India also legislations related to Tax Audit, Cost Audit, Management Audit and operation Audit, etc. are coming up.
- The main purpose of auditing is to certify the correctness of financial statements and to detect errors and frauds.

Qualities of an Auditor

- He must have adequate skills and qualities to conduct his work efficiently.
- An Auditor must be honest, impartial and unbiased.
- He should also be hard-working, have adequate common sense, capacity to hear arguments of others, systematic and methodical.
- An Auditor should ask for clarification on matter on which he is unable to understand the information provided to him.
- His audit report should be correct and clear.
- He must have thorough knowledge of financial management, industrial management and business organizations.

Techniques of Auditing

Following are the common techniques of auditing −

- Checking of posting and casting.
- Physical verification of assets.
- Verification and examination of transactions with available evidences.
- Scrutiny of the books of accounts,
- Checking of various calculations.
- Checking of carried forward balances in next year.
- Checking of Bank reconciliation statements.
- Auditor can get information from inside and outside sources of

Quality Audit

- A systematic and independent examination to determine whether quality activities and related results comply with planned arrangement and whether these arrangements are implemented effectively and are suitable to achieve objectives.

- TYPES OF QUALITY AUDIT:
  1)INTERNAL QUALITY AUDIT
  2)EXTERNAL QUALITY AUDIT

1)INTERNAL QUALITY AUDIT

a) Quality system audit,
b) Management quality audits
c) Process quality audits,
d) Data processing quality audits.
e) Decision Sampling.

2) EXTERNAL QUALITY AUDIT

a)Quality system certification
b)Vendor appraisal
c)Regulatory controls
d)Quality system improvement audit
e)Product certification

Objectives of Audits

There are Three type of objectives of auditing are discussed as follow;-

A) PRIMARY OBJECTIVES

B) SECONDARY OBJECTIVES

C) SPECIAL OBJECTIVES

A) PRIMARY OBJECTIVES

The Primary objectives of auditing are as below;-

1) **FAIRNESS OF STATEMENTS:** The purpose of auditing is determine the fairness of statements. The financial statements show true and fair view after auditing. Due to limitations financial statements it is not possible to provide cent perc accuracy, So an attempt is made to show the fair view of finan statements.

2) **PRESCRIBED LAWS:** The purpose of the audit is to check t prescribed laws have been followed in the preparation of finan statements. There are various laws that govern the working many businesses. The auditor can indicate whether the prescri laws were followed in the preparation of final accou

3) ACCOUNTING POLICIES: The purpose of auditing is to examine the accounting policies. There is need to follow the accounting policies for preparing accounting records. The effective accounting system can provide better results. The auditor can express an opinion on the accounting policies in the best interest of business.

4) INDEPENDENT OPINION: The purpose of the audit is to express an independent opinion. The auditor must be honest in his work. Management and other persons must not influence him There must be high ethical standard for independent reporting.

B) SECONDARY OBJECTIVES

The Secondary objectives of auditing are as below;-

1. **DETECTION OF ERRORS:** The purpose of auditing is to detec the errors. The auditor can use ways to find out errors in the accountin records.

2. **DETECTION OF FRAUDS:** The purpose of auditing is to detec frauds. The management is responsible for the detection of frauds. Th management can take steps to correct the wrong effects of frauds fo the benefit of owners.

3.PREVENTION OF FRAUDS: The purpose of auditing is to Preven frauds. In accounting, it includes manipulation or alteration of record and misappropriation of assets, an omission of the effects of th transaction from records or documents, recording of the transactio without substance and misapplication of accounting policie

C SPECIAL OBJECTIVES

The Special objectives of auditing are as below;-

1. MANAGEMENT AUDIT: The purpose of management audit is to assess the performing, review the organizational structure and suggest the best course of action. It is a voluntary audit.

2. TAX AUDIT: The purpose of auditing is to satisfy the taxation officers. These can be conducted to determine the income. The sole proprietors and partnership firms can settle their tax matters through tax audit

3. SOCIAL AUDIT: The purpose of the social audit is to measure social performance of the business. The society is concerned with the Protection of natu environment The social audit can examine the business performance of the socie

4. PROPRIETY AUDIT. the purpose of propriety audit is to examine the proper use of money. There is a requirement of economic use of resources in the best interest of business. There must be a justification for spending every rupee for th benefit of business. The audit can determine the wise use of money.

5. COST AUDIT: The purpose of cost audit is to verify the correctness of cost accounts. The management must have followed the cost objectives in maintaining books and other records. The cost audit can help the management to improve the efficiency in doing business.

6, OPERATIONS; The purpose of operations audit is to prevent misuse of resources It is a part of social audit The management must use prudence in spending money. There is need of intelligent use of resources for optimum output with lowest possible cost.

7. BID OFFER: The purpose of the audit is to determine the real value of the business forbid offer. The value of net tangible assets becomes the basis of Sales. The bidders can offer bid price on the basis of such price. The audited accounts serve as a guideline to arrive at a certain decision.

8. PURCHASE CONSIDERATION: The purpose of the audit is to determine the purchase price of a business. The audited accounts determine value of assets and liabilities. The buyers and sellers Come to know the real value of a business. They can make a deal to amalgamate or merge business.

9. LOAN: The purpose of the audit may be a loan. The management can approach banks and other lenders. The bankers rely on audited accounts for the supply of money. The audited accounts are legal requirements of the loan facility.

10. ADMISSION: The purpose of the audit may be an admission of a partner. The audited accounts can provide information to new as well as old partners. They can decide terms and conditions for admission. The value of assets and liabilities is agreed upon.

11. PROFITS: The purpose Of audit may be checking variations in profits. The fluctuation in profits can be analyzed by an expert auditor. The file of business depends on reasonable profits.

Management of Audit

- Is a systematic examination of decisions and actions of the management to analyse the performance.
- audit involves the review of managerial aspects like organizational objective, policies, procedures, structure, control and system in order to check the efficiency or performance of the management over the activities of the Company.
- Audit management helps simplify and well-organise the work flow and collaboration process of compiling audits.
- Most audit teams heavily rely on email and shared drive for sharing information between each other.

- Typically task such as submitting client request, sender reminder and following up on findings are all done from using broad tools.
- Audit management oversees the internal/external audit staff, establishes audit programs, and hires and trains the appropriate audit personnel.
- The staff should have the necessary skills and expertise to identify inherent risks of the business and assess the overall effectiveness of controls in place relating to the company's internal controls.

- To manage an audit team in a manner to achieve good results, it is important that audit managers have strong leadership skills,
- Auditing involves planning, method, facts, procedures, controls, risk, and management.
- Communication skills is key to many successful auditors.

Responsibilities

- 1) Appointing responsible individual (s) to work with the auditor(s)
- 2. Providing a work area and facilities for the auditor
- 3. Ensuring auditor(s) access to the necessary facilities
- 4. Attending specific meetings with the auditor (s)
- 5. Reviewing the audit findings to ensure agreement with the facts.

Responsibilities

A. Team Leader Responsibilities

- Make final decision for all phases of the audit.
- Prepare Audit Plan.
- Brief the team.
- Review working documents to ensure adequacy.
- Report critical nonconformities to the auditee immediately.
- Report any major obstacles encountered during the audit.
- Represent the audit team at opening and closing meetings.

B. Team Member Responsibilities:

- Prepare any work documents (Including check-list) necessary to carry out those tasks.
- Review all relevant information related to their assigned tasks.
- Report deficiencies and audit findings to team leader.
- Communicate the audit requirements to the auditee.
- Collect objective evidence from the audit.
- Report the audit findings to auditee.
- Verify corrective actions taken in response to CAR's

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Introduction of Biopharmaceutics

Presented by

Mr. Sujit Kakade

Assistant Professor

Pune District Education Association's
Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre, Kharadi, Pune.

Need of Biopharmaceutics Discipline

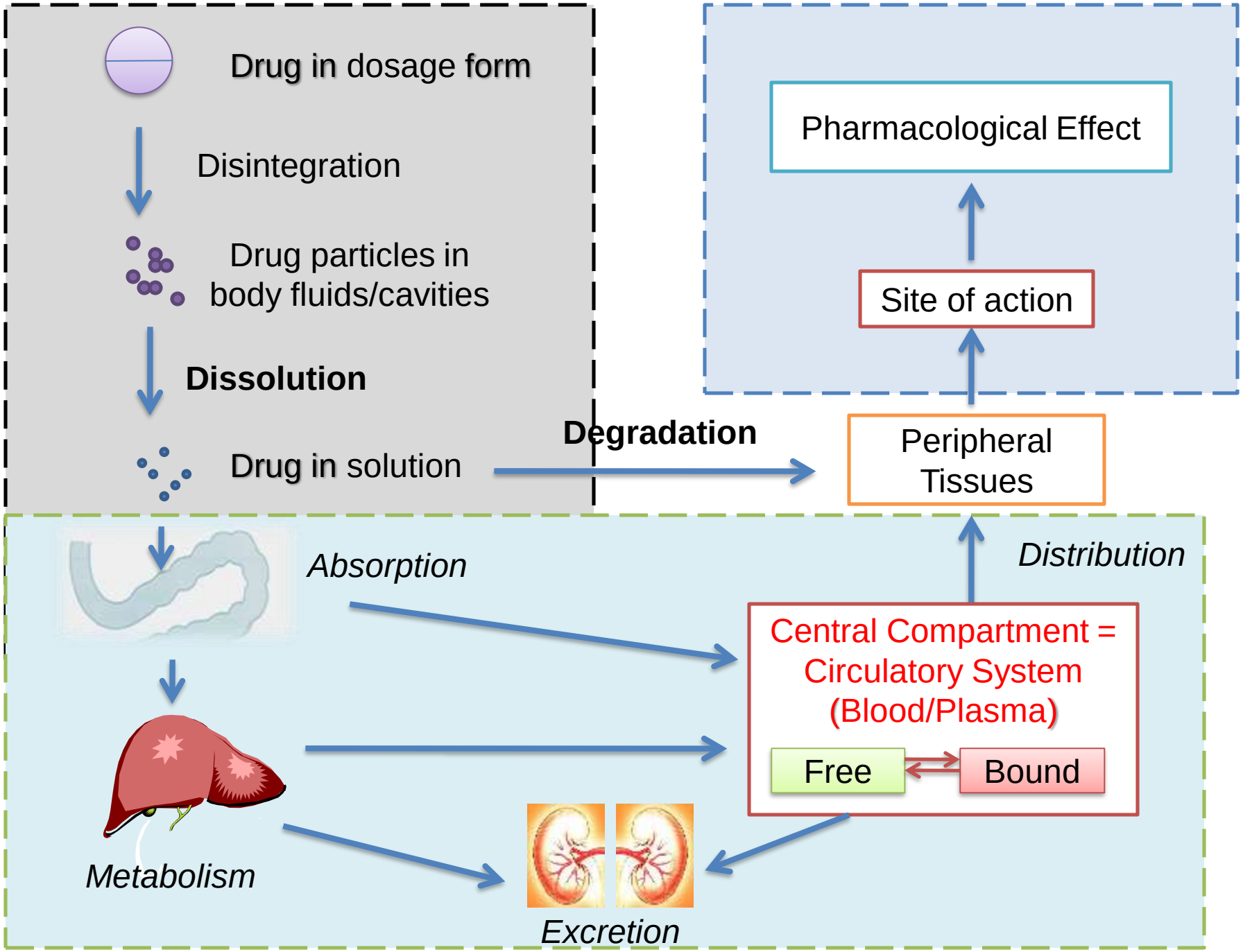
- Earlier, it was believed that the therapeutic response to a drug is an attribute of its intrinsic pharmacologic activity.
- But today, it is very much understood that the dose-response relationship obtained after drug administration by different routes or example, oral and parenteral, are not the same.
- Variations are also observed when the same drug is administered as different dosage forms or similar dosage forms produced by different manufacturers, which in turn depend upon the physicochemical properties of the drug, the excipients present in the dosage form, the method of formulation and the manner of administration.
- A new and separate discipline called *biopharmaceutics* has therefore been developed to account for all such factors that influence the therapeutic effectiveness of a drug.

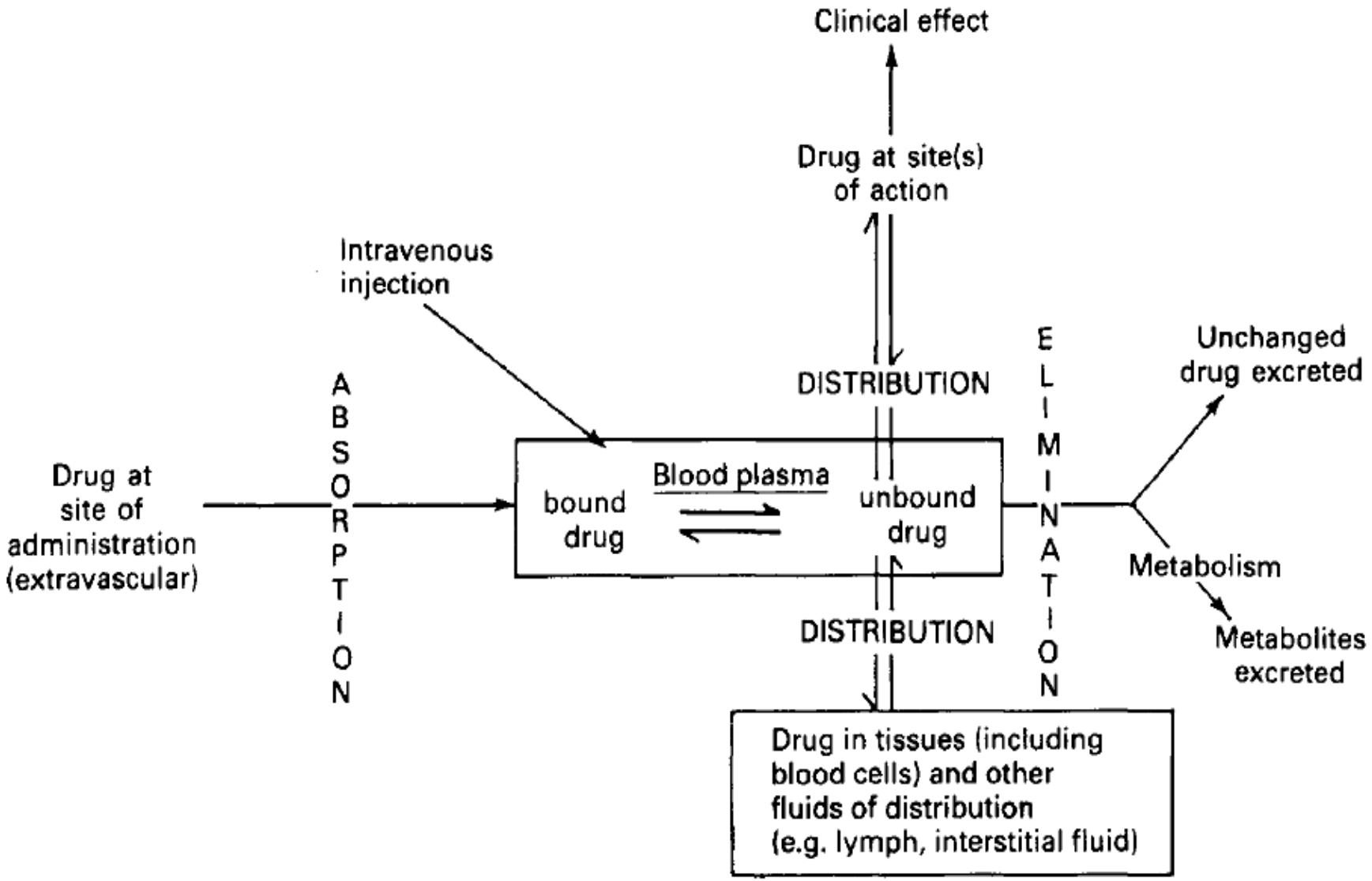
Applications of Biopharmaceutics

- Explain difference in drug effect
- Design of generic drug
- Design drug delivery system

Definition of Biopharmaceutics :

“Biopharmaceutics is defined as the study of factors influencing the rate and amount of drug that reaches the systemic circulation and the use of this information to optimize the therapeutic efficacy of the drug products.”


Drug in dosage form
Disintegration
Drug particles in body fluids/cavities
Dissolution
Drug in solution
Degradation
Pharmacological Effect
Site of action
Peripheral Tissues
Absorption
Distribution
Central Compartment = Circulatory System (Blood/Plasma)
Free
Bound
Metabolism
Excretion


Clinical effect
Drug at site(s) of action
Intravenous injection
DISTRIBUTION
ELIMINATION
Unchanged drug excreted
ABSORPTION
Drug at site of administration (extravascular)
Blood plasma
bound drug
unbound drug
Metabolism
DISTRIBUTION
Metabolites excreted
Drug in tissues (including blood cells) and other fluids of distribution (e.g. lymph, interstitial fluid)

Drug absorption

Drug absorption is defined as the process of movement of unchanged drug from the site of administration to systemic circulation.

Distribution

Distribution is defined as the reversible transfer of a drug between one compartment and another.

Biotransformation / Metabolism

Biotransformation of drugs is defined as the conversion from one chemical form to another.

The term is used synonymously with metabolism.

Excretion

Excretion is defined as the process whereby drugs and/or their metabolites are irreversibly transferred from internal to external environment.

Excretion of unchanged drug is important in the termination of its pharmacologic action.

The principal organs of excretion are kidneys.

Excretion by organs other than kidneys such as lungs, biliary system, intestine, salivary glands and sweat glands is known as nonrenal excretion.

Bioavailability

Bioavailability is defined as the rate and extent (amount) of absorption of unchanged drug from its dosage form.

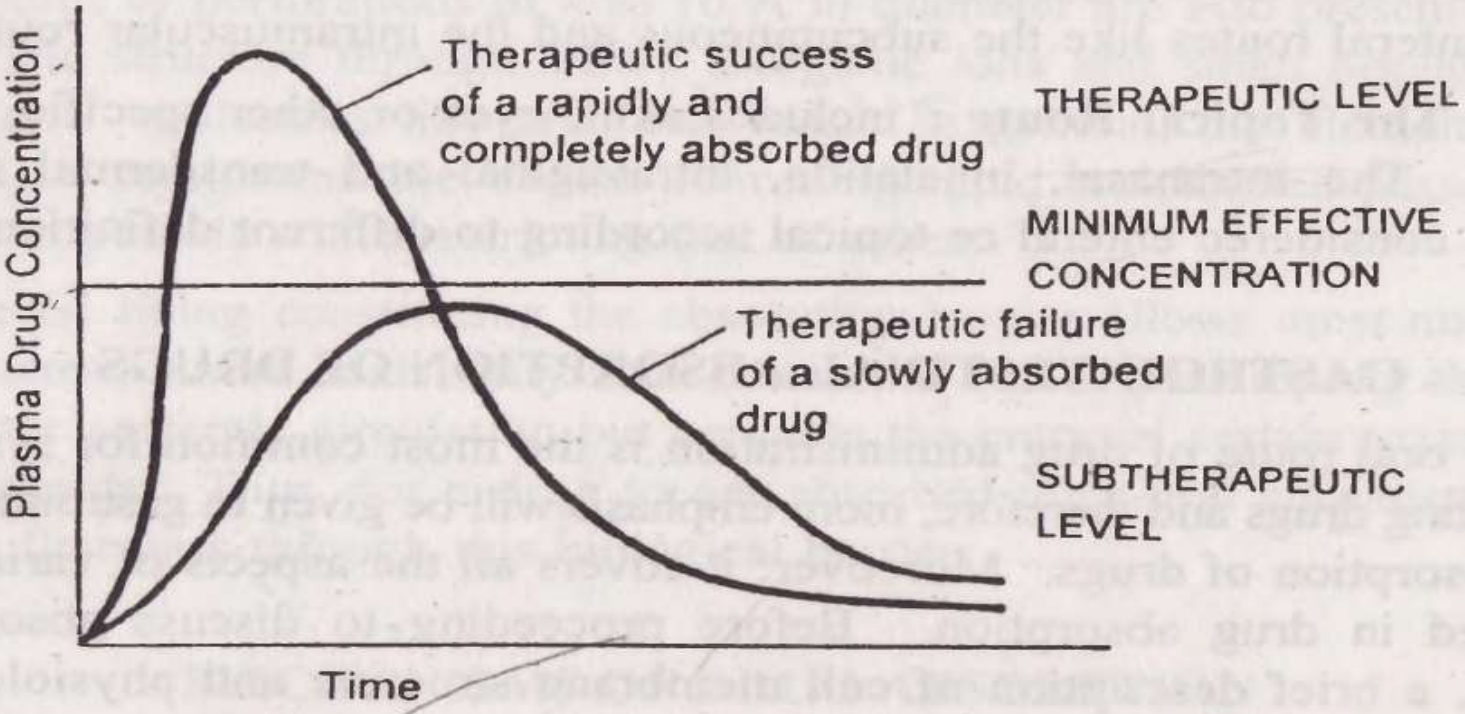


Fig. 2.1 Plots showing significance of rate and extent of absorption in drug therapy

For a drug to be 100% bioavailable…

- The drug must be:
  - completely released from the dosage form
  - fully dissolved in the gastrointestinal fluids
  - stable in solution in the gastrointestinal fluids
  - pass through the gastrointestinal barrier into the mesenteric circulation without being metabolized
  - pass through the liver into the systemic circulation unchanged

- Equivalence : It is a relative term that compares drug products with respect to a specific characteristic or function or to a defined set of standards.

- There are several types of equivalences.

- Chemical Equivalence : It indicates that two or more drug products contain the same labeled chemical substance as an active ingredient in the same amount.

- Pharmaceutical Equivalence : This term implies that two or more drug products are identical in strength, quality, purity, content uniformity and disintegration and dissolution characteristics; they may however differ in containing different excipients.

- Therapeutic Equivalence : This term indicates that two or more drug products that contain the same therapeutically active ingredient, elicit identical pharmacological effects and can control the disease to the same extent.

- **Bioequivalence :**

  It is a relative term which denotes that the drug substance in two or more identical dosage forms, reaches the systemic circulation at the same relative rate and to the same relative extent.

  i.e. their plasma concentration-time profiles will be identical without significant statistical differences.

Pharmacokinetics

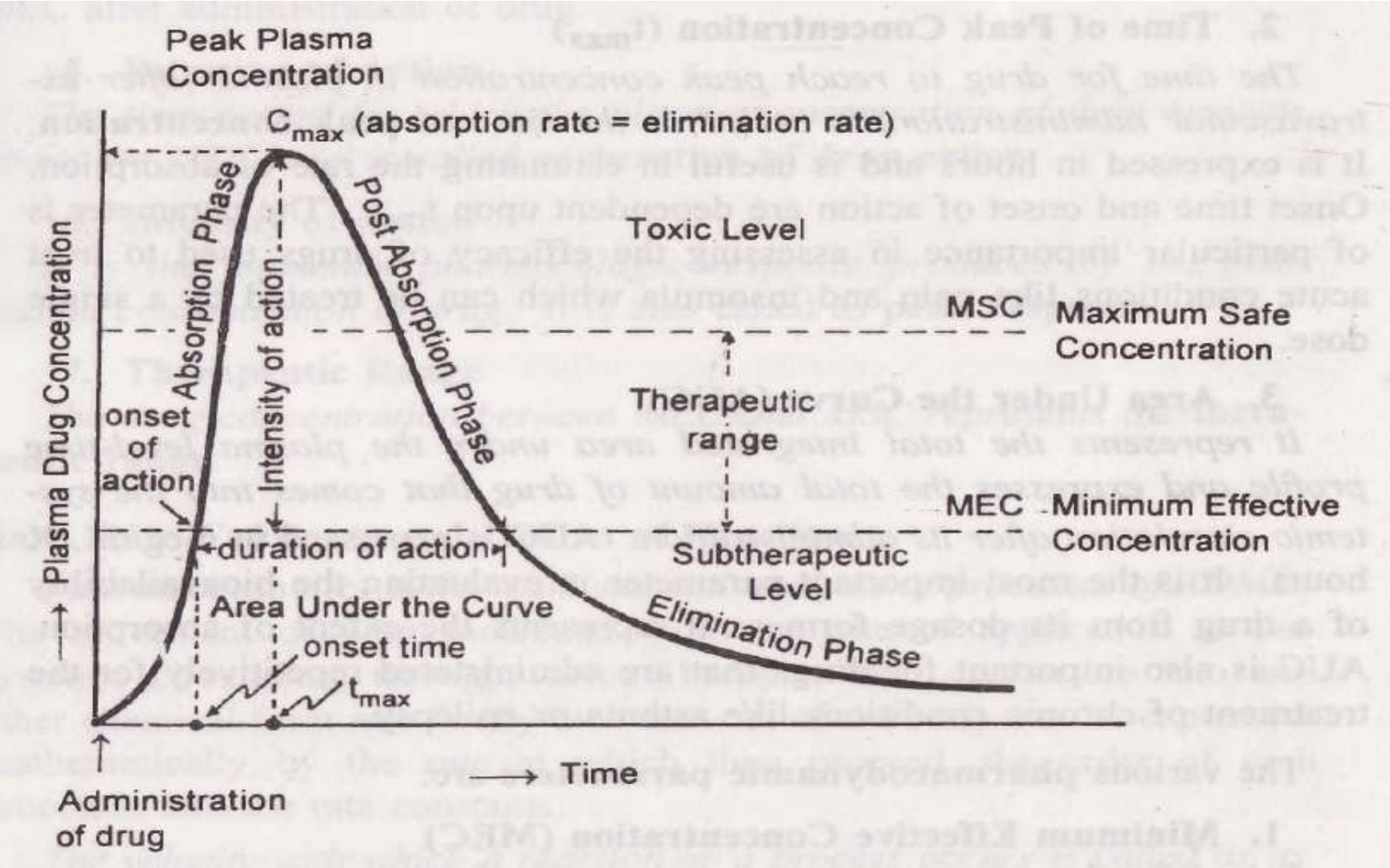
- What the body does to the drug
- The study and characterization of the time course of drug absorption, distribution, metabolism and excretion (ADME) & their relationship with therapeutic & toxic effect of drug.

- Clinical Pharmacokinetics: The use of pharmacokinetics principals in optimizing the drug dosage to suit individual patient needs & achieving maximum therapeutic utility is called clinical pharmacokinetics.

Pharmacodynamics

- What the drugs does to the body
- The study of the relation of the drug concentration or amount at the site of action (receptor) and its pharmacologic response as a function of time.
- It is concerned with the biochemical & physiological effects of the drug & its mechanism of action .

Plasma Concentration-Time Profile



This Profile is generated by obtaining the drug concentration in plasma samples taken at various time intervals after a drug is administered.

Plasma Concentration-Time Profile

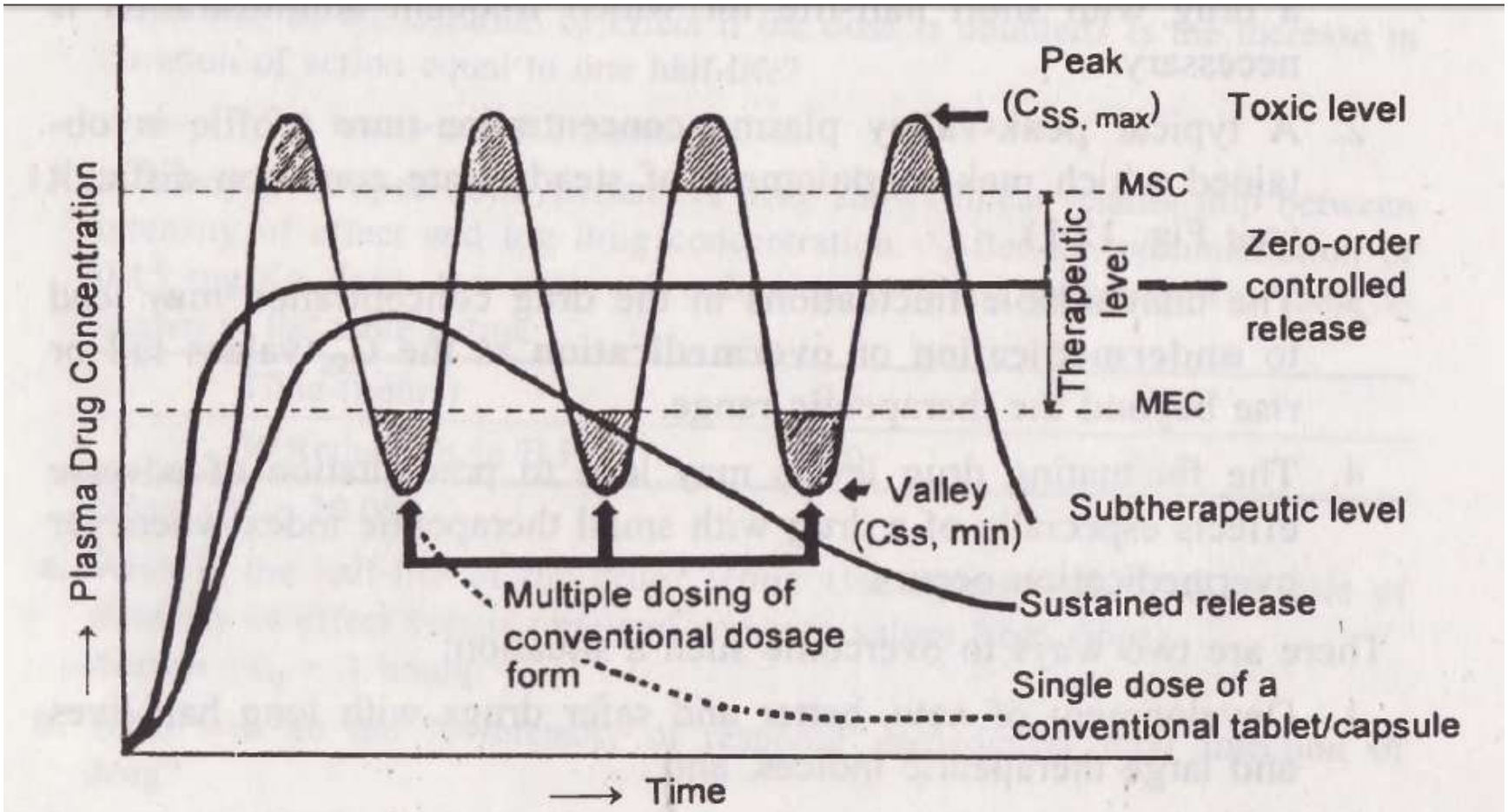


Fig. 15.1 A hypothetical plasma concentration-time profile from conventional multiple dosing and single doses of sustained and controlled delivery formulations

Pharmacokinetic Parameters

1. Peak plasma concentration (C_{max})
2. Time for peak plasma concentration (t_{max})
3. Area under the curve (AUC)

Pharmacodynamic parameters

1. Minimum Effective Concentration (MEC)
2. Maximum Safe Concentration (MSC)
3. Onset of Action
4. Onset Time
5. Duration of Action
6 . Intensity of Action
7. Therapeutic Range

Pune District Education Association's
Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre, Kharadi, Pune.

Introduction to Dosage Forms

Presented by
Mr. Sujit Kakade
Assistant Professor

Learning Outcomes

1. Definition of dosage form.
2. Need of dosage forms.
3. Classification of dosage forms
   - According to route of administration.
   - According to physical state.

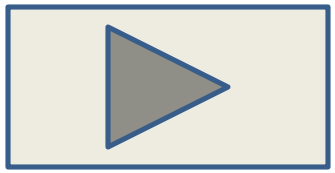
Introduction to Dosage Forms

- Dosage form are the devices into which a drug substance can be incorporated for the convenient and effective treatment of a disease.
- Drugs are rarely administered as in their pure chemical form alone and they are always converted to suitable dosage forms before administration.

Dosage form = Drug + Excipients

Reflection Spot

Que. What is the need of dosage forms?



Need of Dosage Forms

- Offers accuracy in dosing.
- Protection from the environment.
- Masking taste and odor.
- Protection from gastric juice.
- Placement of drugs within blood or body tissues.
- Insertion of drugs into body cavities.
- Modified release medication.
- Optimal drug action.

Classification of Dosage Forms

Dosage Forms

According to Route of administration

1. Oral
2. Topical
3. Rectal
4. Parenteral
5. Vaginal
6. Ophthalmic
7. Nasal
8. Otic

According to Physical State

1. Solid
2. Liquid
3. Semi-solid
4. Gaseous

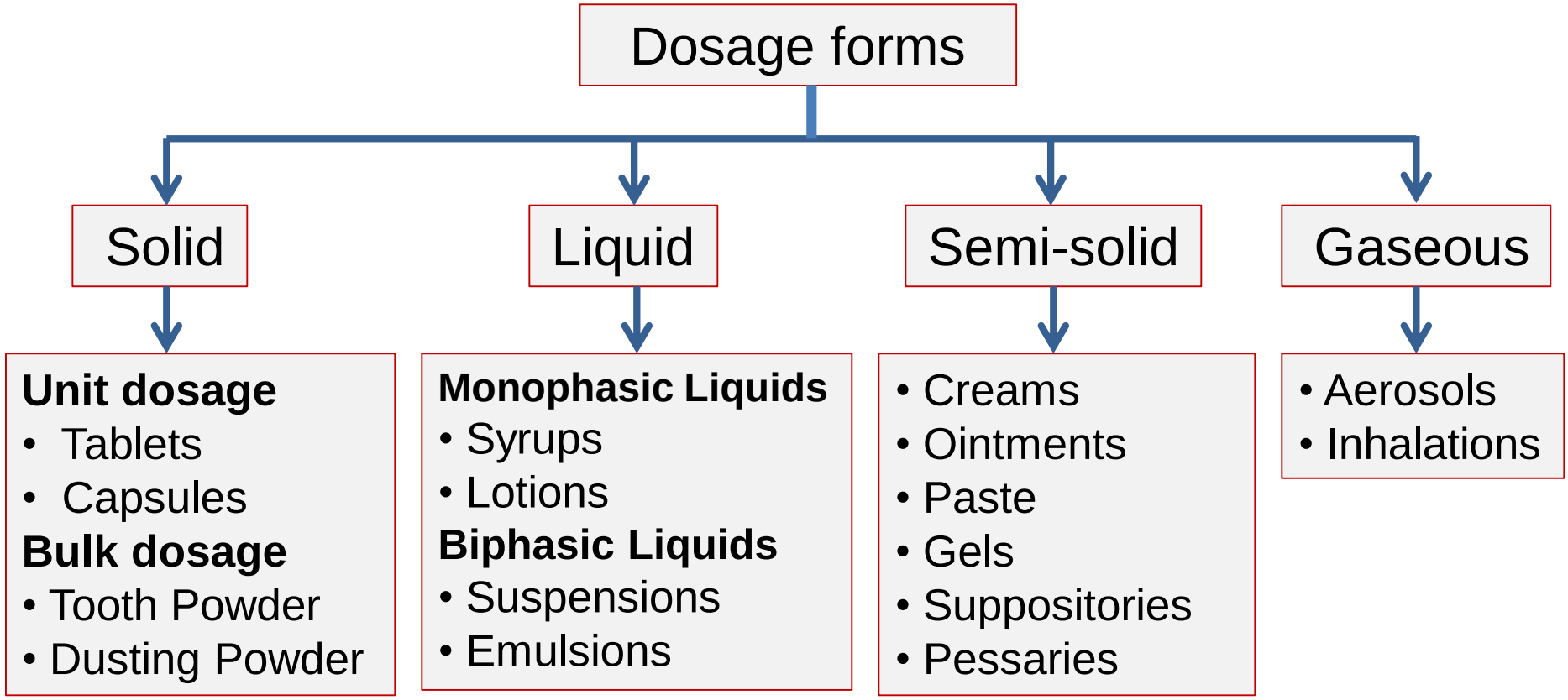
Classification of Dosage Forms

A. According to Route of administration

Sr. No.	Route of administration	Dosage Forms
1.	Oral	Tablet, Capsule
2.	Topical	Ointment, Cream
3.	Rectal	Suppositories
4.	Parenteral	Injectables, Implants
5.	Vaginal	Pessaries
6.	Ophthalmic	Eye drops
7.	Nasal	Nasal drops
8.	Otic	Ear drops

Classification of Dosage Forms

B. According to Physical State



Thank you

Introduction of Microorganisms

Presented by

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Assistant Professor

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& Research Centre, Kharadi, Pune.

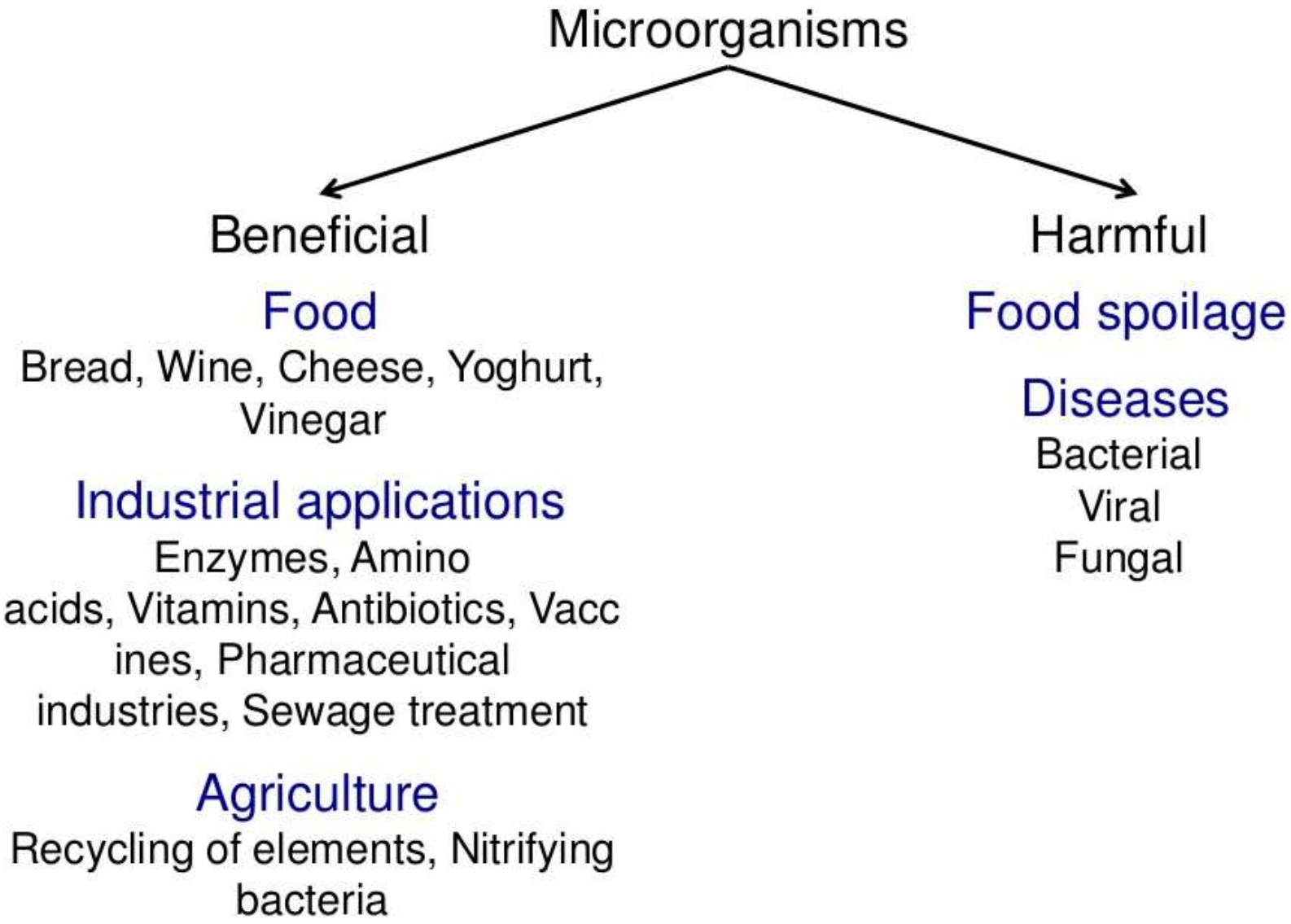
Definition of Microbiology:

“ It is the study of living organisms of microscopic size which include bacteria, fungi, algae, protozoa and viruses. ”

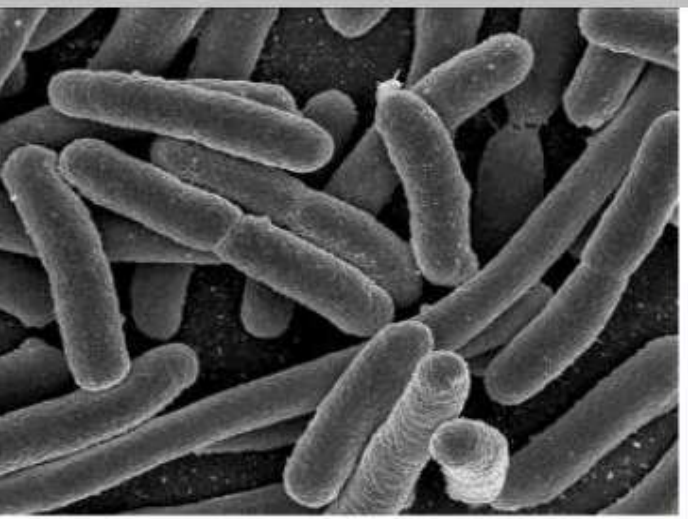
This term is introduced by a French chemist i.e. Louis Pasteur.
He is known as father of microbiology.

Micro-organisms can only be seen by magnifying their image with microscope

Effects on Human beings:

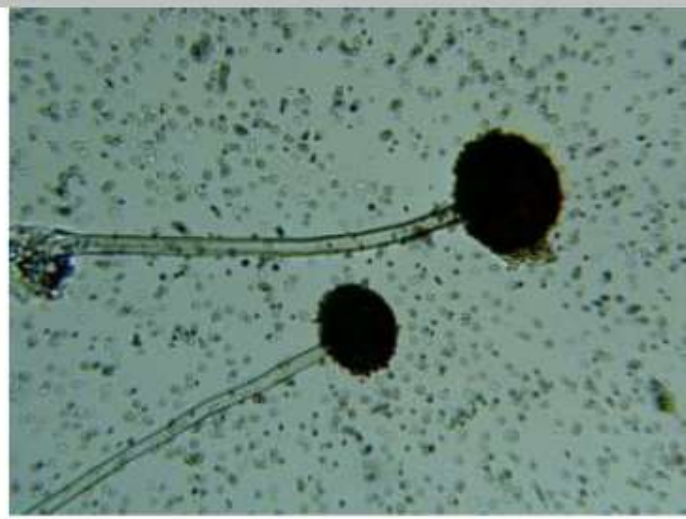


Microbiology includes study of



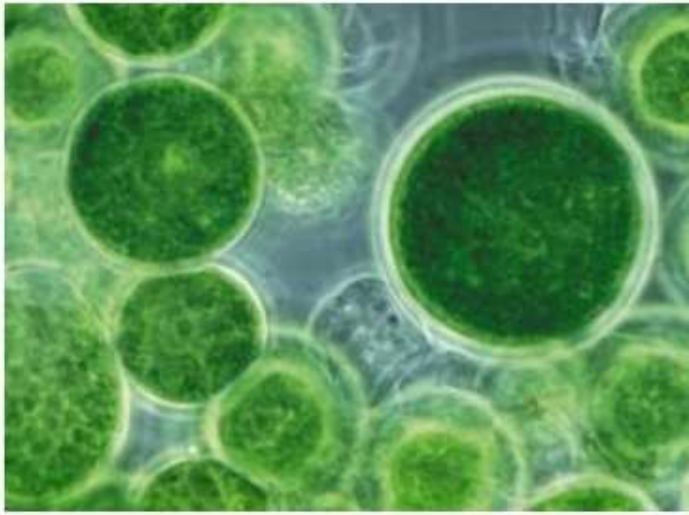
Bacteria

Viruses



Fungi

Protozoa



Algae

Branches of Microbiology

A. Pure Science Branches:

Bacteriology:- study of bacteria

Micobiology :- study of fungi

Protozoology :- study of protozoa

Phycology :- study of algae

Parasitology:- study of parasites

Taxonomy :- study of classification

Genetics:- study of variations and Hereditary

Immunology :- study of mechanism in development of resistance by body to infection

B. Applied Science Branches:

a) **Medical microbiology:** Study of causative agents of infectious disease

b) **Pharmaceutical microbiology:** Study of M. O. which is responsible for production of antibiotics, enzymes, vitamins, vaccines and other pharmaceutical products.

c) **Industrial microbiology:** Study of M. O. which is responsible for production of vitamins, amino acids, alcoholic beverages and other substances.

d) Microbial Biotechnology: Study of scientific manipulation of living M. O. based on genetic and molecular level

e) Food microbiology: Study of M. O. relation to food bioprocessing, food spoilage, food born diseases and preservation of food product

f) Soil microbiology: Study of microbial flora and interaction among soil M. O.

g) Agricultural microbiology: Study of M. O. and relationship of M.O. and crops

h) **Aquatic microbiology:** Study of m.o. and their activities into fresh and marine water.

i) **Air microbiology:** Study which deal with role of aerospora in contamination and spoilage of food and determination of plant and animal through air.

j) **Epidemiology:** Study which deal with consult with monitoring, and spread of disease in community.

Application and Scope of Microbiology

1) Production of Antibiotic: 2/3rd of antibiotics are produced from m.o. Pharmaceutical / industrial microbiology concerned with isolation of antibiotics producing m.o. from environment. E.g: soil, water, Mang antibiotic isolated from natural m.o by process of fermentation e.g: penicillin from penicillium species.

2) Diagnosis of disease and treatment: Different test are used to detect infectious m. o. e.g: ELISA test and Widal test.

Antimicrobial suitability testing is mainly used for selection of antibiotics for treatment of microbial infections.

3) Production of enzyme, vaccine, Organic solvents, biosurfactants, alcoholic and other pharmaceutical product:

Many microbial cell converts raw material or substrate into valuable organic compound such as butanol, ethanol etc microbial cell produce intracellular and extra cellular enzyme those are imp for pharmaceutical fermentation e.g: Amylase & Protease. Different types of biosurfactants are synthesized by no. of m. o. e.g: Acinotobacter.

4) Treatment of Industrial waste material:

- Microbial species are used for decomposition of industrial waste material and organic components.
  e.g: actinomycetes, fungi.
- Anaerobic bacteria decomposes of organic matter by yielding simple organic molecule such as acid, alcohol, glycerol and amines.
- Cynide is degraded by Streptomyces lavendulae.

5) Plant growth promotions:

Many microbial cell are present in soils play an important role in soil fertility, herbicidal resistance, insect resistance, change in protein, oil content, enhance the quality of plant product. e.g: Rhizobium species, Azotobacter species.

Many nitrogen fixing soil M.O. are usefull for providing nitrogen for growth of plants, germination of seeds, flavoring and fruiting of medicinal plants.

Rhizobacter bacteria may release various plant growth promoting substances as secondary metabolites. (PGPR) e.g: Pseudomonas

6) Sterile Product Preparations: It deals with the preparations of sterile rooms, aseptic techniques, and detection of microbes by sampling and testing of different sterile preparations.

7) Sterilization: Different pharmaceutical products are sterilized by killing of M.O.

The process of killing of M.O. is known as sterilization.

All the sterilization process may be validated by using indicator.

A biological indicator preparation of M.O. usually bacterial spores.

8) Steroid Biotransformation: Important steroid can be produced by microbiological transformation of naturally occurring steroid. Microbial conversion of steroids enzyme by M.O. streptomyces.

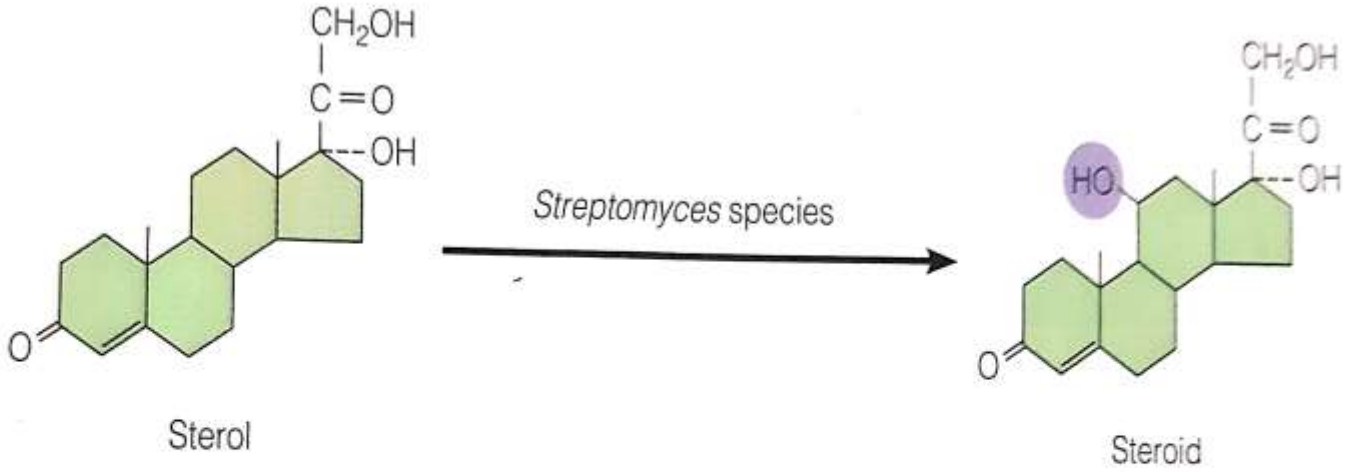


FIGURE 28.13 The production of steroids. Shown here is the conversion of a precursor compound such as a sterol into a steroid by *Streptomyces*. The addition of a hydroxyl group to carbon number 11 (highlighted in purple on the steroid) might require more than 30 steps by chemical means, but the microorganism can add it in only one step.

9) Identification of Microorganisms: Two vital function of microbiology and biotechnology are enumeration and identification of M.O. found in product and Mfg. environment.

10) Testing of Pharmaceuticals:

- Raw material and finished product:
- Microbial assay of antibiotics :
- Evaluation of disinfectant :
- Antimicrobial preservative efficiency testing :
- Endotoxin testing :
- Test for support of the sterility assurance system:

Raw material and finished product:

- Tests for the detection of E. Coli, Salmonella species, Psedomonas aeruginosa & Staphylococcus aureus in the pharmaceutical raw material & in the finished product.
- This test are mainly applied to raw material of natural & biological origin such as starch, gum, gelatin, talc, etc.

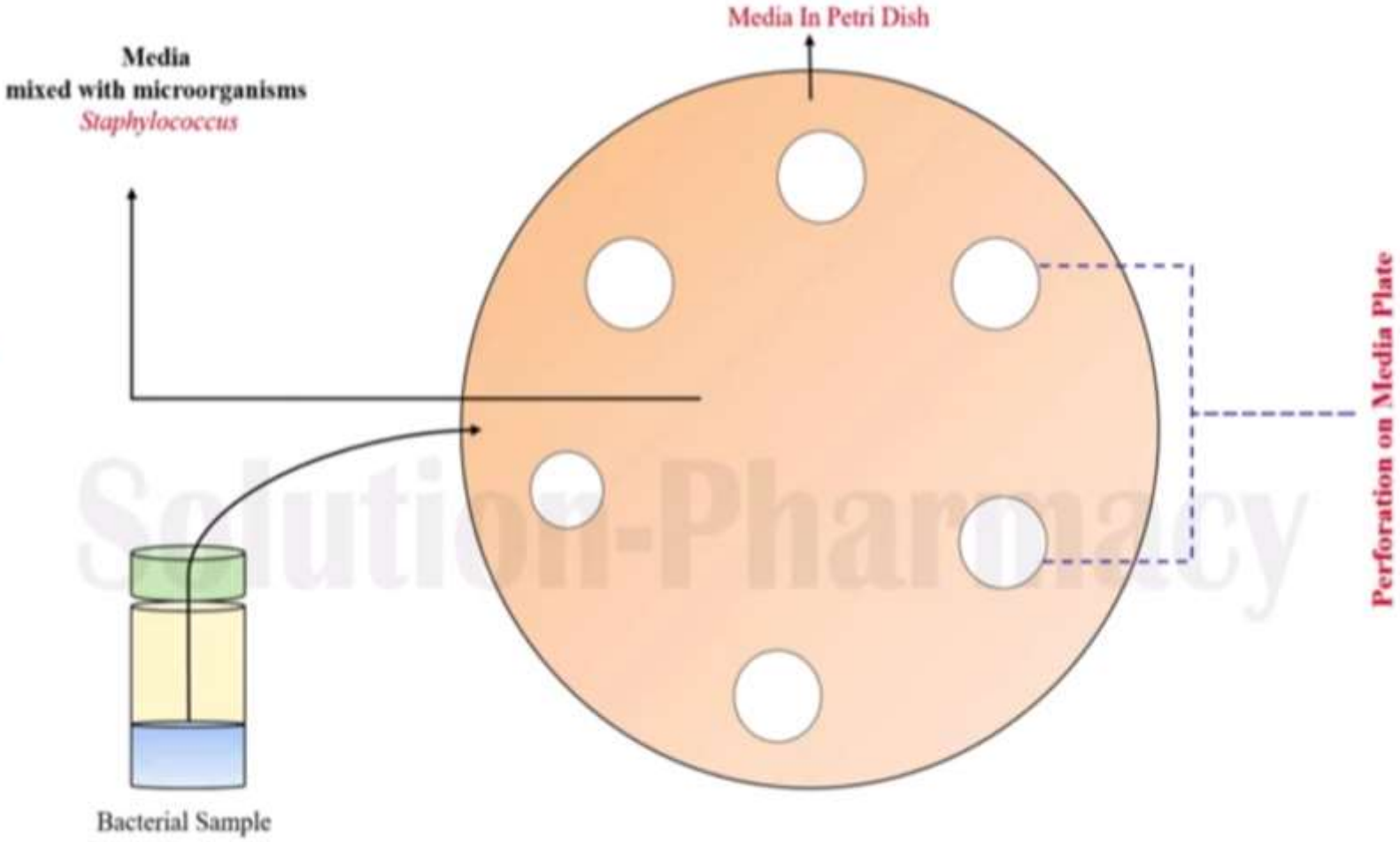
Two test are performed

1. Total number of viable M.O. (Total Viable Count)
2. Exclusion of specific Pathogen.

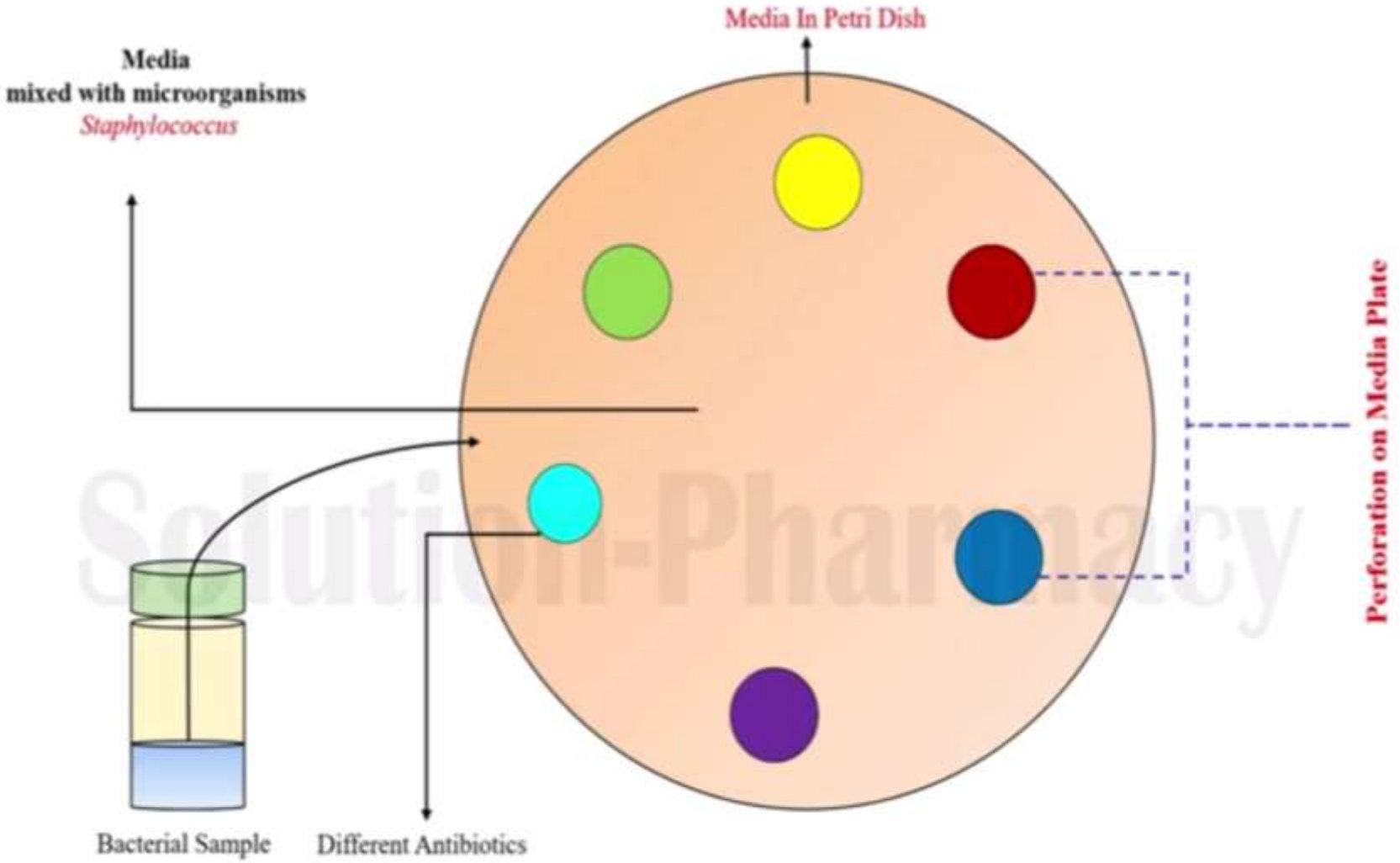
e.g. Water is always tested for TVC & presence of E. Coli

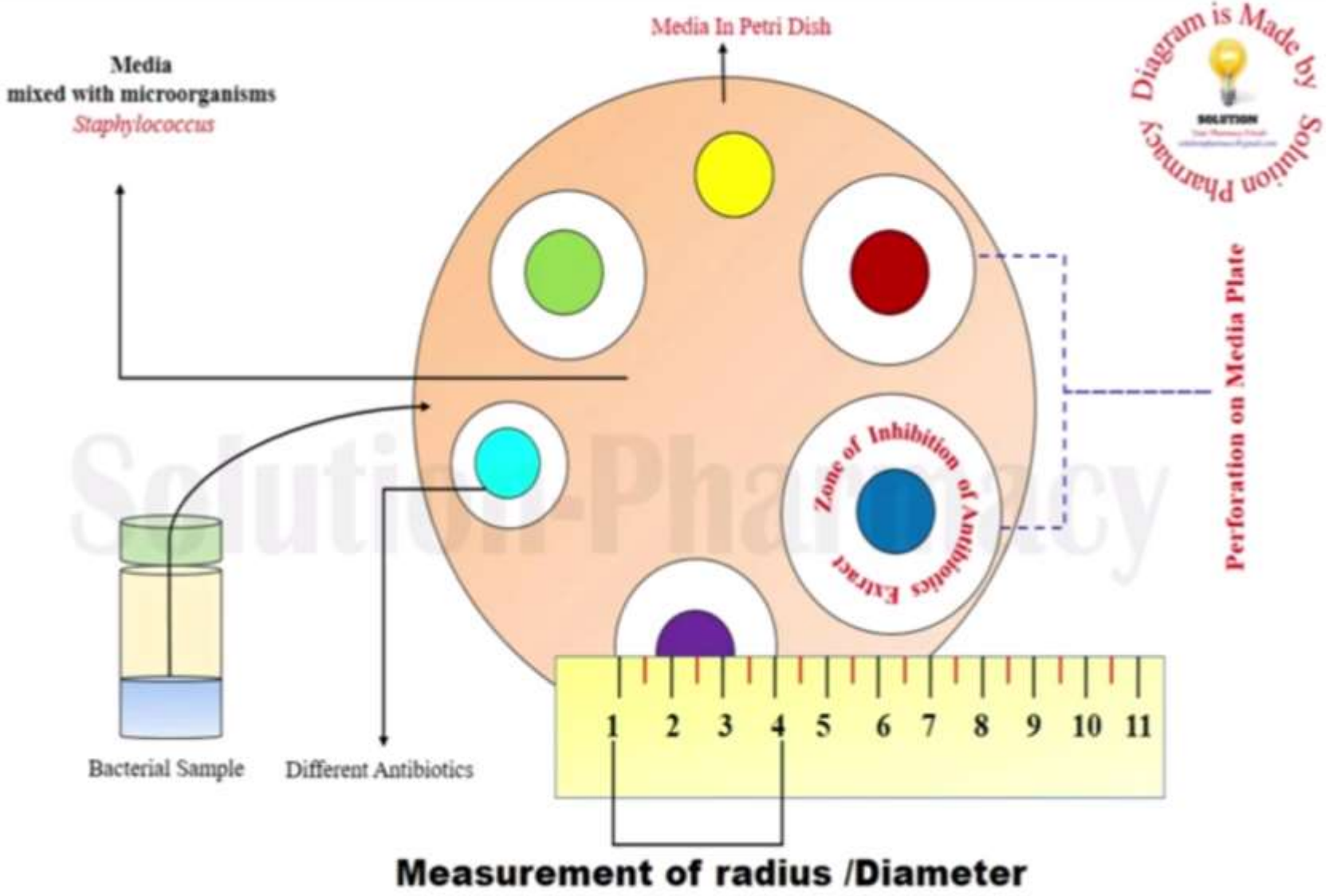
Microbial assay of antibiotics :

Agar Diffusion Method



Solution-Pharmacy

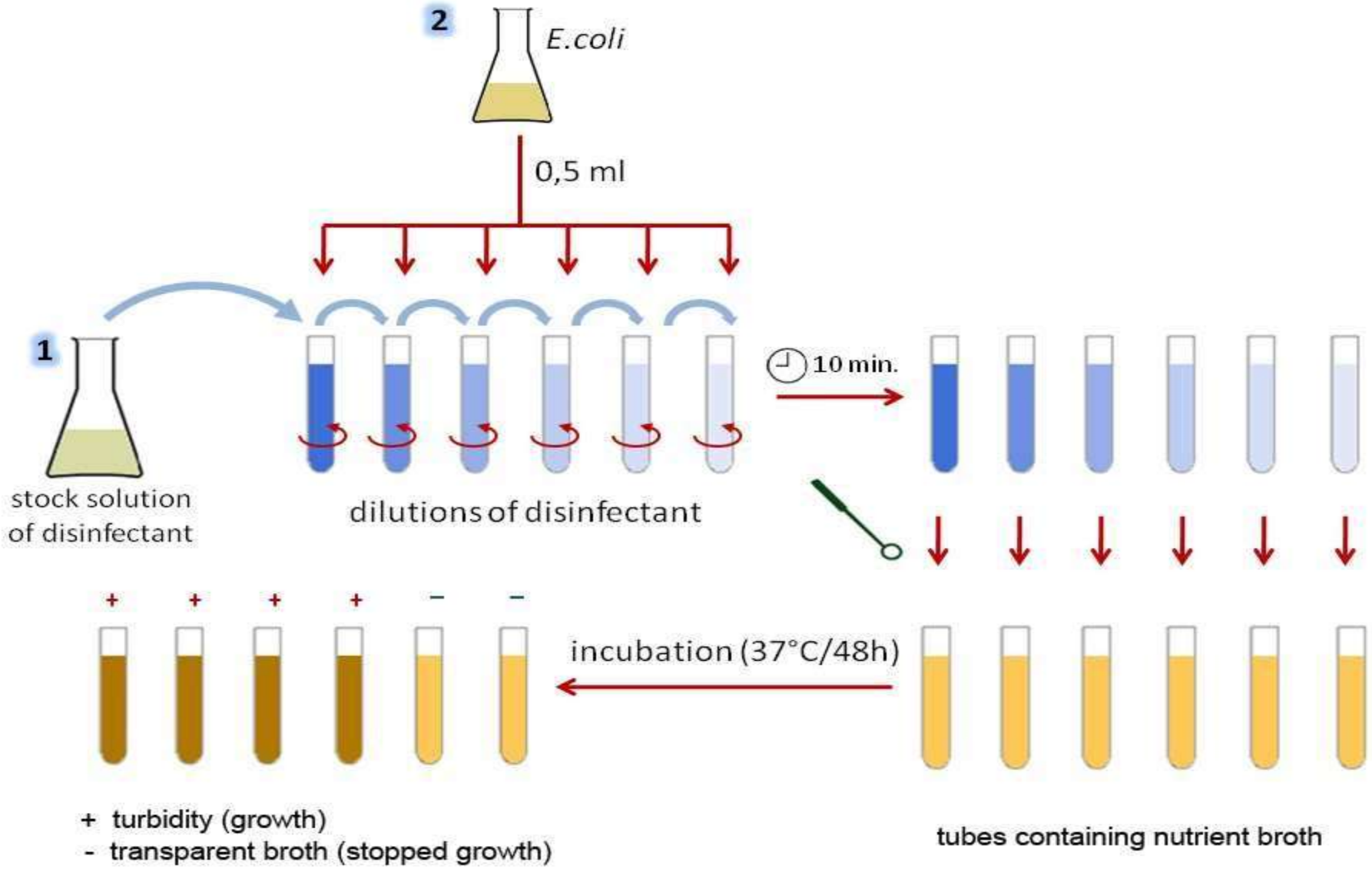

Media In Petri Dish
Media
mixed with microorganisms
Staphylococcus
Perforation on Media Plate
Bacterial Sample
Different Antibiotics


Media In Petri Dish
Media
mixed with microorganisms
Staphylococcus
Diagram is Made by Solution Pharmacy
Perforation on Media Plate
Zone of Inhibition of Antibiotics Extract
Bacterial Sample
Different Antibiotics
1 2 3 4 5 6 7 8 9 10 11
Measurement of radius /Diameter

Actual Image

Evaluation of disinfectant :

Phenol Coefficient Test



Endotoxin testing

If bacterial endotoxins enter patients' bloodstream in sufficient concentrations, this can cause harmful symptoms such as fever and septic shock and can be fatal in the most severe cases.

Methods that can be used to detect bacterial endotoxins.

1. Rabbit Pyrogen Test (RPT)
2. Limulus Amebocyte Lysate (LAL)

Test for support of the sterility assurance system:

Test for sterility:

Sterility Testing is used to demonstrate that a medicinal product or medical device is sterile.

Test method

1. Direct inoculation method
2. Membrane filtration method

Bioburden Testing

Bioburden Testing is used to estimate the number of microorganisms present in the product prior to sterilization.

Bioburden Testing is used to for environmental monitoring in aseptic areas are settle plates, contact plates, swabs for surface.

This method provide an approximate guide to the true microbiological state of a clean room, aseptic & laminar air flow cabinet.

Thank you

PUNE DISTRICT EDUCATION ASSOCIATION'S

SHANKARRAO URSAL COLLEGE OF PHARMACEUTICAL SCIENCES & RESEARCH CENTER KHARADI PUNE

INTELLECTUAL PROPERTY RIGHT

Mr. Nitin Neharkar
Assistant professor,
Department of pharmacutices
Shankarrao ursal college of
Pharmaceutical science & research
Center kharadi pune.

Introduction

- Intellectual property rights is a term used for various legal entitlements which attach to certain types of information, ideas or other intangible in their expressed form.

- The term intellectual property reflects the idea that this subject matter is the product of the mind or the intellect and the intellectual property rights may be protected at law in the same way as any other form of property.

Intellectual Property

- Intellectual property is an intangible creation of the human mind, usually expressed or translated into a tangible form that is assigned certain rights of property.

- Intellectual property include music, literature and other artistic works, discoveries and inventions and words, phrases, symbols and designs.

Categories of Intellectual Property

- Industrial property-

It includes inventions (patents) trademarks, industrial designs and geographical indications of source.

- Copyright-

It includes literary and artistic works such as novels, poem and plays, films musical works, artistic works such as drawing, paintings, photographs and sculptures and architectural designs.

Intellectual Property Rights

- The rights given to people over the creation of their minds. They usually give the creator an exclusive right over the use of his/her creations for a certain period of time.

Types of Intellectual Property Rights

- Patents
- Trademarks
- Copyrights
- Geographical indications
- Industrial designs
- Trade secrets
- Layout designs for integrated circuits
- Protection of new plant variety

Patents

- A patent is an exclusive right granted for an invention.
- In exchange for this right, the patent owner makes technical information about the invention publicly available in the published patent document.
- Patent duration-
- Term of every patent in India is 20 years.

Ministry of Commerce & Industry
Deptt. Of Industrial Policy & Promotion
CGPDTM
Patent Office
Kolkata
Delhi
Mumbai
Chennai
Trade Marks Registry
Mumbai
Kolkata
Delhi
Chennai
Ahmedabad
Geographical Indications Registry
Chennai
Patent Information System
NIIPM
NAGPUR

Trademarks

- A trademark is a sign capable of distinguishing the goods or services of one enterprise from those of other enterprises.
- It protects the public from confusion and deception by identifying the source or origin of products as distinguished some other similar products.
- It may consist of drawings, symbols, three- dimensional signs such as the shape and packaging of goods.

Copyrights

- Copyright is a legal term used to describe the rights that creators have over their literary and artistic works. Works covered by copyright range from books, music, paintings, sculpture and films, to computer programs, databases, advertisements, maps and technical drawings.

Geographical Indications

- Indications which identify a good as originating in the territory of a member, or a region or a locality in that territory, where a given quality, reputation or characteristic of the good is essentially attributable to its geographic origin
- More importantly, it identifies the product's special characteristics, which are the result of the product's origins.

Cont..

Examples-

- Darjeeling tea
- In Rajasthan- Bikaneri Bhujia.
- In Andhra Pradesh- Tirupati Laddu.
- In Maharashtra- Mahabaleshwar strawberry.

Industrial designs

- An industrial design constitutes the ornamental or aesthetic aspect of an article.
- Owners of protected designs must be able to prevent the manufacture, sale or importation of articles bearing or embodying a design which is a copy of the protected design.
- It consists of :
- Three-dimensional features, such as the shape of a product;
- Two-dimensional features, such as ornamentation, patterns, lines or colour of a product.

Cont..

- Industrial designs are applied to a wide variety of industrial products and handicrafts: from technical and medical instruments to watches, jewellery and other luxury items; from house wares and electrical appliances to vehicles and architectural structures.

Trade Secrets

- Usually these are manufacturing or industrial secrets and commercial secrets.
- These include sales methods, distribution methods, consumer profiles, advertising strategies, lists of suppliers and clients, and manufacturing processes. Contrary to patents, trade secrets are protected without registration.

Layout Design for Integrated Circuits

- The aim of the Semiconductor Integrated Circuits Layout-Design Act 2000 is to provide protection of Intellectual Property Right (IPR).

- The main focus of SICLD Act is to provide for routes and mechanism for protection of IPR in Chip Layout Designs created and matters related to it.

Protection of New Plant Variety

- The Plant Variety Protection and Farmers Rights act 2001 was enacted in India to protect the New Plant Variety.

- The act has come into force in 2005 through the Authority.

Benefits of Registered Intellectual Property Rights

- Barrier to market entry- The owner can use the registered intellectual property right to create a barrier preventing or discouraging competition.
- Out-sourcing manufacture- If you plan to use out-sourced manufacturing to a company without first applying for registered intellectual property rights what will stop that company taking your idea and exploiting it themselves? A registered intellectual property right can prevent this.
- Cheap imports and counterfeit and pirated goods- If you have a registered intellectual property right, we can prevent importation of products that infringe your registered intellectual property rights.

Cont..

- Provide protection against a competitor directly copying the idea.
- IP rights help business maintain their long-term competitive edge. Registered IP ensures that entrepreneurs get all the financial benefits from their ideas.
- Registered IP gives consumers confidence that products meet appropriate standards and quality.
- Registered IP is an asset. It helps convince financial institutions to invest in a business, enabling more money to be raised for development.

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THANK YOU

Pune District Education Association's Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre.

INFRARED SPECTROSCOPY

PRESENTED BY: DR.VIJAYA BARGE
(Vice Principal & Professor)

LEARNING OBJECTIVES

Afteí completing this sub-unit the students should be able:

1. l'o study the theoíy of Infíaíed absoíption spectíoscopy .
2. l'o study the modes of vibíations in IR spectíoscopy
3. l'o study the factoís affecting vibíations in IR spectíoscopy
4. l'o study the instíumentation involved in IR spectíoscopy
5. l'o study the applications of IR spectíoscopy

INFRARED SPECTROPHOTOMETER

What is Spectroscopy?

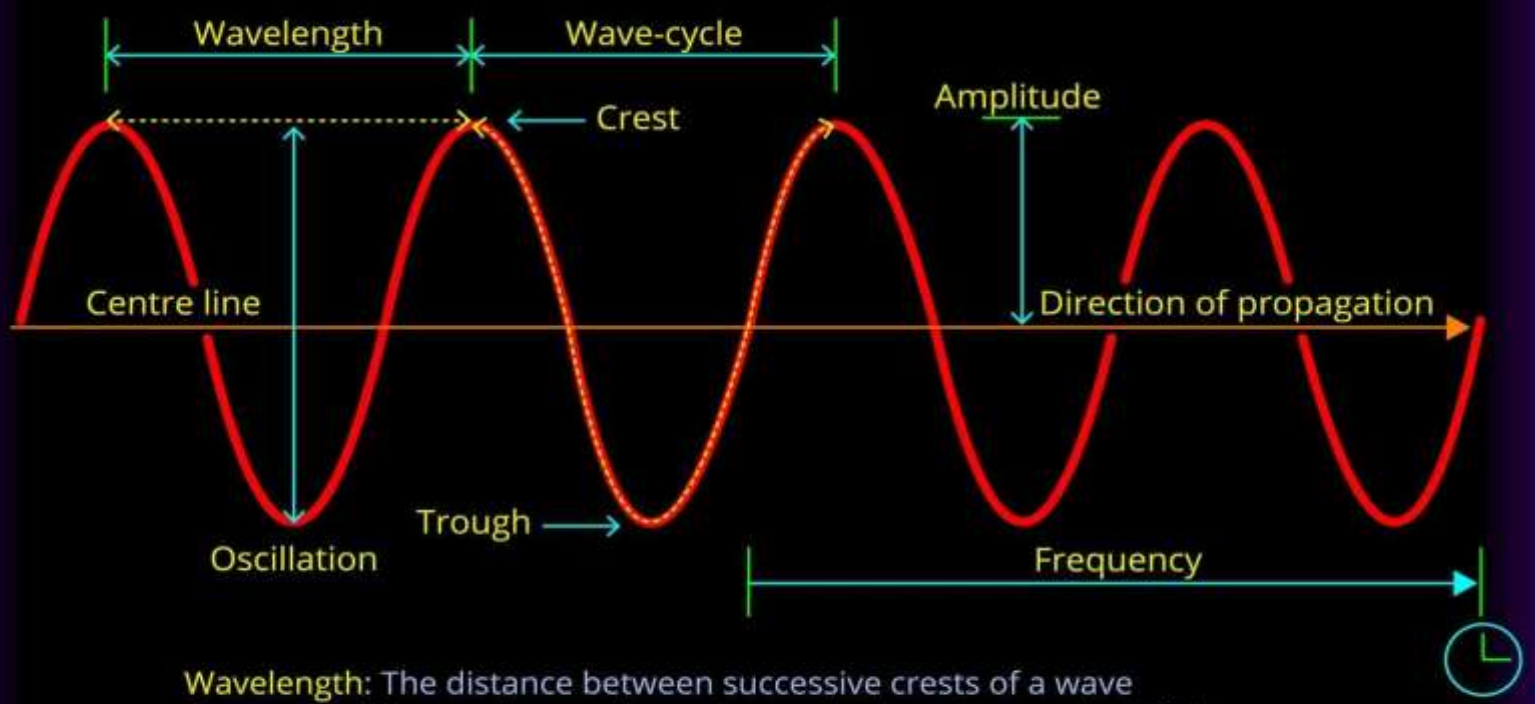
Spectroscopy is the study of the interaction between radiation and matter.

Spectrometry is the measurement of these interactions.

An instrument which performs the measurements is a **spectrometer** or **spectrograph**.

A plot or graph is referred to as a **spectrogram** or **spectrum**.

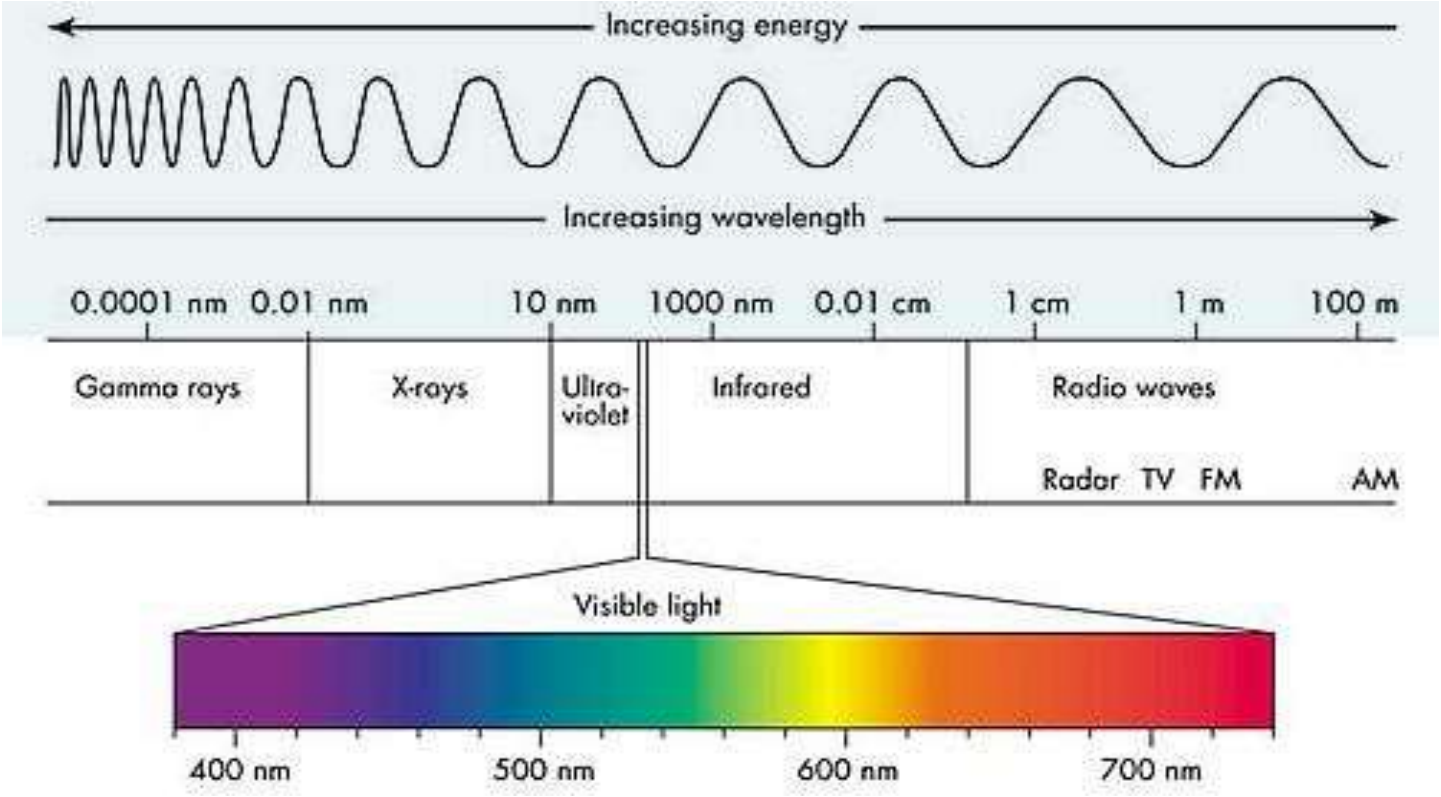
Features of Electromagnetic Waves



Wavelength: The distance between successive crests of a wave
Frequency: Wave cycles passing a given point in a given period of time
Amplitude: Measured between the crest and the mid-point of a wave
Speed of light in a vacuum: Approx. 300,000 km/sec

ELCETROMAGNETIC SPECTRUM

The **electromagnetic** (**EM**) **spectrum** is the range of all types of **EM** radiation



Planck relation:

$$E = h\nu = \frac{hc}{\lambda}$$

where:

E = energy
h = Plank constant
v = frequency
c = speed of light
λ = wavelength

IR SPECTROSCOPY

INTRODUCTION

- Infrared spectroscopy (IR) measures the bond vibration frequencies in a molecule and is used to determine the functional groups.
- The infrared region of the spectrum encompasses radiation with wave numbers ranging from about 12,500 to 50cm-1 (or) wave lengths from 0.8 to 200μ.
- Infrared region lies between visible and microwave region.

IR REGIONS

a) The near IR (0.8 -2.5µm) (12,500-4000cm-1)

b) The middle IR (2.5 -15µm) (4000-667cm-1)

i) Group frequency Region (4000-1500cm-1)

ii) Finger print Region (1500-667cm-1)

c) The far IR (15-200µm) (667-50cm-1)

- most of the analytical applications are confined to the middle IR region because absorption of organic molecules are high in this region.
- Wave number is mostly used measure in IR absorption because wave numbers are larger values & easy to handle than wave length which are measured in µm.
- $E = h\nu = hc/\lambda = hc\bar{\nu}$
- It gives sufficient information about the structure of a compound.

PRINCIPLE OF IR SPECTROSCOPY

- When the energy in the form of IR is applied and if the applied IR frequency = Natural frequency of vibration, the absorption of IR takes place and a peak is observed.
- Molecules are excited to the higher energy state from the ground state when they absorb IR radiation.
- When a compound is exposed to IR radiation, it selectively absorbs the radiations resulting in vibration of the molecules of the compound, giving rise to closely packed absorption bands, called as IR absorption spectrum.
- The bands correspond to the characteristic functional groups and the bonds present in a chemical substance. Thus, an IR spectrum of a compound is considered as the fingerprint for its chemical identification.

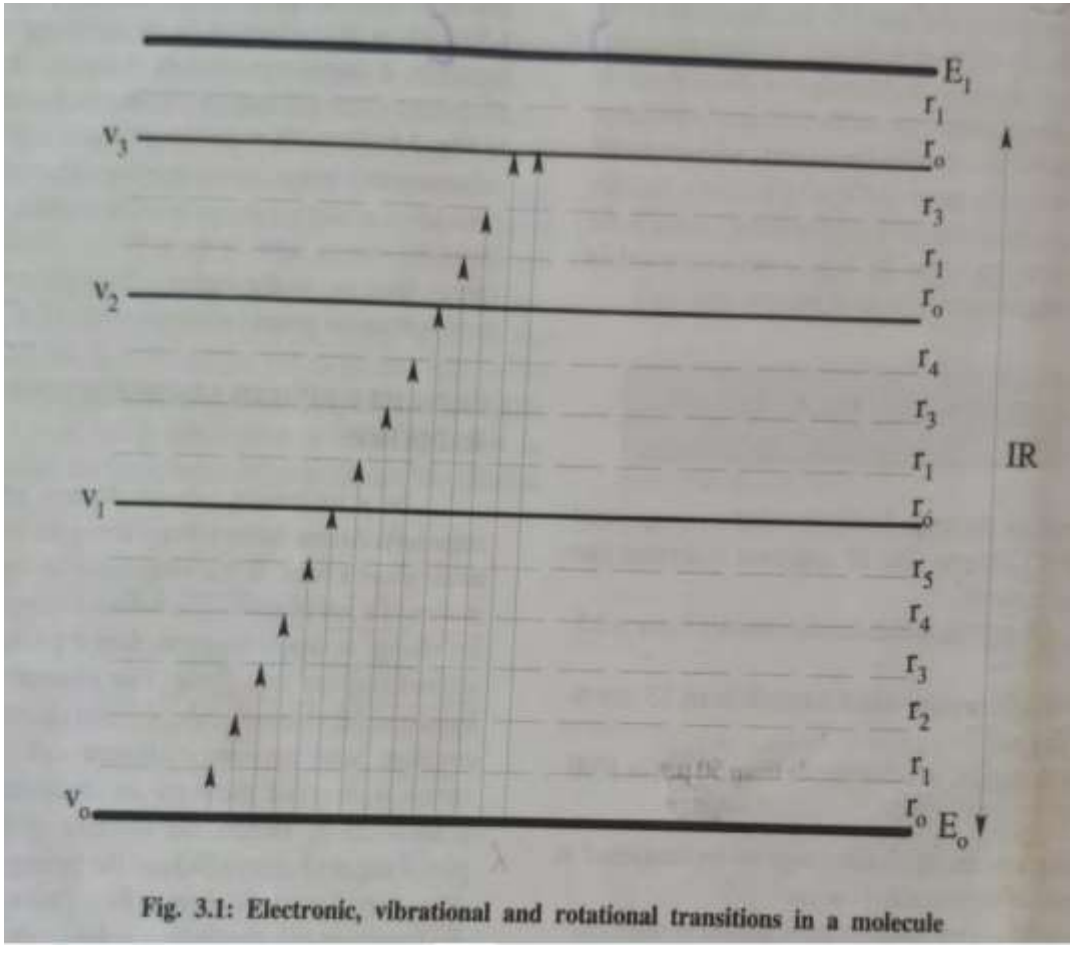
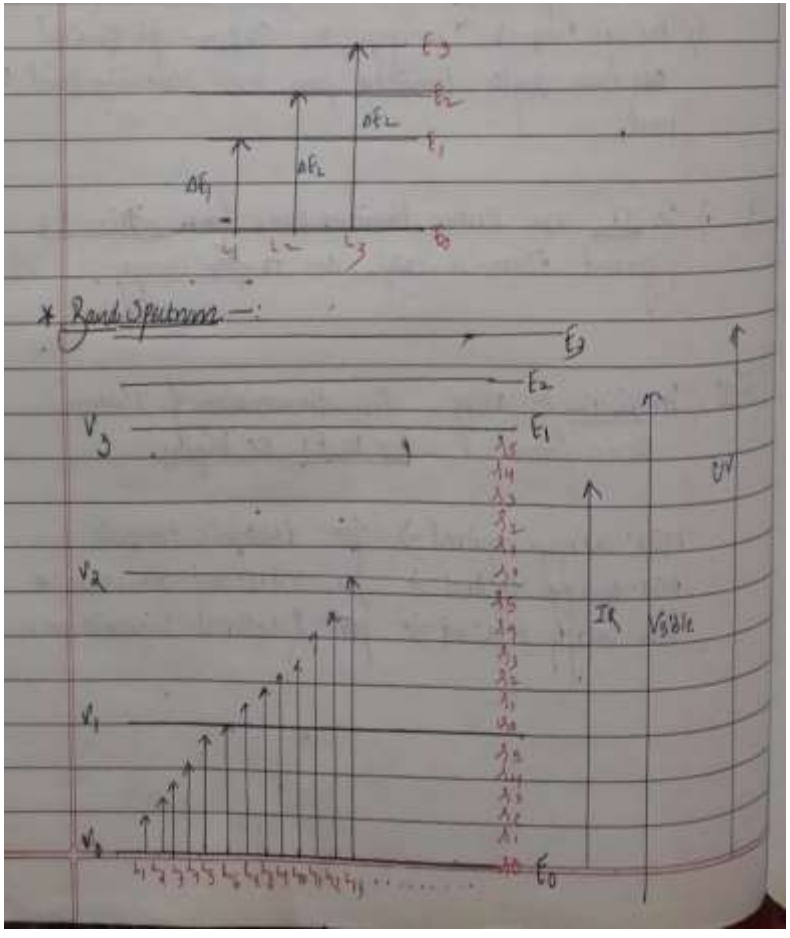


Fig. 3.1: Electronic, vibrational and rotational transitions in a molecule



IR-Active

- The bond undergoing the vibration must be polar
  - A polar bond is usually IR-active.
    - The greater the polarity of the bond, the more intense the absorption
- The bond's vibration must cause a change in dipole
  - Asymmetrical stretching/bending change the dipole moment of a molecule.

IR-Inactive

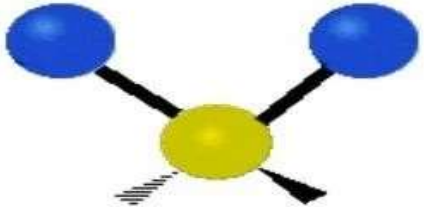
- A nonpolar bond in a symmetrical molecule will absorb weakly or not at all.

MOLECULAR VIBRATIONS

There are 2 types of vibrations.

1) Stretching vibrations
2) Bending vibrations

- 1)Stretching vibrations: in this bond length is altered.
- They are of 2 types
- a) symmetrical stretching: 2 bonds increase or decrease in length.



b) Asymmetrical stretching: in this one bond length is increased and other is decreased.

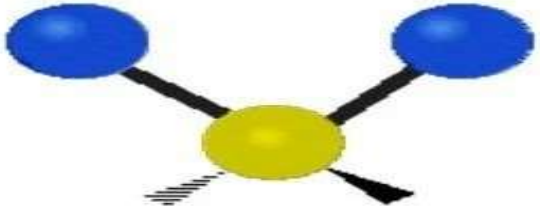
2)Bending vibrations:

- These are also called as deformations.
- In this bond angle is altered.
- These are of 2 types
- a) in plane bending → scissoring, rocking
- b) out plane bending → wagging, twisting

Scissoring:

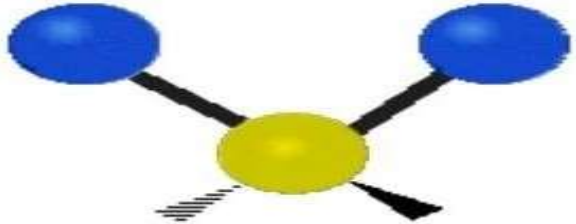
This is an in plane bending.

In this bond angles are decreased.2 atoms approach each other.



Rocking:

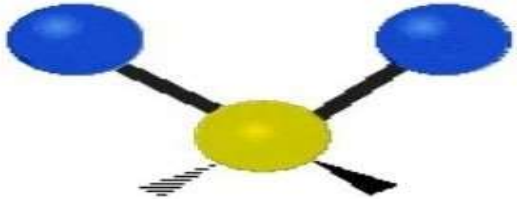
- In this movement of atoms takes place in same direction.



Wagging;

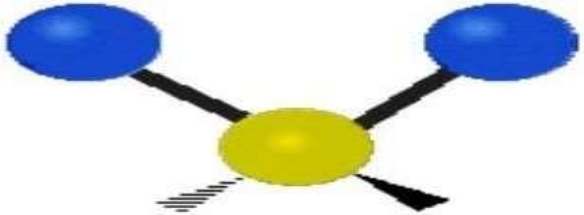
It is an out of plane bending.

In this 2 atoms move to one side of the plane. They move up and down the plane.



Twisting:

- In this one atom moves above the plane and the other atom moves below the plane.



Stretching Vibrations

Symmetrical

Asymmetrical

Bending Vibrations

Scissoring

Rocking

Twisting

Wagging

In plane

Out of plane

FUNDAMENTAL AND OVERTONE BANDS

A **fundamental band** is the spectral band that occurs in a vibrational spectrum of a molecule when the molecule makes a transition from the ground state (v=0) to the first excited state (v=1)

An **overtone band** is the spectral band that occurs in a vibrational spectrum of a molecule when the molecule makes a transition from the ground state (v=0) to the second excited state (v=2)

DEGREE OF FREEDOM

Translation - the movement of the entire molecule while the positions of the atoms relative to each other remain fixed: 3 degrees of translational freedom.

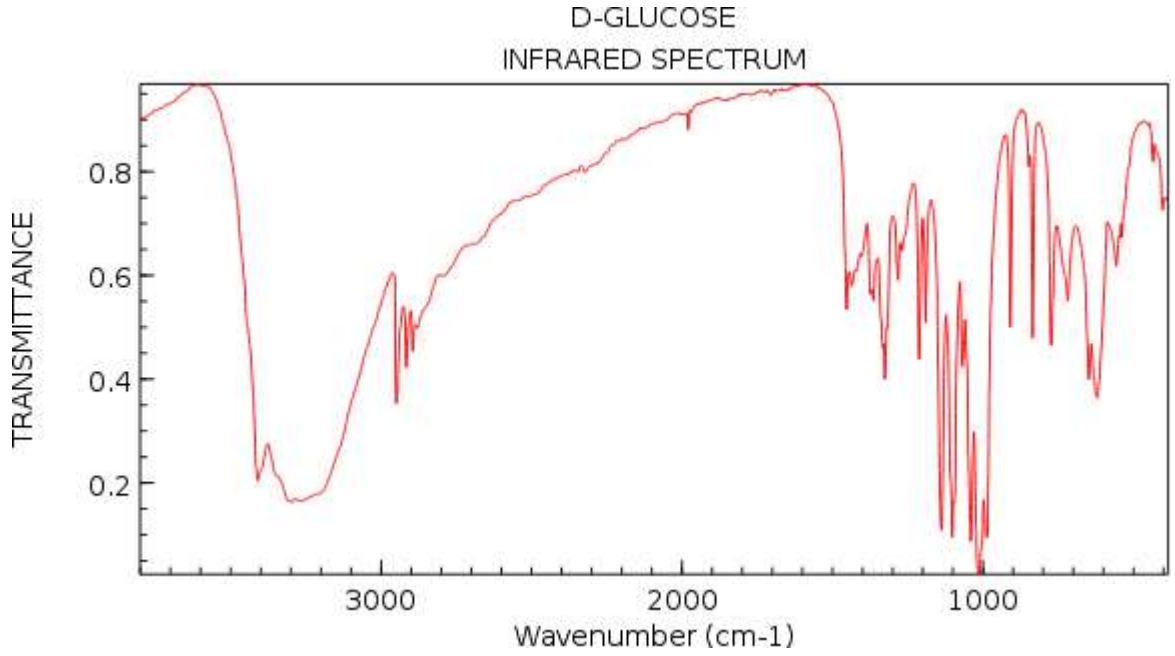
Rotational transitions – interatomic distances remain constant but the entire molecule rotates with respect to three mutually perpendicular axes: 3 rotational freedom (nonlinear), 2 rotational freedom (linear).

Vibrations – relative positions of the atoms change while the average position and orientation of the molecule remain fixed.

Degrees of freedom	linear	non-linear
Translational	3	3
Rotational	2	3
Vibrational	$3N–5$	$3N–6$
Total	$3N$	$3N$

N = number of atoms in molecule

The IR spectrum is **a plot of % transmittance (or absorbance) of the radiation through the molecule** versus wave number of the radiation



IR INSTRUMENTATION

- I R Radiation Source
  - Incandescent lamp
  - Nernst Glower
  - Globar Source
  - Mercury Arc
- Sample Cells & Sampling Substances
  - Sampling of solids
    - Solids run solution
    - Solid films
    - Mull technique
    - Pressed pellet technique
  - Sampling of Liquids
  - Sampling of Gases
- Monochromators
- Detectors
  - Bolometers
  - Thermocouple
  - Thermistors
  - Golay Cells
  - Photoconductivity cell
  - Semiconductor
  - Pyroelectric detectors

INSTRUMENTATION

- Nernst Glower
- Globar
- Incandescent wire source
- Hg Arc

Grating
Filter

Thermal D
Thermocouple
Thermopile
Thermister
Bolometer
Pyroelectric D

Recorder

SOURCE

1. The Nernst Glower

Consist of cylindrical hollow rod or tube having a diameter of 1-2 mm and length of 30 mm.

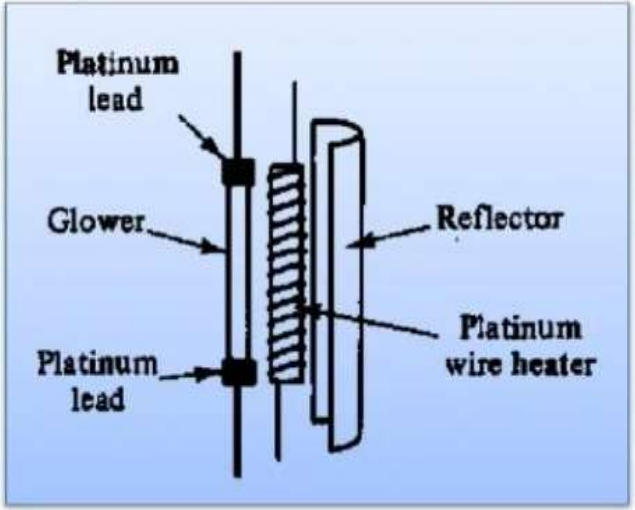
sealed by a platinum leads to the ends to permit electrical connection.

It composed of a mixture of rare earth oxides such as zirconium oxide (ZrO_2), yttrium oxide (Y_2O_3) and thoria.

They electrically heated to about 2000 °C.

It provide maximum radiation about 7100cm^{-1} (1.4 μ)

its lifetime depends on the operating temperature and the care taken in handling it



2. The Globar source

It's sintered silicon carbide rod, usually about 50 mm in length and 5 mm in diameter

Heated to about 2000 °C.

It provide maximum radiation about $5200cm^{-1}$.

The power consumption is normally higher than that of the Nernst Glower

Water cooling is needed to cool the metallic electrodes attached to the rod

Less convenient to use, more expensive & less intense than Nernst Glower.

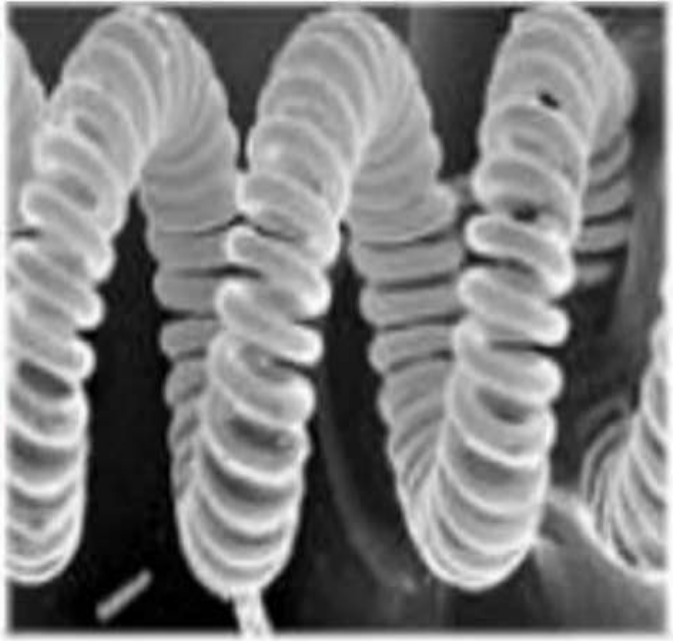
3. Incandescent Wire Source

Lower intensity IR source but longer life than the Globar or Nernst glower.

A tightly wound spiral of nichrome wire heated to about 1100 k by an electric current.

A similar source is a rhodium wire heater sealed in a ceramic cylinder.

Incandescent wire sources are longer lasting but of lower intensity than the glower or globar.



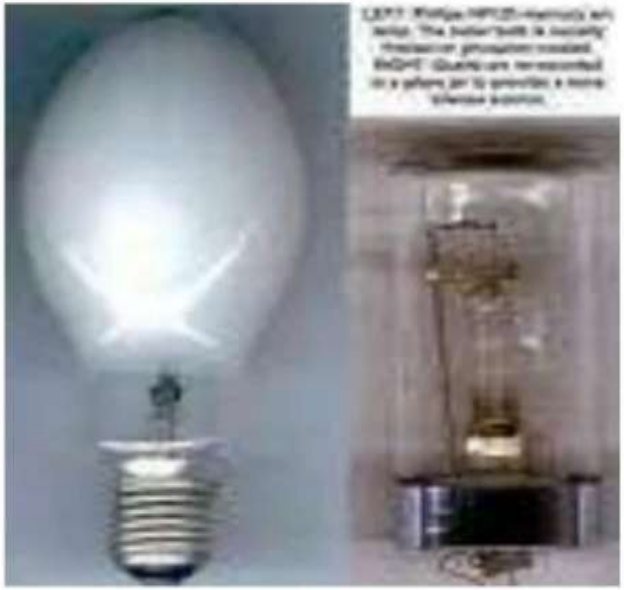
4. The Mercury Arc.

Used for Far IR region (wave no<200cm^{-1}).

It is a high pressure mercury arc which consist of a quartz - jacketed tube containing Hg vapor at P > 1 atm.

When current passes through the lamp, mercury is vaporized, excited, and ionized, forming a plasma discharge at high pressure.

In the UV and visible regions, this lamp emits atomic Hg emission lines that are very narrow and discrete, but emits an intense continuum in the far-IR region.



MONOCHROMATORS

- They convert polychromatic light into mono chromatic light.
- They must be constructed of materials which transmit the IR.
- They are of 3 types.
- a) metal halide prisms
- b) NaCl prisms
- c) gratings

- a) metal halide prisms:
- prisms which are made up of KBr are used in the wavelength region from 12-25µm.
- LiF prisms are used in the wavelength region from 0.2-6µm.
- CeBr prisms used in wavelength region from 15-38
- b) NaCl prisms:
- Used in the whole wave length region from 4000-650cm-1.
- they have to be protected above 20•c because of hygroscopic nature.
- c) gratings:
- They offer better resolution at low frequency than prisms

SAMPLE CELLS

- Sample cells made up of alkali halides like NaCl or KBr are used.
- Aqueous solvents cannot be used as they dissolve alkali halides.
- Only organic solvents like chloroform is used.
- IR spectroscopy has been used for the characterization of solid, liquid, gas samples.

Sampling of solids

Solids run in solution

Solids dissolved in a aqueous solvent

↓

Placed over the alkali metal disk

↓

Solvent is allowed to evaporate

↓

Thin film of solute formed

↓

Entire solution is run in one of the cells for liquids

Note :

This method not used because suitable number of solvents available are less.

Solid films

- Technique used for Amorphous sample.
- Deposited on the KBr / NaCl cell by evaporation of solution.
- Only useful for rapid qualitative analysis.

Mull Technique

- Finely ground solid sample is used.
- Mixed with Nujol (mineral oil)
- Thick paste is made.
- Spread between I R transmitting windows
- Mounted in path of I R beam &
- The spectrum is run.

Disadv. Nujol has the absorption maxima at 2915, 1462, 1376 & 719 cm^{-1}

Press pellet technique

- *Potassium Bromide* is mixed (100 times more)
- Passed through a high pressure *press*
- Small *pellet* formed (1-2 mm thick, 2cm diameter)
- The pellet is *transparent* to I R radiation & is run as such

Adv.

- Pellet can be stored for *long period* of times.
- *Concentration* of sample can be *adjusted* in KBr pellet hence used for quantitative analysis.
- Resolution of spectrum is *superior*.

Disadv.

- Always has a band at *3450 cm-1* (moisture OH-)
- At high pressure *polymorphic changes* occur
- Unsuccessful for polymer which are *difficult to grind* with KBr.

Sampling of liquids

- Liquid samples taken.
- Put it into rectangular *cells of KBr, NaCl* etc.
- I R spectra obtained.

- Sample thickness … such that *transmittance* lies between 15 – 20 % i.e., 0.015 – 0.05 mm in thickness.

- For double beam, *matched cells* are generally employed
- One cell contains *sample* while other has *solvent* used in sample.

Sampling of gases

- Small size particles hence the cells are large.
- 10 cm to 1m long
- Multiple *reflections* can be used to make the effective path length as long as 40 cm
- Lacks sensitivity

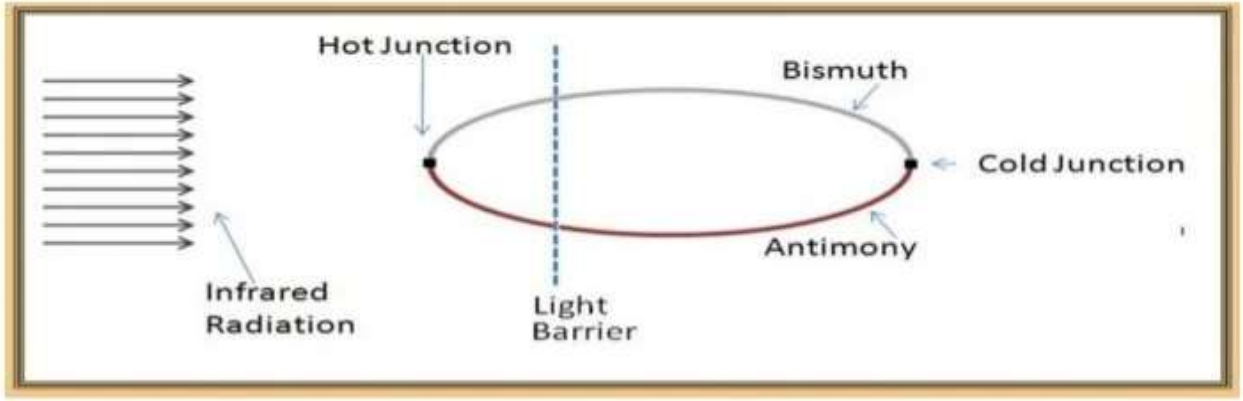
DETECTORS

- **The detectors can be classified into three categories:**

1. **Thermal detectors:-** Their responses depend upon the heating effect of radiation .
2. **Pyroelectric detectors:-** Pyroelectric effect depends on the rate of change of the detector temperature rather than on the temperature itself.
3. **Photoconducting detectors:-** Most sensitive .

Thermocouple

A thermocouple consists of a pair of junctions. Two junctions are formed by connecting two pieces of a similar metal like bismuth (Bi) to either ends of a dissimilar metal such as antimony (Sb) or a semiConducting alloy. One junction is used as a reference junction and another junction is used as a detector junction. A potential difference is developed across the two junctions depending on the difference between their temperatures.



Example : Bismuth & Antimony

1. Reference junction

Temperature of a reference junction need not be kept constant since the difference between the temperatures of the junctions is required to determine the intensity of a signal. Still, most of the times, to get the accurately measurable difference between the temperatures of the two junctions, the temperature of the reference junction is kept more or less constant by cooling it in ice. The greater the difference in the temperatures of the two junctions, the higher is the sensitivity of the method and the higher is the accuracy of the measurement. The reference junction has relatively large heat capacity. i.e. a large amount of heat is associated with the minute change in the temperature of the reference junction.

2. A detector junction

A detector junction or the junction for detecting intensity of IR radiation transmitted after the interaction with an analyte should be designed so as to have complete absorption of heat from the IR radiation incident on it. Hence, special care is taken to design this junction from fine wires of platinum, silver, antimony or bismuth. Fine wires increase the surface area of the junction and help in complete absorption of heat from the IR radiation

Bolometer

- It consists of thin *metallic conductor*, its *resistance* changes due to *increase* in temperature when IR radiation falls on it.
- It is a *electrical resistance thermometer* which can detect and measure feeble thermal radiation.
- The electrical resistance increases approx *0.4%* for every celsius degree increase of temperature .
- The *degree of change* in resistance is regarded as the measure of the amount of IR radiation falling on it.
- A bolometer is made of two *platium strips*, covered with *lamp black*, one strip is sheilded from radiation and one exposed to it. The strips formed two branches of *wheatstone bridge*

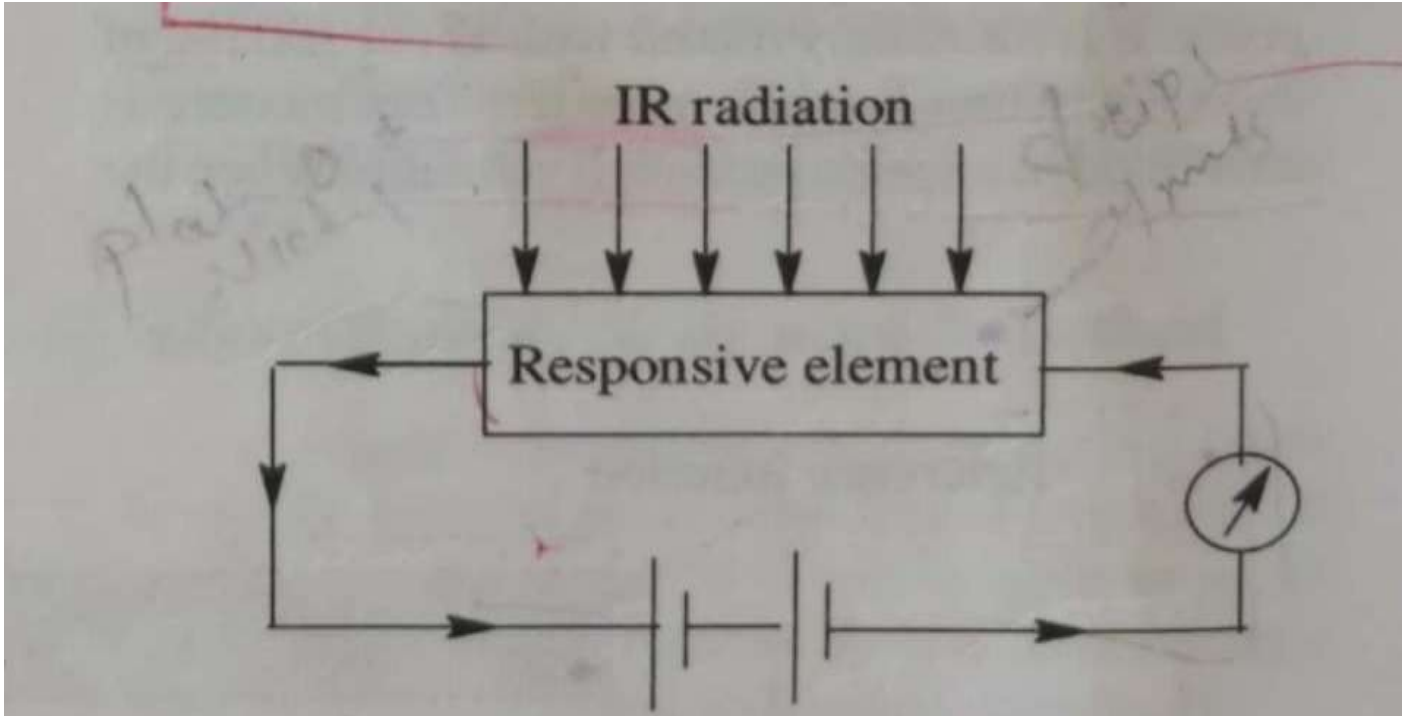


Fig. 3.9: A bolometer

Golay cell

It is also called as gas thermometer. It consists of a cylindrical chamber, filled with xenon gas. Walls of this chamber are blackened for the complete absorption of heat supplied by the IR radiation incident on it. One end of this cylinder is fitted with an IR transparent window. Another end contains a flexible diaphragm which is silvered from outside. The chamber also contains a phototube.When the IR radiation enters this chamber. the blackened walls absorb heat provided by the radiation. This in turn heats the xenon gas inside the chamber. The xenon gas expands and exerts The curvature pressure on the flexible diaphragm depends on the increase in the temperature of the xenon gas, which in turn depends on the heat absorbed by it, which further dépends on the intensity of the incident IR radiation. When the IR radiation falls on the silvered diaphragm, they reflect back in the chamber and fall on the photosensitive surface of a phototube., Fending on the curvature of the flexible diaphragm, a definite fraction of reflected beam strikes the active surface ot a phototube. This leads to a change in the photocurrent. which can related to the power of IR.

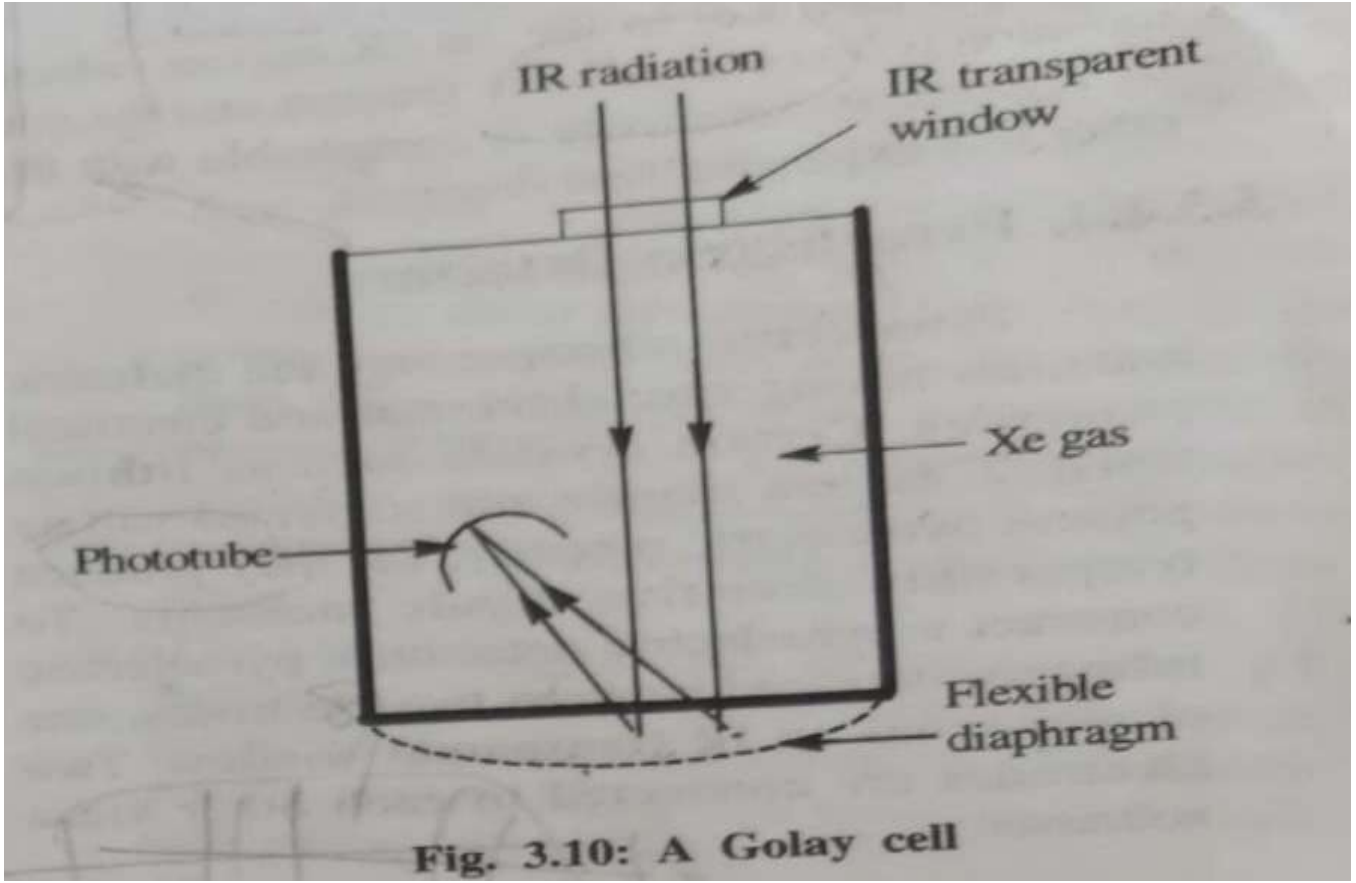
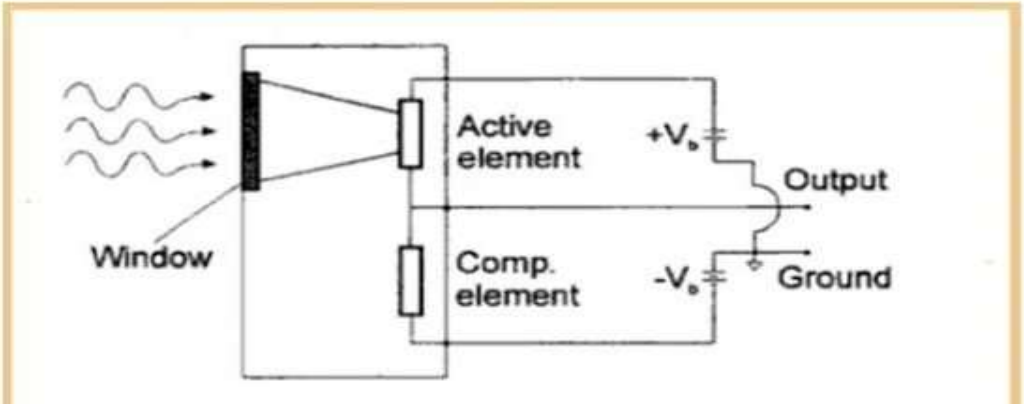


Fig. 3.10: A Golay cell

Thermistors

- It is made up of *metal oxides*.
- It functions by *changing resistance* when heated.
- It consists of two closely placed thermistor flakes, one of the 10 um is an *active detector*, while the other acts as the compensating / *reference detector*.
- A steady *voltage* is applied, due to the temperature increase there is *change* in resistance which is measured and this gives the *intensity* of the IR radiation



Pyroelectric detectors

Pyroelectric substances are the dielectric materials having special thermal and electrical properties. Certain such as lithium Iantatate, barium titanate and triglycine sulfate possess pyroelectric property. They produce temperature sensitive dipole moments.

To construct a pyroelectric detector, a pyroelectric substance is placed between two electrodes, one of which has an IR transparent window. Two electrodes are connected to each other via a voltmeter . Molecular dipoles in a pyroelectric substance are randomly oriented at room temperature. When the IR radiation falls upon a pyroelectric substance, it absorbs energy from the radiation. This energy is utilized in aligning the molecular dipoles of the pyroelectric substance.

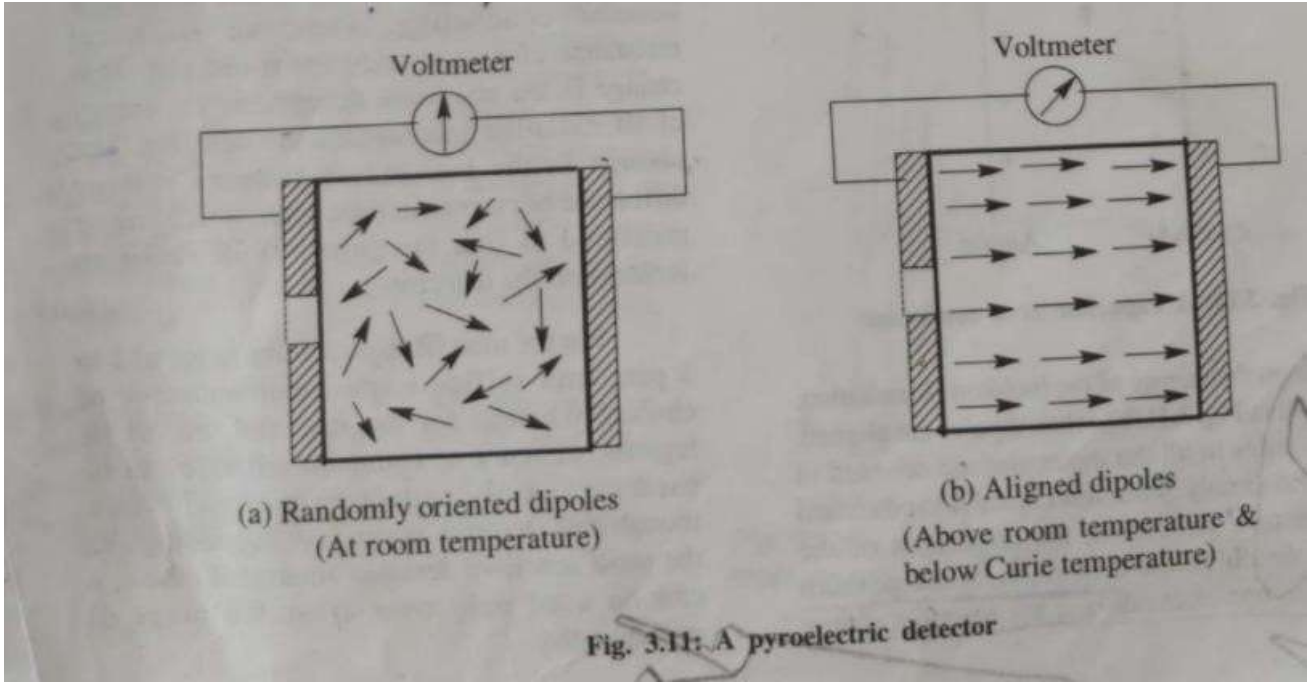


Fig. 3.11: A pyroelectric detector

PHOTOCONDUCTIVITY DETECTORS

1.Infrared light at wavelengths sometimes beyond 5 μmcan be detected with *lead salt detectors*, e.g. containing lead selenide (PbSe) or lead sulfide (PbS). They are *photoconductors* , not photodiodes, i.e., they do not contain a p–n junction but exhibit a reduction in electrical resistance caused by incident light, which induces intraband transitions.

2.Another detector technology, which is widely used, is based on photoresistors containing *mercury cadmium telluride*. Detection at rather long wavelengths (partly beyond 12 μm) is possible.

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Thank you!

Measurement of Bacterial Growth

Presented by

Mr. Sujit Kakade

Assistant Professor

Pune District Education Association's
Shankarrao Ursal College of Pharmaceutical Sciences
& Research Centre, Kharadi, Pune.

Viable Count Method

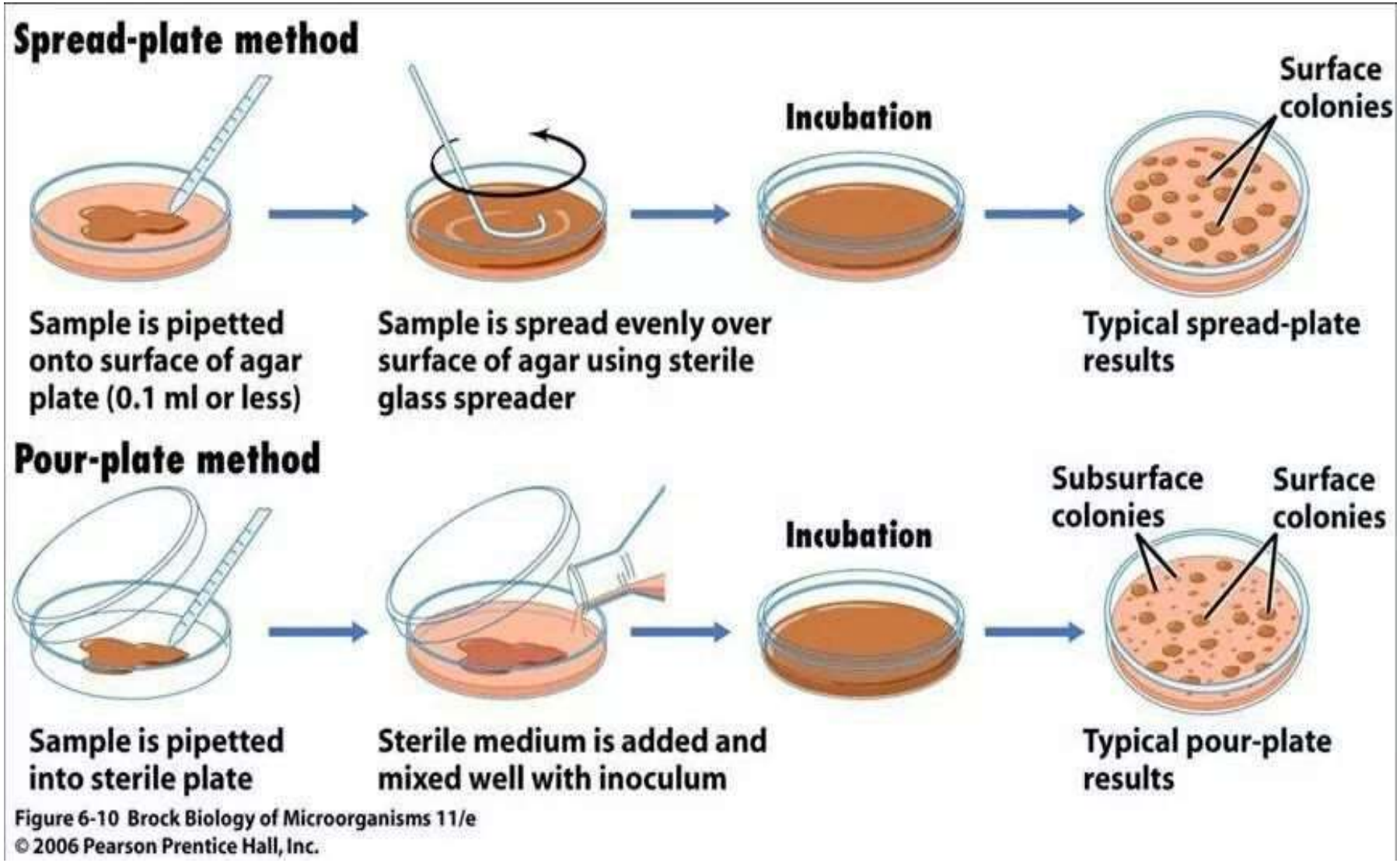

Figure 6-10 Brock Biology of Microorganisms 11/e

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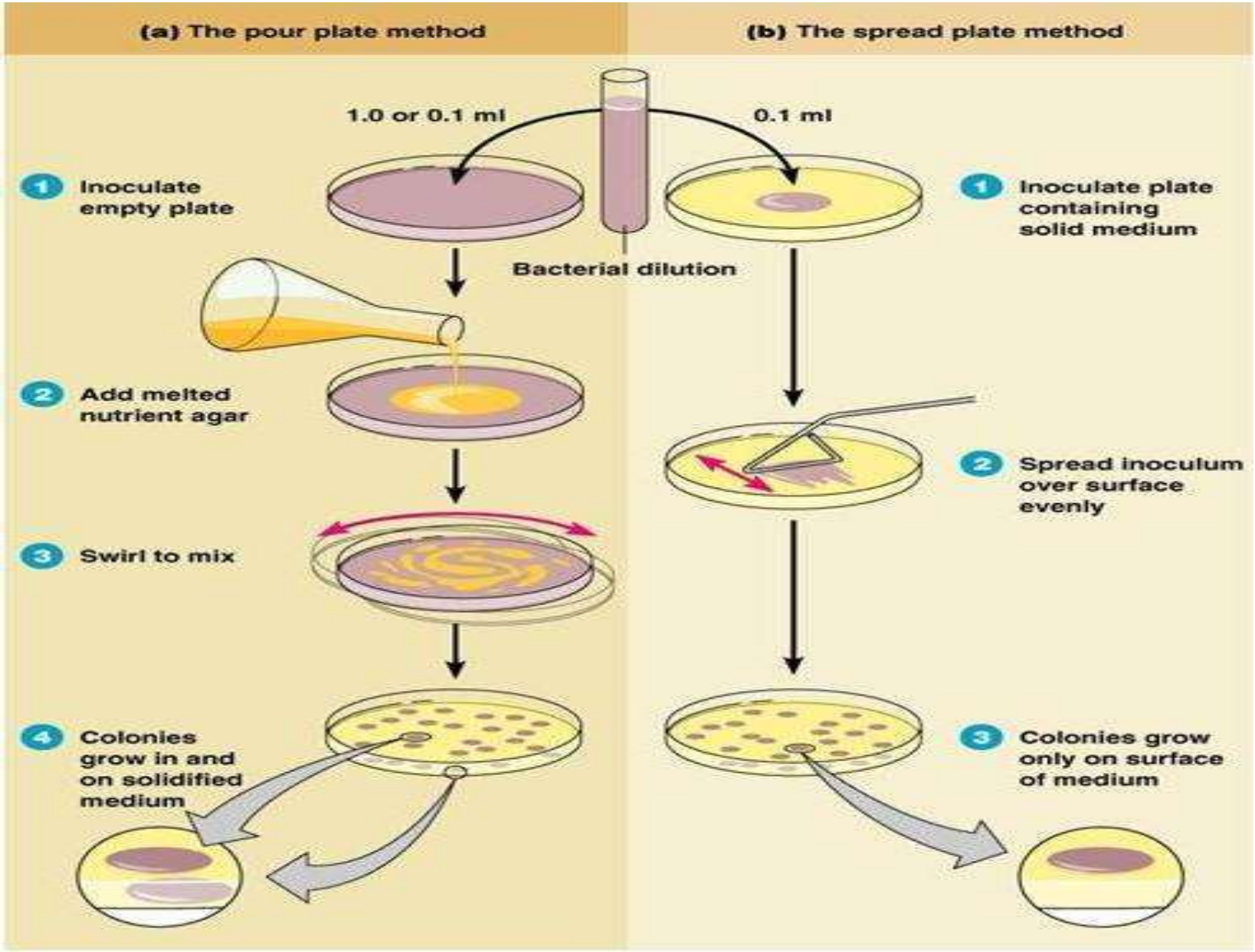

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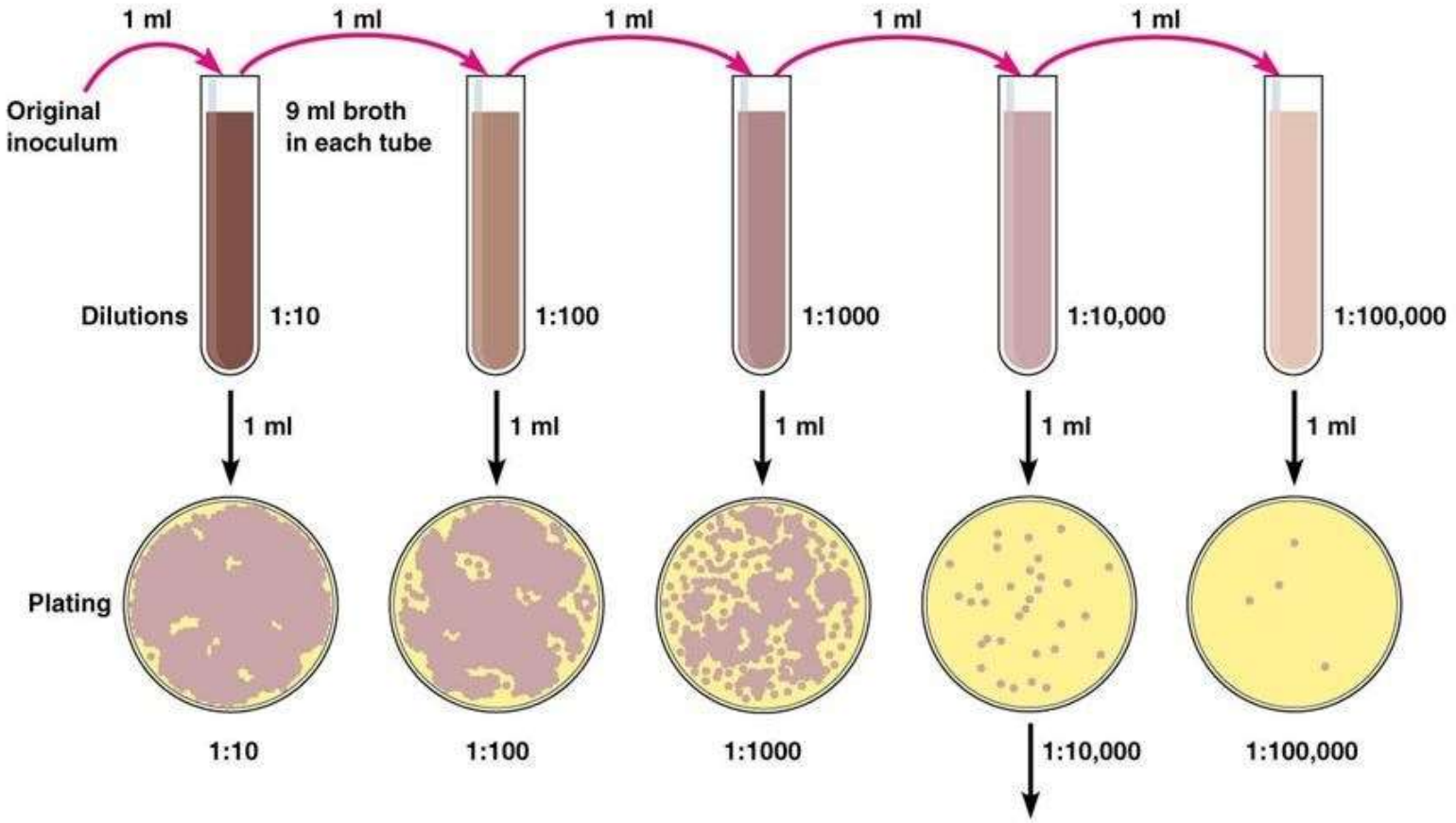

Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000 bacteria/ml in sample.)

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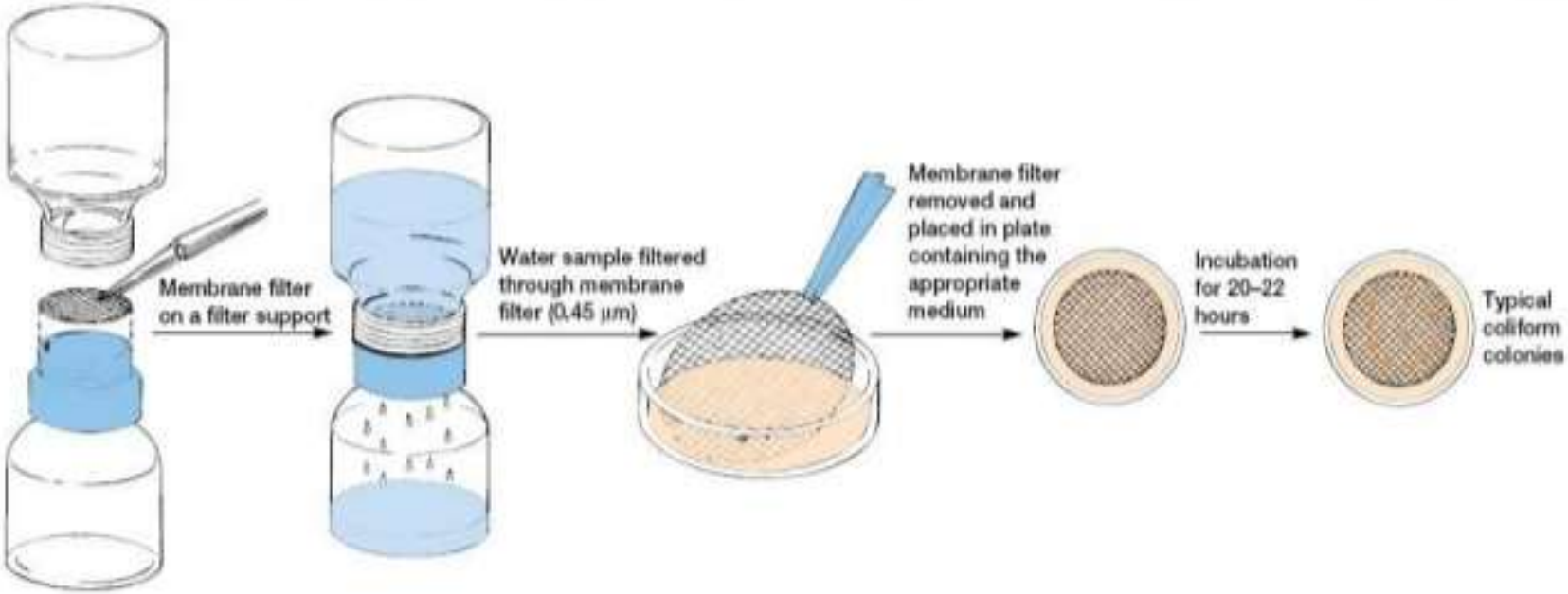
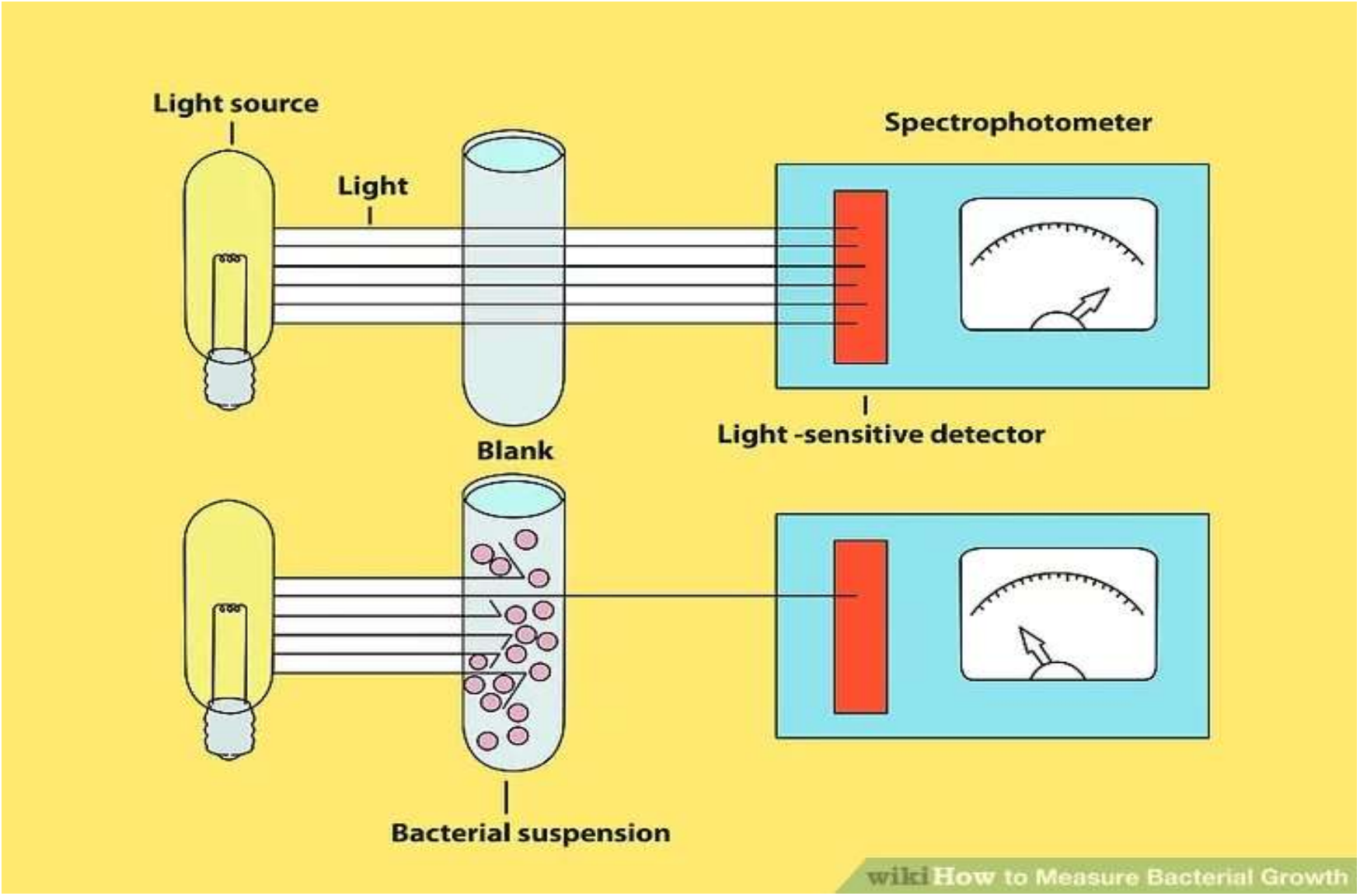
General Procedure for the Membrane Filter Apparatus



Turbidimetric Method



Turbidity

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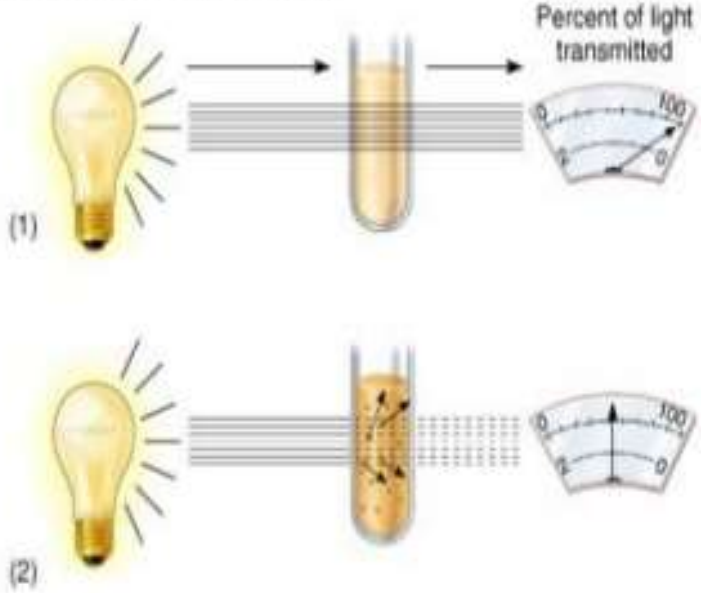
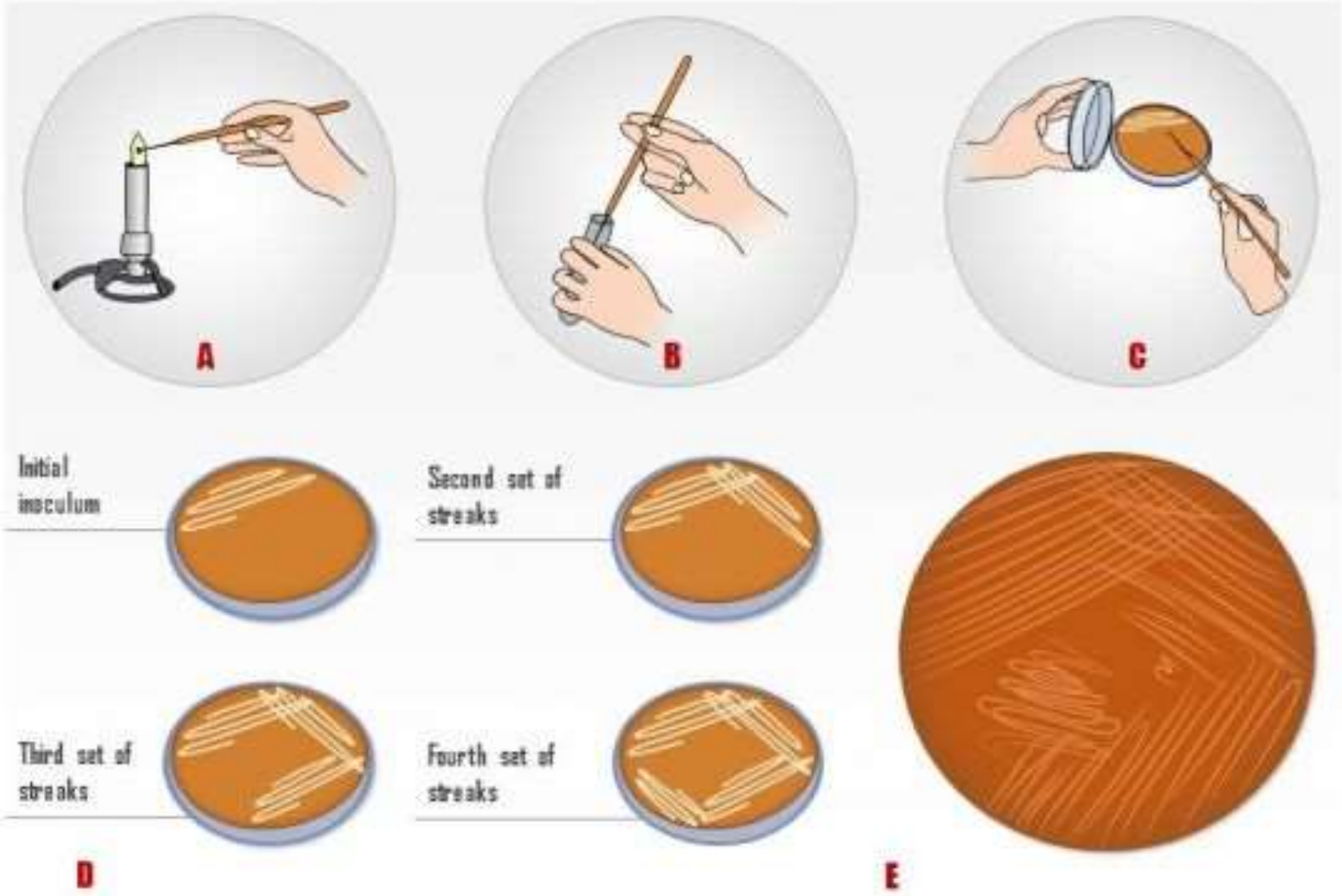
(a)

(b)



The Streak Plate Isolation Method



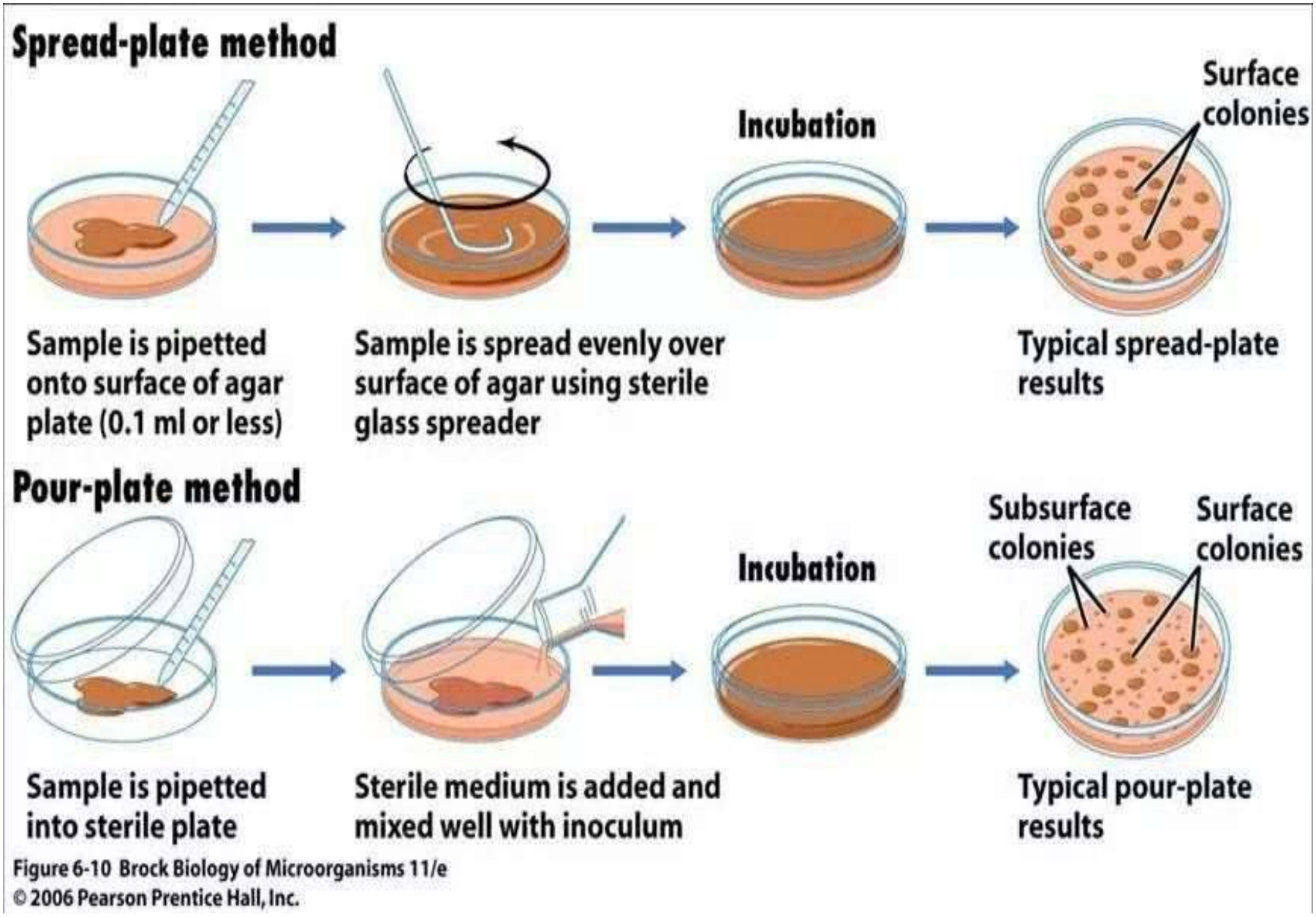


Figure 6-10 Brock Biology of Microorganisms 11/e

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Thank you

Pune District Education Association , Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre, Kharadi, Pune-411014

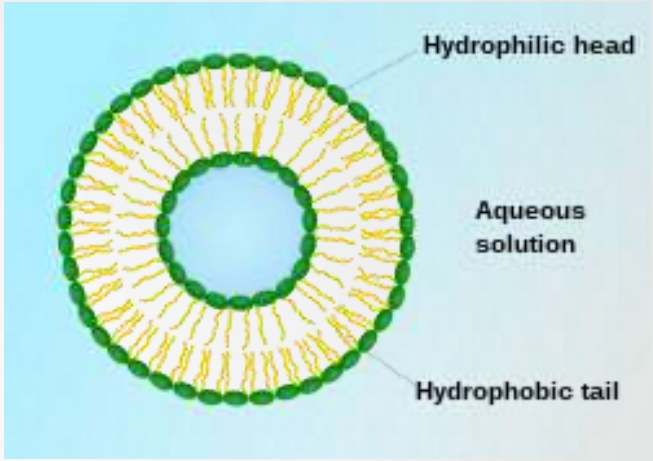
Presentation on

LIPOSOMES

Presented By :-

Mrs. Trusha P. Shangrapawar

LIPOSOMES



- Liposomes are microscopic spheres made from fatty materials, predominantly phospholipids
- Liposomes are made of molecules with hydrophilic and hydrophobic ends that form hollow spheres which can encapsulate water-soluble ingredients (drugs) in their inner water space and oil- soluble ingredients (drugs) in their phospholipid membranes that are made up of one or more concentric lipid bilayers, and range in size from 50 nanometers to several micrometers in diameter.

Advantages of Liposomes

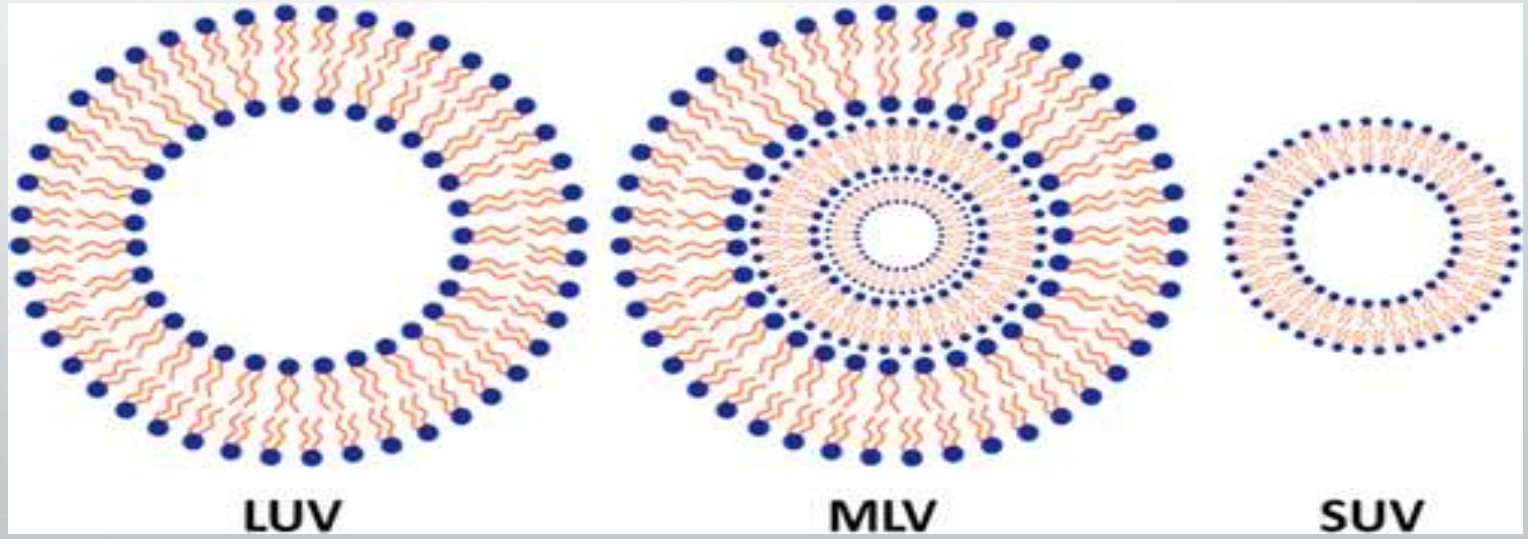
- Liposomes are biocompatible, completely biodegradable, non-toxic, flexible and non-immunogenic for systemic and non-systemic administration.
- Liposomes supply both a lipophilic environment and aqueous in one system and are therefore suitable for delivery of hydrophobic, amphipathic, and hydrophilic drugs and agents.
- Liposomes have ability to protect their encapsulated drug from the external environment and to act as sustained release depots.
- Liposomes can be formulated as a suspension, as an aerosol, or in a semisolid form such as gel, cream and lotion, as a dry vesicular powder (proliposome) for reconstitution or they can be administered through most routes of administration including ocular, pulmonary, nasal, oral, intramuscular, subcutaneous, topical and intravenous.
- Liposomes are increased efficacy and therapeutic index of drug (Actinomycin D-Liposomes help to reduce exposure of sensitive tissues to toxic drugs.

Disadvantages of Liposomes

- Production cost is high.
- Leakage and fusion of encapsulated drug.
- Sometimes phospholipid undergoes oxidation and hydrolysis like reaction.
- Short half-life.
- Low solubility.

Types of Liposomes

- Based on their size and number of bilayers liposomes are classified into three basic types:-
- 1) Multilamellar Vesicles (MLVs)
- 2) Large Unilamellar Vesicles (SUVs)
- 3) Small Unilamellar Vesicles (LUVs)



- Multilamellar Vesicles (MLVs) are of several lipid bilayers separated one another by aqueous space.
- They are heterogenous in size, often ranging from a few hundred to thousands of nanometer in diameter.
- Both Small Unilamellar Vesicles (SUVs) and Large Unilamellar Vesicles (LUVs) consist of single bilayer surrounding the entrapped aqueous space.
- SUVs are less than 100nm in size whereas LUVs have diameter larger than 100nm.

- In terms of composition and mechanism of intracellular delivery liposomes are classified into five types:-
- (i) Conventional liposomes
- (ii) pH sensitive liposomes
- (iii)Cationic liposomes
- (iv) Immuno liposomes
- (v) Long circulating liposomes

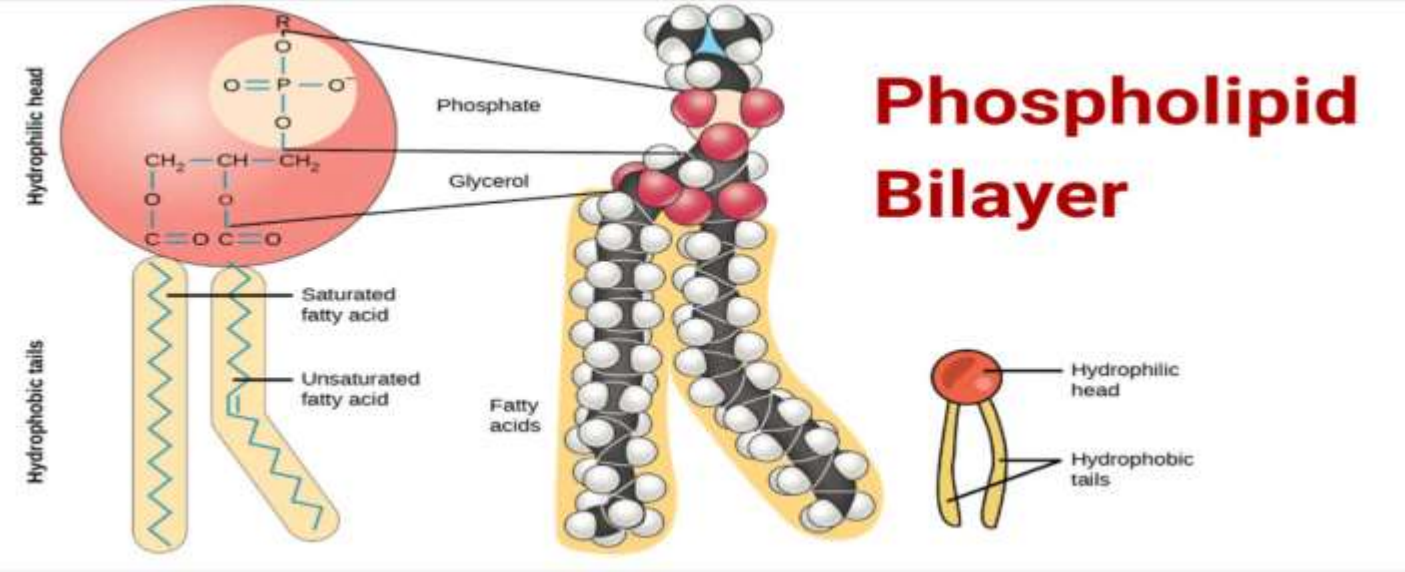
Formulation/Composition of Liposomes.

Material used in the liposomes formulation depends upon the drug nature.-The commonly used material includes phospholipids, glycosphingolipids, sterols, cationic lipid, a variety of other lipids and surfactants.

There are number of structural and nonstructural components of liposomes, major structural components of liposomes are-

1) Phospholipid- It is the major component of the biological membrane; two types of phospholipids are used natural and synthetic phospholipids.

The most common natural phospholipid is the Phosphatidylcholine (PC).It is amphipathic molecule and also known as lecithin. It is originated from animal (hen egg) and vegetable (soya bean).

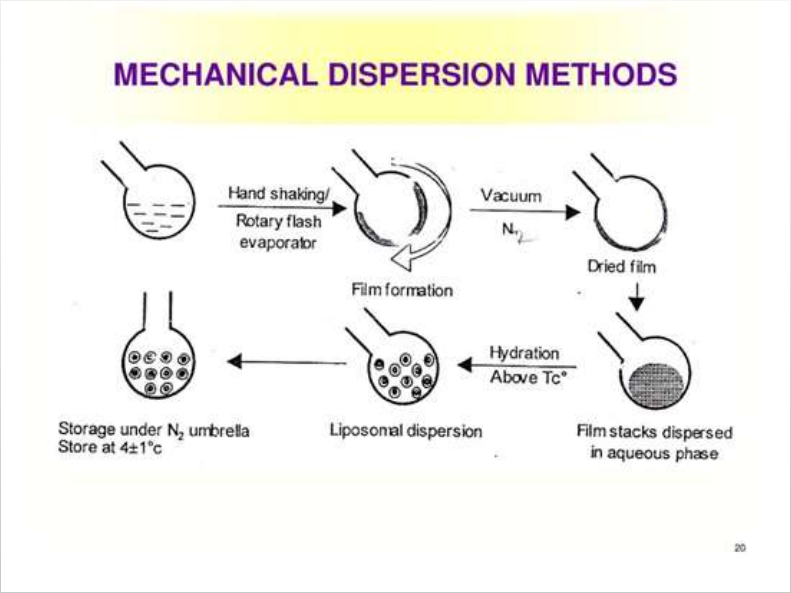


- Cholesterol- Incorporation of cholesterol in liposome bilayer can bring about big changes in the preparation of these membranes.-It can be incorporated into phospholipids membrane in very high concentration up to 1:1 or 2:1 molar ratios of cholesterol to Phosphatidylcholine.
- Being an amphipathic Molecule, cholesterol insert into the membrane with its hydroxyl group of Cholesterol oriented towards the aqueous surface and aliphatic chain aligned parallel to acyl chains in the centre of the bilayers.

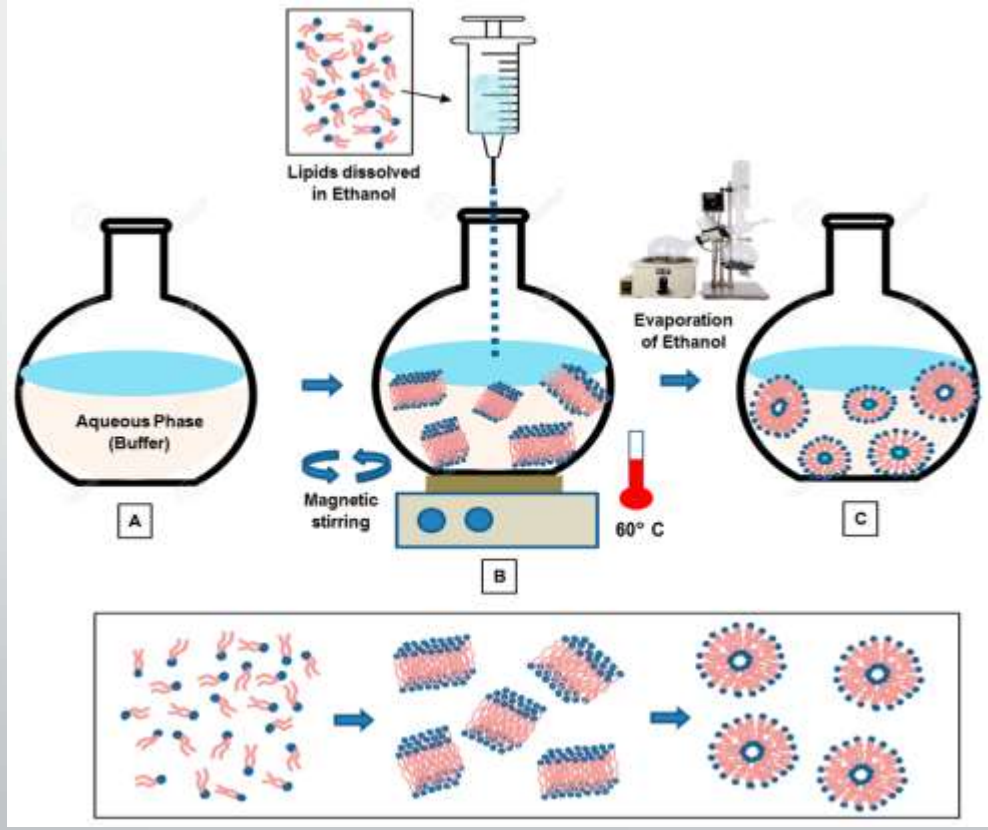
Method of preparation of Liposomes

1) Mechanical Dispersion Method
2) Solvent Dispersion Methods
3) Detergent Removal Method

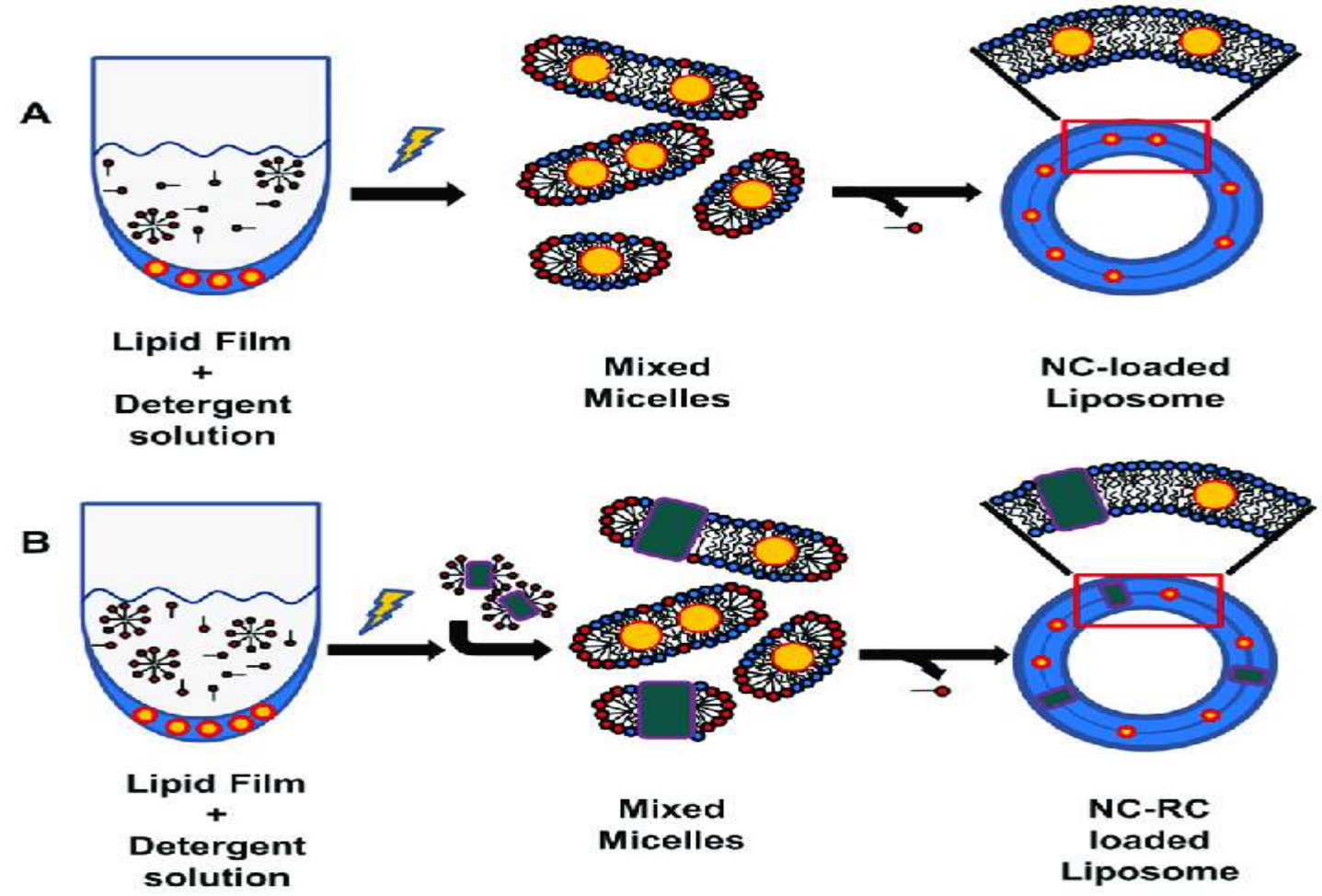
1) Mechanical dispersion methods- Lipid is solubilized in organic solvent, drug to be entrapped is solubilized in aqueous solvent, the lipid phase is hydrated at high speed stirring. Due to affinity of aqueous phase to polar head, it is entrapped in lipid vesicles. for example- Lipid film hydration, Micro-emulsification, Sonication, Dried reconstituted vesicle.



2) Solvent dispersion methods. In this method, lipids are first dissolved in organic solvent, which is then brought in to contact with aqueous phase containing material which is to be entranced in liposome under rapid dilution at rapid evaporation of organic solvent.-for example- Ethanol injection, Ether injection, De-emulsification.



3) Detergent removal method- In this method, phospholipids are brought into intimate contact with the aqueous phase via detergent which associate with phospholipids molecule and serve to screen the hydrophobic portions of the molecules from water.



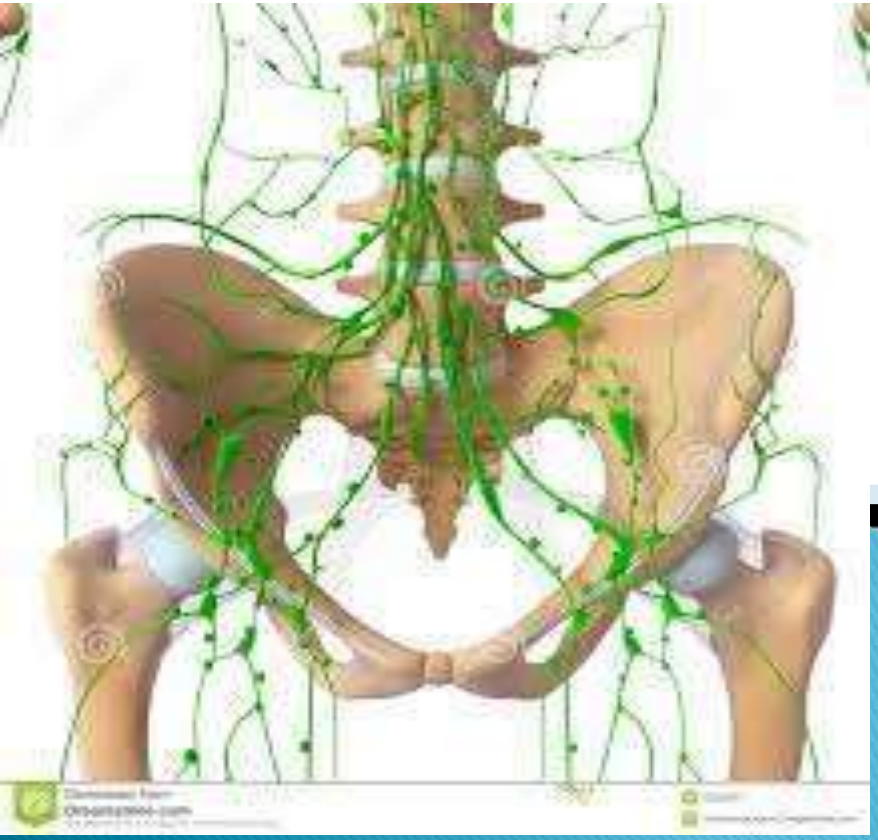
Applications of Liposomes

- Liposomes can target a drug to the intended site of action in the body, thus enhancing its therapeutic efficacy (drug targeting, site-specific delivery).
- Liposomes may also direct a drug away from those body sites that are particularly sensitive to the toxic action of it (site-avoidance delivery).
- Liposomes can act as a depot from which the entrapped compound is slowly released over time. Such a sustained release process can be exploited to maintain therapeutic (but nontoxic) drug levels in the bloodstream or at the local administration site for prolonged periods of time. Thus, an increased duration of action and a decreased frequency of administration are beneficial consequences.

- Drugs incorporated in liposomes, in particular those entrapped in the aqueous interior, are protected against the action of detrimental factors (e.g. degradative enzymes) present in the host.
- Conversely, the patient can be protected against detrimental toxic effects of drugs.
- Liposomes can interact with target cells in various ways and are therefore able to promote the intracellular delivery of drug molecules that in their 'free' form (i.e. non-encapsulated) would be able to enter the cellular interior due to unfavorable physicochemical characteristics (e.g molecules).
- If the drug is an antigen, liposomes can act as immunological adjuvant in vaccine formulation

Thank you!

Lymphatic system



By

Mr. Krunal G. Kanase

HOD & Assistant Professor

Department of Pharmacology

PDEA's SUCOPSRC, Pune

Introduction:-

- All body tissues are bathed in tissue fluid, consisting of the diffusible constituent of blood & waste material from cell. Some tissue fluid returnes to capillaries at their venous end the reminder defuses through the more permeable wall of the lymph capillaries, forming lymph.

- Cardiovascular & lymphatic system both are supply fluid flow in to the body. but bothe are deferent type of fluid..

- Lymphatic system does not having closed circuit & central pump like heart.

It consist of……

Lymph

Lymph vessels

Lymph node

Lymph organ eg. Spleen, Thymus

Diffuse lymphoid tissue eg. Tonsil

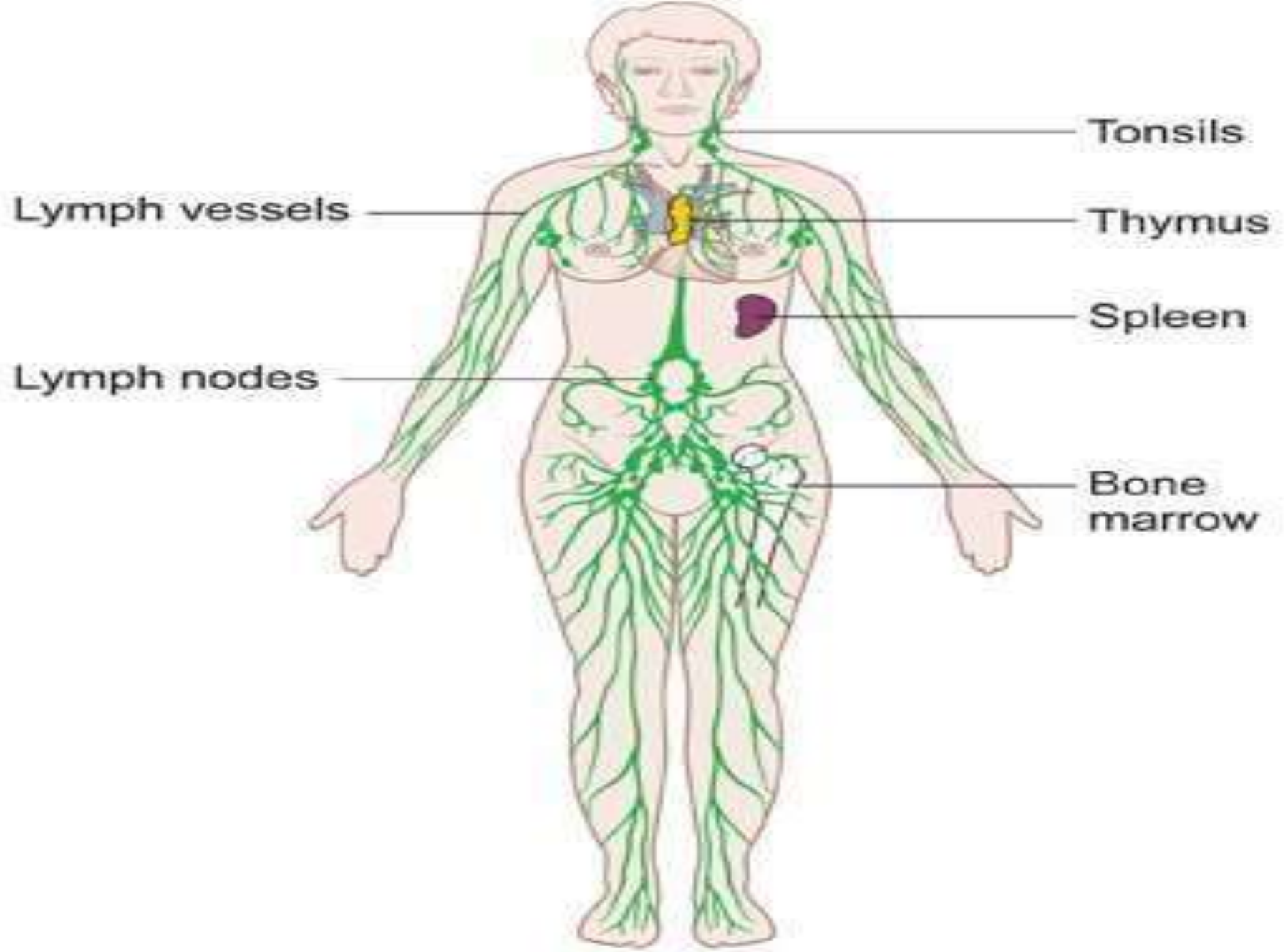


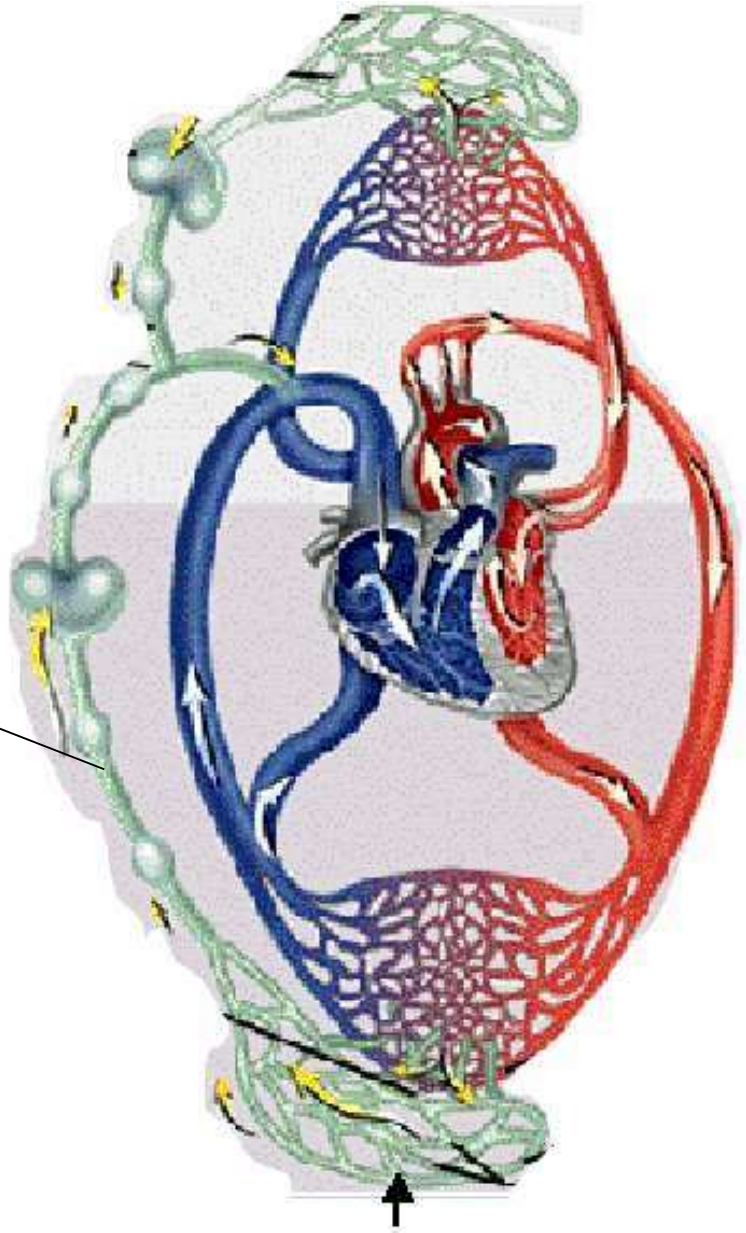
Diagram of the lymphatic system
Copyright © CancerHelp UK

Function of L.S.:-

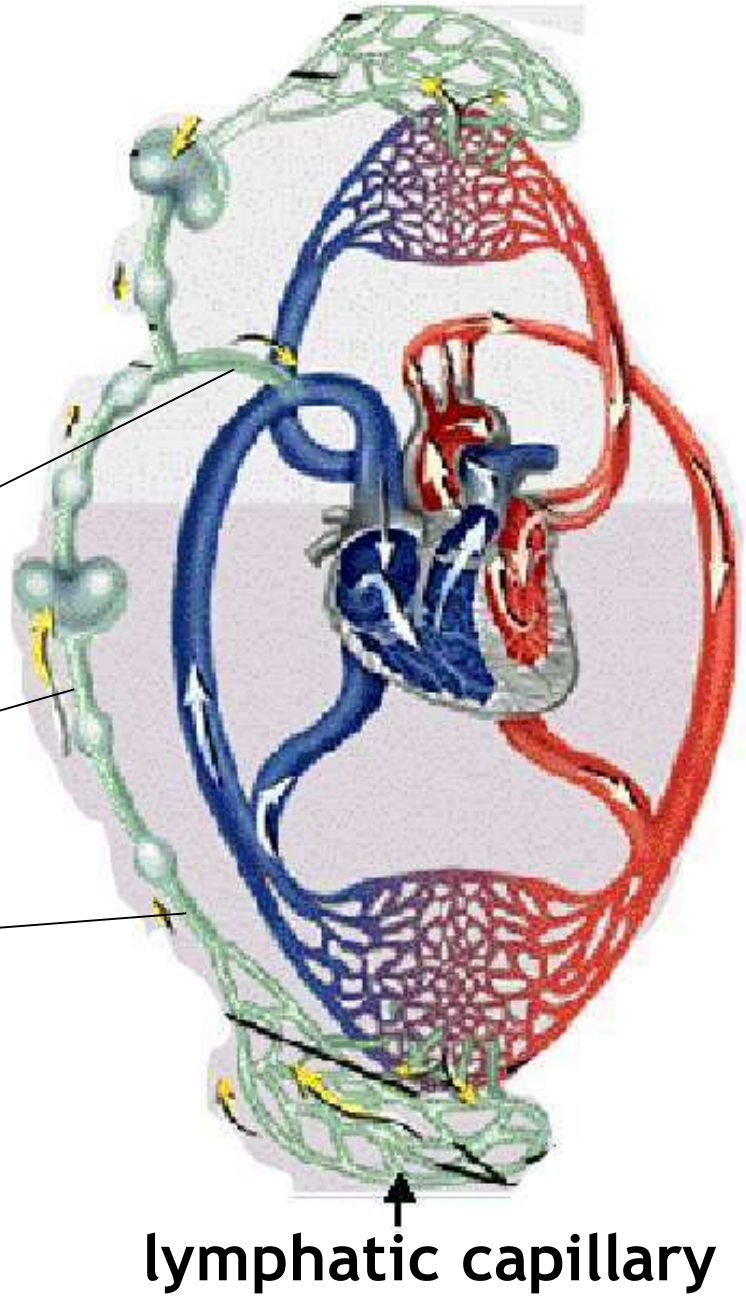
- Return tissue fluid to the bloodstream.
- It absorbs and transports fat and fat soluble vitamins from the intestine..
- Kill the micro-organism.

travel along with blood vessels.

1) lymphatic vessels



Lymphatic vessels start with lymphatic capillaries



Lymph:-

- ***Meaning:-***After blood travels through capillary beds and is moved to the venous system, some of its fluid is left behind in the tissues it called lymph.
- Lymph is a clear, colorless liquid with a composition similar to blood plasma.
- It is nothing but the clear, watery blood plasma leaked out through the capillary walls to flow around the cells.
- It contains oxygen, proteins, glucose and white blood cells.

- All the cells bathed in this fluid receive their nutrients and oxygen from it.
- Tubular vessels transport lymph back to the blood, ultimately replacing the volume lost during the formation of the interstitial fluid.
- Lymph movement occurs despite low pressure due to peristalsis, valves, and compression during contraction of adjacent skeletal muscle and arterial pulsation

The origin on lymph :-

the cardiovascular system pumps blood through its system but it cannot return all the fluid from the body cells.

The lymph system picks up 60%of the fluid dropped off at the cellular level,

at this point we are talking about interstial fluid, the IF picks up plasma and becomes tissue fluid

The tissue fluid is then picked up by lymph capillaries,

the tissue fluid is called *lymph*

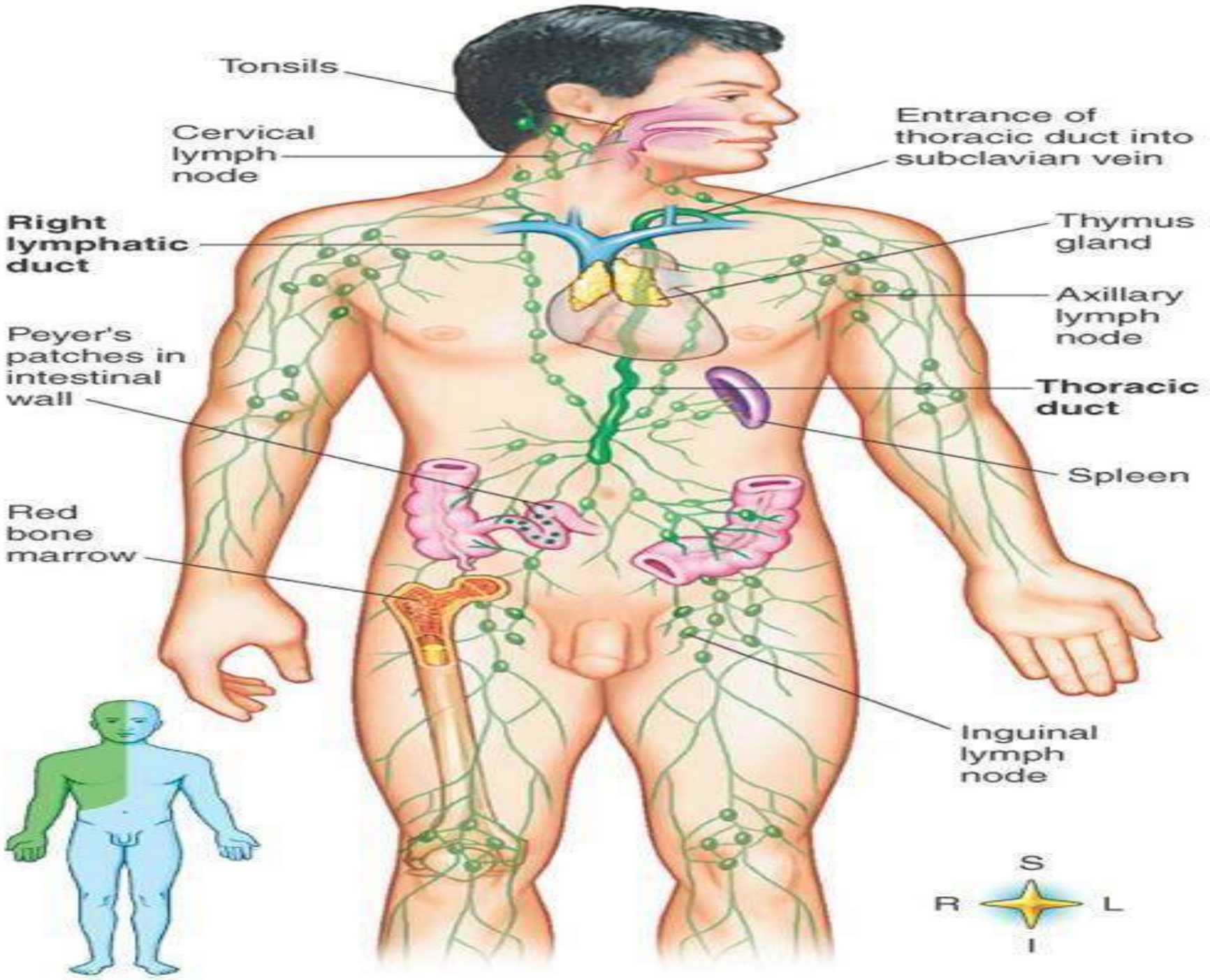
- **Lymph** is an alkaline (**pH** > 7.0)

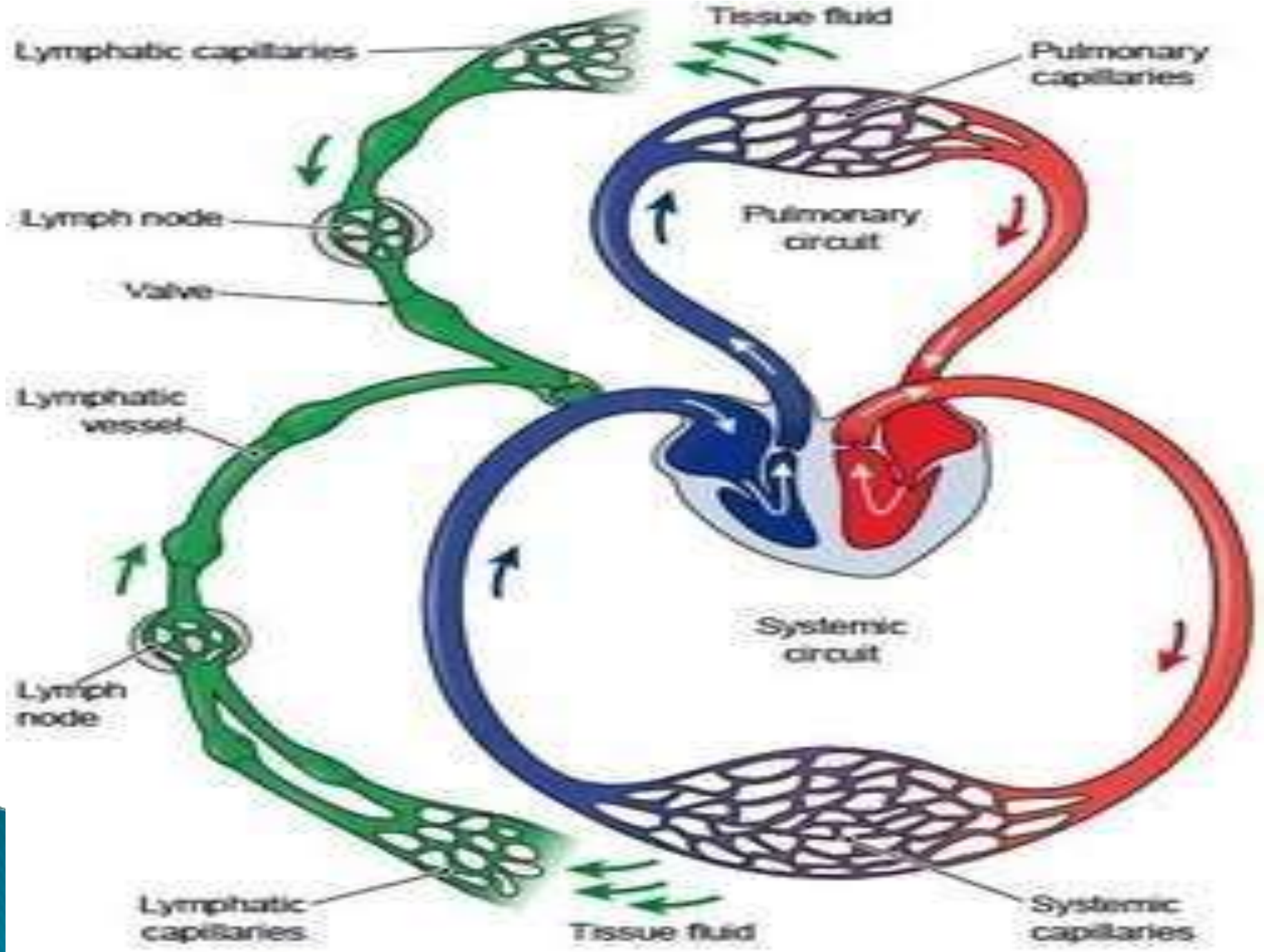
Function of lymph:-

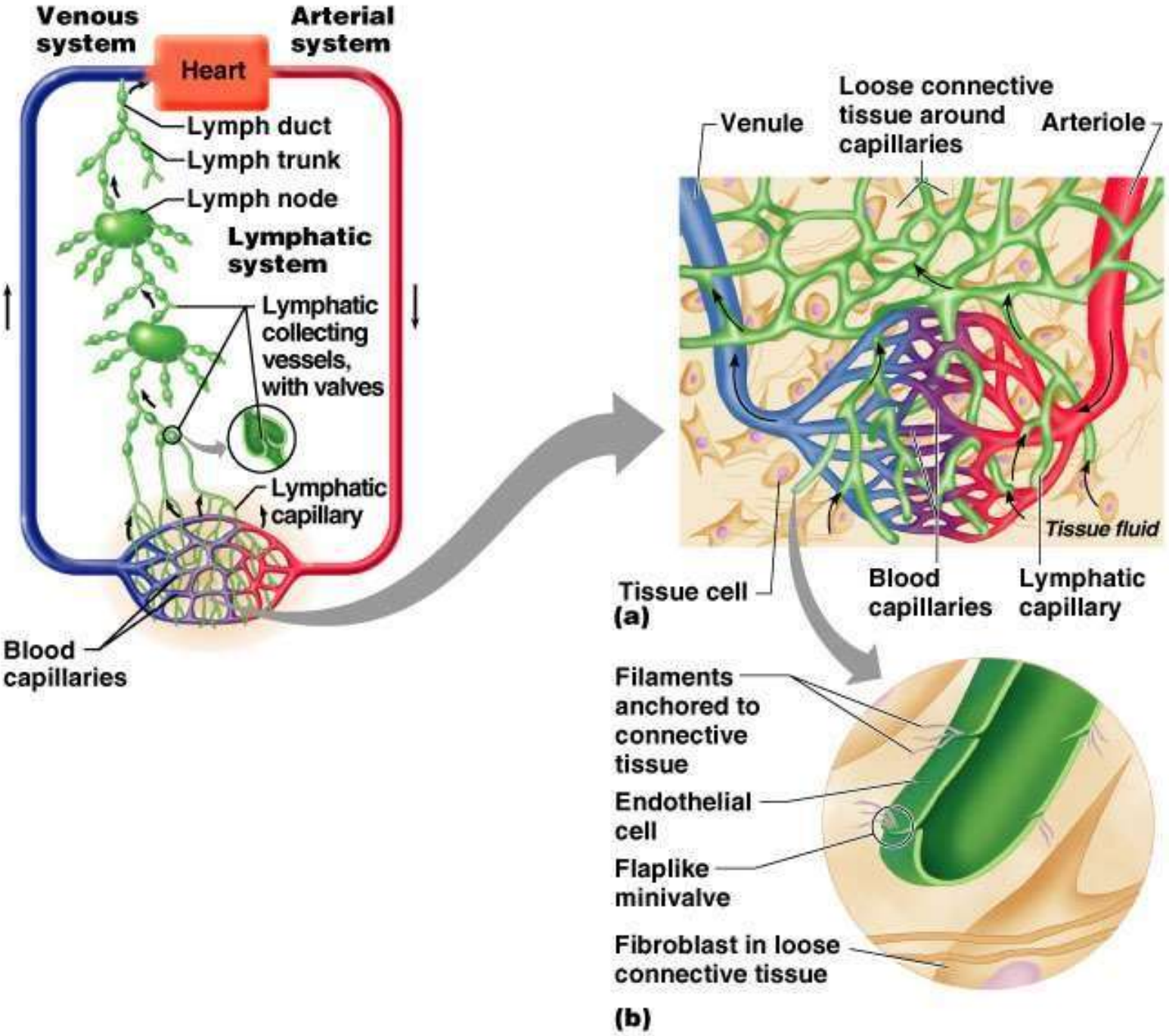
- Lymph acts as a "middle man" which transports oxygen, food materials, hormones, etc., to the body cells
- And brings carbon dioxide and other metabolic wastes, from the body cells to blood and then finally pours the same into the venous system.
- Body cells are kept moist by the lymph.
- It destroys the invading microorganisms and foreign particles in the lymph nodes

Lymph vessels:-

- Return to the blood of any fluids that have escaped from the circulation.
- Lymphatic vessels are intimately connected with blood vessels so that they can perform this function.
- Distribution of lymphatic vessels
  - Lymphatic vessels travel alongside blood vessels
  - Lymphatic vessels are absent from bones, teeth, bone marrow, and the central nervous system


Tonsils
Cervical lymph node
Entrance of thoracic duct into subclavian vein
Right lymphatic duct
Thymus gland
Axillary lymph node
Peyer's patches in intestinal wall
Thoracic duct
Spleen
Red bone marrow
Inguinal lymph node
S
R
L
I


Tissue fluid
Lymphatic capillaries
Pulmonary capillaries
Lymph node
Pulmonary circuit
Valve
Lymphatic vessel
Systemic circuit
Lymph node
Lymphatic capillaries
Tissue fluid
Systemic capillaries



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Circulation of lymph:-

Lymphatic trunks from the union of collecting vessels.

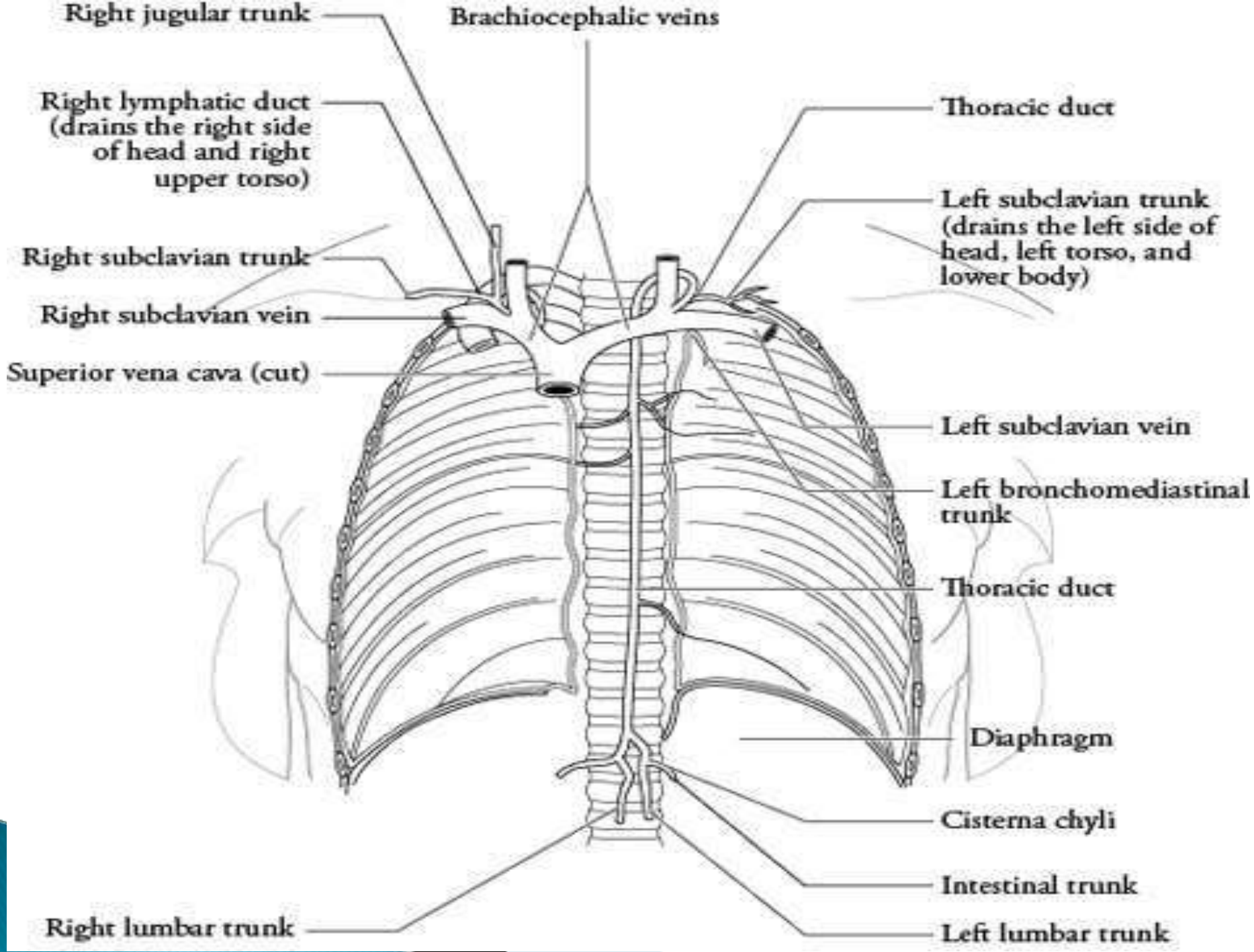
The nine major trunks, draining lymph from regions for which they are named, are the 1.lumbar,2. jugular, 3.subclavian, 4.bronchomediastinal trunks, each of which occurs in pairs (left and right, for each side of the body), and a (5)single intestinal trunk.

Lymphatic ducts are the largest lymphatic vessels.

These two ducts drain lymph into veins in the neck (the right and left subclavian veins at their junctures with the internal jugular veins). Valves in the lymphatic ducts at their junctions with the veins prevent the entrance of blood into the lymphatic vessels.

- The left thoracic duct collects lymph from the left side of the body and regions of the right side of the body below the thorax. It ultimately drains lymph into the left subclavian vein. It begins at the cisterna chili, an enlarged region of the lymphatic vessel that forms following the union of the intestinal trunk and right and left lumbar trunks.

- The right thoracic duct collects lymph from the upper right side of the body (right arm and right regions of thorax, neck, and head), a much smaller area than that serviced by the thoracic duct. It ultimately drains lymph into the right subclavian vein.


Right jugular trunk
Brachiocephalic veins
Right lymphatic duct (drains the right side of head and right upper torso)
Thoracic duct
Left subclavian trunk (drains the left side of head, left torso, and lower body)
Right subclavian trunk
Right subclavian vein
Superior vena cava (cut)
Left subclavian vein
Left bronchomediastinal trunk
Thoracic duct
Diaphragm
Cisterna chyli
Intestinal trunk
Right lumbar trunk
Left lumbar trunk

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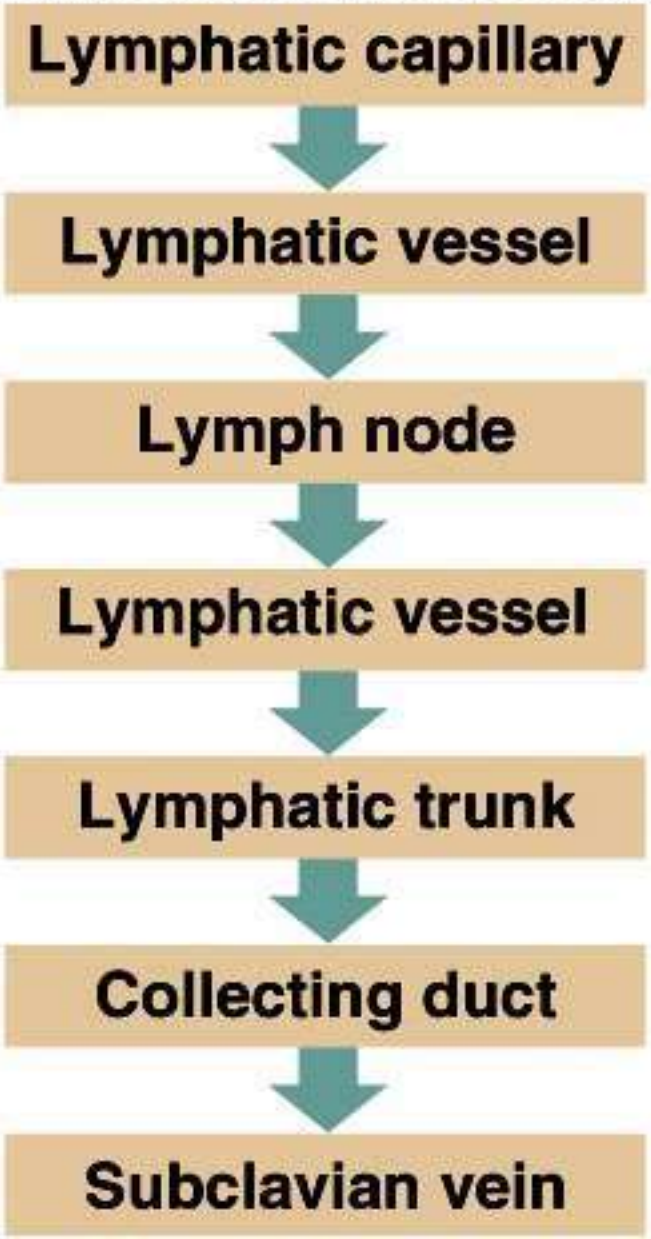

Lymph nodes:-

- They are widely distributed throughout the body along the lymphatic pathways.
- Lymph nodes are not present in the central nervous system.
- Composed of lymphoid tissue.

Structure of lymph nodes

- Small bean-shaped structures
- Usually less than 2.5 cm (1 inch) in length.
- Three superficial regions where lymph nodes tend to cluster
    1. Inguinal nodes in the groin
    2. Axillary nodes in the armpit
    3. Cervical nodes in the neck

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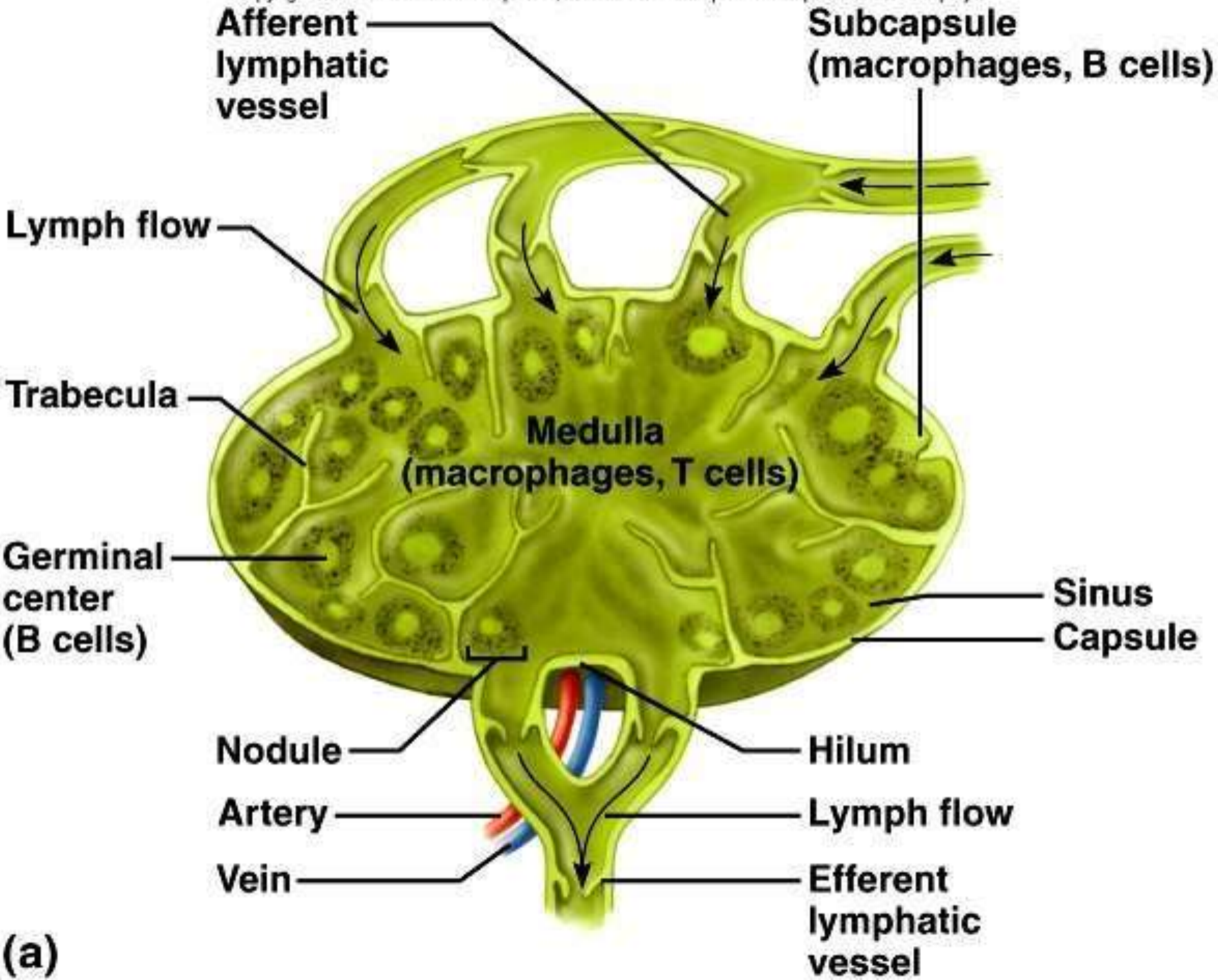
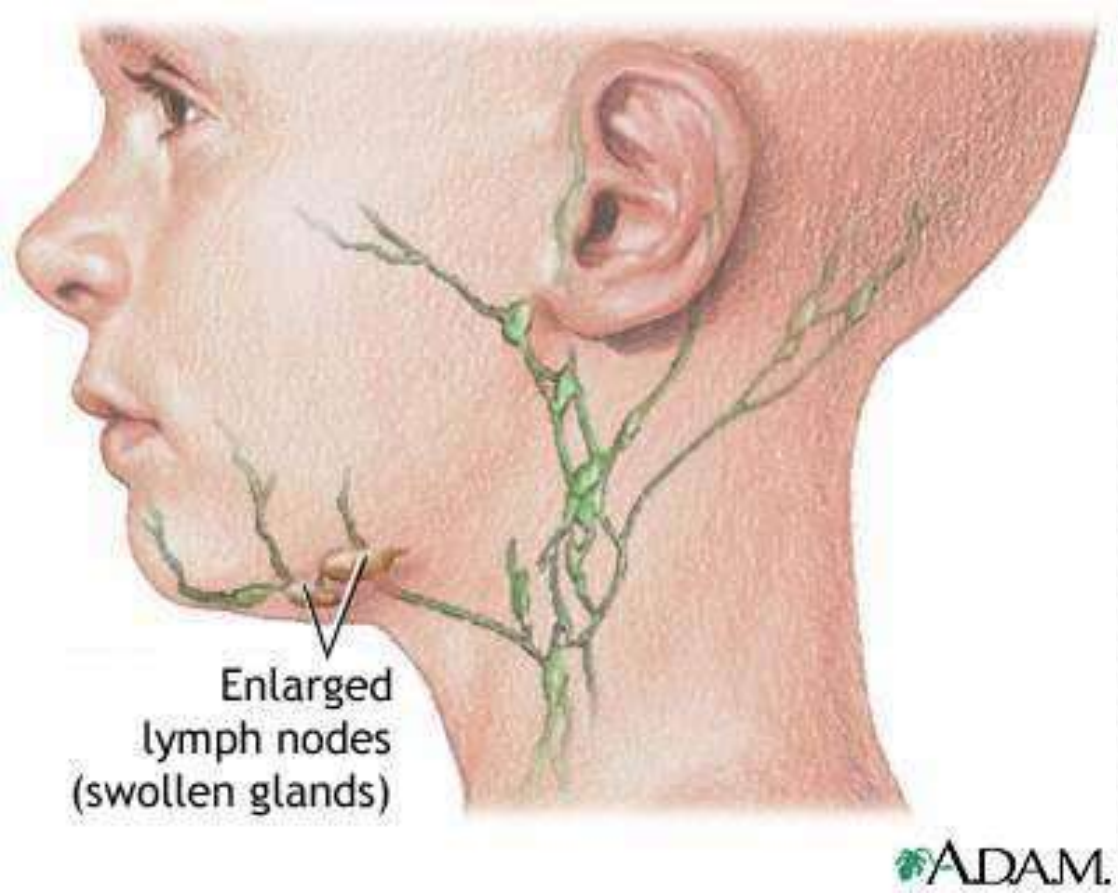


Function of Lymph nodes

- Filter the lymph before it is returned to the blood.
- Preventing foreign particles from entering the bloodstream
- They also produce lymphocytes

Swollen Lymph Glands



Tonsils:-

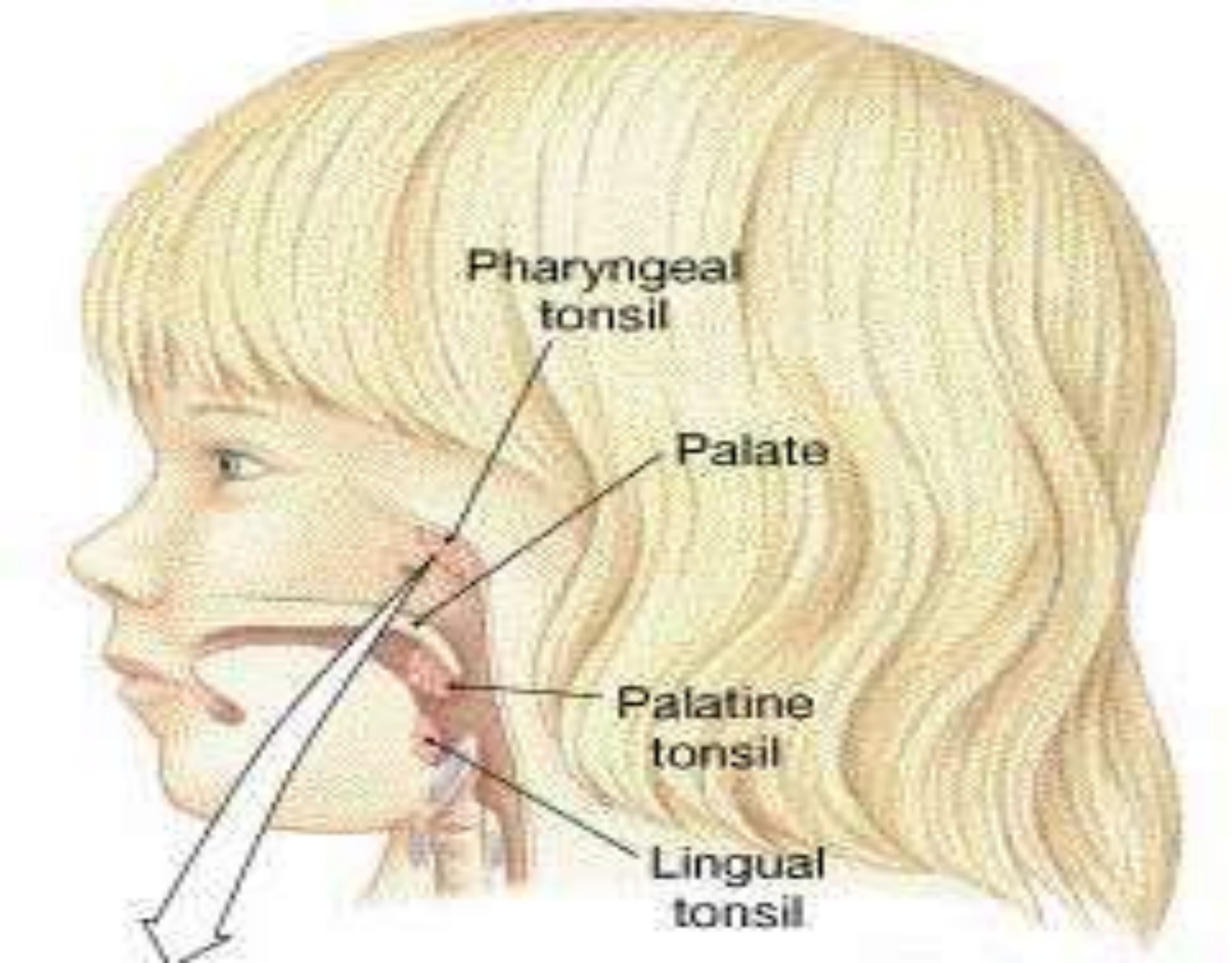
- Cluster of lymphatic tissue just under the mucous membranes that line the nose, mouth, and pharynx (throat) called tonsils.

- There are three groups of tonsils.
    1. Pharyngeal tonsils
    2. Palatine tonsils
    3. Lingual tonsils

Tonsils

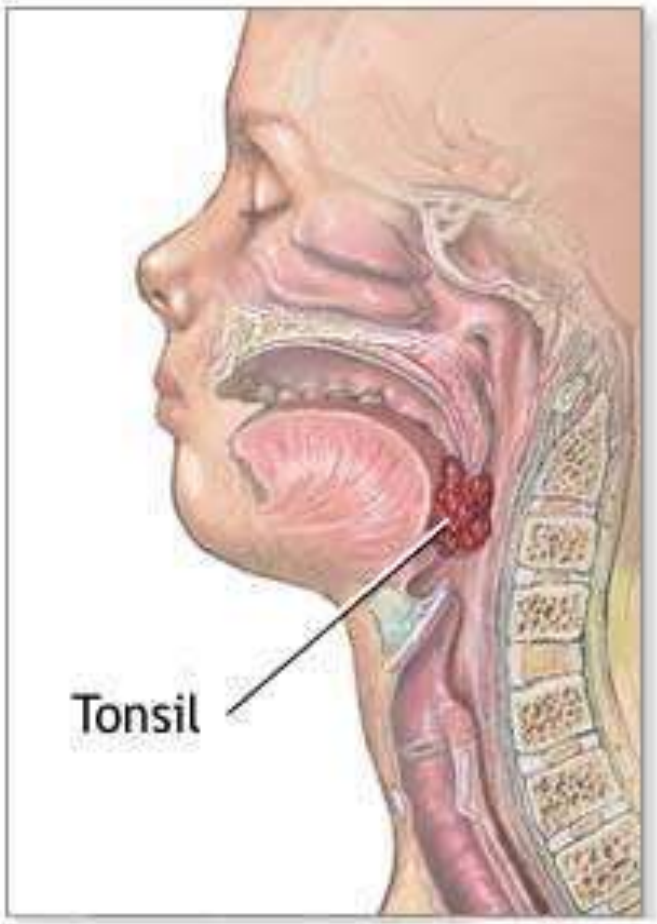
Function:-

- Provide protection against harmful substances and pathogens that may enter the body through the nose or mouth.
- Blood supply by:-Tonsillar branch of facial artery
- Nerve supply by:-glossopharyngeal nerve


Pharyngeal tonsil
Palate
Palatine tonsil
Lingual tonsil

Enlarge Tonsil

Greatly enlarged tonsil causing obstructive sleep apnea



Lymph node:-

Pune District Education Association's Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre.

Mass spectrometry

Presented by : Dr. Vijaya U. Barge
(Vice Principal & Professor)

Learning Objectives :

- Mass spectrometry is sensitive analytical technique with potential applications in the field of analytical and bio-analytical chemistry.
- This chapter covers all aspects of this versatile technique including instrumentationdetails and newer avenues being explored.
- Soft ionization techniques are described, including chemical ionization Cl, electrospray ionization ESI, FAB, MALDI, etc.
- Different types of ionic species are described, such as molecular ions, isotopic ions,metastable ions, rearrangement ions, etc.
- Recognition of molecular ion peak is an important aspect of mass spectrometry and this has been described in detail along with application of nitrogen rule, index of unsaturation, ring rule, etc.
- General rules for fragmentation are described including application of even electron rule.
- Application of the technique for elucidating molecular formulae of the compounds is described.
- Mass fragmentation pattern of different chemical classes of compounds is highlighted.
- Common fragment ions and fragments lost from the molecular ion have been tabulated at the end of the chapter.

Mass spectrophotometry:

Mass spectrometry is an analytical technique that is used to measure the mass-to-charge ratio of ions. The results are presented as a mass spectrum, a plot of intensity as a function of the mass-to-charge ratio

mass spectrometers can be used to identify unknown compounds via molecular weight determination

Ionisation in mass spectroscopy:

The atom is ionised by knocking one or more electrons off to give a positive ion. (Mass spectrometers always work with positive ions).

The particles in the sample (atoms or molecules) are bombarded with a stream of electrons to knock one or more electrons out of the sample particles to make positive ions.

- The choice of ionization method depends on the nature of the sample and the type of information required from the analysis. So-called 'soft ionization' methods such as field desorption and elec- trospray ionization tend to produce mass spectra with little or no fragment-ion content.

Classification according to hard and soft source:

Hard source: It imparts sufficiently energy to analyte molecule

- Relaxation then involves rupture of bonds producing fragment ions that have mass to charge ratio less than that of molecular ion.

Peaks in hard source provide useful information about the kind of functional groups and thus structural information about analytes.

E.g. Chemical ionization

Soft source:

Little fragmentation.

- Supply accurate information about the molecular weight of the analyte molecules.

E.g.

1)Matrix Assisted Laser Desorption Ionization (MALDI)

2) Electron impact ionization

Basic Type	Name and Acronym	Ionizing Agent
Gas Phase (for Volatile compounds)	Electron Ionization (EI) √	Energetic electrons
	Chemical Ionization (CI) √	Reagent gaseous ions
	Field ionization (FI)	High-potential electrode
Condensed Phase Desorption (for nonvolatile compounds)	Field desorption (FD)	High-potential electrode
	Plasma desorption (PD)	Fission fragment from ^{252}Cf
	Secondary ion mass spectrometry (SIMS)	Energetic beam of ions
	Fast atom bombardment (FAB) √	Energetic atomic beam
	Electrospray ionization (ESI) √	High electrical field
	Atmospheric pressure chemical ionization (APCI) √	High electric field Laser
	Matrix-assisted desorption/ionization (MALDI) √	Laser Beam

- Electron Impact (El-Hard method)
- - small molecules, 1-1000 Daltons, structure

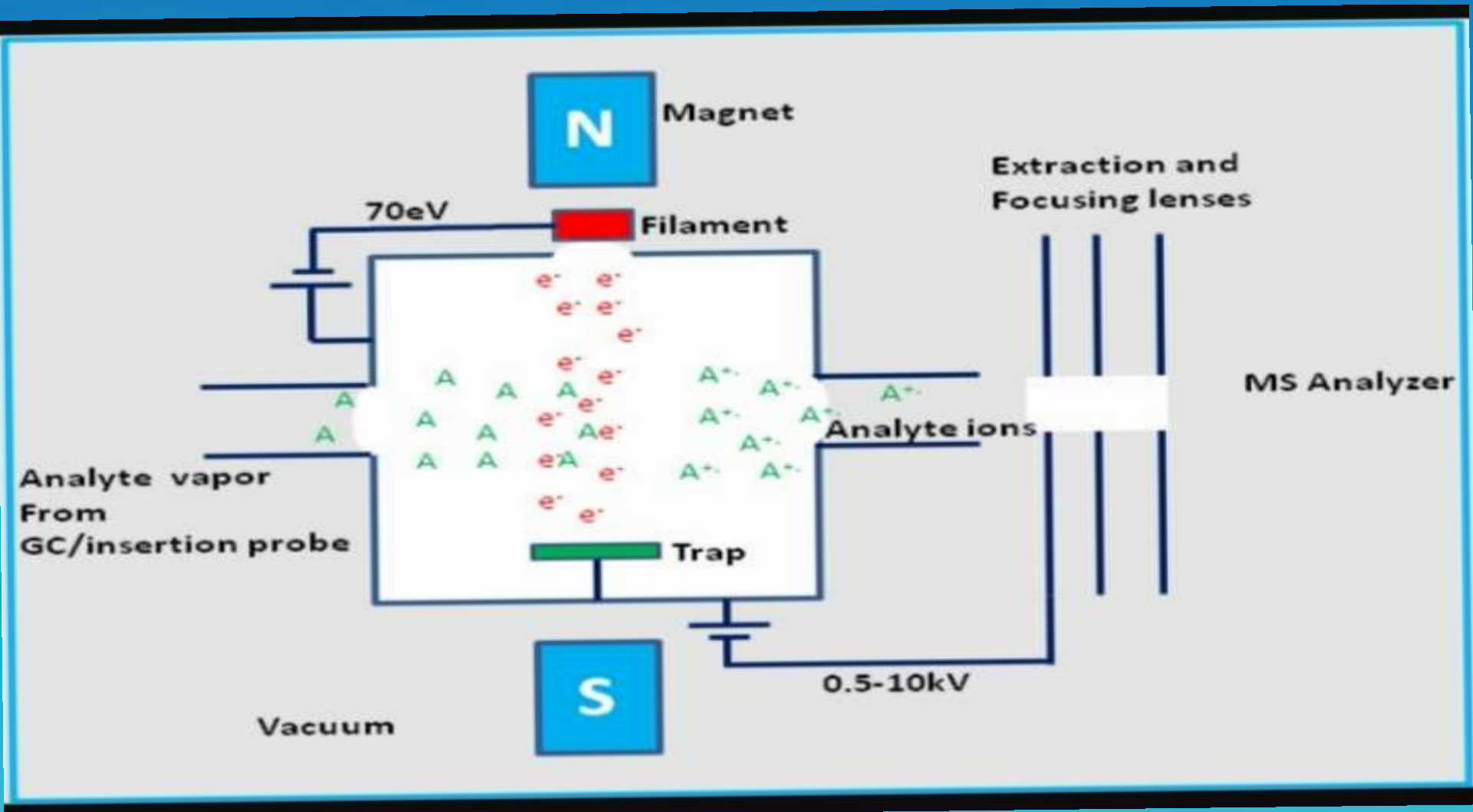
- Fast Atom Bombardment (FAB - Semi-hard) peptides, sugars, up to 6000 Daltons -

- Electrospray lonization (ESI - Soft) peptides, proteins, up to 200,000 Daltons

- Matrix Assisted Laser Desorption (MALDI-Soft) - peptides, proteins, DNA, up to 500 kD

Electron Impact ionization (EI) -

- EI is done by volatilizing a sample directly in the source that is contained in a vacuum system directly attached to the analyzer.
- The gas phase molecules are bombarded by a beam of electrons formed by
- heating a filament bias at a negative voltage compared to the source. The bias voltage is most commonly at -70 volts.
- The electron beam ejects an ion from the gas phase molecule producing a radical ion.
- This technique is considered a hard ionization technique, because it causes the
- ion to fragment. EI is also the method that is most commonly used for GC-MS.


N
Magnet
Extraction and
Focusing lenses
70eV
Filament
MS Analyzer
Analyte ions
Analyte vapor
From
GC/insertion probe
Trap
S
0.5-10kV
Vacuum

Chemical ionization:

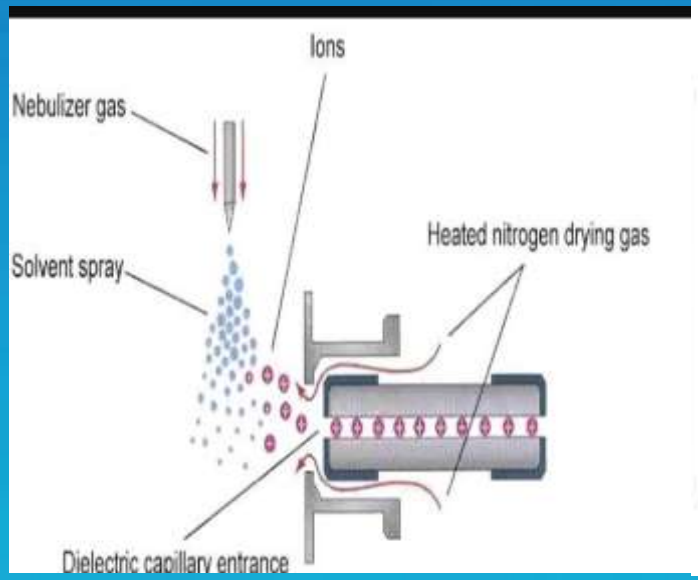
- Chemical ionisation is a lower energy alternative to EI for volatile analytes.
- methane is ionised by an electron beam in the same way as with EI. The ionised methane gas then reacts with un-ionised reagent gas to form the methanium carbocation (protonated methane).
- Methanium can then udergo proton transfer to the analyte [M] to form the protonated analyte molecule [M+H]+. If the pressure of the reagent gas is too high, then the side reactions can also occur, leading to formation of the analyte adduct ion - this is seen as an [M+C2H5] peak in the spectrum (occurring 28 m/z higher than the [M+H]+).

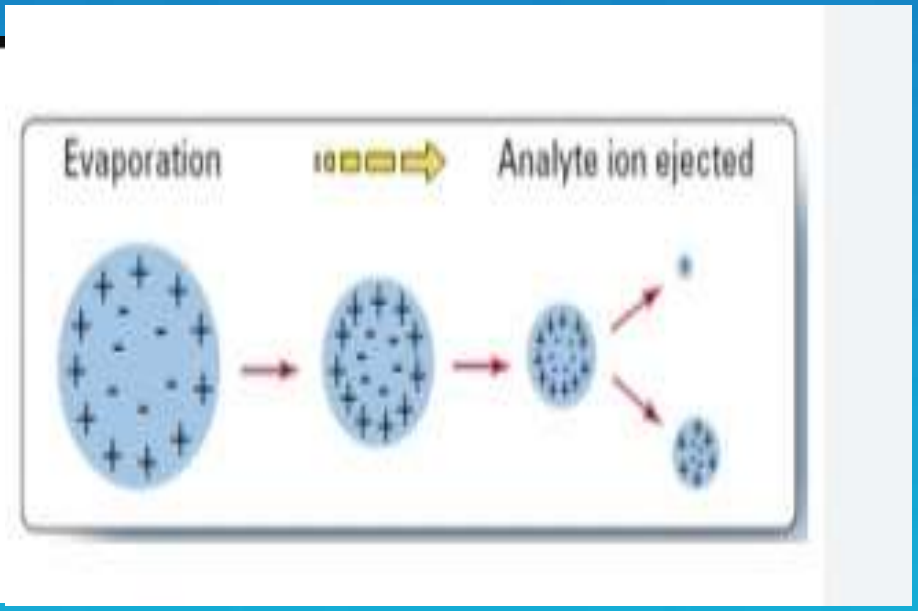
In CI mass spectrometry the molecules of a vaporized sample are ionized
by a set of reagent ions (reagent plasma) in a series of ion-molecule
reactions

Generally hydrogen (H_2), methane (CH_4), isobutane (iso-C_4H_{10}) and
ammonia (NH_3) are used as reagent gases in CI mass spectrometry;
with all these CI gases the compounds form protonated molecule
ion in their CI spectra.

- Electrospray relies in part on chemistry to generate analyte ions in solution before the analyte reaches the mass spectrometer.
- The LC eluent is sprayed (nebulized) into a chamber at atmospheric pressure in the presence of a strong electrostatic field and heated drying gas. The electrostatic field causes further dissociation of the analyte molecules. The heated drying gas causes the solvent in the droplets to evaporate. As the droplets shrink, the charge concentration in the droplets increases. Eventually, the repulsive force between ions with like charges exceeds the cohesive
- forces and ions are ejected (desorbed) into the gas phase.
- These ions are attracted to and pass through a capillary sampling orifice into themass analyzer

Diagram of spray:





Atmospheric pressure photoionization:

- Atmospheric pressure photoionization (APPI) for LC/MS is a relatively new
- technique. As in APCI, a vaporizer converts the LC eluent to the gas phase.

- A discharge lamp generates photons in a narrow range of ionization energies.
- The range of energies is carefully chosen to ionize as many analyte molecules
- as possible while minimizing the ionization of solvent molecules.
- The resulting ions pass through a capillary sampling orifice into the mass analyzer.

Fast Atom Bombardment (FAB) -

- FAB is a technique that was popular in
- the 80's to early 90's because it was the first technique that allowed
- ionization of non-volatile compounds that could be done simply.
- It was developed by Michael Barber at the University of Manchester in 1980.
- It was done by bombarding a sample in a vacuum with a beam of atoms,
- typically Ar or Xe, accelerated to Kilovolt energies. The sample was
- typically mixed in a matrix. The two most common matrixes were glycerol
- and 3 Nitro-benzoic acid.

Electrospray mass spectrometry (ESI-MS)

- - Liquid containing analyte is forced through a steel capillary at high voltage to electrostatically disperse analyte. Charge imparted from rapidly evaporating liquid. If the sample has functional groups that readily accept H+ .(such as amide and amino groups found in peptides and proteins) then positive ion detection is used-PROTEINS
- If a sample has functional groups that readily lose a proton (such as carboxylic acids and hydroxyls as found in nucleic acids and sugars) then negative ion detection is used-DNA

Matrix-assisted laser desorption ionization (MALDI)

- - Analyte (protein) is mixed with large excess of matrix (small organic molecule)
- Irradiated with short pulse of laser light. Wavelength of laser is the same as absorbance max of matrix.
- Sample is ionized by bombarding sample with laser light
- Sample is mixed with a UV absorbant matrix
- Light wavelength matches that of absorbance maximum of matrix so that the matrix transfers some of its energy to the analyte (leads to ion sputtering)

Mass Analyzers

Although in theory many type of mass analyzer could be used for LC/MS, four types are used most often

- Quadrupole
- Time-of-flight
- Ion trap
- Fourier transform-ion cyclotron resonance (FT-ICR or FT-MS)

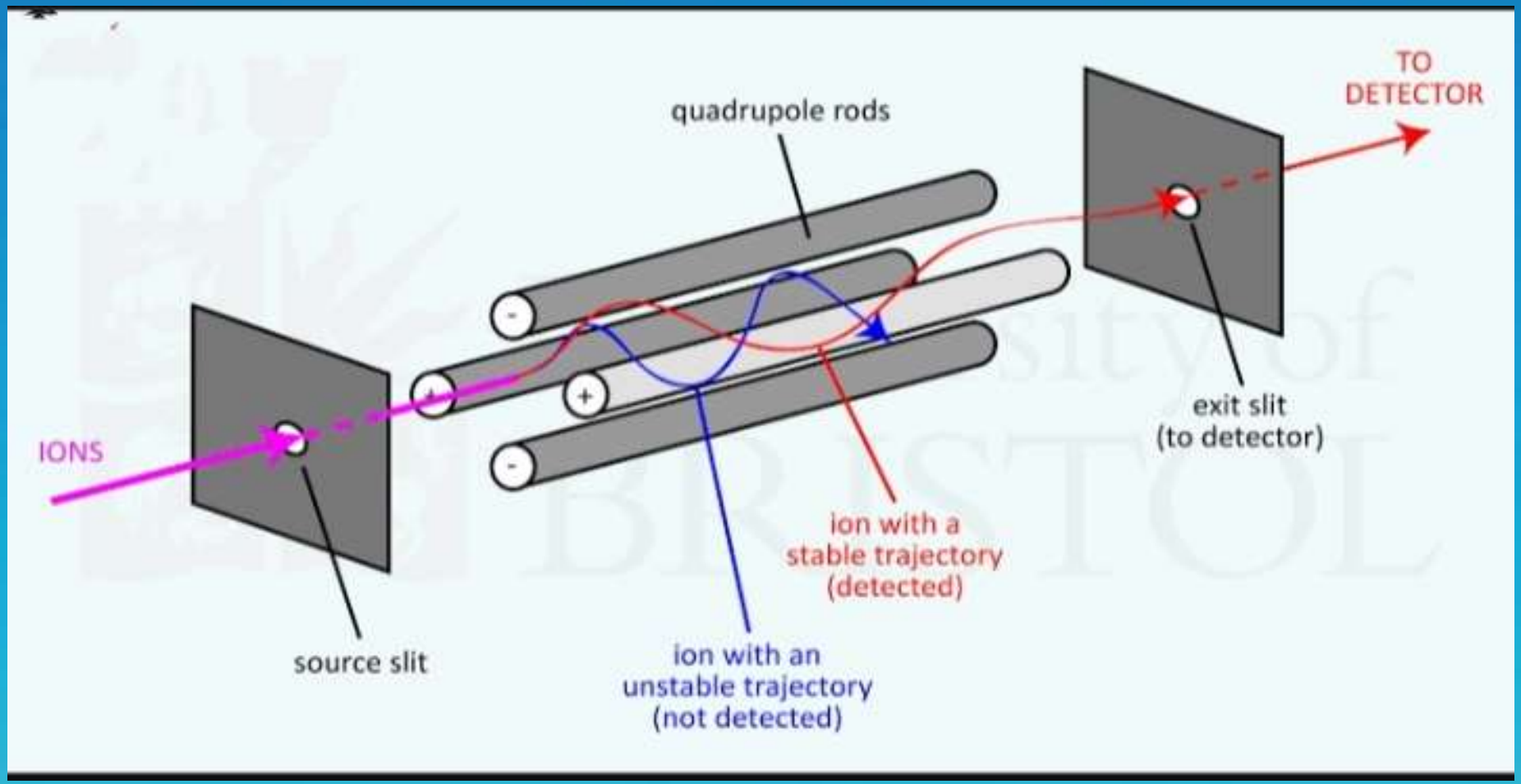
Quadrupole

- A quadrupole mass analyzer consists of four parallel rods arranged in a square. The analyte ions are directed down the center of the square

- Voltages applied to the rods generate electromagnetic fields. These fields determine which mass-to-charge ratio of ions can pass through the filter at a given time

- Quadrupoles tend to be the simplest and least expensive mass analyzers.

Diagram of quadruple



Quadrupole mass analyzers can operate in two modes:

- Scanning (scan) mode
- Selected ion monitoring (SIM) mode

In **scan** mode, the mass analyzer monitors a range of mass-to-charge ratios.

In **SIM** mode, the mass analyzer monitors only a few mass to-charge ratios.

SIM mode is significantly more sensitive than scan mode but provides
information about fewer ions.

Scan mode is typically used for qualitative analyses or for quantitation when all analyte masses are not known in
advance.

Time-of-flight

- In a time-of-flight (TOF) mass analyzer, a uniform electromagnetic force is applied
- to all ions at the same time, causing them to accelerate down a flight tube.
- Lighter ions travel faster and arrive at the detector first, so the mass-to-charge
- ratios of the ions are determined by their arrival times. Time-off light mass
- analyzers have a wide mass range and can be very accurate in their mass measurements.

Metastable ion

- Metastable ion in mass spectrometry, An ion which is formed with sufficient excitation to dissociate spontaneously during its flight from the ion source to the detector.

- Fragment of a parent ion will give rise to a new ion (daughter) plus either a neutral molecule or a radical. M1 + M2 + + non charged particle .An intermediate situation is possible; M1 + may decompose to M2 + while being accelerated.
- The resultant daughter ion M2 + will not be recorded at either M1 or M2, but at a position M* as a rather broad, poorly focused peak. Such an ion is called a metastable ion.

Significance of Metastable lons:

- Metastable ions are useful in helping to establish fragments routes.
- Metastable ion peak can also be used to distinguish between fragmentation Processes, which occur in few microseconds

Applications

Many disciplines use mass spectroscopy for chemical identification.

1. **Astronomy**: analysis of astronomical components of the solar system
2. **Electronics**: analysis of microchips
3. **Environmental**: detection of toxic chemical, monitoring of nuclear facilities, analysis of petroleum products, etc.
4. **Forensics**: toxicology, trace metals, biological materials, etc.
5. **Medical**: drug abuse diagnosis, analysis of pharmaceuticals and products of genetic engineering
6. **Military**: mobile mass spectrometers are used to detect liquid chemical warfare agents

Metabolic Pathways In Higher Plants and Their Determination

Prof. Dr.R.Y.Patil
PDEAs Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre Kharadi

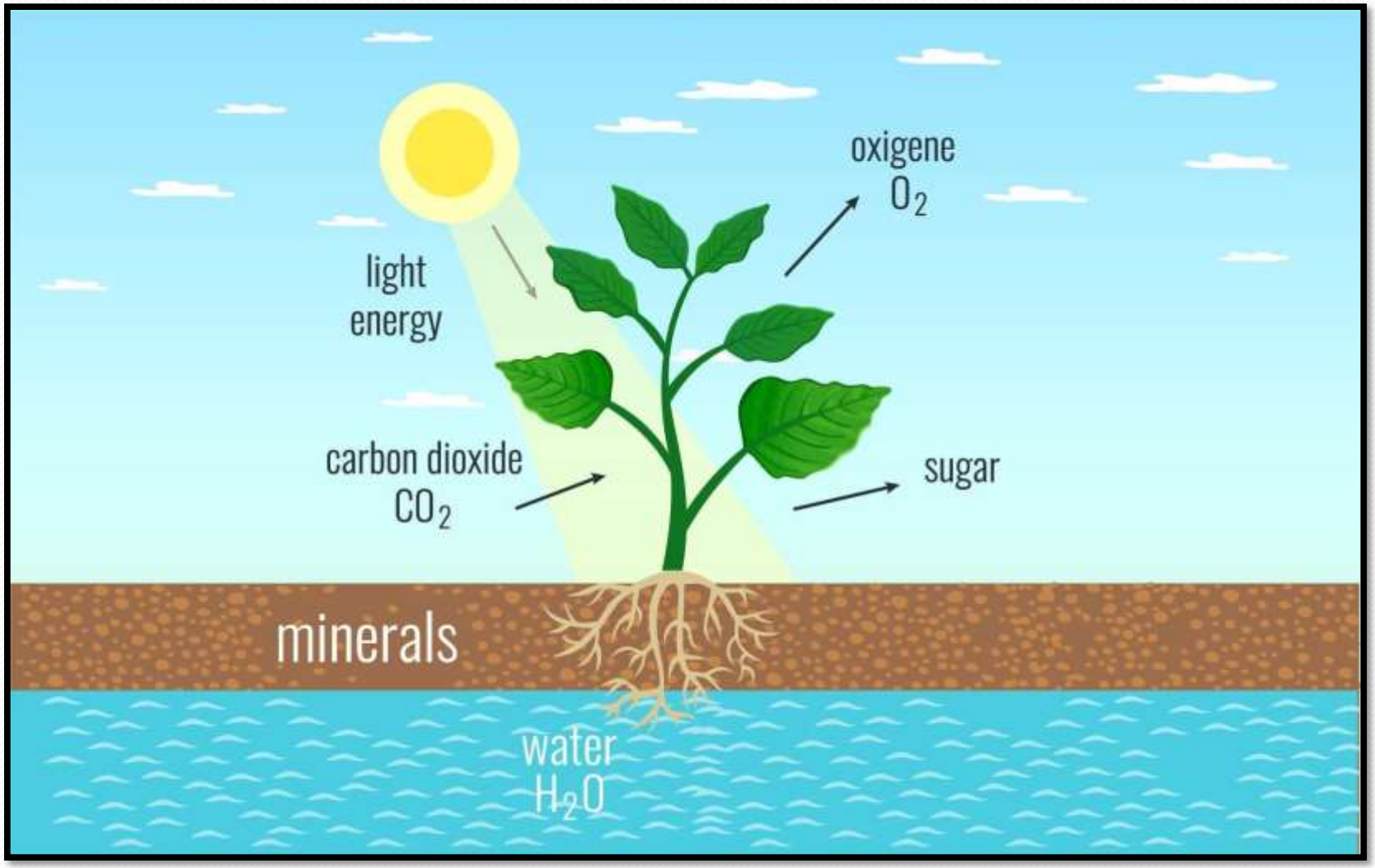
Learning outcome

After completion of this chapter student should be able to

1. Explain the meaning of primary and secondary metabolites with their examples
2. Understand the metabolic pathways in formation of secondary metabolites
3. Understand the applications of biogenetic studies

Introduction

- Plants are dependent on water and sunlight for their food and growth
- The root system absorb the water while leaves utilizes the light energy for biosynthetic reactions.
- Biosynthetic reactions produces organic compounds
- Plant metabolism is complex process involves photosynthesis, respiration and chemical events of synthesis and degradation of organic compounds.
- Photosynthesis produces the substrate for respiration and different building blocks as starting organic compounds for biosynthesis of primary and secondary metabolites.


oxigene
O_2
light
energy
carbon dioxide
CO_2
sugar
minerals
water
H_2O

Metabolic Pathways

- *Metabolism* comprises all chemical reactions that occur within living organisms.
- The processes that involve degradation of compounds correspond to *catabolism*
- Those leading to synthesis of new molecules correspond to *anabolism*
- *Metabolic pathways* refer to the sequence of enzyme catalyzed reactions that lead to the conversion of a substance into a final product.

Primary and Secondary Metabolites

- A higher plant is solar powered biochemical factory which manufactures both primary and secondary metabolites.
- Primary Metabolites- Primary metabolites are substances that are widely distributed in nature, occurring in form or another in virtually all organisms and are needed for general growth and physiological development.
- Secondary Metabolites- Secondary metabolites are biosynthetically derived from primary metabolites, but are more limited in distribution,

- Secondary metabolites may represent chemical adaptations to environmental stresses, or they may serve as defensive, protective or offensive chemicals against microorganisms, insects and higher herbivorous predators.

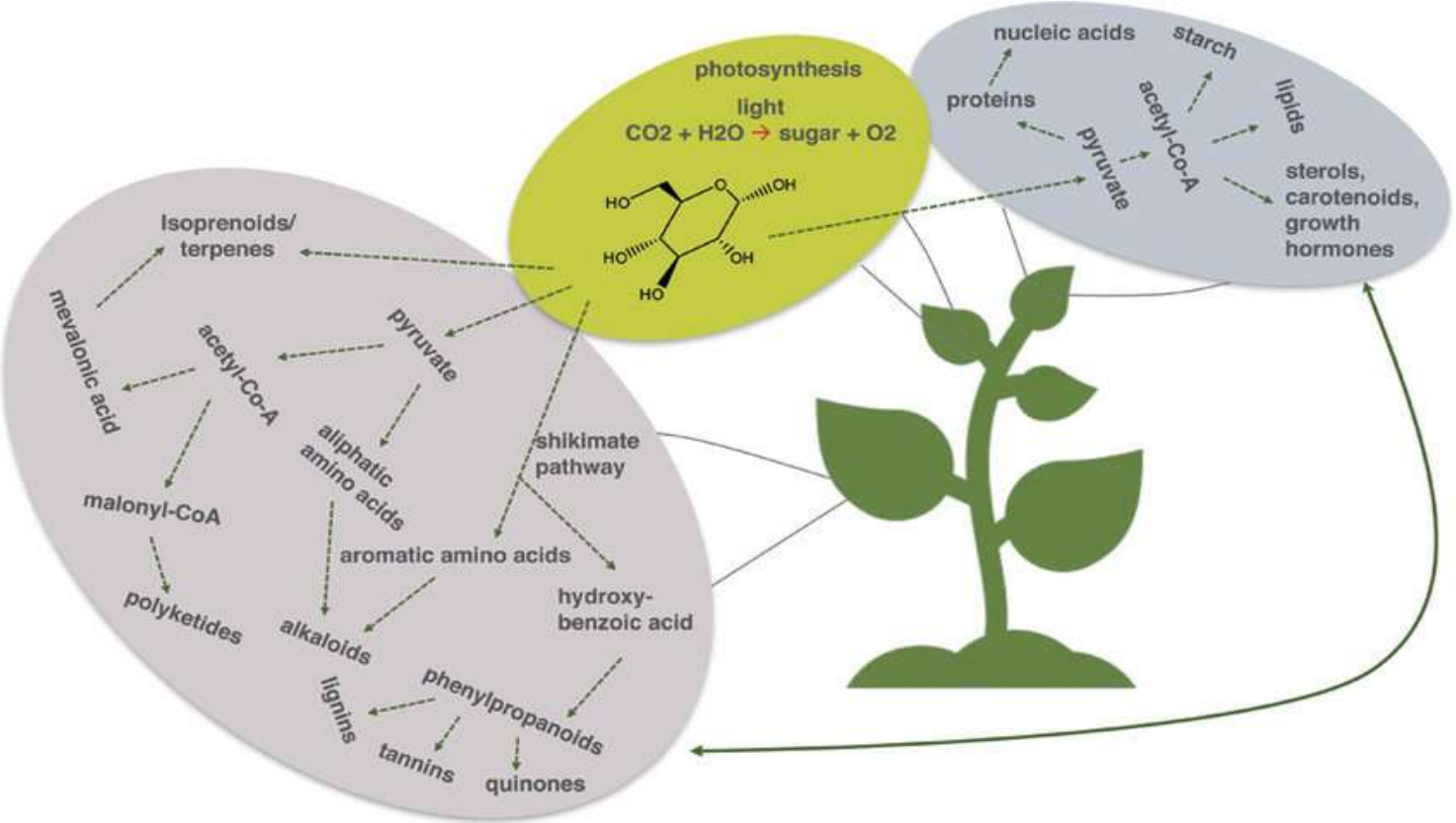
Primary Vs Secondary metabolites

Primary metabolites	Secondary metabolites
• They perform physiological functions of the body. • They play the direct role in growth and development of the organism. • They are same in all plant species. • They are produced in large amount and extraction is comparatively easy.	• They are the derivatives of the primary metabolites. • They take part in ecological functions. • They are unique to each plant species. • They are produced in small amount and extraction is comparatively tough.

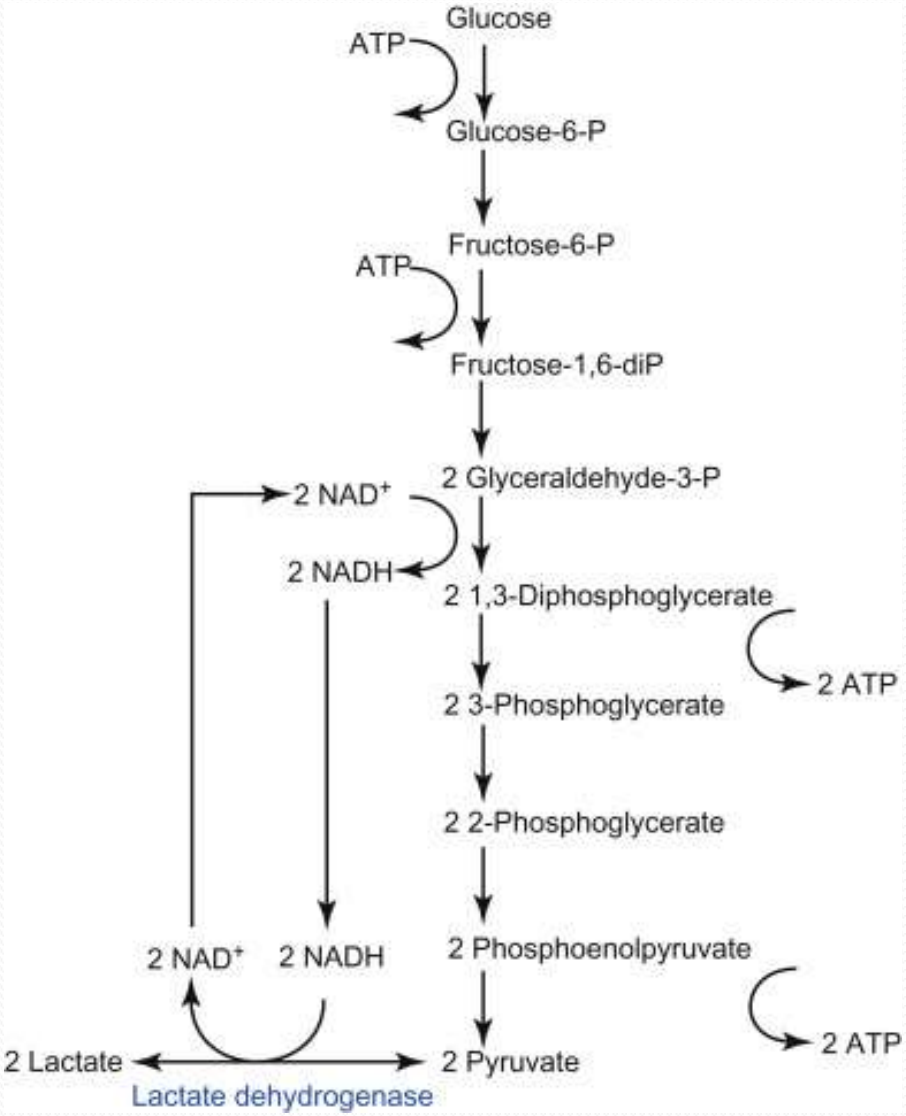
Building Blocks

- Very important in biosynthesis and production of secondary metabolites as they acts precursor for it
- These are tiny chemical molecules which are produced from primary metabolites mostly synthesized from photosynthesis, glycolysis and kreb's cycle.
- Despite of few numbers they are involved in synthesis of thousands of secondary metabolites.
- e.g. Acetyl coenzyme A (Acetyl -CoA), Shikimic acid, mevalonic acid, 1-deoxy xylulose 5-Phosphate

A simplified schematic diagram of carbon metabolism in plants.
Atmospheric carbon dioxide is fixed to glucose which is converted to sugars, starch, amino acids, fatty acids etc.
These primary metabolite products are fed into pathways to form secondary metabolites such as terpenes, tannins, lignins, alkaloids etc.



Glycolysis


Glucose
ATP
Glucose-6-P
Fructose-6-P
ATP
Fructose-1,6-diP
2 Glyceraldehyde-3-P
2 NAD+
2 NADH
2 1,3-Diphosphoglycerate
2 ATP
2 3-Phosphoglycerate
2 2-Phosphoglycerate
2 Phosphoenolpyruvate
2 NAD+
2 NADH
2 ATP
2 Lactate
2 Pyruvate
Lactate dehydrogenase

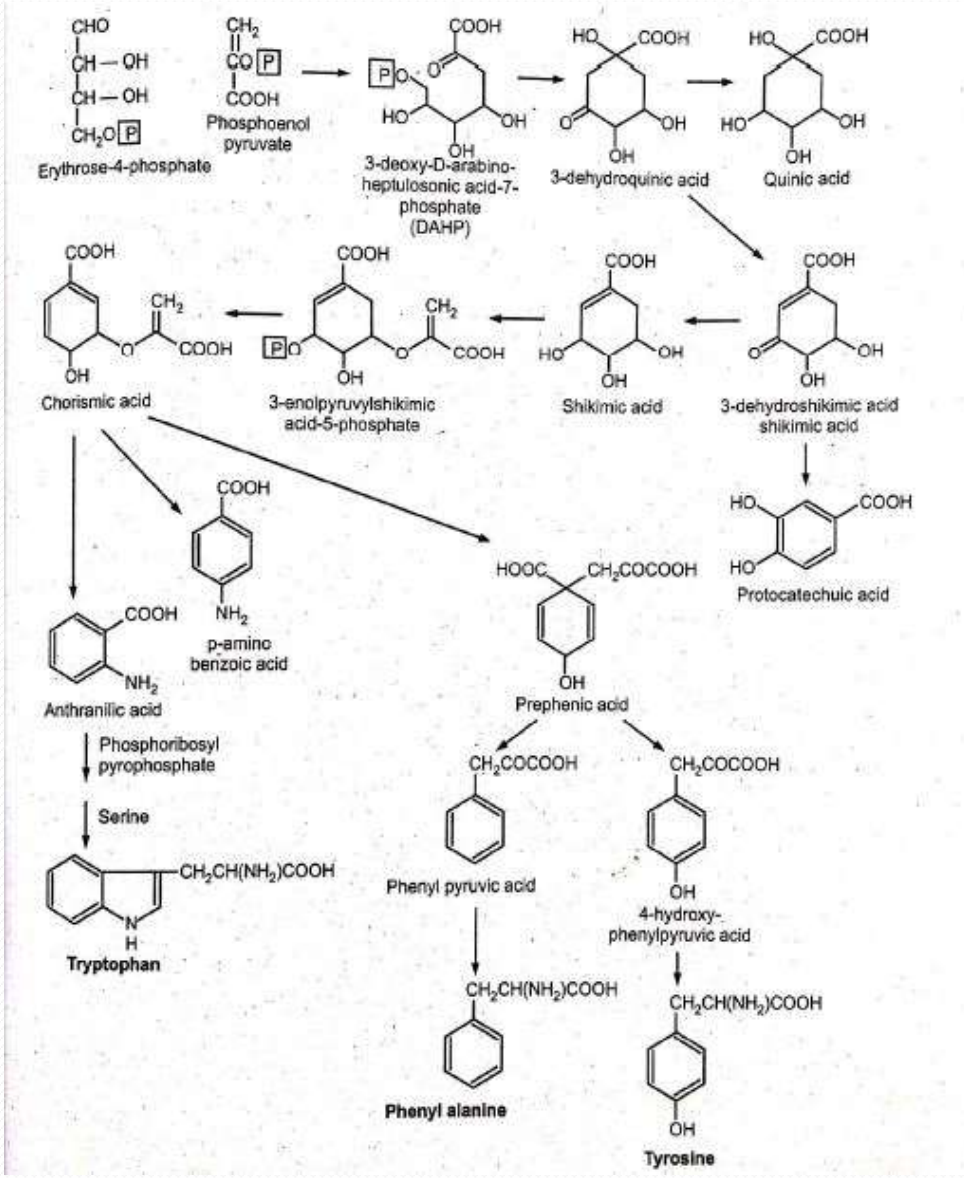
Shikimic Acid Pathway

- Plays a very significant role in the biosynthesis of various secondary metabolites in higher plants.
- Shikimic acid acts as an essential biochemical intermediate (precursor) for the synthesis of a wide variety of secondary metabolites.
- Animals are devoid of Shikimic acid, hence they can not synthesize essential amino acids such as phenylalanine, tryptophan, tyrosine etc.
- The acid exists as anionic shikimic, was first isolated from Japnaese plant Shikimi *(Illicium anisatum)*.

Shikimi plant (*Illicium anisatum)*

- Shikimic acid pathway is most popular as biosynthetic pathway for aromatic amino acid such as *l-phenylalanine, l-tyrosin, l-tryptophan.*
- Pathway begins with two building blocks from different biosynthetic pathway involves *phosphoenol pyruvate* from glycolysis and D-erythrose 4-phosphate from pentose phosphate cycle.
- As a first step these precursors combine to form 3-deoxy D-arabino heptulosonic acid-7-phosphate (DAHP), a reaction catalysed by phospho -2-oxo-3 deoxy heptonate aldolase by removal of H_2PO_4 molecule

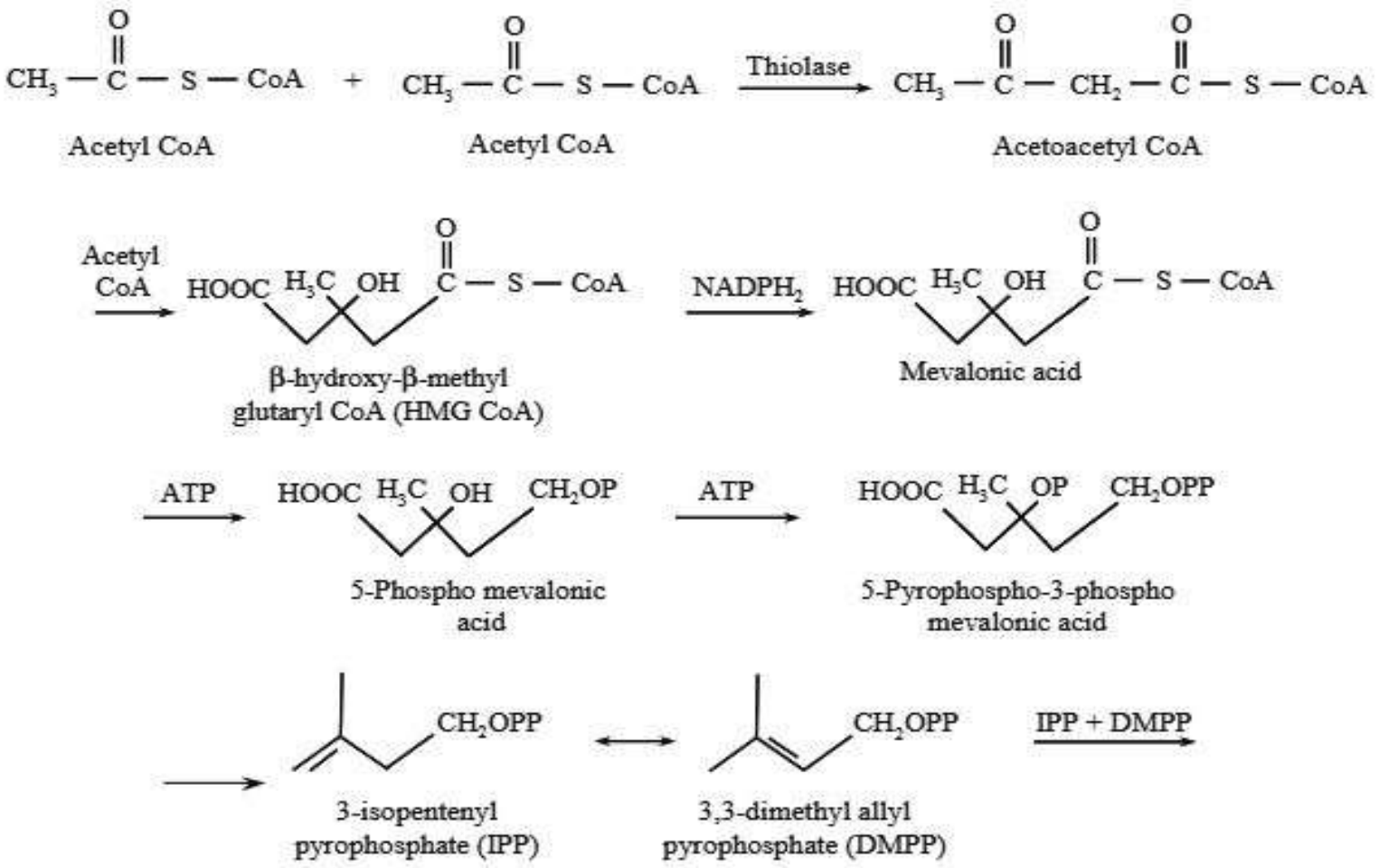
Shikimic Acid Pathway

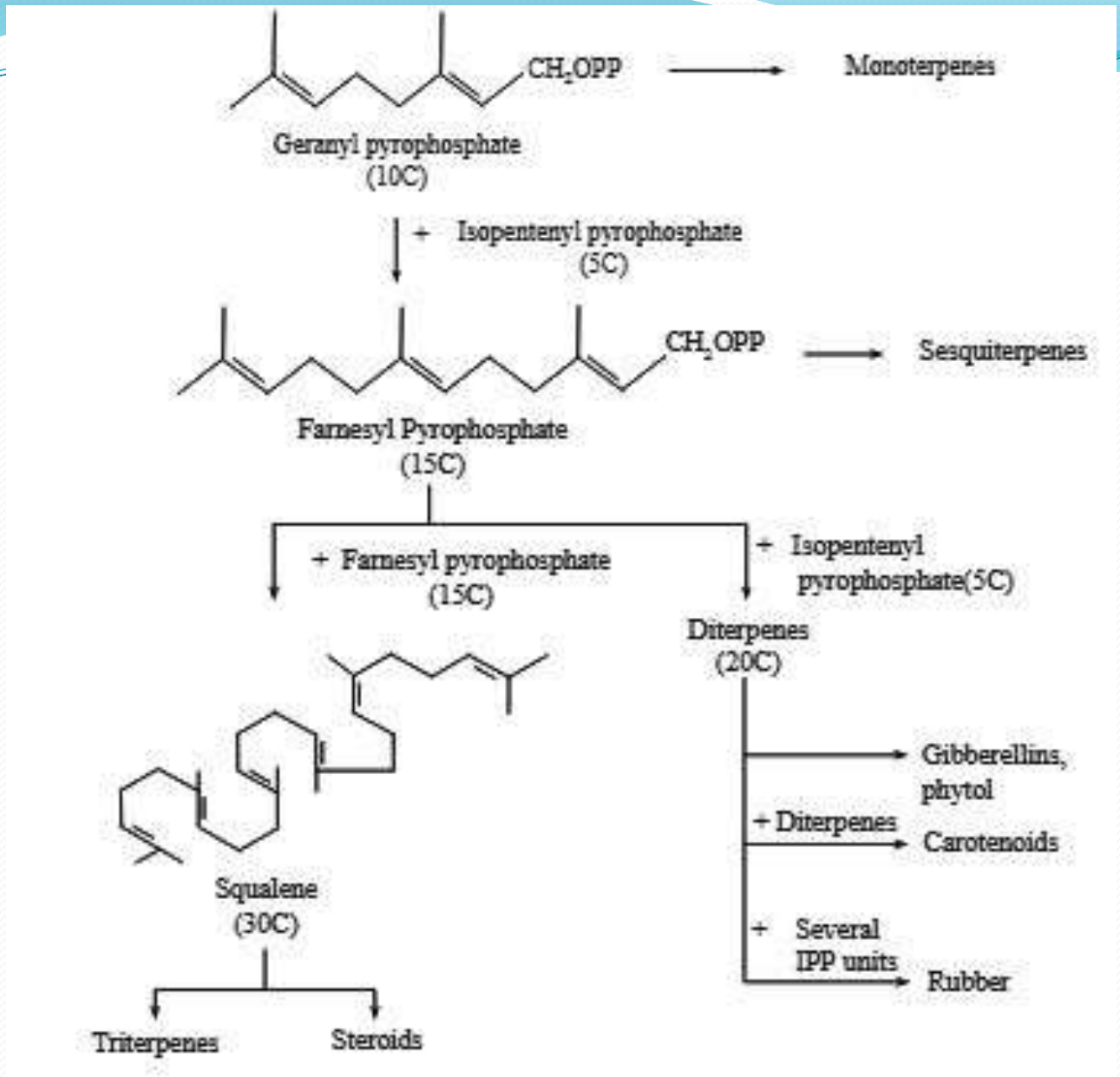


Acetate Pathway

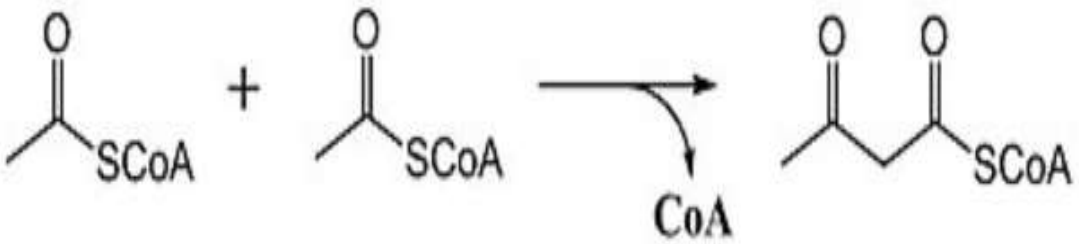
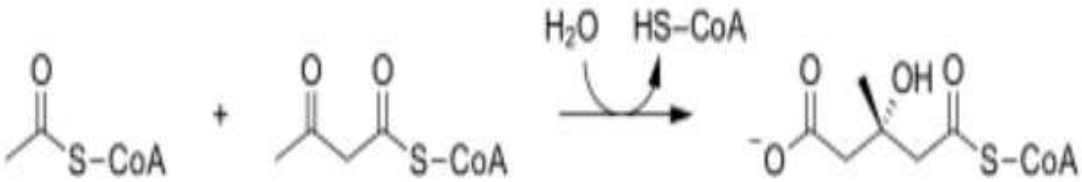
- The Mevalonate pathway was discovered in 1950's when radioactively labeled mevalonic acid was found to be incorporated in cholesterol.
- The further study confirm the involvement of Acetate as a precursor.
- Also known as Acetate- mevalonate pathway
  Mevalonic acid pathway
  HMG CoA reductase pathway
- Responsible for biosynthesis of many secondary metabolites such as isoprenoids and steroids

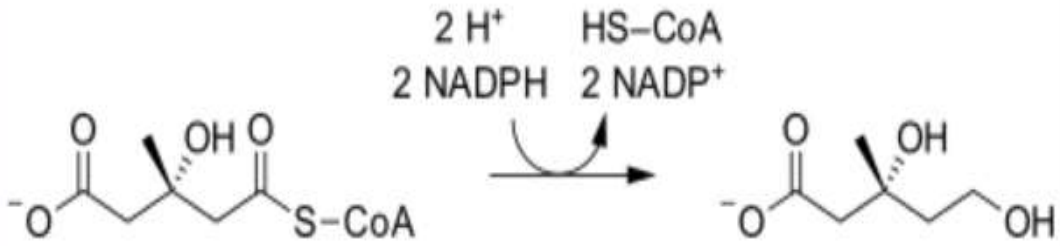
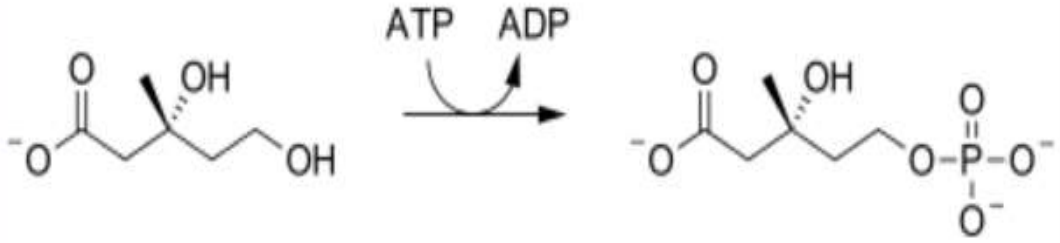
Acetate Pathway

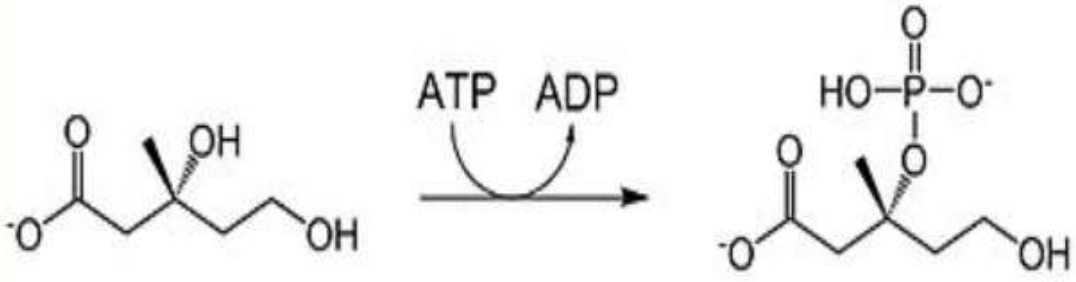
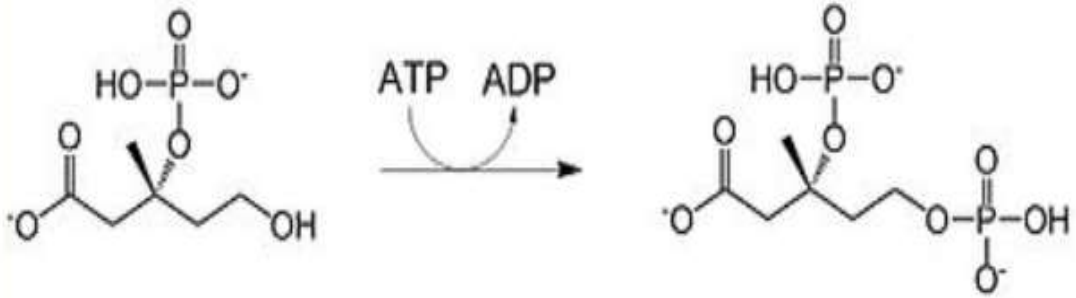


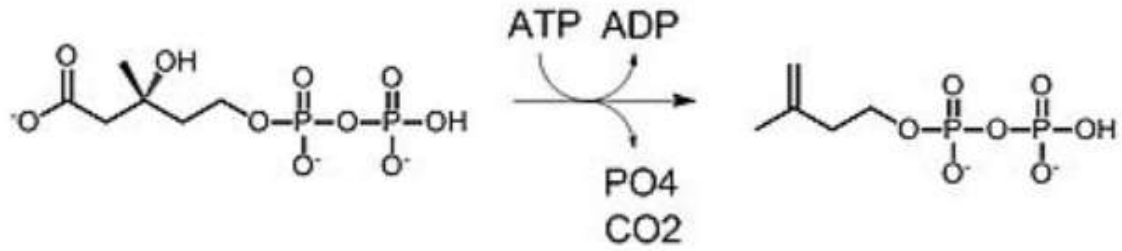
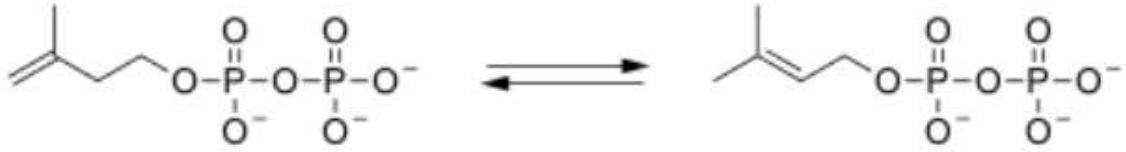

CH2OPP
Monoterpenes
Geranyl pyrophosphate
(10C)
+ Isopentenyl pyrophosphate
(5C)
CH2OPP
Sesquiterpenes
Farnesyl Pyrophosphate
(15C)
+ Farnesyl pyrophosphate
(15C)
+ Isopentenyl
pyrophosphate(5C)
Diterpenes
(20C)
Gibberellins,
phytol
+ Diterpenes
Carotenoids
+ Several
IPP units
Rubber
Squalene
(30C)
Triterpenes
Steroids

Enzymatic reactions [edit]

Enzyme	Reaction	Description
Acetoacetyl-CoA thiolase		Acetyl-CoA (citric acid cycle) undergoes condensation with another acetyl-CoA molecule to form acetoacetyl-CoA
HMG-CoA synthase		Acetoacetyl-CoA condenses with another Acetyl-CoA molecule to form 3-**h**ydroxy-3-**m**ethyl**g**lutaryl-CoA (HMG-CoA).

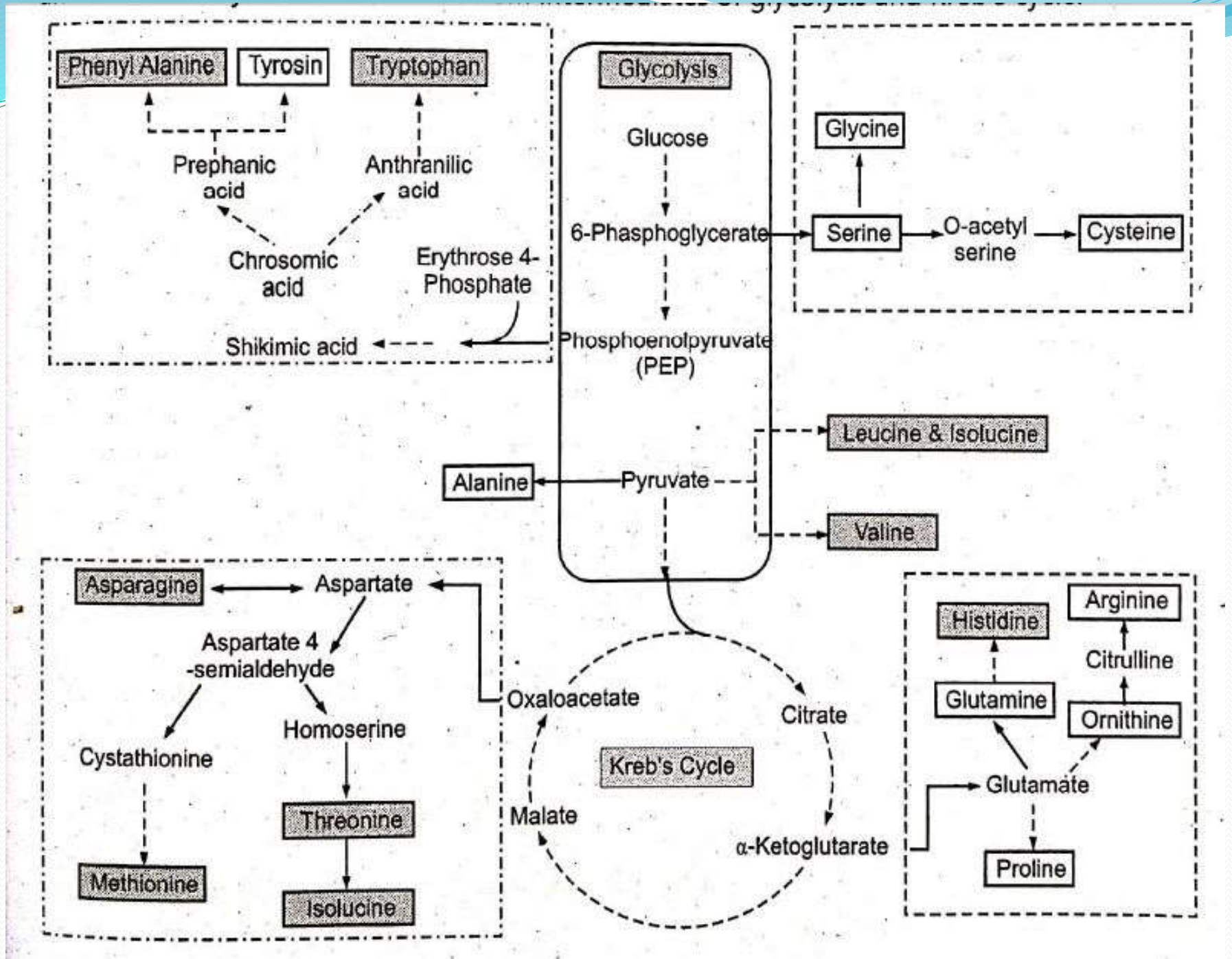
HMG-CoA reductase	 Nicotinamide adenine dinucleotide phosphate oxidase (NADPH)	HMG-CoA is reduced to mevalonate by NADPH. This is the rate limiting step in cholesterol synthesis, which is why this enzyme is a good target for pharmaceuticals (statins).
mevalonate-5-kinase		Mevalonate is phosphorylated at the 5-OH position to yield mevalonate-5-phosphate (also called *phosphomevalonic acid*).

mevalonate-3-kinase		Mevalonate is phosphorylated at the 3-OH position to yield mevalonate-3-phosphate. 1 ATP is consumed.
mevalonate-3-phosphate-5-kinase		Mevalonate-3-phosphate is phosphorylated at the 5-OH position to yield mevalonate-5-phosphate (also called *phosphomevalonic acid*). 1 ATP is consumed.

phosphomevalonate kinase		mevalonate-5-phosphate is phosphorylated to yield mevalonate-5-pyrophosphate. 1 ATP is consumed.
mevalonate-5-pyrophosphate decarboxylase		Mevalonate-5-pyrophosphate is decarboxylated to yield isopentenyl pyrophosphate (IPP). 1 ATP is consumed.
isopentenyl pyrophosphate isomerase		isopentenyl pyrophosphate is isomerized to dimethylallyl pyrophosphate.

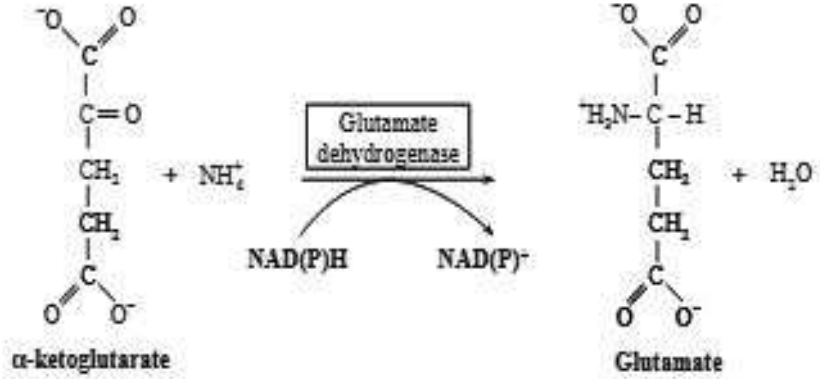
Amino acid Pathway

- Plants and microorganisms have the ability to synthesize all amino acids necessary for protein synthesis.
- Human beings can not generate half number of amino acids, therefore they are categorized as essential and remaining as non essential.
- The essential amino acid need to be included in our daily diet in order to lead healthy life.
- The absence of even single amino acid results in synthesis of wrong protein.

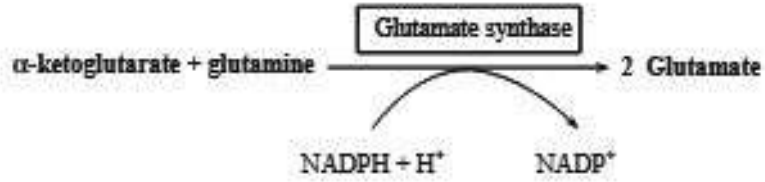

Phenyl Alanine
Tyrosin
Tryptophan
Prephanic acid
Anthranilic acid
Chrosomic acid
Erythrose 4-Phosphate
Shikimic acid
Glycolysis
Glucose
6-Phasphoglycerate
Phosphoenolpyruvate (PEP)
Glycine
Serine
O-acetyl serine
Cysteine
Leucine & Isolucine
Alanine
Pyruvate
Valine
Asparagine
Aspartate
Aspartate 4 -semialdehyde
Homoserine
Cystathionine
Threonine
Methionine
Isolucine
Oxaloacetate
Citrate
Kreb's Cycle
Malate
α-Ketoglutarate
Histidine
Arginine
Citrulline
Glutamine
Ornithine
Glutamate
Proline

1. **Biosynthesis of Glutamate**

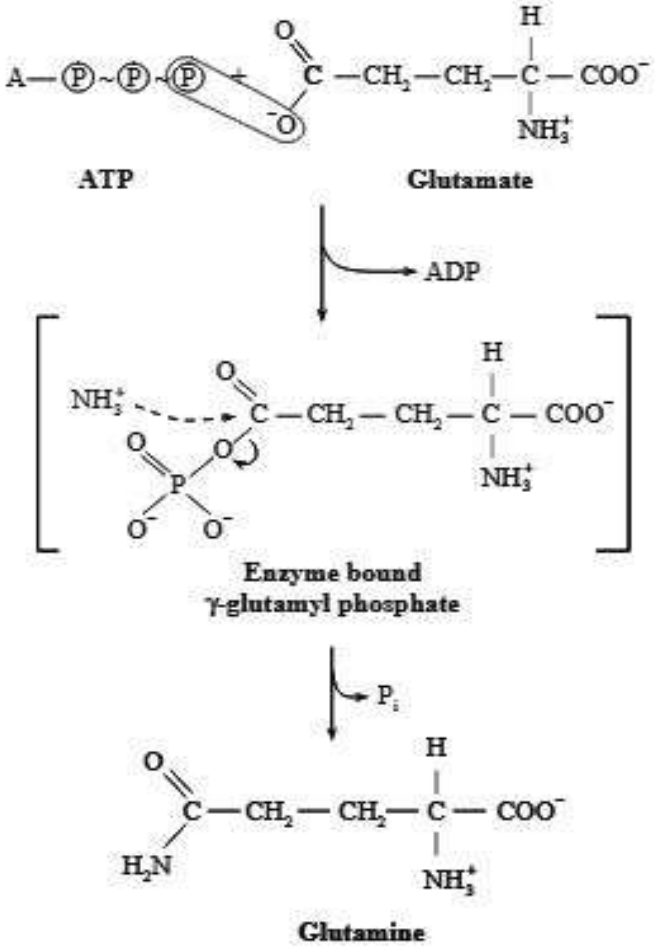
α-ketoglutarate and NH_4^+, a Krebs cycle intermediate, undergo reductive amination in a reaction catalyzed by glutamate dehydrogenase to synthesize glutamate. (Recall that glutamate dehydrogenase is present in the matrix of mitochondria in eukaryotic cells)



In *E.coli*, the synthesis of glutamate is slightly different. The δ-amino group of glutamine is incorporated into α-ketoglutarate in a reaction catalyzed by NADPH dependent glutamate synthase resulting in the formation of 2 molecules of glutamate.



Glutamine is synthesized by the incorporation of ammonia into glutamate in a reaction catalyzed by the enzyme ATP-dependent glutamine synthetase. The γ-phosphoryl group of ATP and γ-carboxyl group of glutamate interact with each other to form an energy rich enzyme bound intermediate i.e., γ-glutamyl phosphate. In the subsequent step, the phosphate group of γ-glutamyl phosphate is replaced by ammonia to form glutamine as shown below.



Investigation of Biosynthetic Pathway

- Different techniques used for the investigation of biosynthetic pathway of primary and secondary metabolites are

1. Tracer technique
2. Use of isolated organ and tissues
3. Grafting method
4. Use of Mutant strains
5. Enzymatic studies

Study of utilization of Radioactive isotopes in the investigation of Biogenetic studies

- Plants are live biosynthetic laboratory, where multitude of biosynthetic reactions or pathways takes place at single time.
- This leads to production of simple to chemically complex diverse compounds.
- It is not a simple process, it involves genetically defined enzymes, cofactors.
- The intermediates of biosynthetic pathway may be precursor for more than one phytomolecules.

- Thus, study of biosynthetic pathways are complicated and not easy task.
- When it was realized that elements existed with identical chemical properties but with different atomic weights, the possibility of its utilization for the study of biosynthetic pathways has been increased.
- The elements with different isotopes found as stable isotope (^{2}H, ^{13}C, ^{15}N,^{18}O) and unstable isotopes (^{1}H, ^{14}C) which decay with emission.
- The use of such unstable radioisotopes for the study of biosynthetic pathway are known as radiotracers, Radioactive tracers or radioactive label.

Tracer technique

- Tracer techniques utilizes radioactive isotope labelled compound to find out or to trace the different intermediates and various steps in biosynthetic pathways in plants, at a given rate & time.
- When the labelled compounds are administered to the plants, they become a part of metabolic pool (Collective term for all the substances involved in the metabolic process in a biological system) and undergo reaction characteristics.

- Isotopes-

✓ two or more forms of chemical elements which have the same number of proton but different number of neutron.

✓ they have different physical properties but same chemical properties.

Tracer technique can be defined as technique which utilizes a labelled (radioactive) compound to find out or to trace the different intermediates and various steps in biosynthetic pathways in plants.

Significance of tracer techniques-

- Applicable for living systems. Wide ranges of isotopes are available.
- High sensitivity
- More effective
- Simple administration and isolation.
- Shows accurate results when enough metabolic time & technique is used.

Radioactive isotopes: The array of radioactive isotopes preferred for specific biosynthetic pathway studies such as ^{1}H, ^{14}C, ^{24}Na, ^{42}K, ^{35}S, ^{35}P, ^{131}I amongst them carbons and hydrogens are widely used for biological investigations, similarly for metabolic studies, the sulphur, phosphate, and alkali and alkaline earth metals are preferred. The labelled nitrogen due to their specific distribution used for studies on protein, alkaloids, and amino acid.

[e.g. ^{1}H, ^{14}C, ^{24}Na, ^{42}K, ^{35}S, ^{35}P, ^{131}I decay with emission of radiation]

The carbon with mass number 10, 11 and 14 are, among them ^{10}C has a half-life of 8.8 s and ^{11}C has half-life of 20 min., which limits their usefulness in biological research. While, ^{14}C with more than 5000 years half-life preferred for biological investigation studies. Tritium (^{3}H) is preferred hydrogen radioactive isotope with half-life 12.43 years. Tritium (^{3}H) labelling is effected by catalytic exchange (platinum catalyst) in aqueous media, by irradiation of organic compounds with tritium gas and by hydrogenation of unsaturated compounds with tritium gas. Tritium is a pure β-emitter of low toxicity.

Consideration for Tracer technique

- The physical and chemical nature of presumed precursor of targeted biochemical pathway should be known for radiolabelling.
- The assumed labelled compound should involve in the biosynthetic reaction
- The concentration of radio labelled compounds incorporated in biological system should be sufficient to reach in targeted biogenetic pathway
- The radio labelled compound should not damage the system for which it is used.

Steps in Tracer techniques

1. Preparation of labelled compounds

The radioactive carbon and hydrogen are incorporated into presume precursor for investigation of biogenetic pathway. The radioactive ^{14}C can be produced synthetically and naturally.

The labelled ^{14}C produced by the bombardment of ^{14}N with slow neutrons, on the target material usually being aluminium or beryllium nitride in nuclear reactor. The purity of ^{14}C used for investigation of biosynthetic reaction is of great importance. The radio labeled carbon produced by these method may contaminate with inorganic radioactive carbon compounds. Small proportion of impurity during investigation of biosynthesis reaction may hamper the results of the experiments.

In another technique of producing the ^{14}C labelled compounds, algae *Chlorella* grows in an atmosphere containing $^{14}CO_2$. As a result, all compounds possessing a uniform labelling of its carbon atoms. This method is suitable for preparing natural compounds such as certain amino acids (obtained by hydrolysis of proteins).

Tritium (^3H) labelling is effected by catalytic exchange (platinum catalyst) in aqueous media, by irradiation of organic compounds with tritium gas and by hydrogenation of unsaturated compounds with tritium gas.

2. Introduction of labelled/ radioactive compound

Before selection of method for introduction following points are required to be considered

(a) The presumed precursor should introduce at specific site so that it takes parts in the biosynthetic reaction.

(b) The time of the introduction of labelled compounds should be correct so that the presumed labeled precursor should be available during the biosynthesis of the compound under investigation.

(c) The dose should be as minimum as possible, to avoid the involvement of labelled compound in other biosynthetic reaction.

Methods for introduction of Radioactive compounds

Root Feeding: This method is preferred in the plant in which roots are the biosynthetic sites for production of targeted compounds. Such plants are hydroponically cultivated to avoid microbial contamination along with labelled compound in it e.g., Tobacco alkaloid, Datura alkaloid.

Stem feeding: In this method, the stems of sufficient size are cut and cut ends immersed in water with necessary nutrients along with labelled compounds. This method is not suitable for plants of which stem ooze out the latex at cut ends.

Direct injection: It most easy method for introduction of labelled compounds, it is possible for those plants which bears the hollow stem e.g., umbelliferous and opium.

Infiltration: Suitable for plant rooted in soil or other support. The infiltration method enable to introduce the labelled compounds without disturbing the roots (Wick feeding).

Floating method: This technique is preferred when small amount of material is available. This technique is used in conjugation with vacuum infiltration to remove gases.

Spray technique: The radiolabelled compound mixed in water, spread over the leaves. The compound get absorbed in the leaves, e.g., Steroids.

3. Separation and determination of labelled compound in various biochemical fractions

Different methods of extraction are used depending on nature of drug and its source.

- Soft tissue (Fresh)- Infusion, Maceration
- Hard tissue- Decoction and hot percolation
- Unorganized drug- Maceration with solvent
- Fat and oil- Non-polar solvent
- Alkaloids, Glycosides, Flavonoids- Slightly polar solvent
- Plant phenol- Polar solvent

Detection and assay of radioactivity labelled compound

When radioactive tracers are used in biogenetic studies, adequate methods for the detection and estimation of the label are essential.

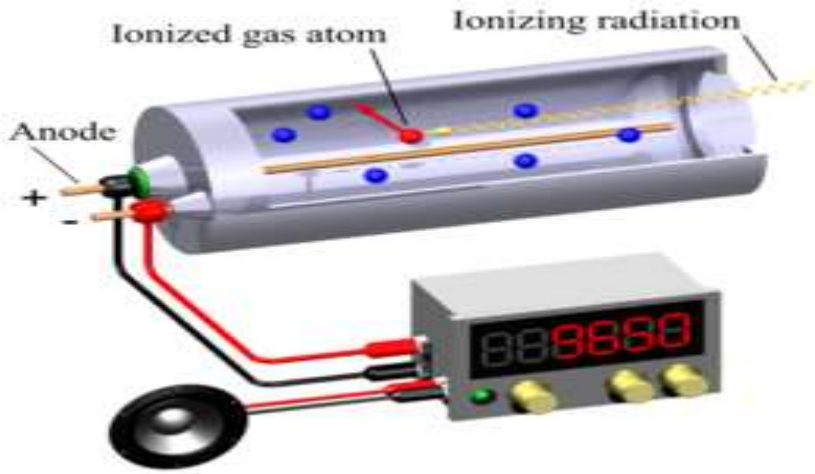
- For soft and easily absorbed radiation from ^{3}H, ^{14}C labelled compounds- Liquid scintillation counter.
- Modern instruments are used for mixed radiation like ^{3}H and ^{14}C. This is possible because both are β-emitters and different radiation energy.
- With all counters, the instrument is connected to a suitable rate meter which records the count over a given time.
- Different instruments are used to determine nature of metabolites. e.g- GM Counter, Scintillation or liquid scintillation counter and ionization chamber.

Different types of detectors are used

- Geiger-muller counter
- Liquid scintillation counter
- Gas ionization chamber
- Mass spectroscopy
- NMR electrodemeter
- Autoradiography

Geiger-muller counter

- Used for detection and measurement of all types of radiation i.e. alpha, beta, gamma
- Composed of pair of electrodes surrounded by gas usually helium or argon
- The electrodes have high voltage across them.



- When radiation enters the tube, it ionizes the gas , the ions (and electrons) get attracts towards the electrode and an electric current is produced
- A scaler counts the current pulses and obtains a ‘count’ whenever radiation ionizes the gas.
- Counts individual particle at rates upto 10,000/sec

Advantages

relatively inexpensive

Durable

easily portable

detects all types of radiation

Disadvantages

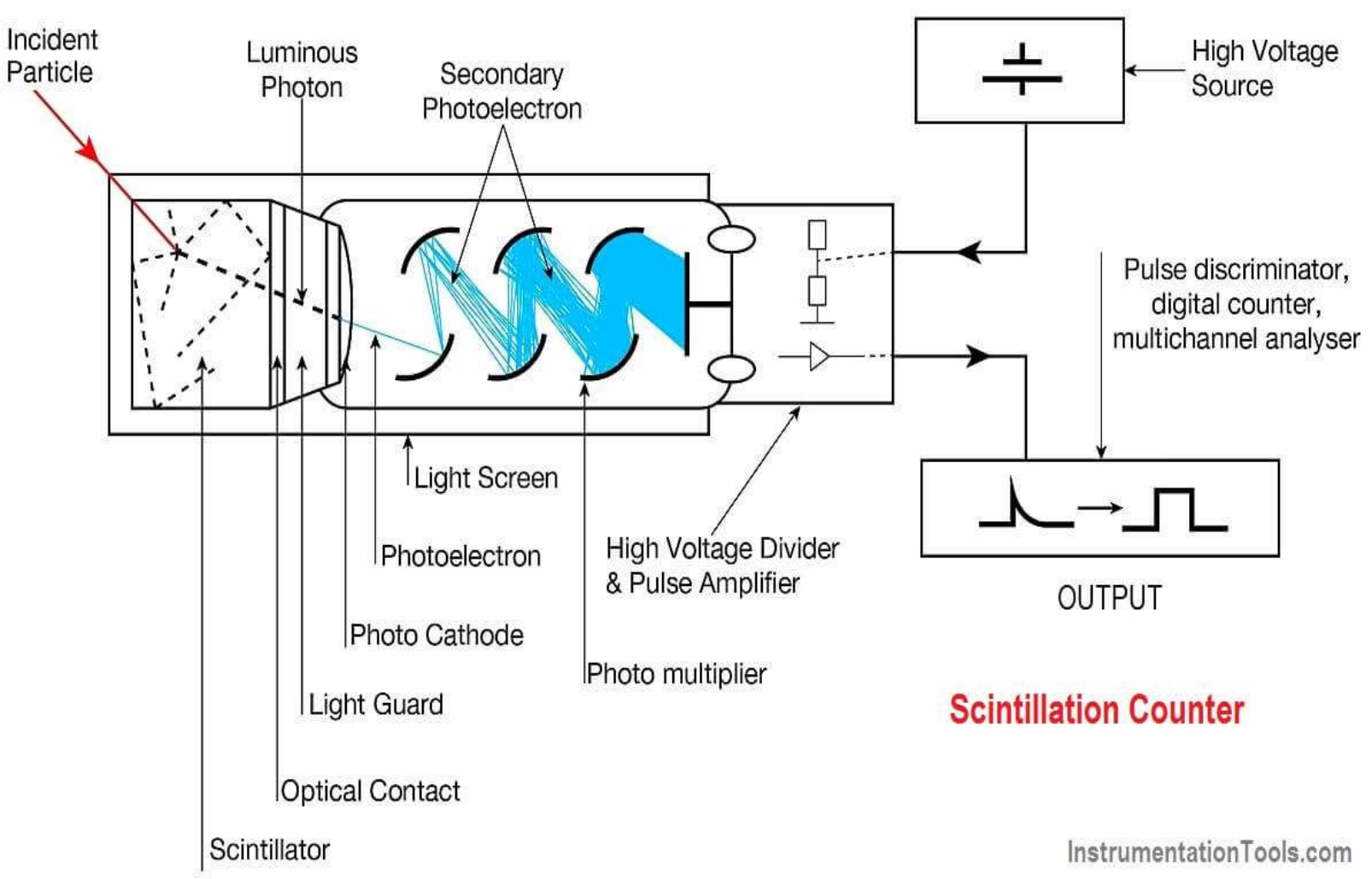
cannot differentiate type of radiation

Less sensitive

Scintillation Counter

- A scintillation detector or scintillation counter is formed when the scintillation detector is coupled to an electronic light sensor such as a photomultiplier tube (PMT) or a photodiode.
- A scintillator is a material that exhibits scintillation- a luminescence property that is stimulated by ionizing radiation.
- Samples shall be dissolved or suspended in a "cocktail" containing a solvent (aromatic organics such as benzene or toluene), typically some form of a surfactant, and small amounts of scintillators.

- The conversion of kinetic energy of a particle into a fleeting pulse of light as a result of its penetrating a suitable luminescent substance.
- Rutherford successfully used this method.
- The flashes of light produced bombardment with alpha particles on fluorescent screen prepared from zinc sulphide.


Incident Particle
Luminous Photon
Secondary Photoelectron
High Voltage Source
Pulse discriminator, digital counter, multichannel analyser
Light Screen
Photoelectron
High Voltage Divider & Pulse Amplifier
OUTPUT
Photo Cathode
Photo multiplier
Light Guard
Optical Contact
Scintillator
Scintillation Counter
InstrumentationTools.com

Gas ionization chamber

- The ionization chamber is the simplest of all gas-filled radiation detectors and is commonly used for ionizing radiation, including x-rays, gamma rays and beta particles.
- Conventionally, the term "ionization chamber" is used solely to describe those detectors that collect all the charges caused by direct ionization of the gas using an electrical field

- Mass Spectrophotometer-
- Mass spectrometry (MS) is an analytical technique used to measure the mass-to-charge ratio of charged particles.

  It is used to determine the mass of the particles, to determine the elemental composition of the sample or molecule, and to elucidate the chemical structures of the molecules, such as peptides and other chemical compounds.

- NMR Spectrophotometer-
- NMR spectroscopy is a research technique that exploits the magnetic properties of certain atomic nuclei to determine the physical and chemical properties of the atoms or molecules they contain.
- It relies on the phenomenon of nuclear magnetic resonance and can provide detailed information on the structure, dynamics, reaction status and chemical environment of the molecules.

Autoradiography

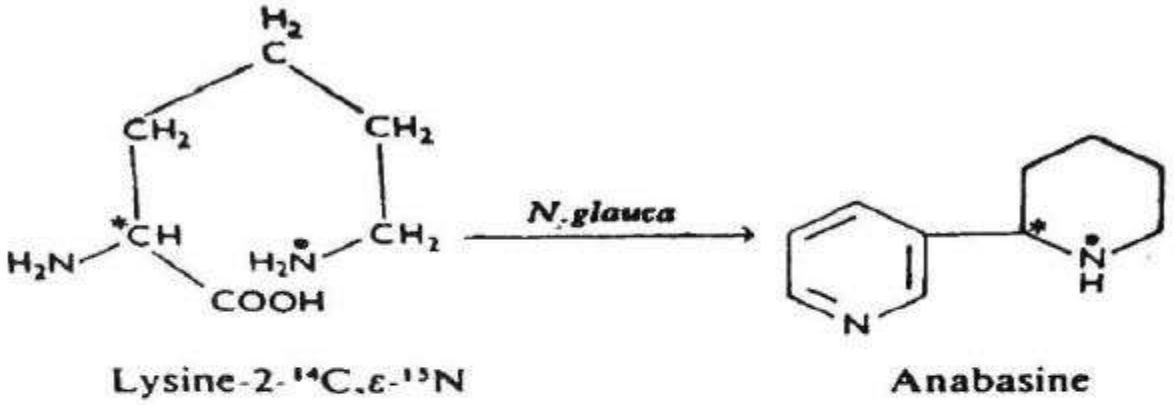
- It is a tool for examining the distribution of radioactive material in a plant object, e.g. histological tissue, chromatography sheet.
- This method uses a photographic film or emulsion as an ionizing radiation detector.
- The specimen is in close contact with the emulsion for a period (exposure duration).
- In this technique, a sample containing a radiolabelled metabolite is placed in direct contact with suitable photosensitive material such as x-ray (photographic) film for a specific period.
- The pattern of delivery of radioactive substances can be elucidated with the aid of the autograph collected.

Methods of Tracer Technique

1. **Precursor Product sequence-**

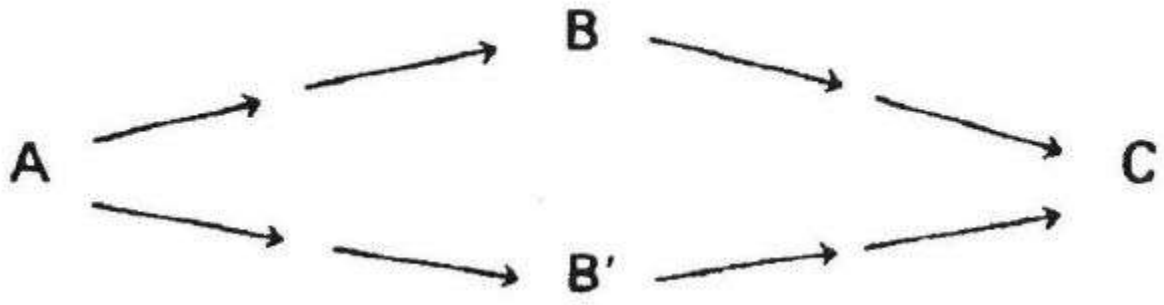
- ✓ Commonly employed method of investigation of biogenetic pathway
- ✓ The properly labelled precursor of the constituent under investigation on a labelled form is fed into the plant and after a suitable time the constituent is isolated, purified and radioactivity is determined.
- ✓ Radioactivity of the isolated compound is not sufficient evidence that the particular compound fed is a direct precursor, because the compound may enter the general metabolic pathways and distributed randomly through a whole range of products.

- Further evidence can be made by double and triple labelling experiments by using either different isotopes of specific labelling by one isotope at two or more positions in the molecule.
- In the double labelled lysine for the investigation of biogeniesis of anabesine confirms which nitrogen of lysine molecule involved in formation of piperidine ring of anabasine in *Nicotiana glauca*


N.glauca
Lysine-2-¹⁴C,ε-¹⁵N
Anabasine
N.glauca
Lysine-2-¹⁴C,α-¹⁵N
Anabasine

Competitive Feeding

- It is another tracer technique, in which the labelled compounds used to determine precursor and exact intermediates.
- In plants, biosynthesis of phytochemical involved the vast possibilities for their precursor and intermediates, it is always unpredictable and confusing to identify them.
- This technique has great value in confirming which of possible intermediate is normally used amongst two possible by the plant


A
B
B′
C

Sequential Analysis

- Plant is grown in atmosphere of $^{14}CO_2$ and plant parts are subjected for analysis at given interval to obtain the sequence in which various related compounds becomes labelled.
- Most of the biosynthetic pathways are long sequence of related compounds.
- From the results obtained certain biosynthetic routes confirmed and others rejected.
- This method has been very successfully used in the elucidation of the path of carbon in photosynthesis.

Investigation of biosynthetic Pathways

- Different techniques used for the investigation of biosynthetic pathway of primary and secondary metabolites are

1. Tracer technique
2. Use of isolated organ and tissues
3. Grafting method
4. Use of Mutant strains

Overview

Natural excipients

* Able to know After completion of this chapter Natural excipients...

1. Definition.
2. Functions.
3. Ideal properties.
4. Types of natural excipients.
5. Natural binders & its examples.

Types of excipients

- Natural colorants
- Natural sweeteners
- **Natural binders**
- Natural diluents
- Viscosity builders
- Disintegrating agents
- Natural perfumes & flavoring agents

PHARMACEUTICAL EXCIPIENTS..

• Accordingto International P**harmaceutical Excipients Council:-**

"substance other than the active drug substance which has been appropriately evaluated for safety & are included in a drug delivery system to either aid the processing of the drug delivery system during its manufacture, protect, support or enhance stability, bioavailability, assist in product identification, or enhance any other attributes of the overall safety & effectiveness of the drug delivery system during storage or use."

• Pharmaceutical excipients are inactive substances added to medicinally active compounds to formulate dosage form.

FUNCTION OF EXCIPIENTS :

- Add bulk to the formulation
- During manufacturing, helps to handle API.
- Assist in drug administration
- Enhance patient compliance
- Enhance drug solubility & bioavailability.
- Avoid drug degradation
- Modify the pH & osmolarity of liquid dosage form.
- Helps in particle dispersion
- Helps to mask unpleasant taste, odor, color.
- Helps to maintain stability.

IDEAL PROPERTIES OF EXCIPIENTS

- • They should be used practically.
- • They should be non toxic & nonirritant
- • Should be non volatile in nature.

- • Should not be affected by temperature, light & hydrolysis.
- • Should be easily available & cheap.
- • Should not have specific color, odor & taste.

- • Should possess good water & lipid solubility.
- • Should be compatible with active ingredient in the Preparation
- • Should be pharmacologically inert.

Natural excipients --

NATURAL BINDERS

• Binders are excipient which binds all ingredients used in formulation to form suitable dosage form.

Binders

Solution binder

eg. Gelatin, cellulose

Dry binders

eg. MC, PEG.

• Natural binders are less toxic, can be degraded naturally, economic, available easily & abundantly & enhance stability & texture to dosage form.

◆ Natural binders

1. ACACIA

- Synonym: Indian gum, Babul.
- B/S: it is dried gummy exudation from the stems & branches of Acacia arabica, A. senegal, Fam- Leguminoseae.

- **Description**: it occurs as yellowish white or white flakes, spherical spheres, powder, granules, or spray dried powder. .

C/C: Arabinose, galactose, rhamnose, & glycuronic acid.

- **Chemical test**: When acacia powder is treated with lead sub acetate, it gelatinizes the aqueous solution.

Use: As a natural binder for tablets, suspending agent, emulsifying agent, thickener. As base in pastilles & lozenges.

◆ Natural binders

2.TRAGACANTH

• **Synonym**: Gum tragacanth, Persian tragacanth•

B/S: dried gum obtained from plant Astragalus gummifer, Fam- Fabaceae.

• **G/S:** Iraq, Iran, Syria, Turkey, Afghanistan, Pakistan & Russia.

• **Description**: Tragacanth has flat, lamellated, curved fragments, or straight or spirally twisted linear pieces. It is white to yellowish in colour, translucent, odourless with mucilagenous taste.

2.TRAGACANTH

- C/C: water soluble
& water insoluble
polysacharides. Bassorin, cellulose, starch, protein & ash

- **Chemical test:** when strong iodine solution is added to tragacanth, it developes olive green colour.

- **Use:** used to make various
pharmaceutical formulations like creams, gels, & emulsions.

3. STARCH

Synonym: Amido, Amidon, Amylum

- **B/S**: it is polysaccharide obtained from grains like corn (Zea mays) Fam- Gramineae, potato (Solanum tuberosum) Fam-Solanaveae, wheat (Triticum aestivum) & rice (Oryza sativa), fam- Poaceae.

- **Description**: odourless, tasteless, fine white coloured powder. Practically insoluble in cold ethanol & in cold water.

- **C/C**: Amylose & amylopectin & a-glucose.

STARCH

- **Chemical test:** starch solution is heated with HCl & then neutralized by NaOH, the solution is heated with Fehling's solution, brick red ppt is produced.

- **Use:** it is a binder of choice in wet granulation & tablet formulation. Has wide applications in nasal, oral, periodontal novel drug delivery system & to get site specific drug delivery.

NATURAL BINDERS..

4.GELATIN

• **Synonym**: Byco, Cryogel, Instagel, Solugel

• **B/S:** it is a protein obtained by partial acid or alkaline hydrolysis of animal collagen tissues like bone, skin, tendon & ligaments. • Description: faintly yellow amber coloured vitreous, brittle solid. Odourless & tasteless. Practically insoluble in water, most of organic solvents, fixed oils & volatile oils. soluble in hot water, glycerol, & acetic acid.

• **C/C:** Glycine (25-27%), alanine, glutamic acid, proline, arginine, aspartic acid, hydroxy proline, isoleucine, leucine, phenylalanine & tryptophn.

4.GELATIN

- **Chemical test:** gelatin powder, when heated with soda lime, evolves
ammonia gas.

- **Use**: used in implantable delivery systems, mainly hard & soft gelatin capsule. In formulation of suppositories, pastilles, pessaries, pastes. Absorbable gelatin sponge acts as haemostatic agent.also used in food industry & in photographic emulsion.

Natural Colorants:

Natural colourants are highly coloured compound capable to impart colour to fabric paper, leather, drugs, cosmetics and plastic.

Dye is compound which gets absorbed by the material and gives permanent colour.

While pigment is coloured compound only applied to the surface.

Natural colourants should have intense colour, should be highly water soluble. it should possess reactivity and substantivity. The colourant should have fastness property.

The colourant have chromophore and auxochrome properties. Chromophore imparts colour to compound while auxochrome makes the dye water soluble and helps to bind it to substrate. The main function of colouring agent is to impart attractive colours to dosage forms, with patient compliance and for easy identification of dosage forms.

Advantages

1. *Natural colourants are soft, shining and soothing.*
2. *Wide ranges of colours are available. Natural dyes are usually renewable so no disposal problem.*
3. *Ecofriendly and stable.*
4. *Non-hazardous to human health.*
5. *Show some medicinal properties.*

Disadvantages

1. *Can produce allergies.*
2. *May be carcinogenic in nature. Can produce toxic effects and teratogenic effects.*
3. *As per WHO report, synthetic colourants can cause problem like immune system problems, ADHD, low temperature to frustration, impulsivity etc.*

Some Natural colorants are

1.HENNA

- **Synonyms**: *Mehendi, Egyptian private*.
- **Biological Source***: It consists of dried leaves of Lawsonia inermis belonging to*
- **Family:** *Lythraceae*.
- **Geographical Source:** *North Africa,India, Srilanka*.
- **Constituents:** *It contains coloring principle Lawsone. Other constituents are phenolic glycosides, xanthene, flavonoids, fats, resin and tannin etc*.
- **Applications:** *It is used as hair dye & hair care products and other cosmetics*.

HENNA PLANT

2.TURMERIC

- **Synonyms:** *Haldi, Indian saffron*.
- **Biological Source:** *It consists of dried, as well as fresh rhizomes of the plant Curcuma longa Linn belonging to*
- **Family:** *Zingiberaceae*.
- **Geographical Source:** *It mainly grows in India and also in China, Malaysia, Italy and Australia.*
- **Constituents:** *It contains 5% of volatile oil, resin and curcuminoids. Curcumin is the main component of curcuminoids. It is responsible for producing yellow coloured shades. Other phytoconstituents are sugars, resins, proteins, vitamins and minerals etc*.
- **Applications:** *It is used as colourants in various pharmaceutical dosage forms, cosmetics And food products*.

TURMERIC

3.ANNATTO

- **Synonyms:** *Lipstick tree; Annotta*.
- **Biological Source***: It consists of dried seeds of Bixa orellana belonging to*
- **Family**: *Bixaceae*.
- **Geographical Source:** *It is indigenous to central and South America and grown in India also*.
- **Constituents:** *It consists of major colourant bixin. It consists of minor constituents like trans-bixin and cis-norbixin. The seeds contain 4.5-5.5% pigment, which consists of 70-80% bixin*.
- ***Applications:*** *It acts as colouring and coating agent for solid and liquid pharmaceutical dosage forms. It is used as colouring agent in food and dairy products*.

ANNATTO

THANK YOU

•Diluents

Diluents are those substance which enhances the bulk of the solid formulation or dilute any liquid formulation.
Major Function of diluent Or filler is that they provide structural form and make them suitable for administration by enhancing bulk volume.
It is used to
•Improve the cohesion.
•Enhance the flow.
•Adjust weight of tablet as per die cavity.

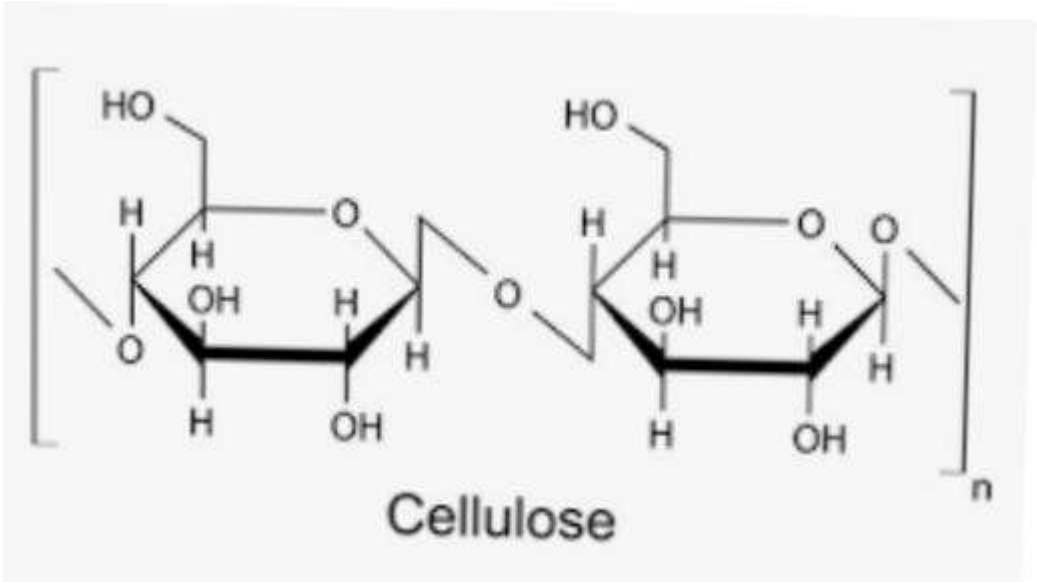
- *Anhydrous lactose, lactose monohydrate and sugar alcohols such as sorbitol , xylitol and mannitol are the commonly used Diluents.*

Classification :

- **Water Soluble – They are readily soluble in water. Ex- Lactose, Sucrose, mannitol**
- **Water Insoluble – They are insoluble in water. Ex- Starch, powdered cellulose,Microcrystalline cellulose**

Cellulose

- **Synonyms –** Arabocel, Elcema,Sanacel.
- **Biological Source-** It is pollysachharide and is structural compound found in cells of Plants.
- **Description –** It is white colourless and tasteless powder varying particle sizes.It may be fine freeflowing powder or granular dense powder or coarsefully and nonflowing material.



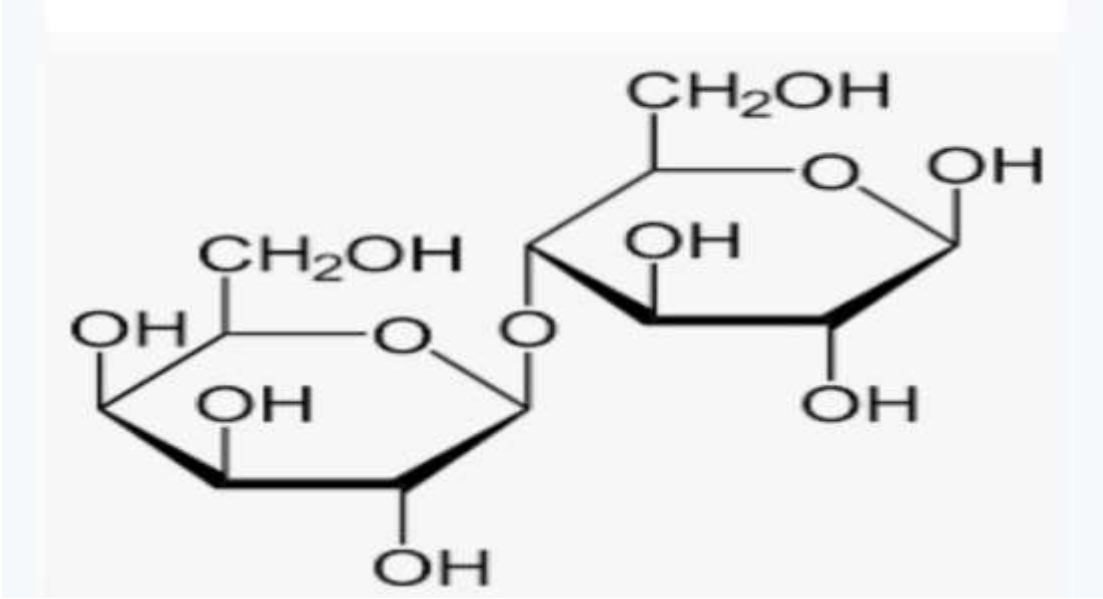
Cellulose

- **Chemical Test-** With Schulze reagent cellulose shows purple colour.
- **Application-.**

Diluent for tablet and fillers for hard gelatin capsules , also used in mfg of pellets and in cosmetics as well food industry , cellulose derivatives like Microcrystalline cellulose (MCC) acts as stabalizer and thickening agent.

Lactose

- **Synoyms-** Milk sugar,Lactin,Lactosum.
- **Biological Source-** Natural diassacharide animal origin consisting of galactose , glucose and obtained from milk of most of mammals.
- **Discription-** white crystalline powder, odourless and faintly sweet taste, available in several forms like anhydrous alpha monohydrate and anhydrous beta lactose.It is completely soluble in water and slightly soluble in ethanol as well as hygroscopic .



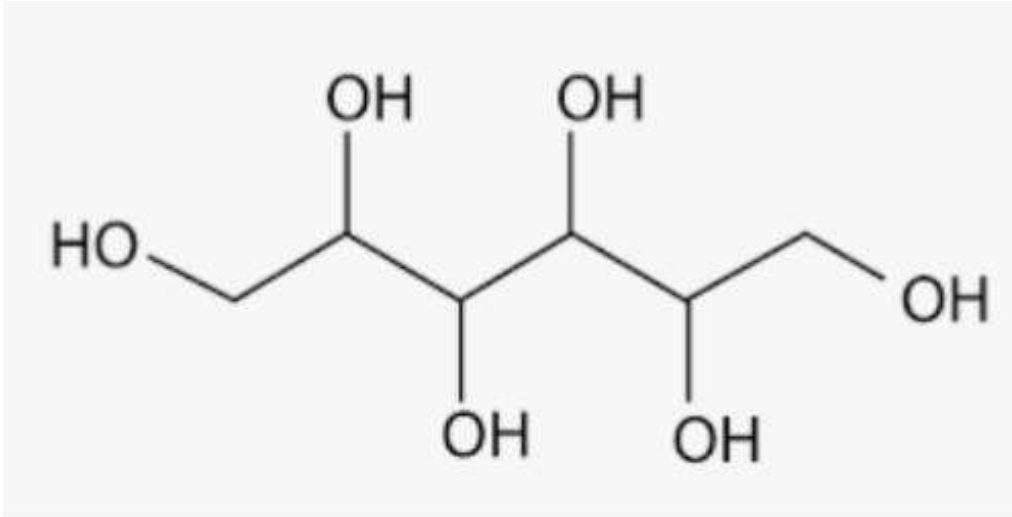
- **Chemical Test –** Lactose mixed with water and heated on water bath with Ammonia shows red colour.
- **Uses-** It used as Diluents in tablets, capsule. It is used as coating solution with Sucrose. Lactose also used in lyophilized product.

Mannitol

- **Synonyms-** Cordycepic acid,Manna sugar.
- **Biological Source-** It is exudation from the stem of Fraxinus ornus linn.
- **Family-** Oleceaea.
- **Discription-** White crystalline powder or free flowing granules. Odourless, nonhygroscopic and sweet powder. It develops cooling sensation in mouth.

- **Chemical constituents-**

mannotriose,mannatetrose, dextrose,mucilage and small amount of fluroscent substance fraxin .



- **Uses-**

Most suitable diluent for moisture sensitive drugs.
It is also used as sweetning agent,widely used in chewable tablets.
Therapeutically acts as osmotic diuretic as a diagnostic agent for kidney.

-

*Topic Name :

Disintegrants

Disintegranting agents are those which helps in disintegration of tablets and release contents of tablets or capsule into smaller fragments. Disintegrating agents when added to the drug formulations it causes quick dissolution.

Disintegrating agents act by various mechanisms like:

1. Swelling : Disinfectants swell and break tablet into pieces
2. Porosity : Water starts penetrating inside table by capillary action and break interarticulate bonds.
3. Deformation : During compression, disintegrating agents deformed.When they come in contact with water , they get back to their normal structure. It facilitates break up of tablet.
4. Repelling : Particle repel each other causing tablet to disintegrate.

#Advantages of Natural Disintegrants:

1. They are effective in lower concentrations.
2. Good Compressibility and flowability.
3. They are more effective at intragranular region.

#Disadvantages of Natural Disintegrants

1. They are more Hygroscopic
2. Some are anionic and may cause Some slight in-vitro binding with cationic drugs.

#There are some Natural Disintegrants :

1. CHITOSAN
2. PREGELATINIZED STARCH
3. MICROCRYSTALLINE CELLULOSE

1. CHITOSAN :

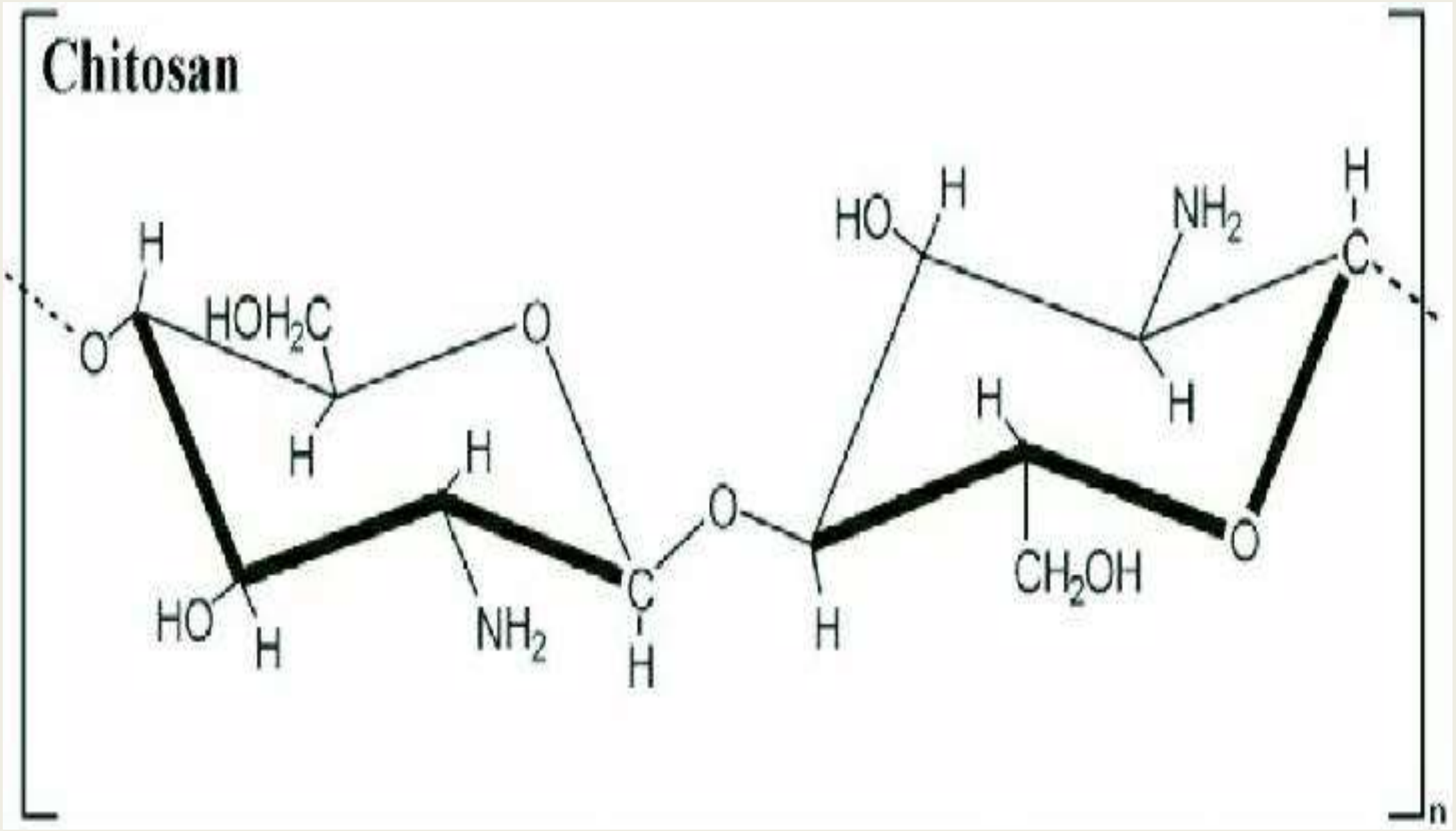
Synonym : Deacetylated chitin

Biological source : It is a natural polysaccharide obtained from crab and shrimp shells.

Description :

Chitosan is odourless, white or creamy white powder or flakes. During precipitation chitosan may form cotton like fibre. Chitosan is sparingly soluble in water; practically insoluble in ethanol (95%). It is soluble in dil.&Conc. organic acids & to some extent in mineral inorganic acids except Phosphoric acid & H_2SO_4

STRUCTURE :



Chemical constituents : It contains two units

1. B-(1-4) D-glucosamine (deacetylated unit)
2. N-acetyl-D-glucosamine (acetylated unit)

Chemical Tests :

1.With iodine and 10% sulphuric acid solution, chitosan forms deep violet colour.

USES :

1. Chitosan is used in various pharmaceutical dosage forms such as controlled drug delivery, mucoadhesive dosage forms, improved peptide delivery, colonic drug delivery systems and for gene delivery.
2. It can be used in gels, films, beads, micro spheres, tablets and coatings for liposomes.
3. It acts as coating agent, disintegrant, film forming agent, tablet binder, viscosity increasing agent.

2. PREGELATINIZED STARCH :

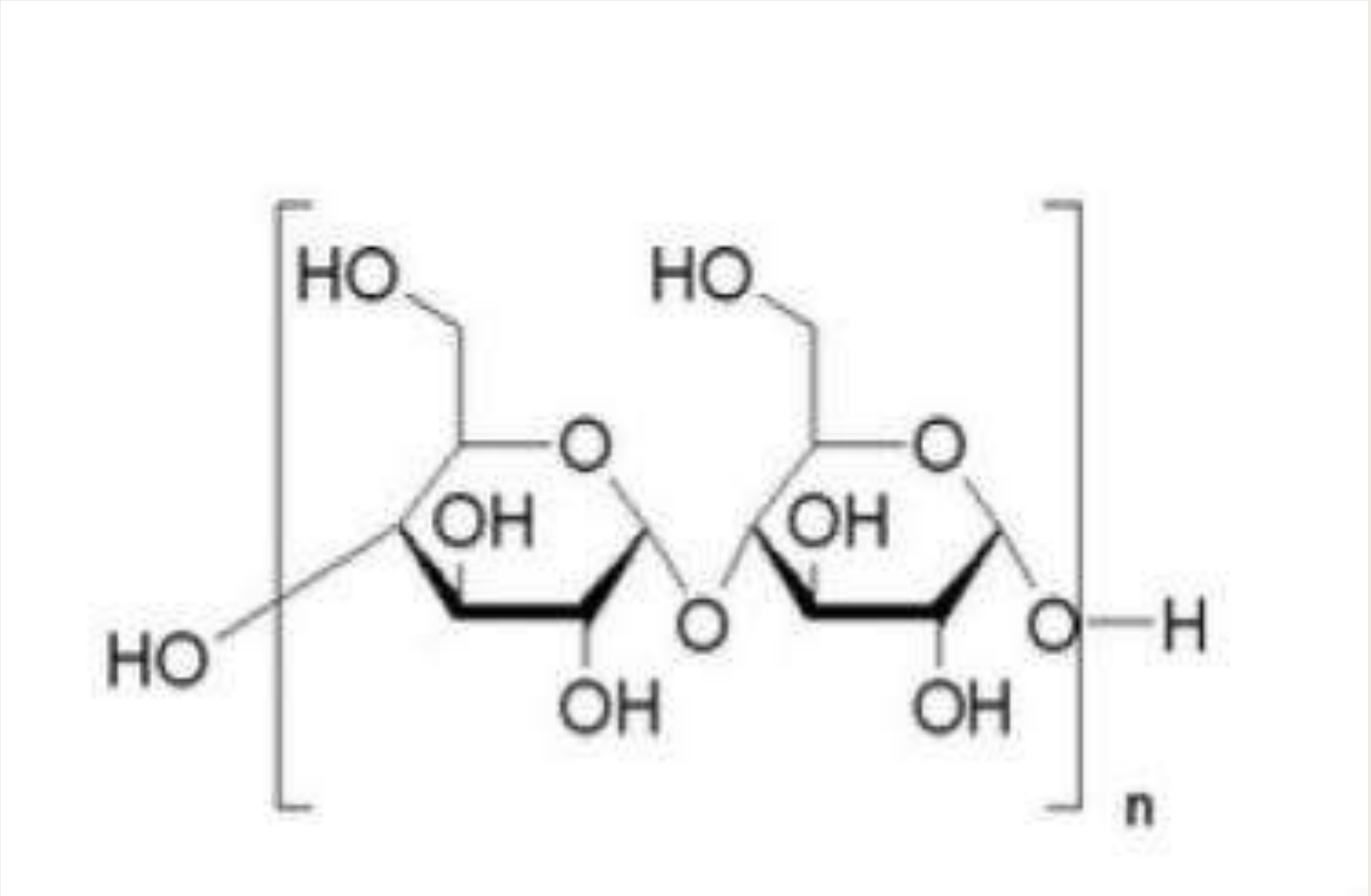
Synonym : Compressible starch, Instastarch, Pharma-Gel, Prejel

Biological Source : Pregelatinized starch is obtained from modification of Potato starch.

Description :

It is occurs as a moderately coarse to fine, white to offwhite colored powder which is odorless and has characteristic taste. It is practically insoluble in organic solvents, slightly soluble in cold water.

Structure :



Chemical Constituents : It mainly consists of 5% free amylose, 15% of free amylopectin and 80% unmodified starch.

Chemical Tests : When solution to pregelatinized starch is treated with iodine solution, it forms reddish violet to blue colour.

USES :

1. It is mainly used as disintegrants, binder and diluents in tables and capsules.
2. It is suitable binder for tables made by direct compression method.
3. It acts as disintegrating agent between 5 to 10% concentration.

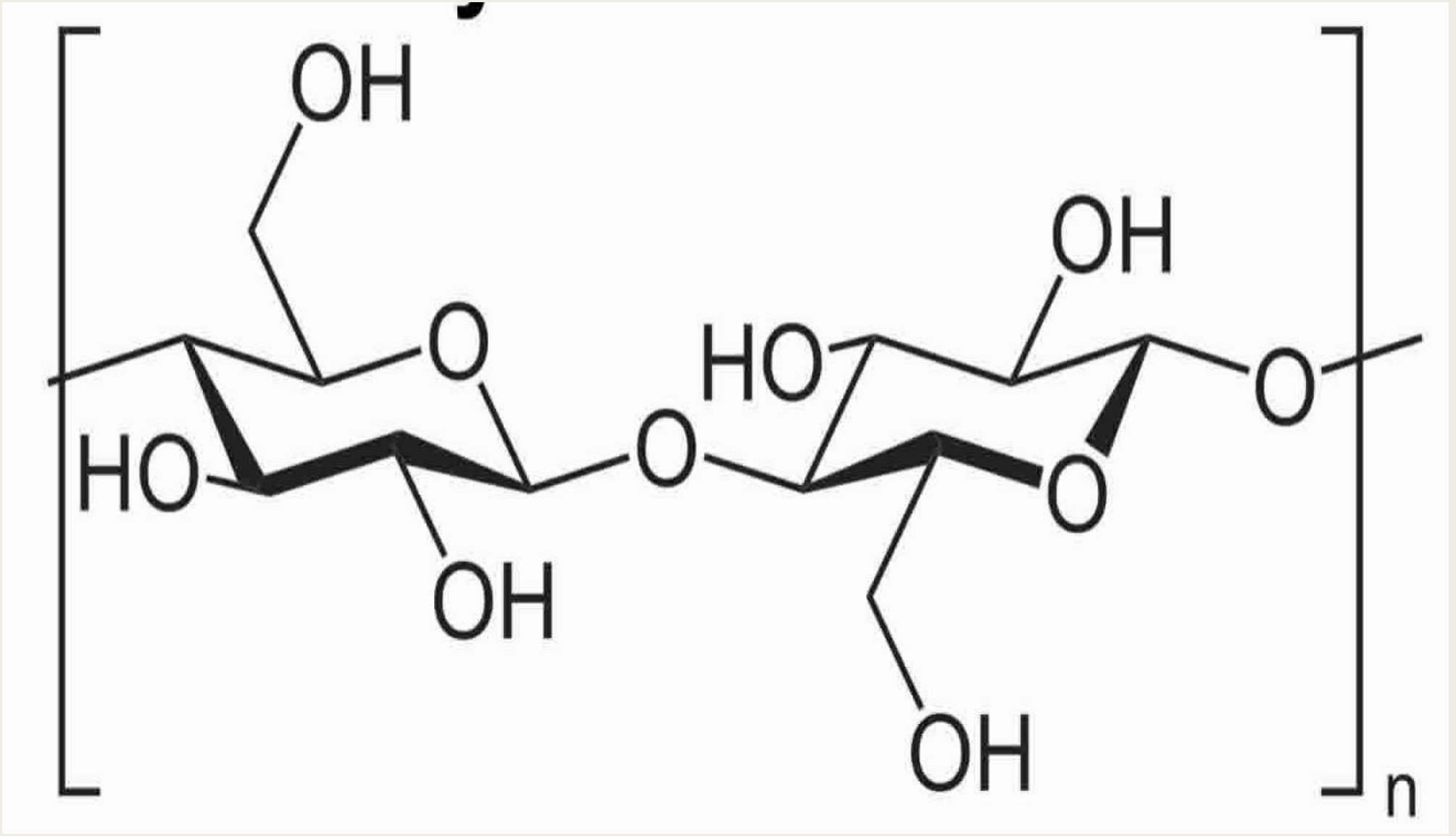
3. MICROCRYSTALLINE CELLULOSE :

Synonym : Avicel, Celex, Cellulose gel.

Biological source : It is obtained from partial depolymerization of cellulose.

Description : It is a white, odourless, tasteless, crystalline powder having porous particles. It is commercially available in different particle size and moisture grades. It is slightly soluble in 5% w/v sodium hydroxide solution and practically insoluble insoluble water, dilute acids and organic solvents.

Structure :



Chemical constituents : It consists of linear chain of B (1, 4) – linked glucose residue.

Chemical Tests : MCC is heated with phosphoric acid and solution of catechol in phosphoric acid is added. On heating for 30min, it produces red colour.

Uses : It is used as Lubricant and disintegrating agents. It is applied as binder or diluent in oral tables and capsules formulations and suitable for both wet granulation and direct compression method.

THANK YOU

Pune District Education Association's
Shankarrao Ursal College of
Pharmaceutical Sciences & Research Center
kharadi pune

Narcotic Drugs and Psychotropic Substances Act, 1985

MR. NITIN NEHARKAR
ASSISTANT PROFESSOR,
DEPARTMENT OF PHARMACUTICES
SHANKARRAO URSAL COLLEGE OF
PHARMACEUTICAL SCIENCE & RESEARCH
CENTRE KHARADI PUNE.

Content

- Introduction
- Objective
- Definition's
- Authorities and officers
- Offences and penalties
- NDPS Rules

Introduction

- There are many act related to this act in the past which have many drawbacks and which are modified by NDPS act,
- Like, opium act 1857,
  opium act 1878,
  dangerous drug act 1930.etc

- These above act have many drawbacks and related only with punishments and hence urgent needs have come to overcome these drawbacks.

- Therefore; Government of India withdraw these acts and passed NDPS Act 1985 with modification or to minimize deficiencies in old acts.

Continue.

- The NDPS Act has since been amended thrice –in 1988, 2001, 2014.
- **Background of the NDPS:**
- India had no legislation regarding narcotic until 1985.
- Cannabis smoking in india has been known since at laest 2000 BC and is mentioned in Atharvaveda.
- The US began to campaign for a worldwide law against all drugs, following the adaption of single Convention on Narcotic drugs in 1961.
- However India opposed and withstand American pressure to make cannabis illegal for nearly 25 years.
- American pressure increased in 1980 and in 1985. the government decided and pass NDPS act, banning all narcotic drugs in India.

Objective

- To consolidate(combine) and amend(modify) the law relating to narcotic drugs.
- To make stringent provision for control and regulation of operations relating to NDPS.
- To prevent illicit traffic in NDPS ordinance, 1988 was passed to provide detention in certain cases.

Definition's

- **Addict:** A person habitual to regular use of any narcotic drug or psychotropic substance is called as Addict.
- **Cannabis:** (Hemp)cannabis consist of dried flowering tops of cannabis sativa

  family: cannabinaceae
- cannabis is plant genus which includes charas, ganja.

  it includes the following;
- **Charas** is a resin substance obtained from cannabis plant, is a concentrated preparation and liquid known as hashish oil or liquid hashish.
- **Ganja** is a flowering or fruited top of cannabis plants.

Continue..

- **Coca derivatives (dried leaf)**

  crude cocaine; used directly or indirectly for manufacture of cocaine.

  ecgonine;

  cocaine(chemical constituent) which is methyl ester of benzoyl Ecgonine and its salt.

- **coca leaf :** it includes;
  - The leaf of coca(erythroxylon) plant.
  - Any mixture thereof with or without any neutral material (not include any preparations containing less than 0.1% cocaine.

- **Manufacture:**
  - All processes other than production by which NDPS are obtained.
  - Refining and making of such drugs.

Continue..

- **Manufactured drugs** : it includes
- all coca derivatives, medicinal cannabis, opium derivatives and poppy straw concentrate.
- **Medicinal cannabis**: (medicinal hemp)
- Any extract or tincture of cannabis.
- **Narcotic drugs** :
- It includes coca leaf, cannabis, opium and poppy straw and all manufactured drugs.
- Opium: (latex)
- It includes coagulated juice of opium poppy and its mixture with or without neutral(not affect main action of drug) material.

Continue..

- Opium derivative:
- It includes medicinal opium

  prepared opium

  phenanthrene alkaloids like morphine, codeine, thebaine and there salts.

  diacetyl morphine (heroine and its salts)

- Opium Poppy :

  it includes,

  plant of papaver somniferum and its other species.

- Poppy straw: (khaskhas)

  all the parts(husk) of opium poppy (except seeds) after harvesting.

Continue.

- **Psychotropic substances:**
- It means any substance natural or synthetic or any salt or any preparation of such substance which include in list of psychotropic substances specified in schedule.
- **Illicit(illegal) traffic;(supplement of NDPS act passed in 1988)**
- It includes
- Cultivating any coca plants or gathering any portion of it.
- Cultivating the opium poppy or any cannabis plant.
- Engaging in production, manufacturing, possession, sale, purchase, transport, warehousing, consumption, interstate export, import, import to and export from India of NDPS.
- Giving premises for the above activities except as permitted under the act.
- Financing directly or indirectly for any above activities.
- Harboring the person engaged in the above activities.

Authorities and officers

1. Measure for preventing and combating abuse of narcotic drugs and illicit traffic therein (central authorities)
2. Officers of central government
3. NDPS consultative committee

- prohibition, control and regulation.

CONTINUE.

1. **Measure for preventing and combating abuse of narcotic act and illicit traffic therein.**

- To prevent illegal activities and misuse of NDPS act central government can take measure in following matters

- Work management by various officers of the state government and other authorities under this act and all officers who are involved in enforcement of the provision of this act.
- Government major require as per international convention.
- Helping of concerned authorities in foreign countries to prevent the illicit traffic of NDPS.
- Identification, treatment, education, aftercare, rehabilitation, and social reintegration of addicts.
- Other matter regarded as suitable by central government for purpose of this act and preventing use of NDPS and illegal traffic.

Continue.

2. Officers of central government

- ✓ Apart from central authorities central government appoints narcotic commissioner.

- Narcotic commissioner himself or with the help of other officers shall perform the function related to,
- Supervision of cultivation of opium poppy
- Production of opium
- Other function which may charge to him by central government.

Continue.

3. NDPS consultative committee : (advisory)

- This committee is constituted to provide advice to central government on matters involving the administration of this act.
- Committee consist of chairmen and other member not more than 20 as appointed by central government.
- For efficient work committee may approach one or more committees and committee will advice central government on matter related to this act.

❑ Prohibition control and regulation

- According to **section 8** of NDPS Act anyone can not cultivate, manufacture, posses, stores, import, export of these drugs except for medicinal purpose.
- As per **section 9**, of this act empower the central government to permit control and regulate by rules and certain operation of NDPS Drugs.

- Prohibition of certain operation as per **section 8**
- Power of **central government** to permit, control and regulation.
- Power of **state government** to permit control and regulation.

1.Prohibition of certain operation as per section 8,

- **No person shall,**
- Cultivate coca plant and gather any portion of coca plant.
- Cultivate opium poppy or any cannabis plant.
- Produce, manufacture, posses, sell, purchase, transport, ware house, use, consume, import and export inter state, import and export from India or transfer of any NDPS expect for medical and scientific purposes and to extent and in the manner provided by other provision of this act.
- **Power of central government to permit control and regulate:**

1. Central government by making rules, controls and regulates following operation.

- Cultivation of coca plant are gathering any portion of coca plant and also production sales purchase, transport, import and export of coca plant.

Continue..

- Cultivation of opium poppy only on behalf of central government.
- Production and manufacture of opium and production of poppy straw.
- The manufacture, possession, transport, import, export, purchase, sale, consumption or use of psychotropic substance.
- The sale of opium and its derivatives from central government for export from India or sell to the state government or manufacturing chemists.

2. Central government controls on certain operation.

- Government self fix time to time the limit within which license may be given the cultivation of opium poppy.
- The communication between cultivator and authorized officer control by central government.
- Central government fixes the price of opium time to time.

Continue..

- Central government may prescribe the form and condition of license and permit for manufacturing, possession, transport, import inter state, sale, purchase, consumption, or use of NDPS.

- Rules are provide for weighment, examination and classification according to quality and consistence of the opium received at factory.

- If opium delivered by cultivator was found adulterated during examination in the central government it may comes under the rules which is taken by authorized officers.

Continue..

- **Power of state government to permit, control and regulate:**

1. **Permission and regulation of certain operation by state government:**

- Possession, transport, interstate import, export, warehousing, sale, purchase, consumption and use of poppy straw.
- Cultivation of any cannabis plant production, manufacturing, possession, transport, import, export, sale, purchase, and use of cannabis.
- Possession, transport, purchase, sale, interstate import, export, use and consumption of manufactured drugs other than prepared opium.
- The manufacture and possession of prepared opium from opium lawfully processed by an addict registered with state government on medical advice are his personal conception.

- **Conditions To Grant License For Cultivation And Collection Of Opium Under NDPS Act:**

1. The land for poppy cultivation shall be free from litigation.
2. The licence for cultivation of opium poppy shall specify the area and designate the plots on which cultivation take place.
3. The licensee shall not transfer his licence and cultivate poppy for production of opium or poppy show over the area of land and plots specified in the license.
4. The licensee shall bring the opium and deliver at place fixed for weighment all opium collected by him from the crop and he should accept that the prize of opium he fixed by central government.
5. The delivery and weighment of opium shall be under the supervision of district opium officer or any other officer authorized by narcotic commissioner.

Continue..

6. The licensee shall extract as much as opium as possible.
7. Final payment for opium delivered by him shall be made of appropriate time.
8. He should obey the provisions and order issued by component authorities.
9. The license shall be canceled making him disqualified for grant of license. If he breaks any law for any reason of the condition's, he may punishable.
10. He district opium officer designate one of cultivator of opium poppy as lambardor in each village where opium poppy is cultivated.
11. Lambardor shall perform his given function and on such terms and conditions as given by narcotic commissioner.

✓ Offences and penalties

Sr.No.	Offenses	Penalties	Section Of Act
1	Cultivation of opium, cannabis or coca plants without license	Rigorous imprisonment-up to 10 yrs. + fine up to 1 lakh	Opium-18 (c) Cannabis- 20 Coca- 16
2	Embezzlement of opium by licensed farmer	Rigorous imprisonment-up to 10 yrs.to 20 yrs. + fine up to 1to 2 lakh	19
3	Production, manufacture, possession, sale, purchase, transport, import or export interstate, or use of NDPS	Small quantity- Rigorous imprisonment-up to 6 months or fine up to 10,000 or both Rigorous imprisonment-up to 10 + fine up to 1 lakh Commercial quantity – Rigorous imprisonment-up to 10 yrs.to 20 yrs. + fine up to 1to 2 lakh	Prepared opium section 8 Opium- 18 Cannabis – 20 Psychotropic substance- 22

continue

Sr. No.	Offenses	Penalties	Section Of Act
4	Import, export or transshipment	Small quantity- Rigorous imprisonment-up to 6 months or fine up to 10,000 or both Rigorous imprisonment-up to 10 + fine up to 1 lakh Commercial quantity – Rigorous imprisonment-up to 10 yrs.to 20 yrs. + fine up to 1to 2 lakh	Prepared opium section 8 Opium- 18 Cannabis – 20 Psychotropic substance- 22
5	External dealing in NDPS i.e. engaging in or controlling trade whereby drugs are obtained from outside India are supplied to a person outside India	Rigorous imprisonment-up to 10 yrs.to 20 yrs. + fine up to 1to 2 lakh	Section 24
6	violation pertaining to controlled substance	Rigorous imprisonment-up to 10 yrs.to 20 yrs. + fine up to 1to 2 lakh	25A

Continue..

Sr. No.	Offenses	Penalties	Section Of Act
7	Financing traffic and harboring offenders	Rigorous imprisonment-up to 10 yrs.to 20 yrs. + fine up to 1to 2 lakh	27A
8	Attempts, abetment and criminal conspiracy	Rigorous imprisonment-up to 10 yrs.to 20 yrs. + fine up to 1to 2 lakh	28 Abetment and criminal consirancy-29

NDPS Rules

- **Schedule- I**
- **Narcotic drugs-**

1. Coca leaf
2. Cannabis
3. Acetorphan
4. Diacetylmorphine(heroin)
5. Etrophine

Preparation, admixture extract and other substance containing any of above drugs.

- **Psychotropic substance:**

1. Tenocyclidine
2. Alprazolam
3. Bromazepam
4. Camazepam

Continue..

- **Schedule-II**

Psychotropic substance

- Amphetamine
- Mecloqualone
- Methaqualone

- **Schedule –III**

- Psychotropic substance
- Amobarbital
- Cyclobarbital
- pentazocine

❑ Amendment

- **1988 amendment**
  - NPDS act 1988 get approval from president ramaswami venkantraman on 8 January 1989

- **2001 amendment:**
  - NDPS Act 2001get approval from k.r narayanan on 9 may 2001.

- **2014 amendment:**
  - NDPS Act 2014 get approval from president pranab Mukherjee on 7 march 2014.

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2. A TEXTBOOK OF FORENIS PHARMACY", (10th Ed.) Vallabh Prakashan , Delhi, 2007, 31-47. References

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Thank you

The Nervous System

By

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HOD & Assistant Professor

Department of Pharmacology

PDEA's SUCOPSRC, Pune

Functions of the Nervous System

1. Sensory input – gathering information
   - To monitor changes occurring inside and outside the body (changes = stimuli)
2. Integration –
   - to process and interpret sensory input and decide if action is needed.
3. Motor output
   - A response to integrated stimuli
   - The response activates muscles or glands

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Structural Classification of the Nervous System

- Central nervous system (CNS)
  - Brain
  - Spinal cord
- Peripheral nervous system (PNS)
  - Nerve outside the brain and spinal cord

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Functional Classification of the Peripheral Nervous System

- Sensory (afferent) division
  - Nerve fibers that carry information *to* the central nervous system

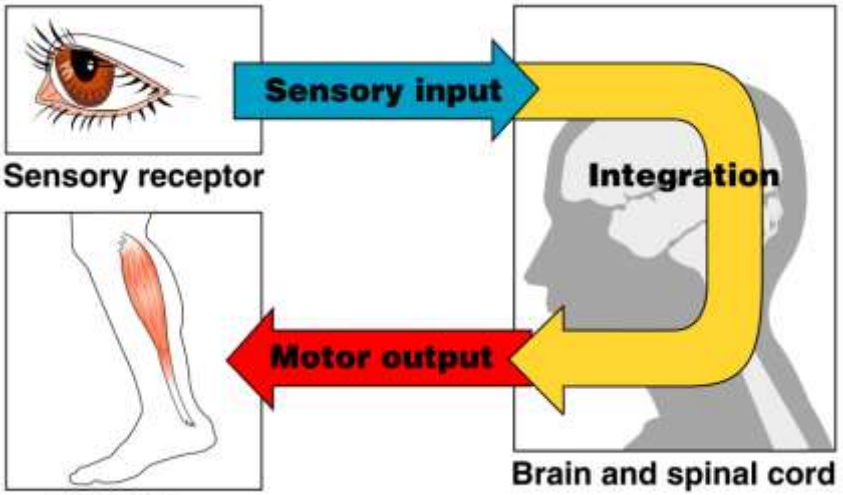

Figure 7.1

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Functional Classification of the Peripheral Nervous System

- Motor (efferent) division
  - Nerve fibers that carry impulses *away from* the central nervous system

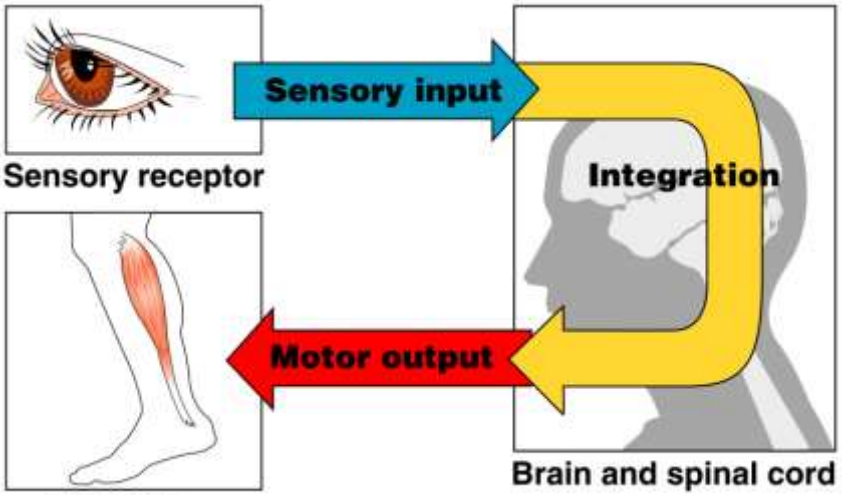

Figure 7.1

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Functional Classification of the Peripheral Nervous System

- Motor (efferent) division
  - Two subdivisions
    - Somatic nervous system = voluntary
    - Autonomic nervous system = involuntary

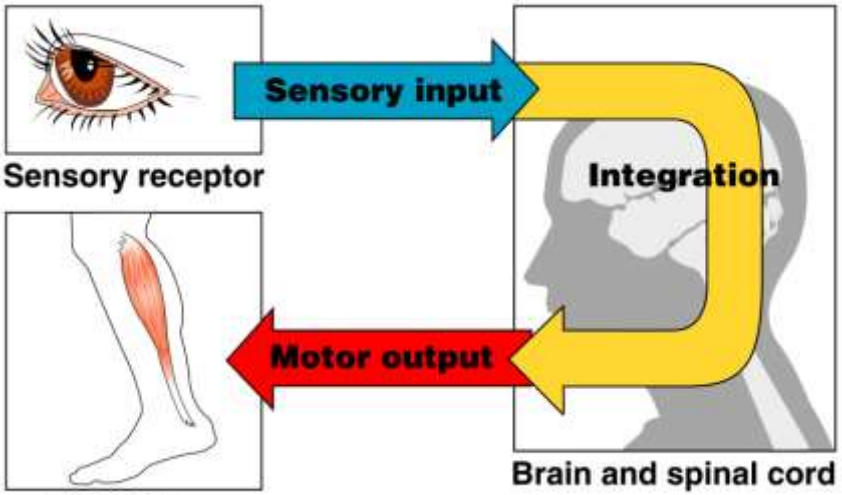

Figure 7.1

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Organization of the Nervous System

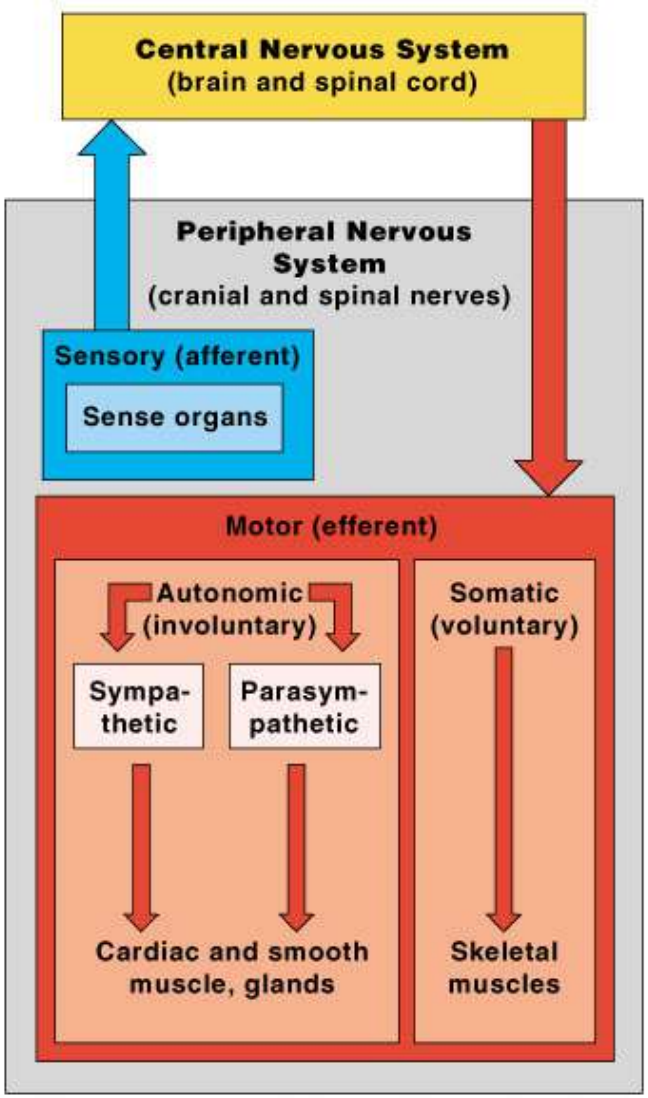

Figure 7.2

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Nervous Tissue: Support Cells (Neuroglia or Glia)

- Astrocytes
  - Abundant, star-shaped cells
  - Brace neurons
  - Form barrier between capillaries and neurons
  - Control the chemical environment of the brain (CNS)

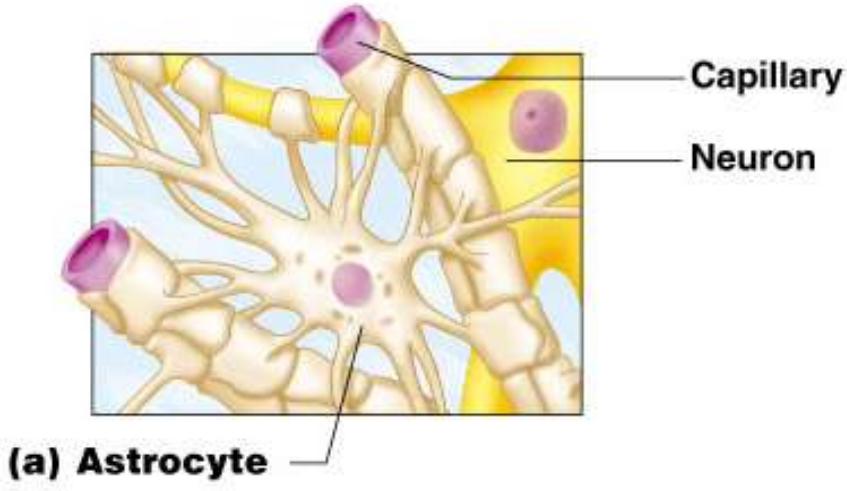

Figure 7.3a

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Nervous Tissue: Support Cells

- Microglia (CNS)
  - Spider-like phagocytes
  - Dispose of debris
- Ependymal cells (CNS)
  - Line cavities of the brain and spinal cor
  - Circulate cerebrospinal fluid

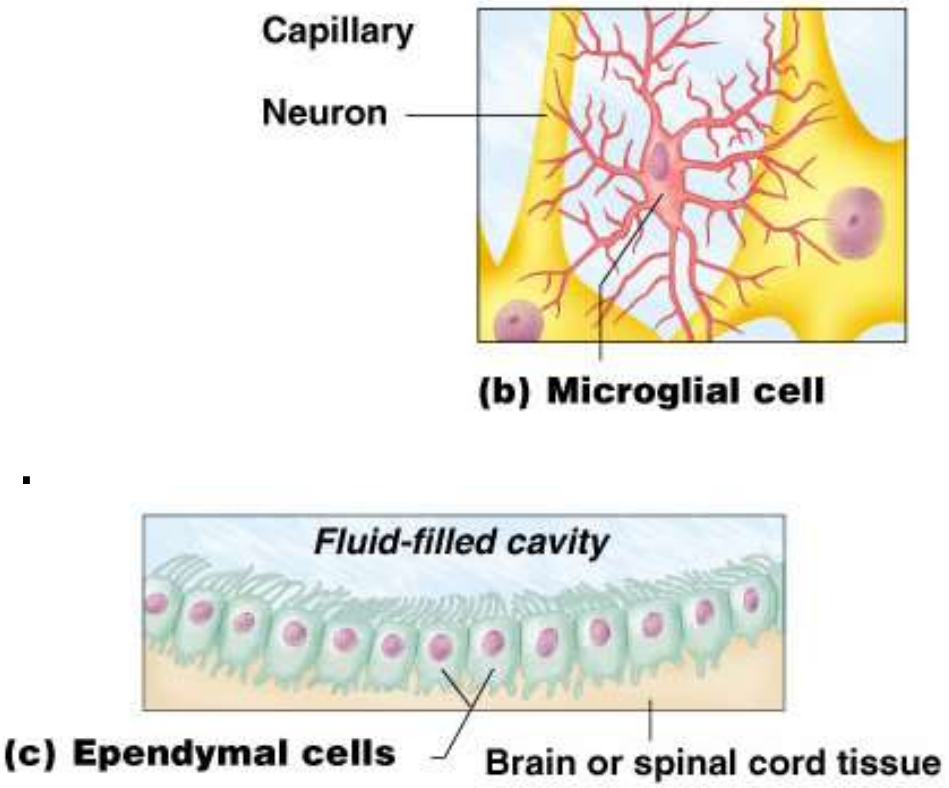

Figure 7.3b, c

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Nervous Tissue: Support Cells

- Oligodendrocytes (CNS)
  - Produce myelin sheath around nerve fibers in the central nervous system

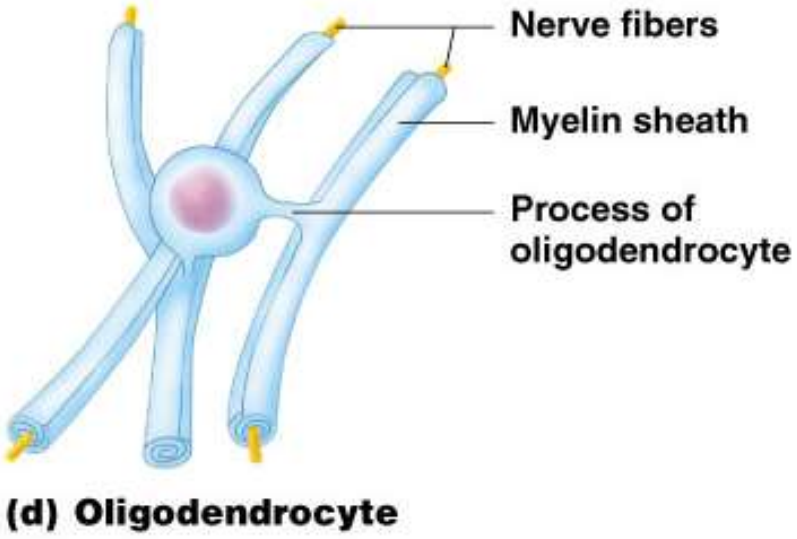

Figure 7.3d

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Neuroglia vs. Neurons

- Neuroglia divide.
- Neurons do not.
- Most brain tumors are “gliomas.”
- Most brain tumors involve the neuroglia cells, not the neurons.
- Consider the role of cell division in cancer!

Support Cells of the PNS

- Satellite cells
  - Protect neuron cell bodies
- Schwann cells
  - Form myelin sheath in the peripheral nervous system

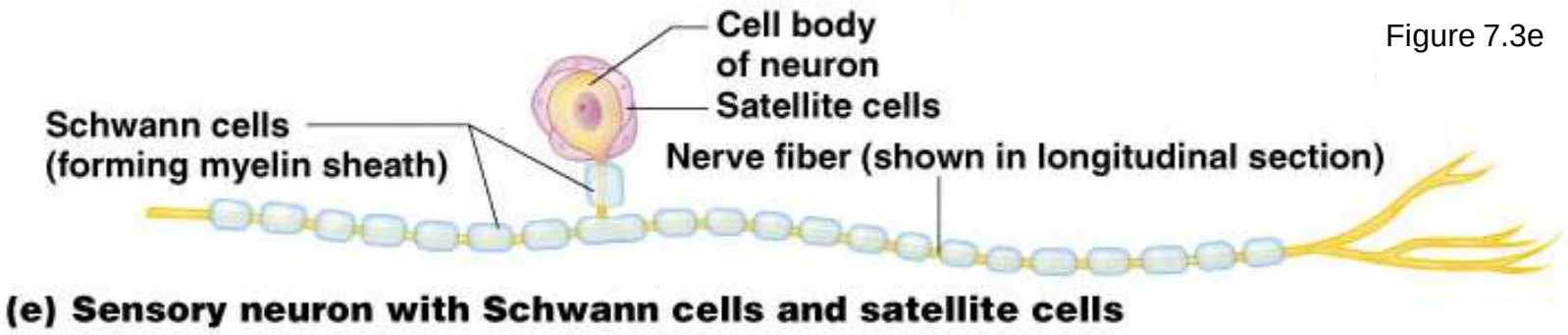

Figure 7.3e

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Nervous Tissue: Neurons

- Neurons = nerve cells
  - Cells specialized to transmit messages
  - Major regions of neurons
    - Cell body – nucleus and metabolic center of the cell
    - Processes – fibers that extend from the cell body (dendrites and axons)

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Neuron Anatomy

- Cell body
  - Nucleus
  - Large nucleolus

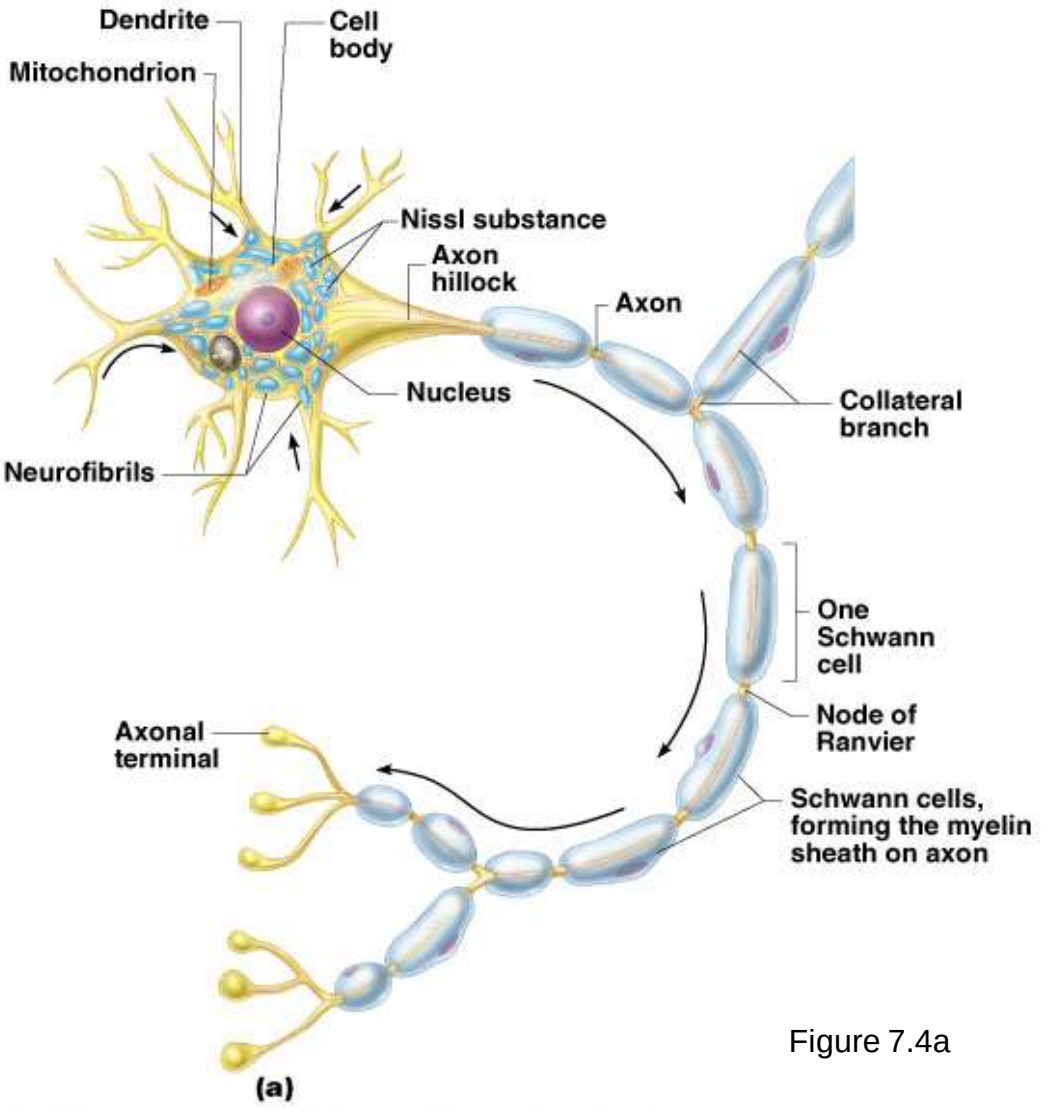

Figure 7.4a

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Neuron Anatomy

- Extensions outside the cell body
  - Dendrites – conduct impulses toward the cell body
  - Axons – conduct impulses away from the cell body (only 1!)

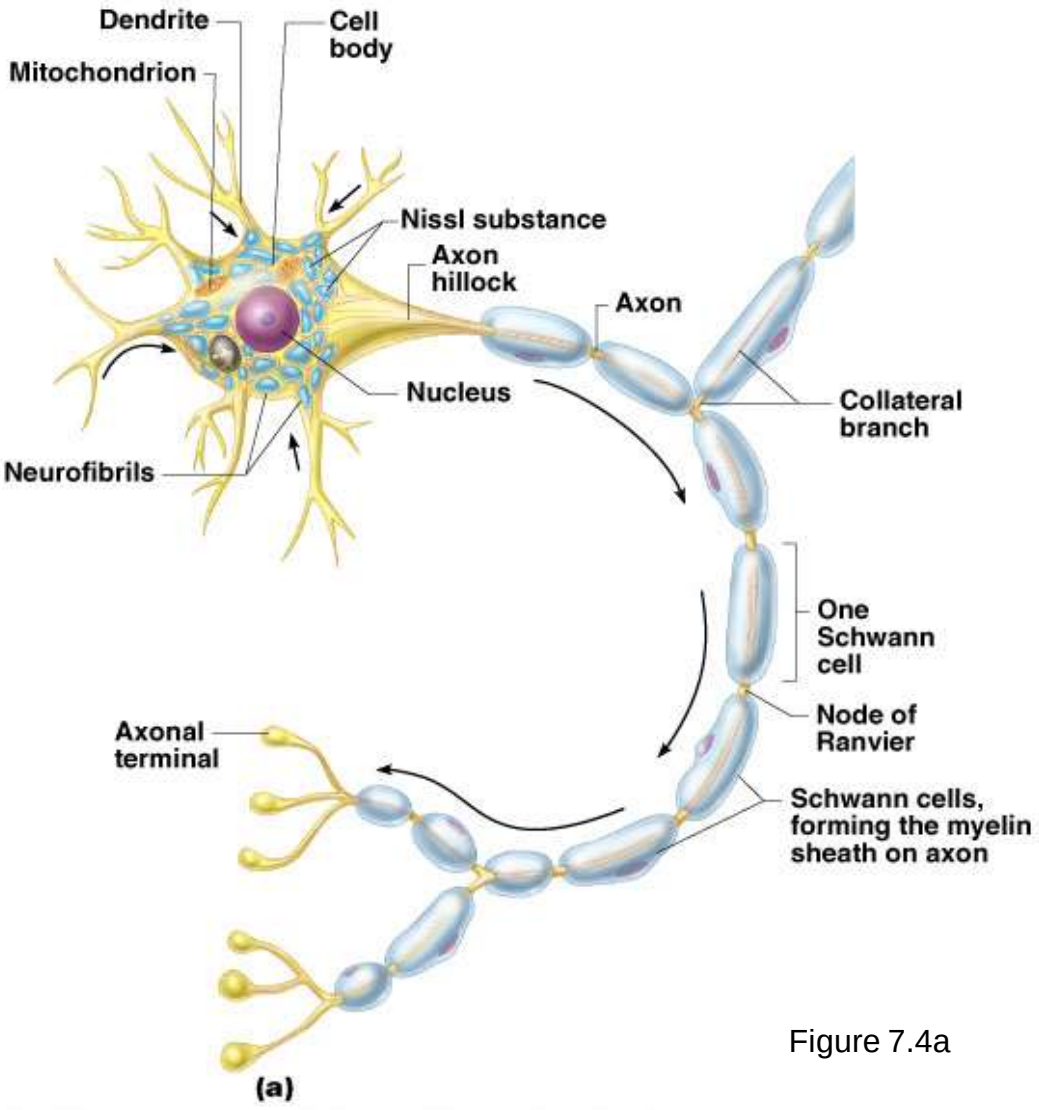

Figure 7.4a

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Axons and Nerve Impulses

- Axons end in axonal terminals
- Axonal terminals contain vesicles with neurotransmitters
- Axonal terminals are separated from the next neuron by a gap
  - Synaptic cleft – gap between adjacent neurons
  - Synapse – junction between nerves

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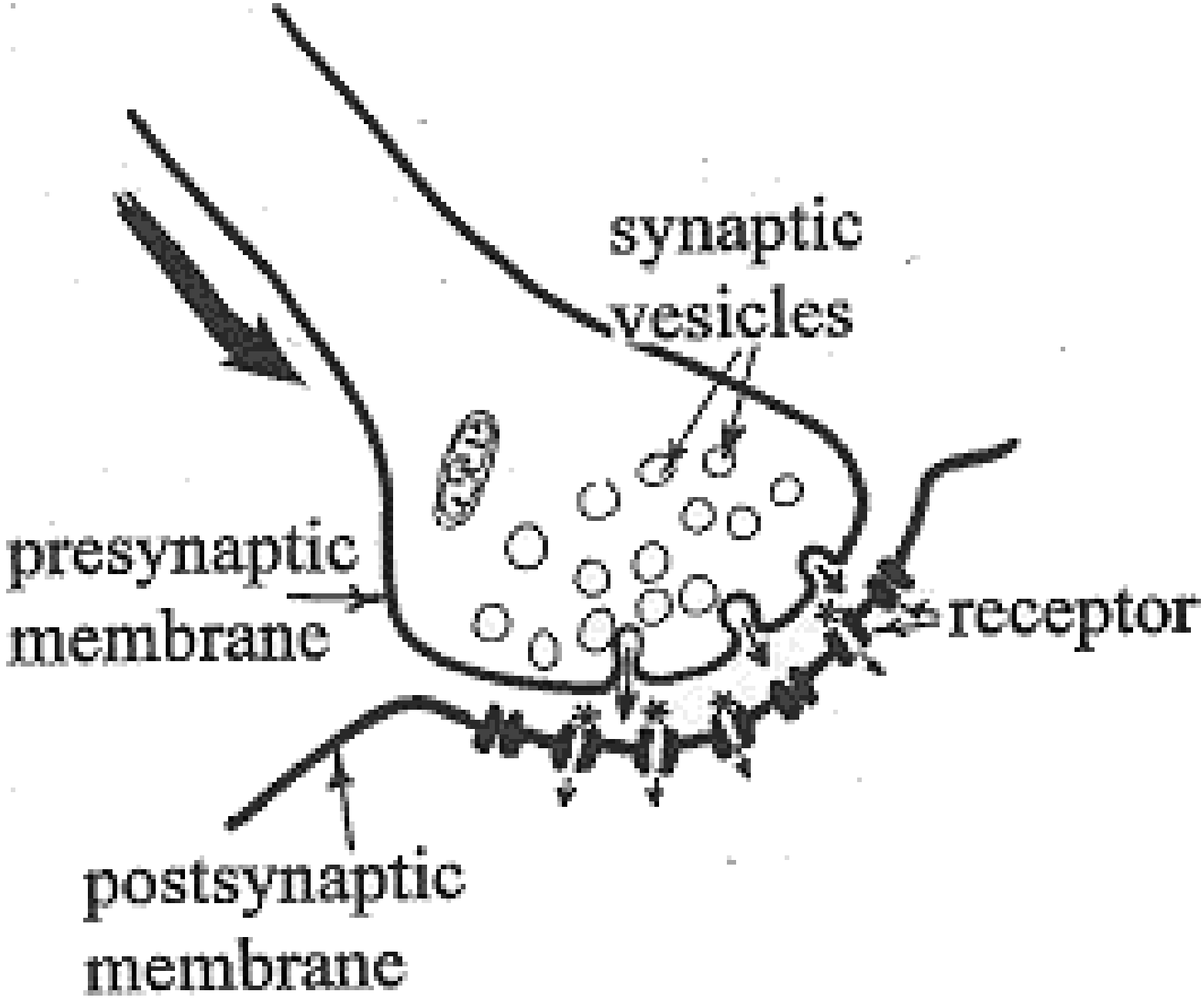
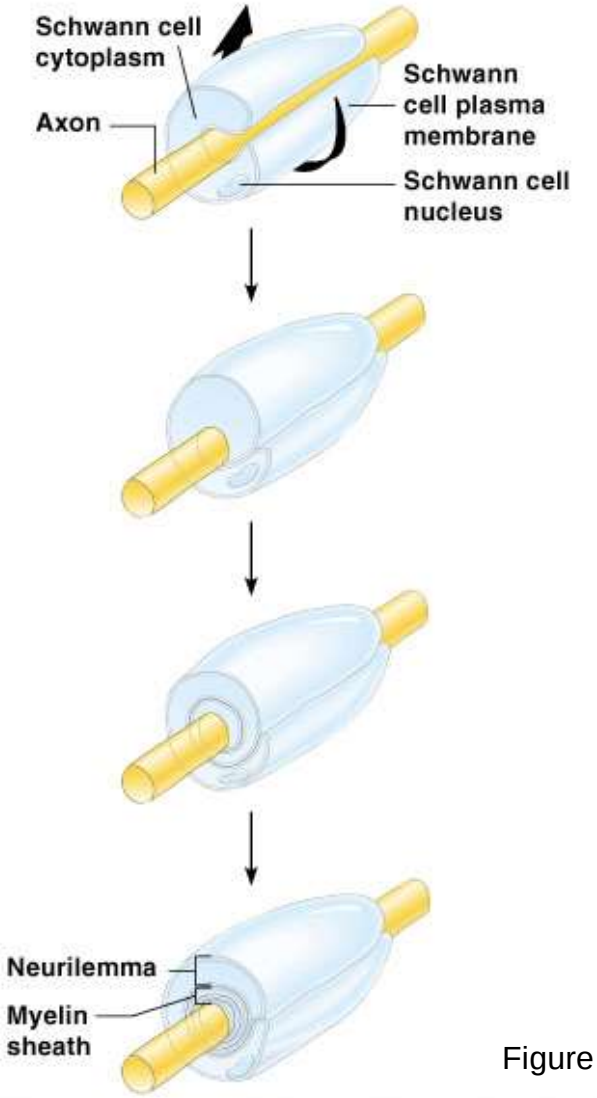

synaptic
vesicles
presynaptic
membrane
receptor
postsynaptic
membrane

Nerve Fiber Coverings

- Schwann cells – produce myelin sheaths in jelly-roll like fashion
- Nodes of Ranvier – gaps in myelin sheath along the axon



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Application

- In Multiple Scleroses the myelin sheath is destroyed.
- The myelin sheath hardens to a tissue called the scleroses.
- This is considered an autoimmune disease.
- Why does MS appear to affect the muscles?

Neuron Cell Body Location

- Most are found in the central nervous system
  - Gray matter – cell bodies and unmylenated fibers
  - Nuclei – clusters of cell bodies within the white matter of the central nervous system
- Ganglia – collections of cell bodies outside the central nervous system

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Functional Classification of Neurons

- Sensory (afferent) neurons
  - Carry impulses from the sensory receptors
    - Cutaneous sense organs
    - Proprioceptors – detect stretch or tension
- Motor (efferent) neurons
  - Carry impulses from the central nervous system

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Functional Classification of Neurons

- Interneurons (association neurons)
  - Found in neural pathways in the central nervous system
  - Connect sensory and motor neurons

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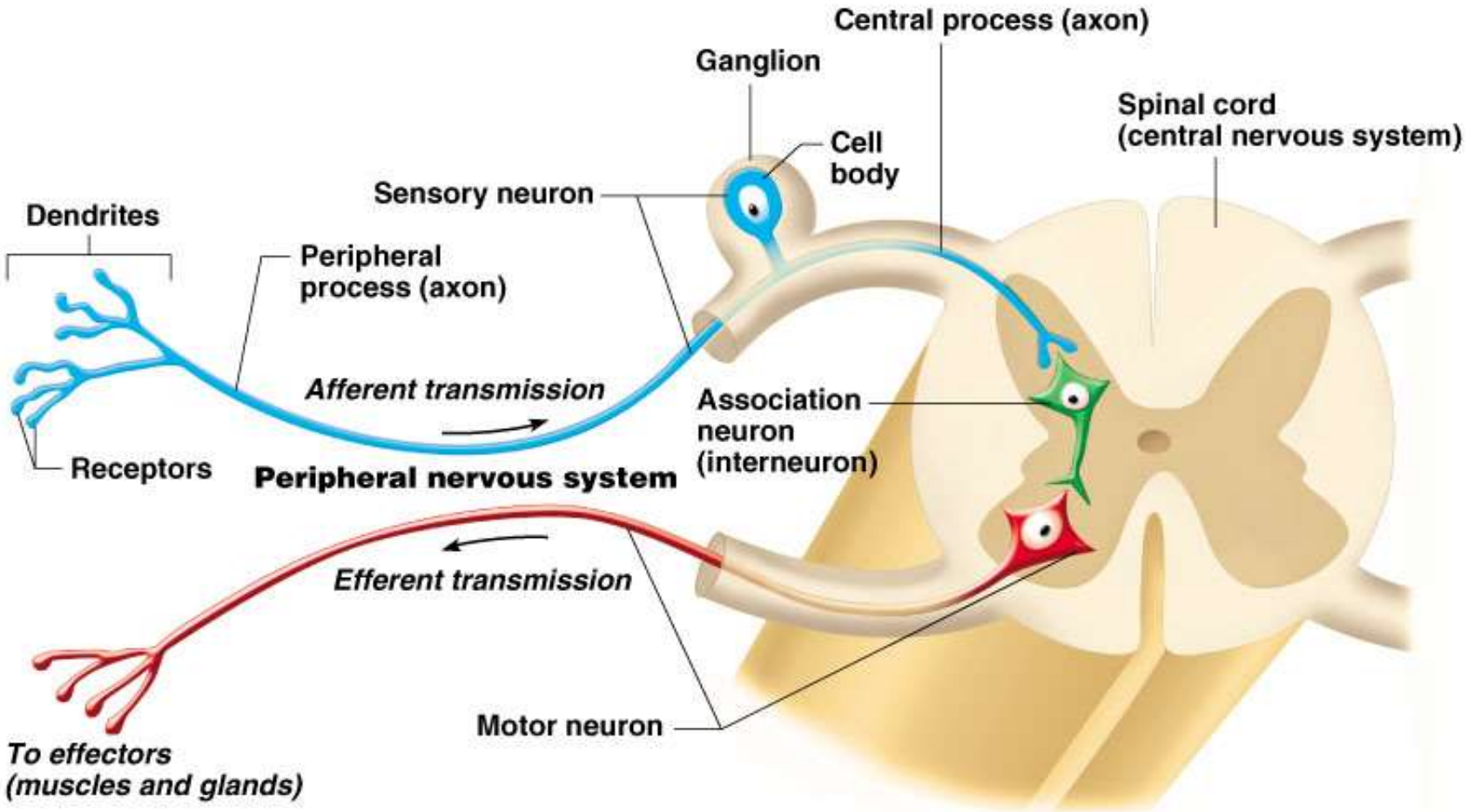
Neuron Classification



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Structural Classification of Neurons

- Multipolar neurons – many extensions from the cell body

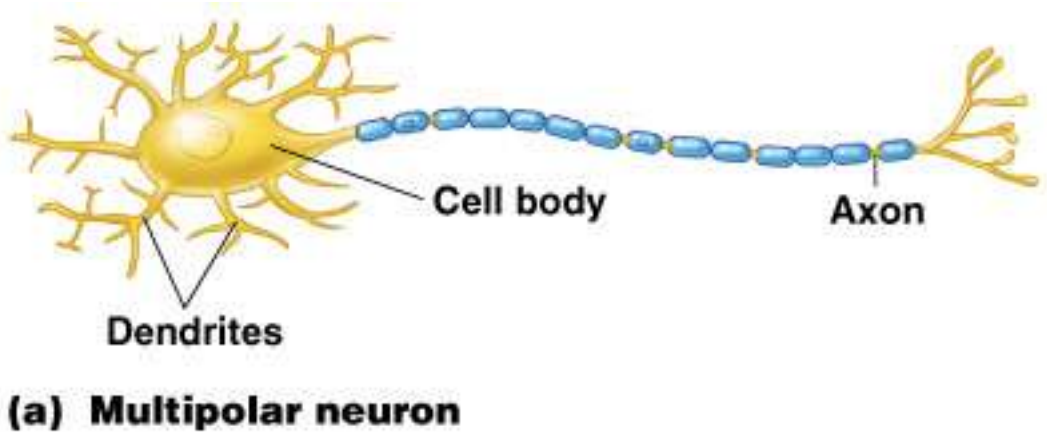

Figure 7.8a

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Structural Classification of Neurons

- Bipolar neurons – one axon and one dendrite

(b) Bipolar neuron

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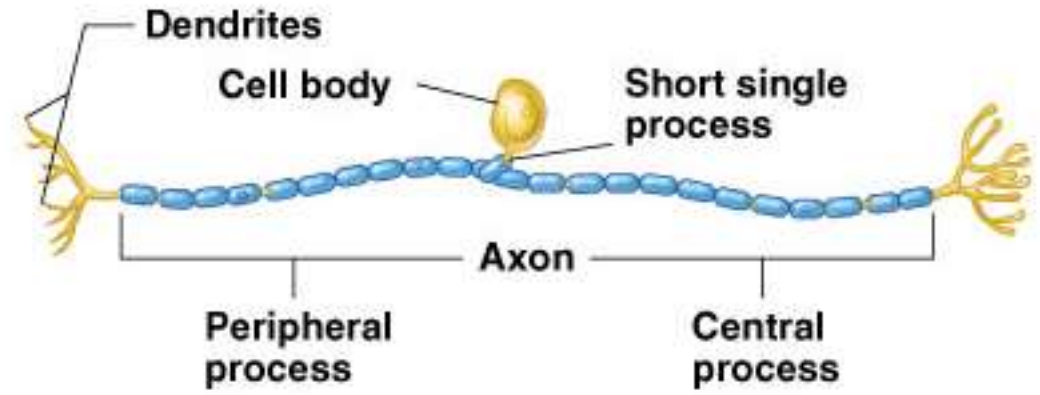
Structural Classification of Neurons

- Unipolar neurons – have a short single process leaving the cell body



(c) Unipolar neuron

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How Neurons Function (Physiology)

- Irritability – ability to respond to stimuli
- Conductivity – ability to transmit an impulse
- The plasma membrane at rest is polarized
  - Fewer positive ions are inside the cell than outside the cell

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Starting a Nerve Impulse

- Depolarization – a stimulus depolarizes the neuron's membrane
- A deploarized membrane allows sodium (Na^+) to flow inside the membrane
- The exchange of ions initiates an action potential in the neuron

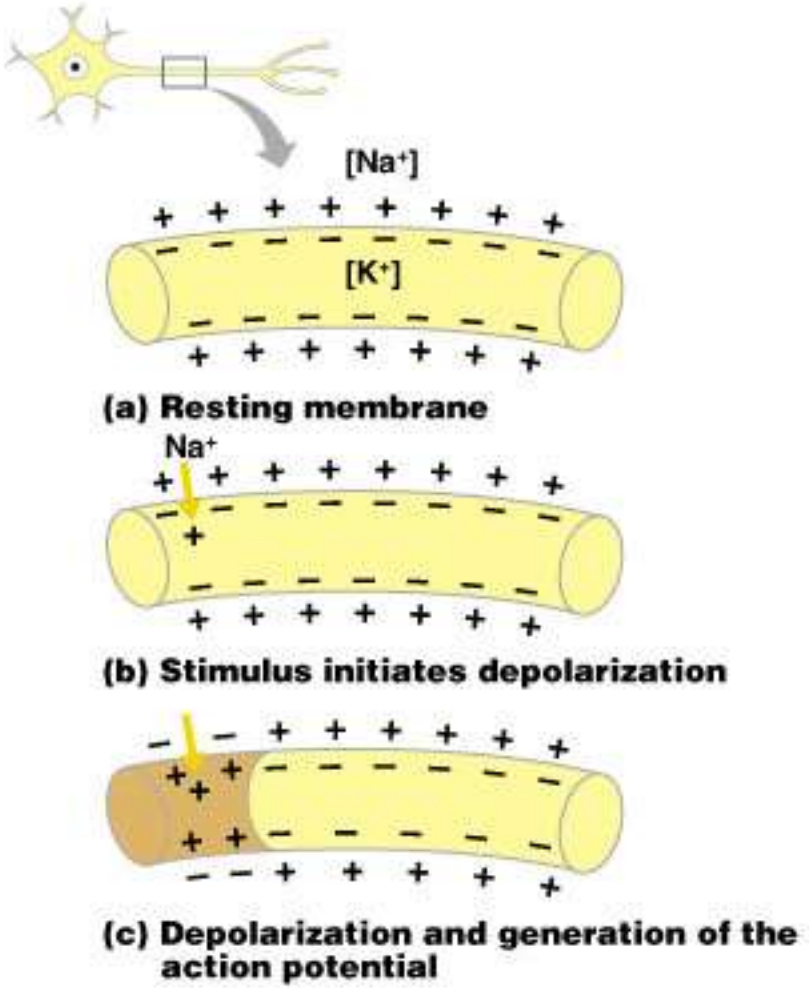

Figure 7.9a–c

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The Action Potential

- If the action potential (nerve impulse) starts, it is propagated over the entire axon
- Potassium ions rush out of the neuron after sodium ions rush in, which repolarizes the membrane
- The sodium-potassium pump restores the original configuration
  - This action requires ATP

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Nerve Impulse Propagation

- The impulse continues to move toward the cell body
- Impulses travel faster when fibers have a myelin sheath

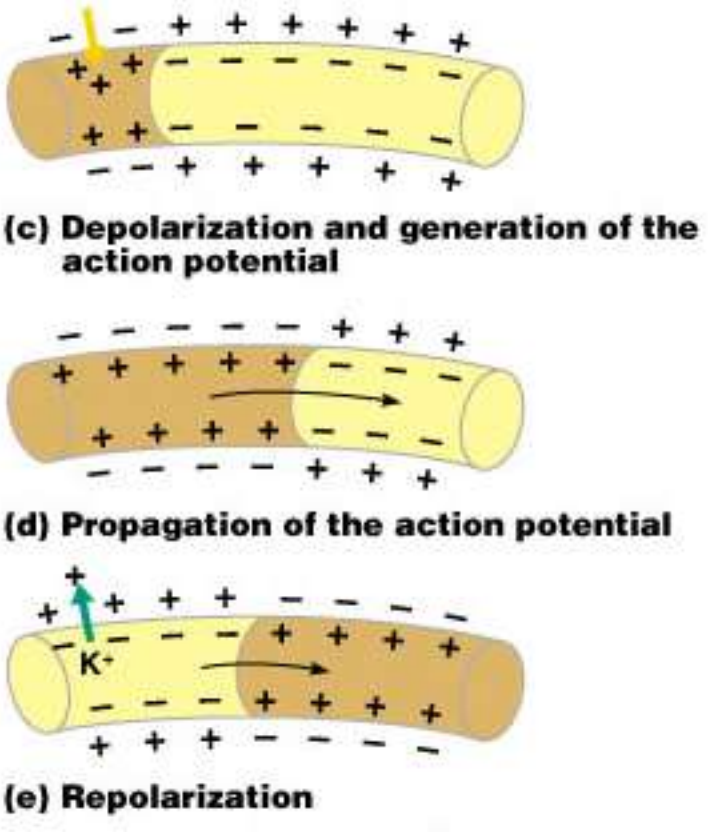

Figure 7.9c–e

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Continuation of the Nerve Impulse between Neurons

- Impulses are able to cross the synapse to another nerve
  - Neurotransmitter is released from a nerve's axon terminal
  - The dendrite of the next neuron has receptors that are stimulated by the neurotransmitter
  - An action potential is started in the dendrite

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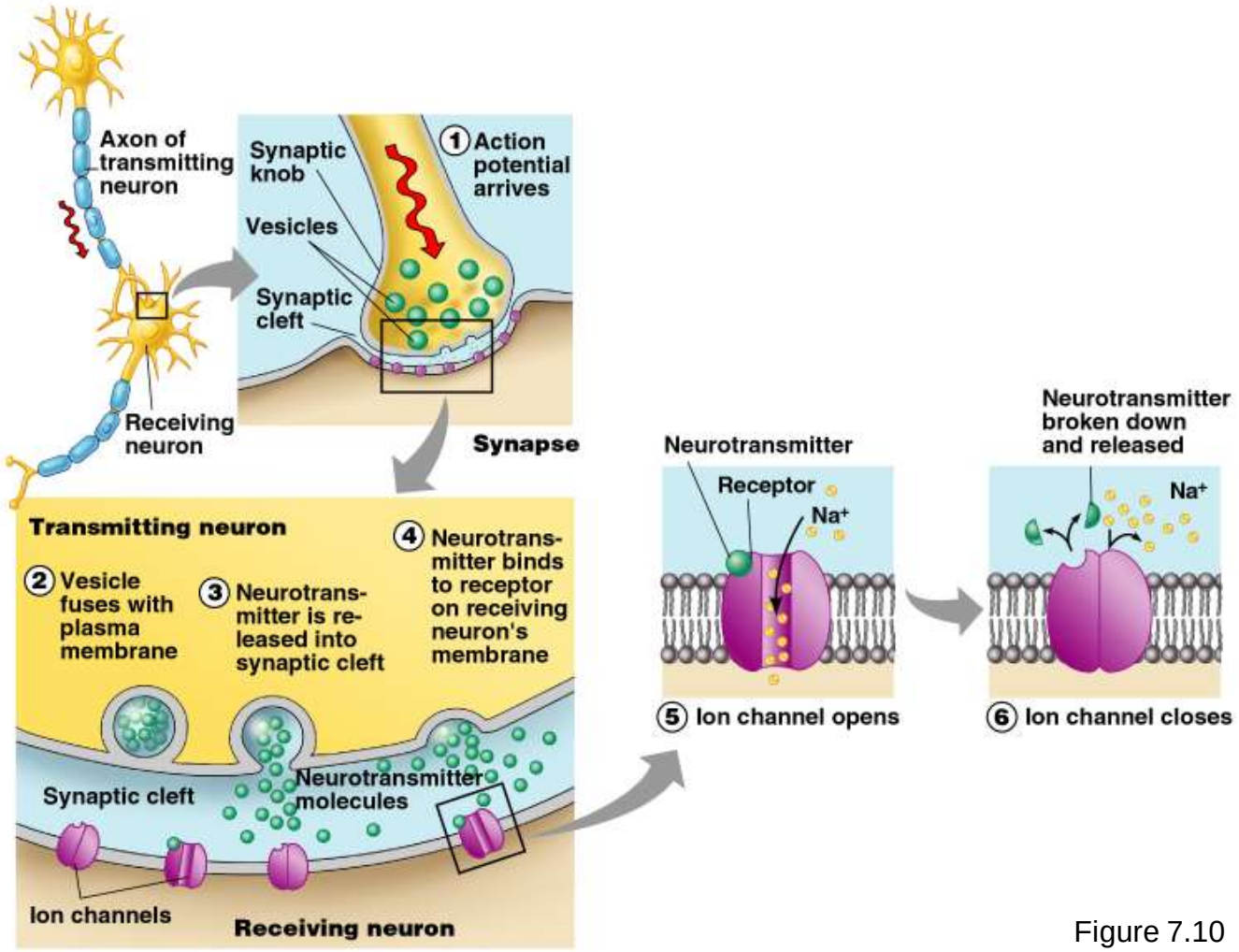
How Neurons Communicate at Synapses



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The Reflex Arc

- Reflex – rapid, predictable, and involuntary responses to stimuli
- Reflex arc – direct route from a sensory neuron, to an interneuron, to an effector

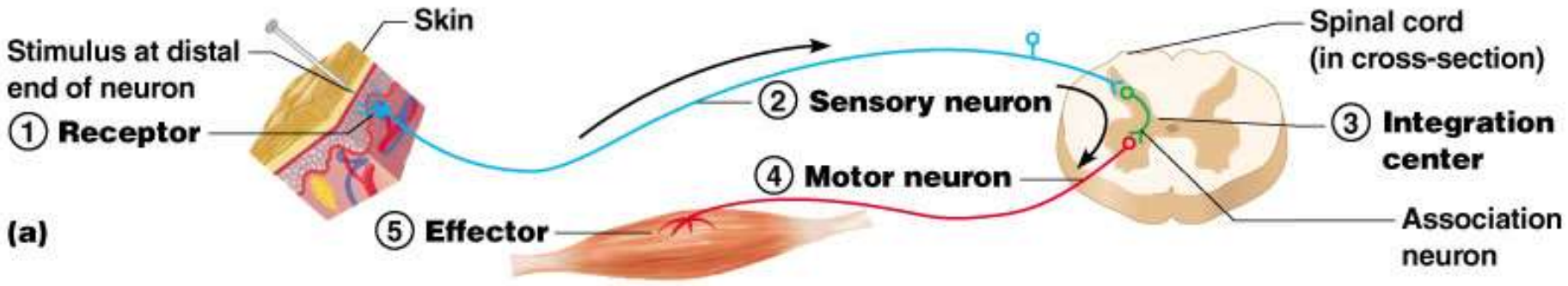

Figure 7.11a

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Simple Reflex Arc

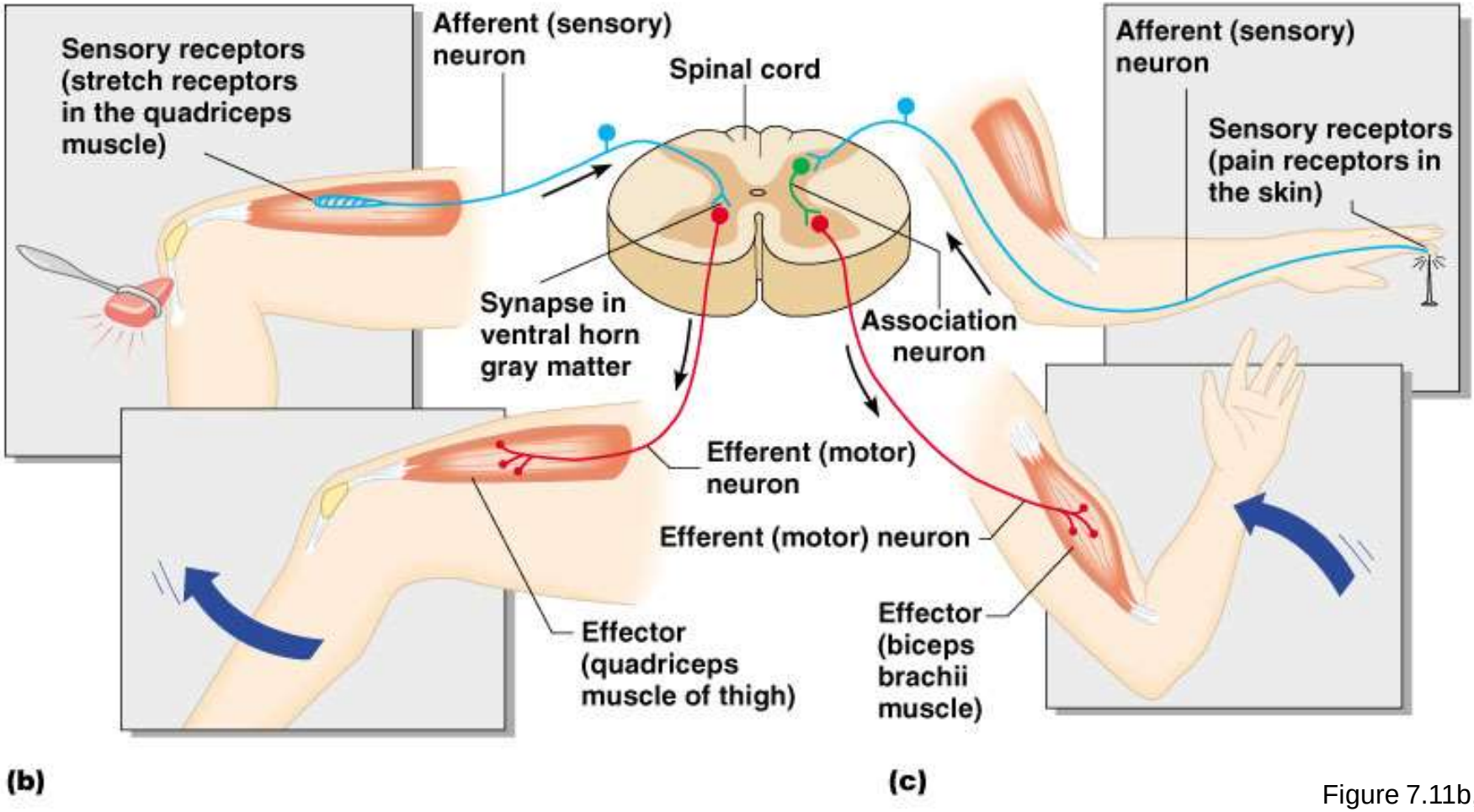

Figure 7.11b, c

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Types of Reflexes and Regulation

- Autonomic reflexes
  - Smooth muscle regulation
  - Heart and blood pressure regulation
  - Regulation of glands
  - Digestive system regulation
- Somatic reflexes
  - Activation of skeletal muscles

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Central Nervous System (CNS)

- CNS develops from the embryonic neural tube
  - The neural tube becomes the brain and spinal cord
  - The opening of the neural tube becomes the ventricles
    - Four chambers within the brain
    - Filled with cerebrospinal fluid

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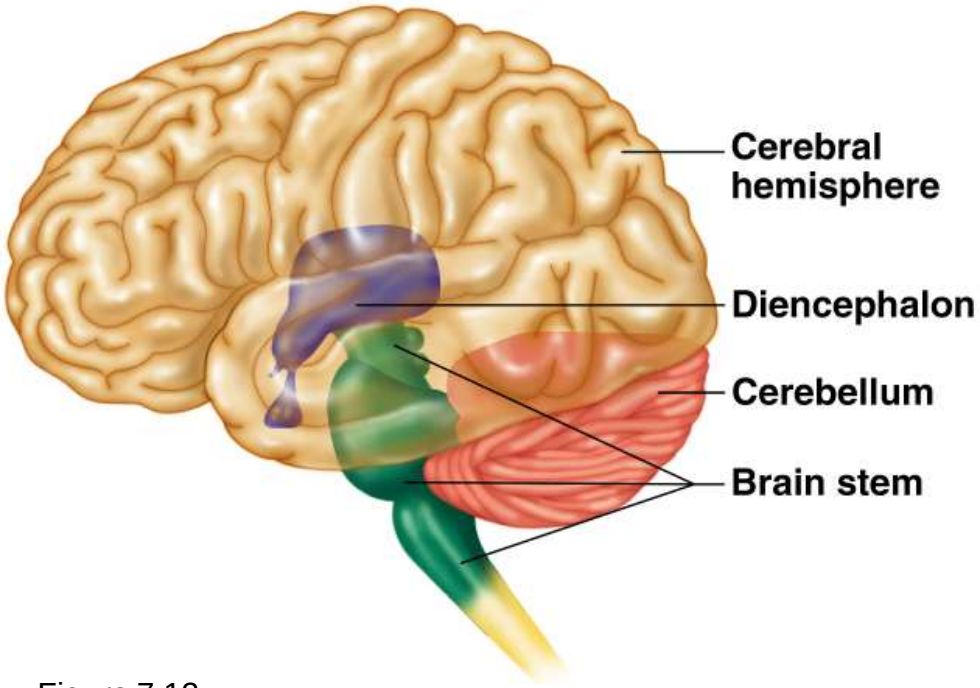
Regions of the Brain

- Cerebral hemispheres
- Diencephalon
- Brain stem
- Cerebellum



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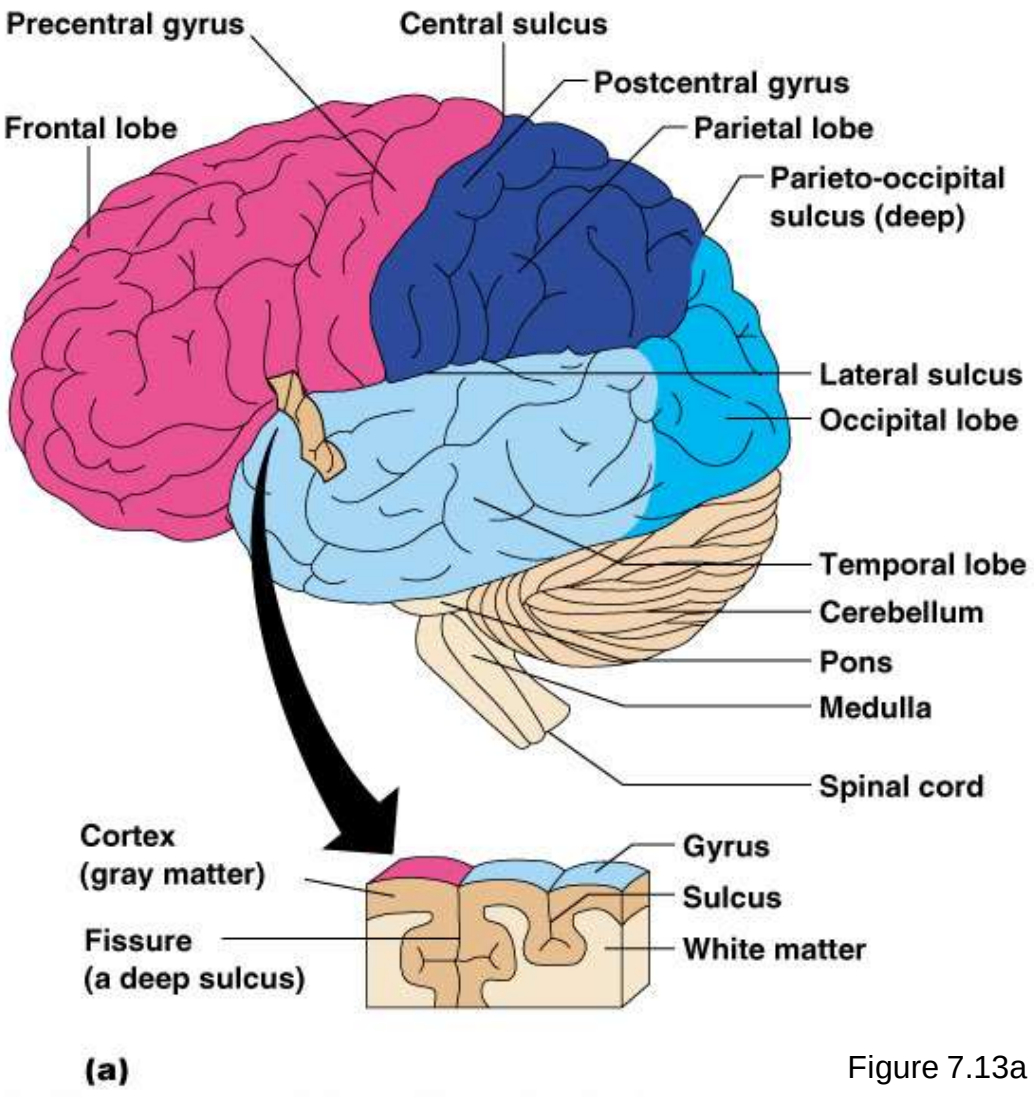
Cerebral Hemispheres (Cerebrum)

- Paired (left and right) superior parts of the brain
- Include more than half of the brain mass



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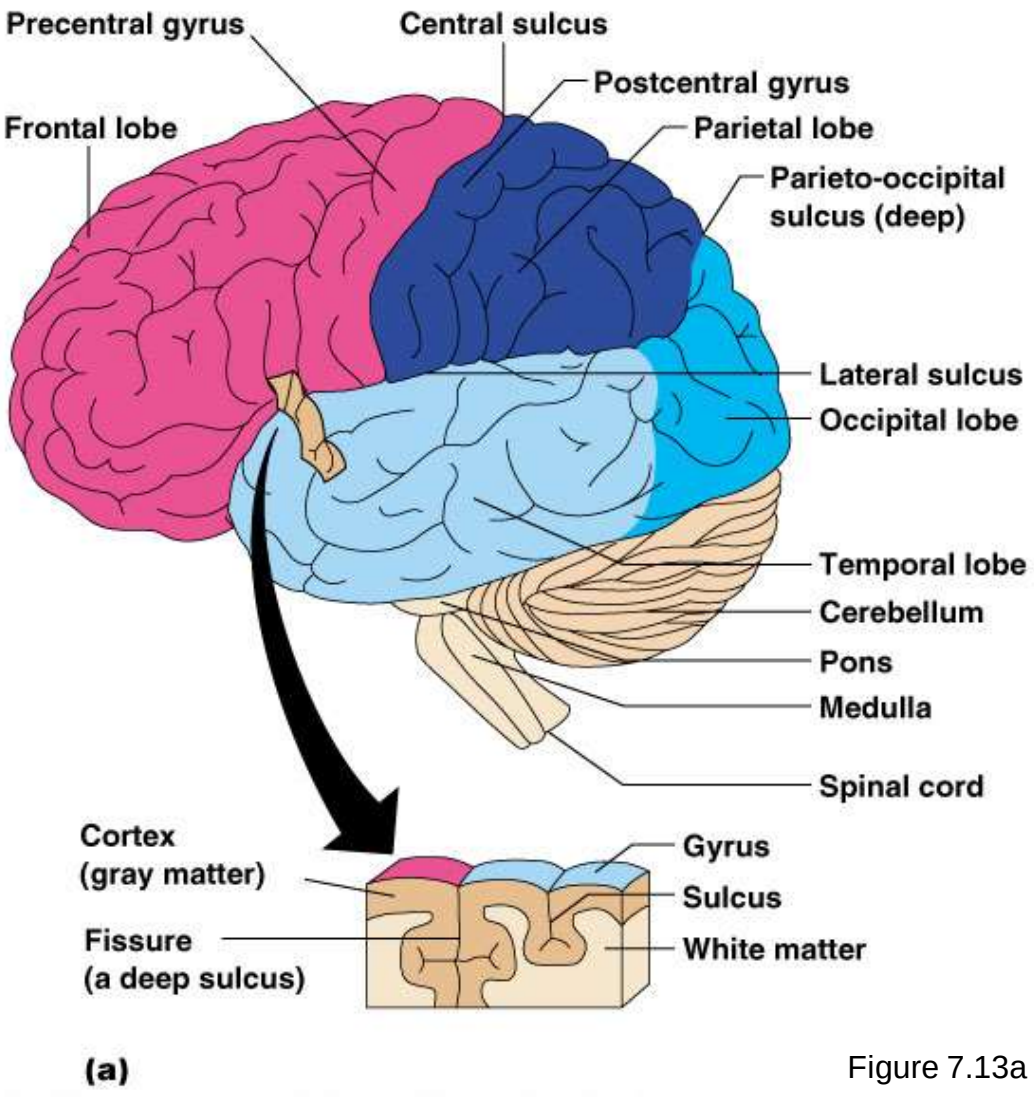
Cerebral Hemispheres (Cerebrum)

- The surface is made of ridges (gyri) and grooves (sulci)



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Lobes of the Cerebrum

- Fissures (deep grooves) divide the cerebrum into lobes
- Surface lobes of the cerebrum
  - Frontal lobe
  - Parietal lobe
  - Occipital lobe
  - Temporal lobe

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Lobes of the Cerebrum

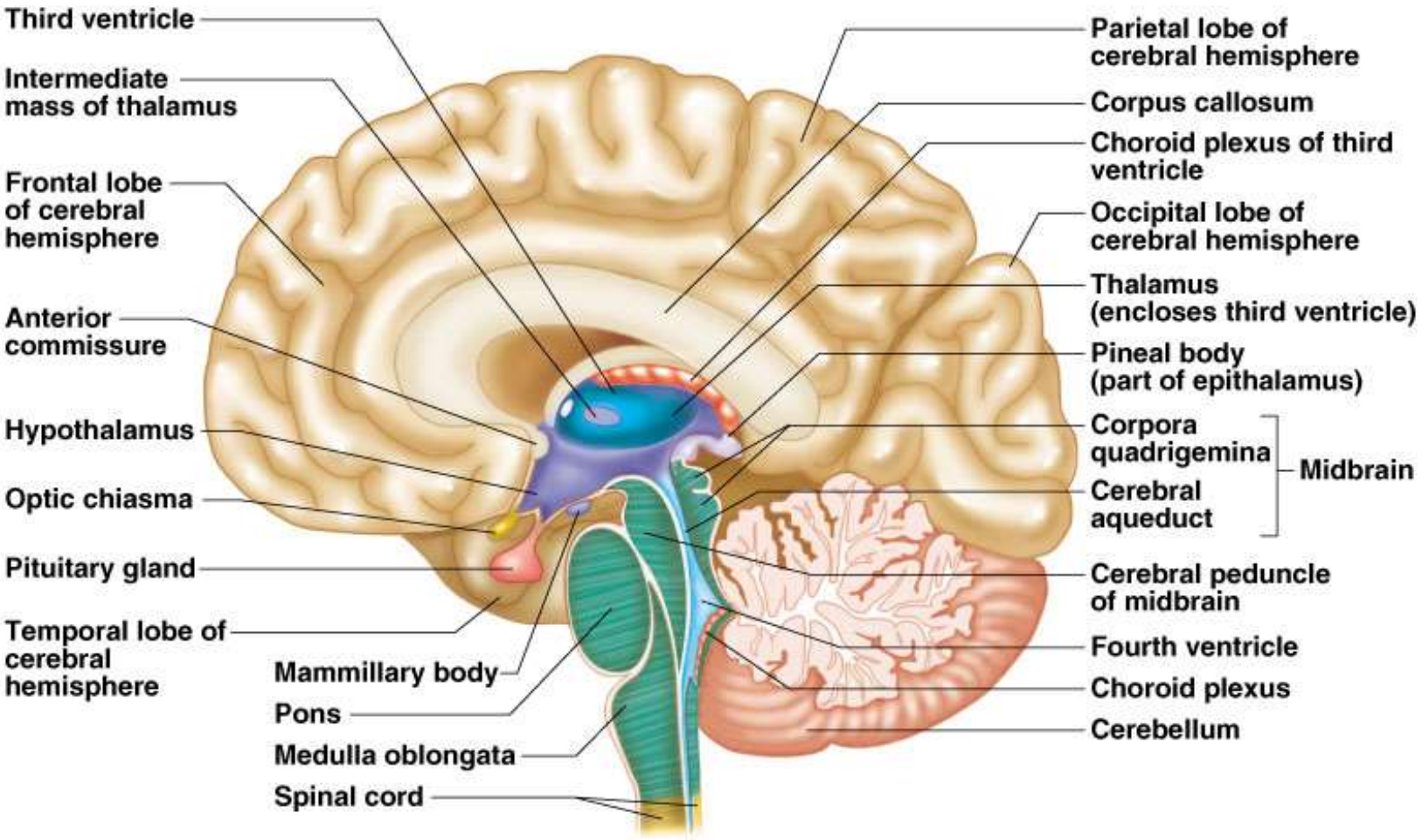

Figure 7.15a

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Specialized Areas of the Cerebrum

- Somatic sensory area – receives impulses from the body's sensory receptors
- Primary motor area – sends impulses to skeletal muscles
- Broca's area – involved in our ability to speak

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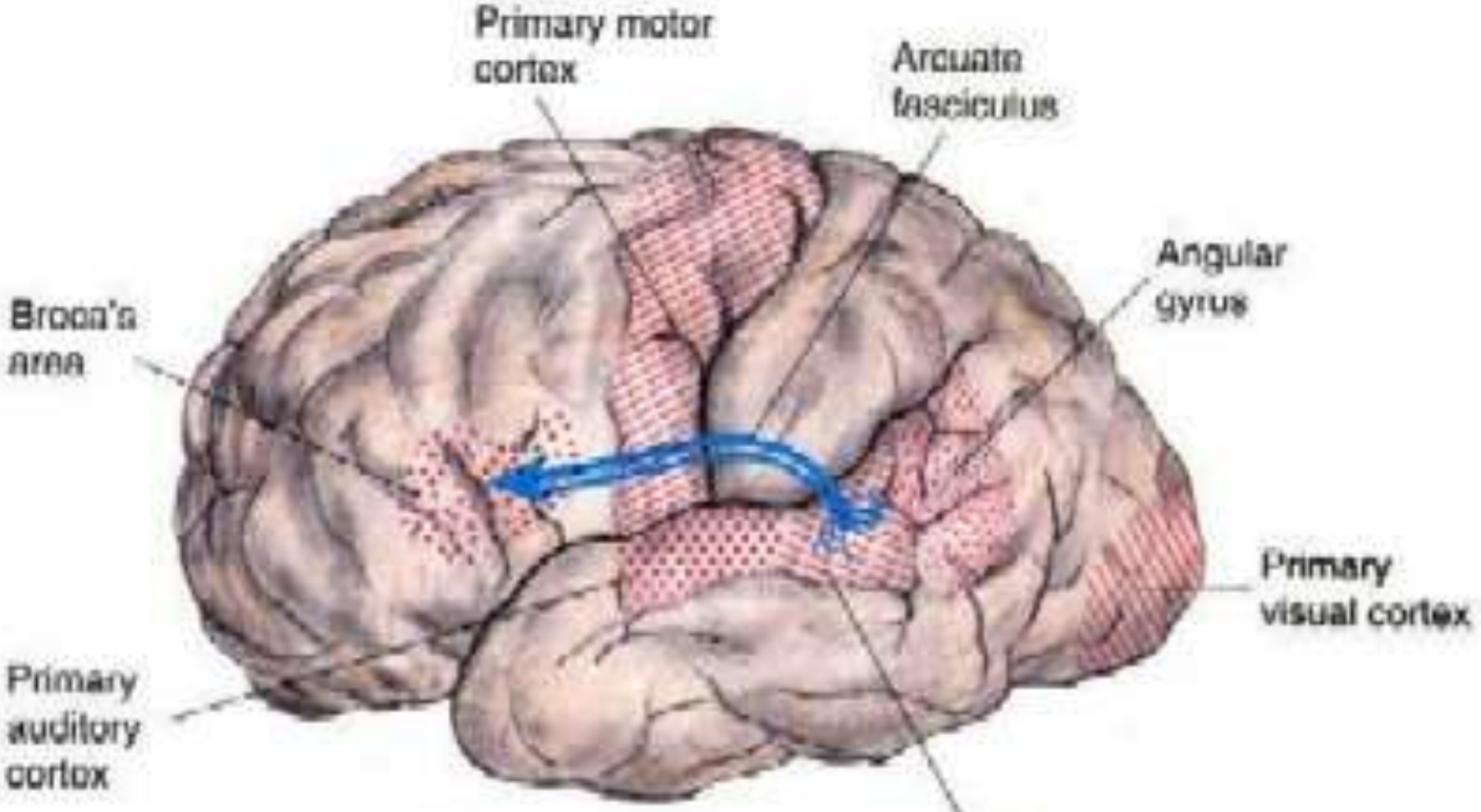
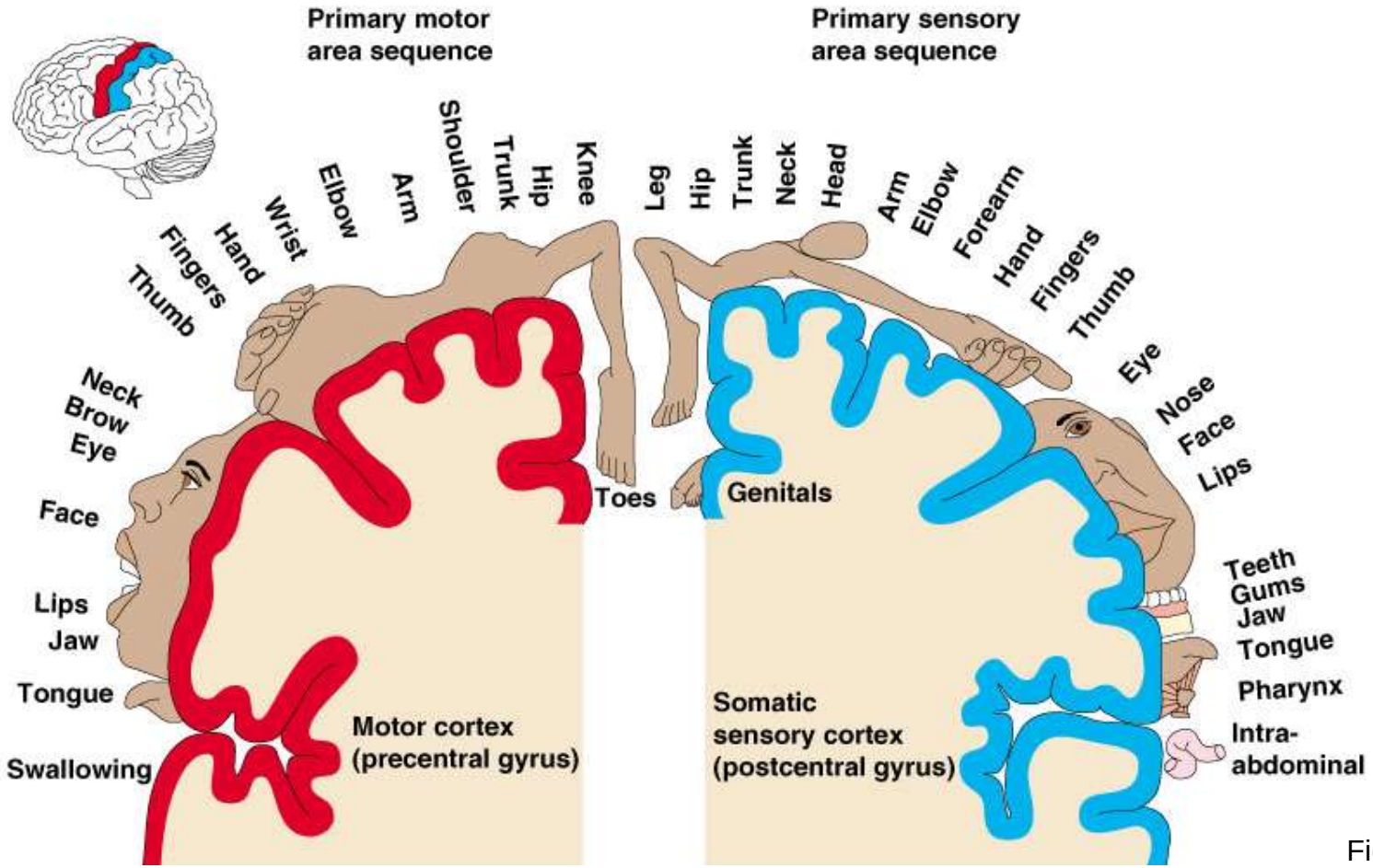


Figure 8. Components of the Wernicke-Geschwind Model.

Sensory and Motor Areas of the Cerebral Cortex



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Specialized Area of the Cerebrum

- Cerebral areas involved in special senses
  - Gustatory area (taste)
  - Visual area
  - Auditory area
  - Olfactory area

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Specialized Area of the Cerebrum

- Interpretation areas of the cerebrum
  - Speech/language region
  - Language comprehension region
  - General interpretation area

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Specialized Area of the Cerebrum

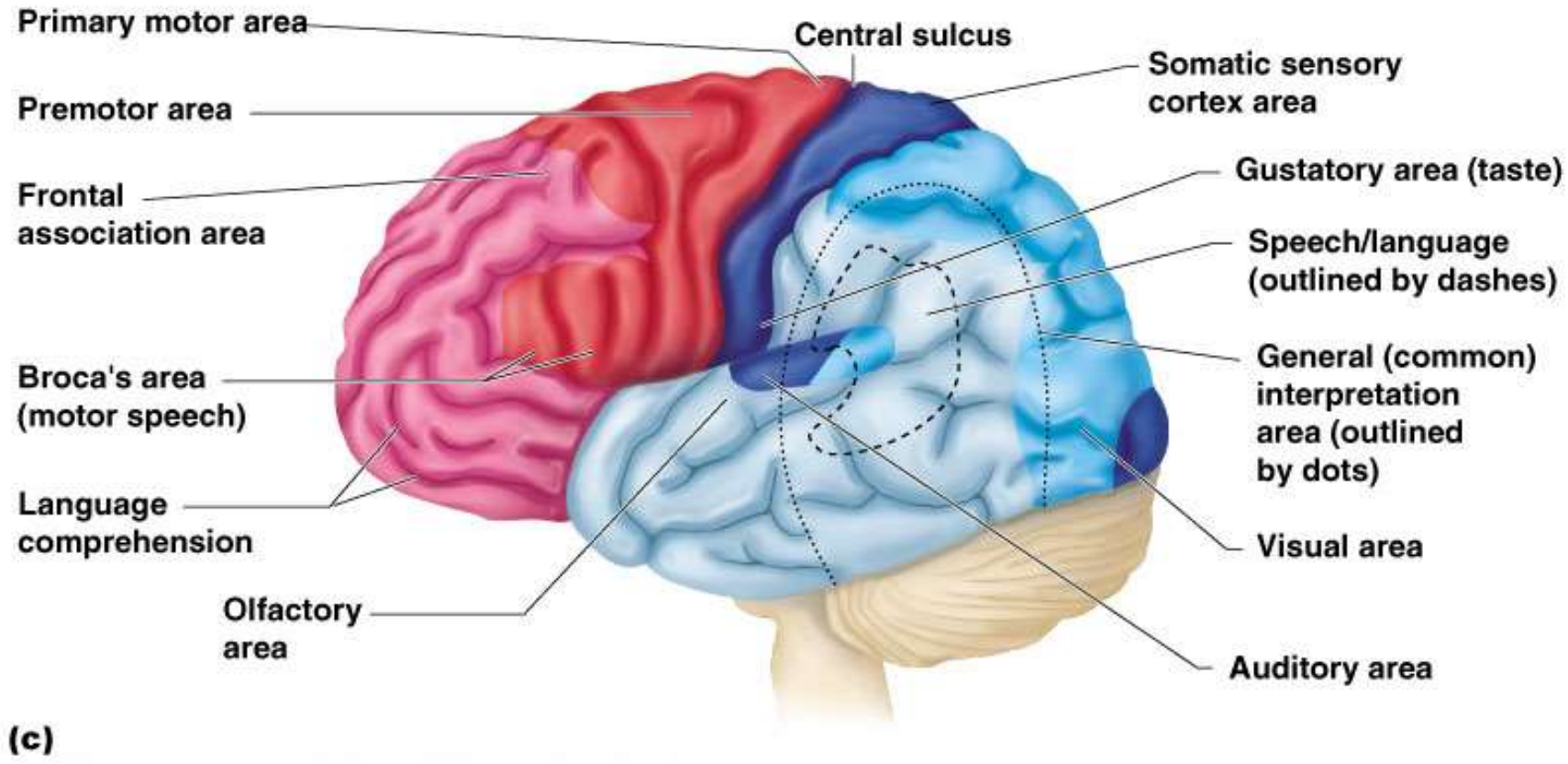

Figure 7.13c

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Layers of the Cerebrum

- Gray matter
  - Outer layer
  - Composed mostly of neuron cell bodies

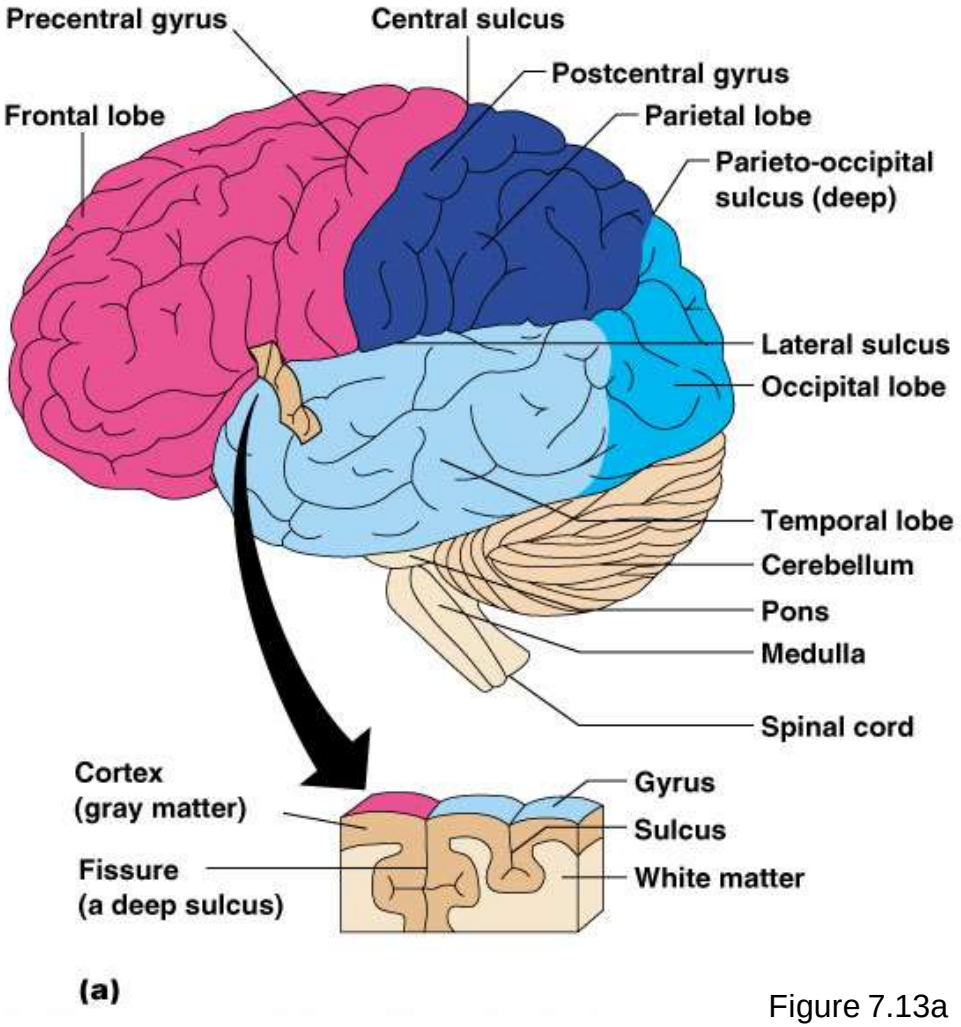

Figure 7.13a

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Layers of the Cerebrum

- White matter
  - Fiber tracts inside the gray matter
  - Example: corpus callosum connects hemispheres

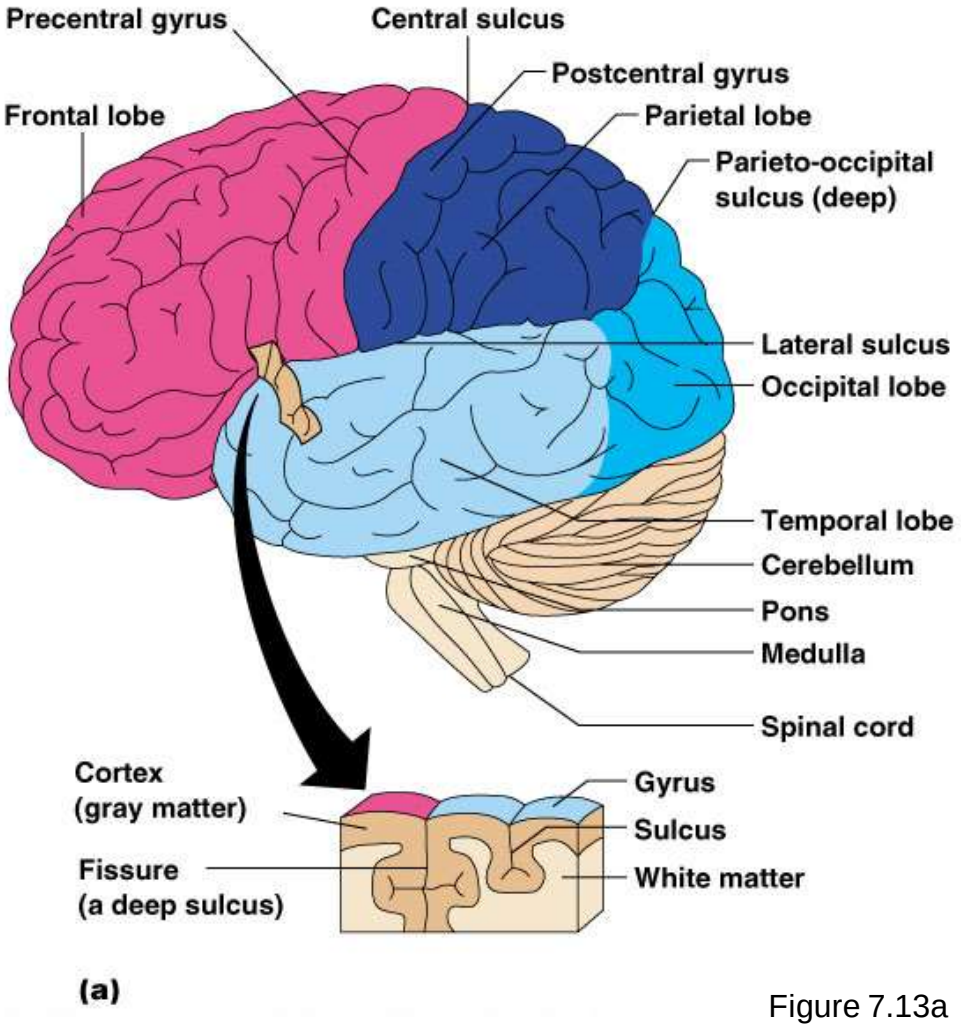

Figure 7.13a

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Layers of the Cerebrum

- Basal nuclei – internal islands of gray matter
- Regulates voluntary motor activities by modifying info sent to the motor cortex
- Problems = ie unable to control muscles, spastic, jerky
- Involved in Huntington's and Parkinson's Disease

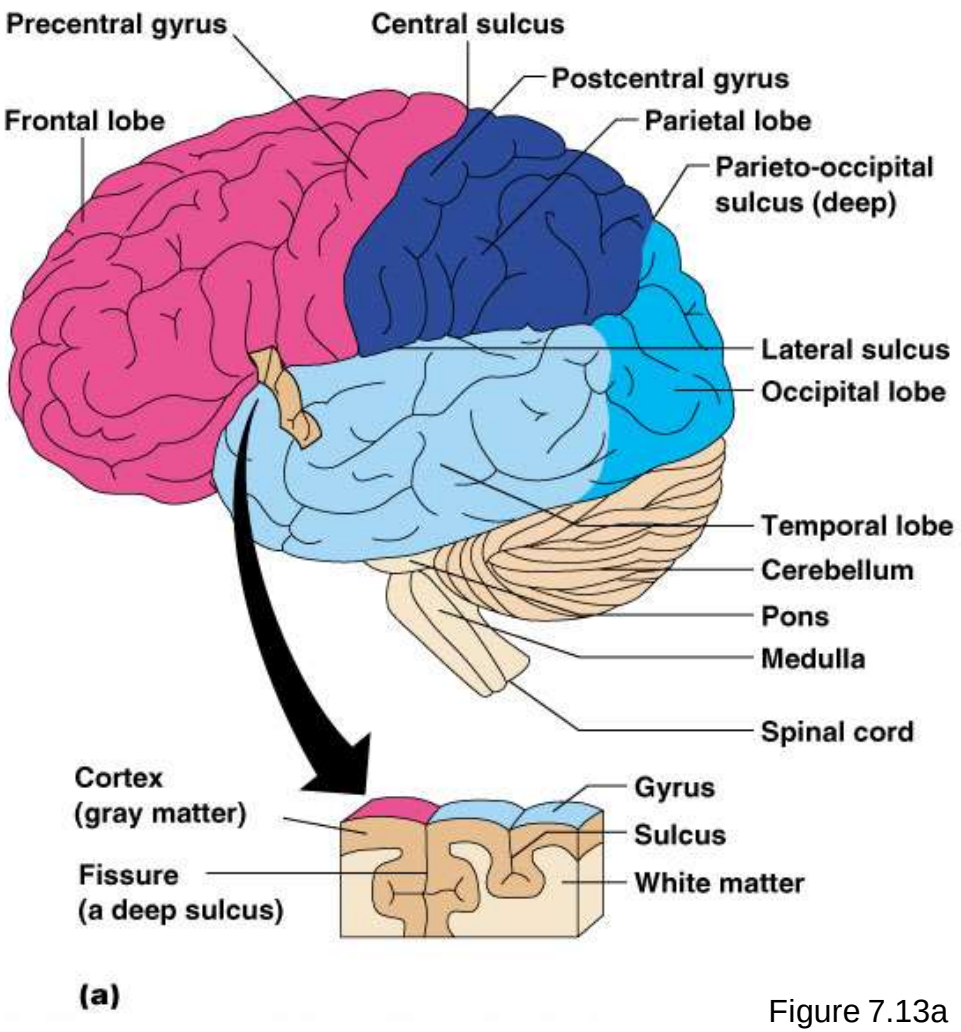

Figure 7.13a

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Diencephalon

- Sits on top of the brain stem
- Enclosed by the cerebral heispheres
- Made of three parts
  - Thalamus
  - Hypothalamus
  - Epithalamus

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Diencephalon

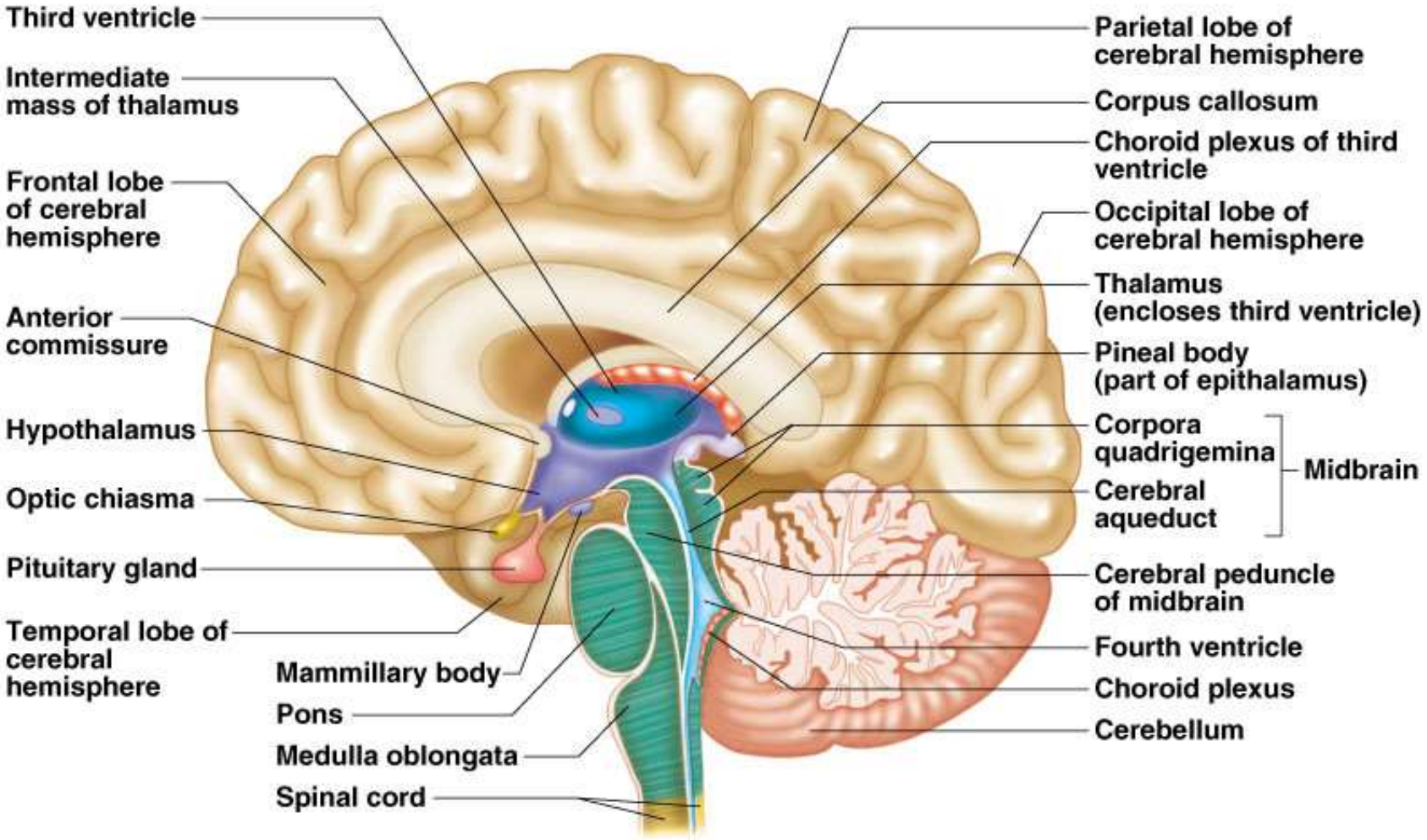

Figure 7.15

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Thalamus

- Surrounds the third ventricle
- The relay station for sensory impulses
- Transfers impulses to the correct part of the cortex for localization and interpretation

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Hypothalamus

- Under the thalamus
- Important autonomic nervous system center
  - Helps regulate body temperature
  - Controls water balance
  - Regulates metabolism

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Hypothalamus

- An important part of the limbic system (emotions)
- The pituitary gland is attached to the hypothalamus

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Epithalamus

- Forms the roof of the third ventricle
- Houses the pineal body (an endocrine gland)
- Includes the choroid plexus – forms cerebrospinal fluid

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Brain Stem

- Attaches to the spinal cord
- Parts of the brain stem
  - Midbrain
  - Pons
  - Medulla oblongata

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Brain Stem

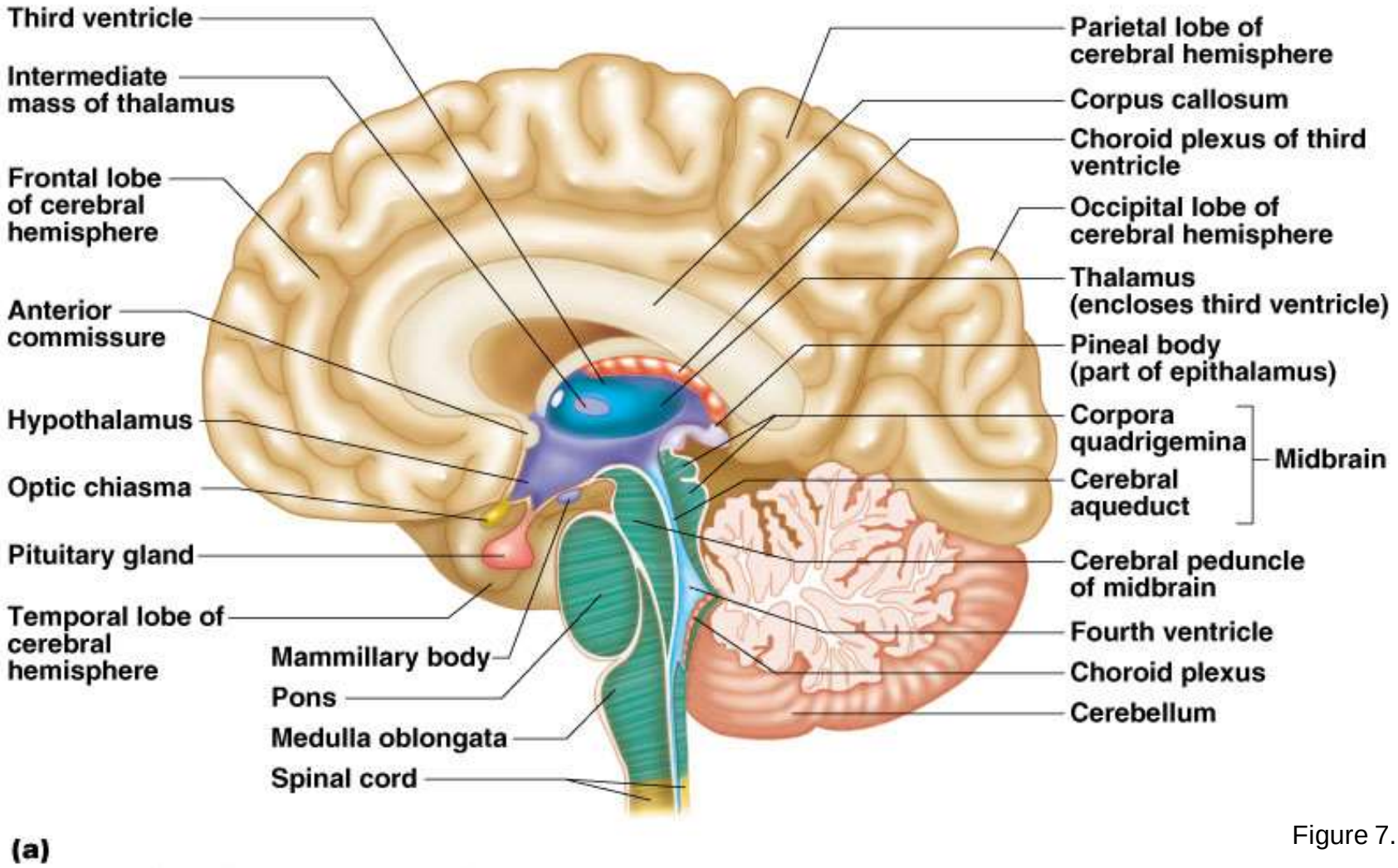

Figure 7.15a

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Midbrain

- Mostly composed of tracts of nerve fibers
  - Reflex centers for vision and hearing
  - Cerebral aquaduct – 3rd-4th ventricles

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Pons

- The bulging center part of the brain stem
- Mostly composed of fiber tracts
- Includes nuclei involved in the control of breathing

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Medulla Oblongata

- The lowest part of the brain stem
- Merges into the spinal cord
- Includes important fiber tracts
- Contains important control centers
  - Heart rate control
  - Blood pressure regulation
  - Breathing
  - Swallowing
  - Vomiting

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Cerebellum

- Two hemispheres with convoluted surfaces
- Provides involuntary coordination of body movements

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Cerebellum

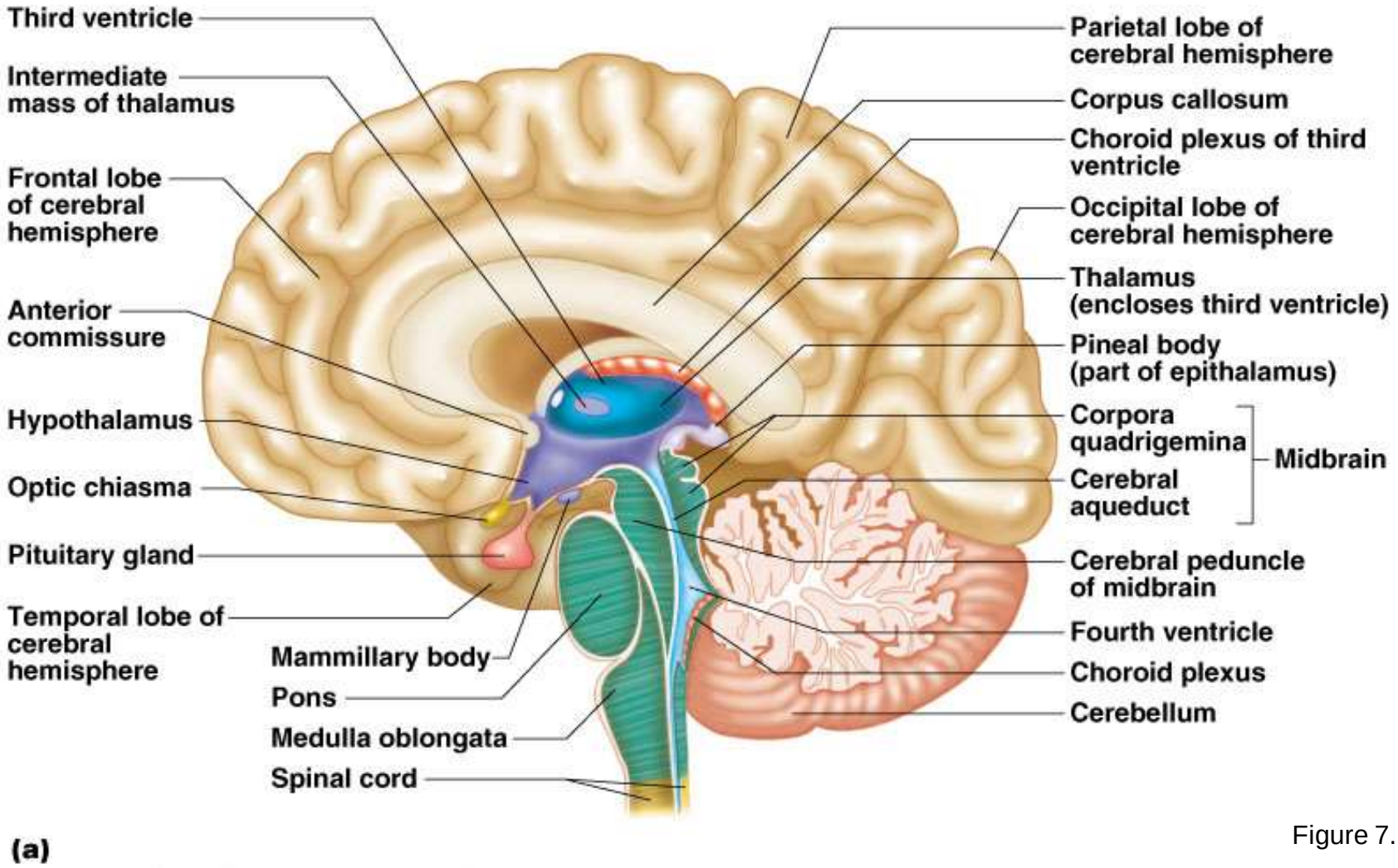

Figure 7.15a

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Protection of the Central Nervous System

- Scalp and skin
- Skull and vertebral column
- Meninges

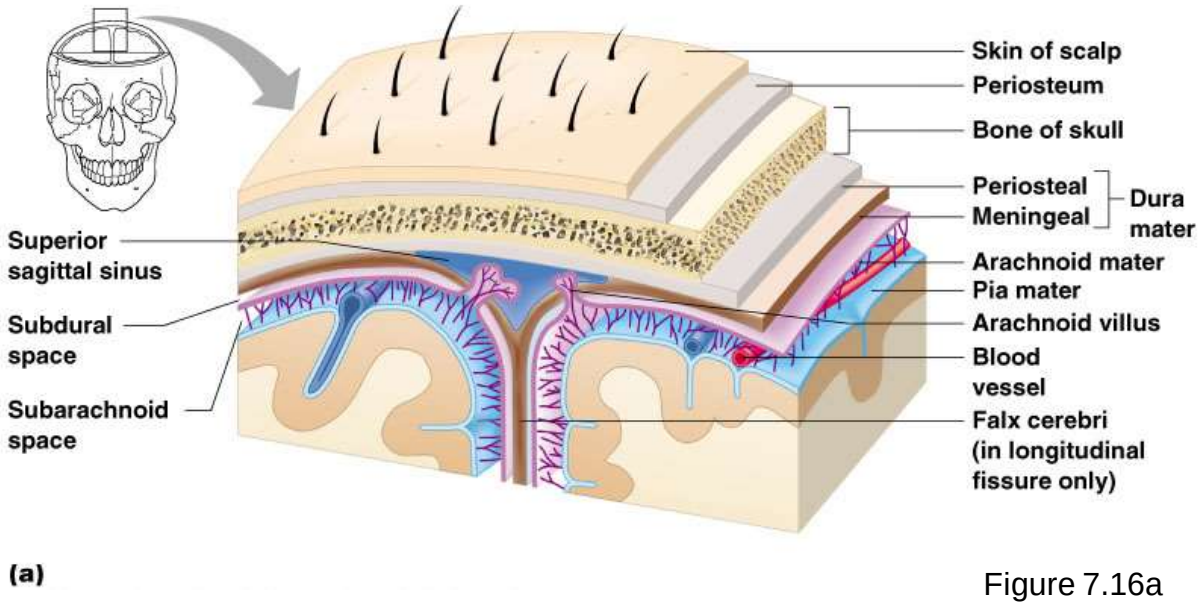

Figure 7.16a

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Protection of the Central Nervous System

- Cerebrospinal fluid
- Blood brain barrier

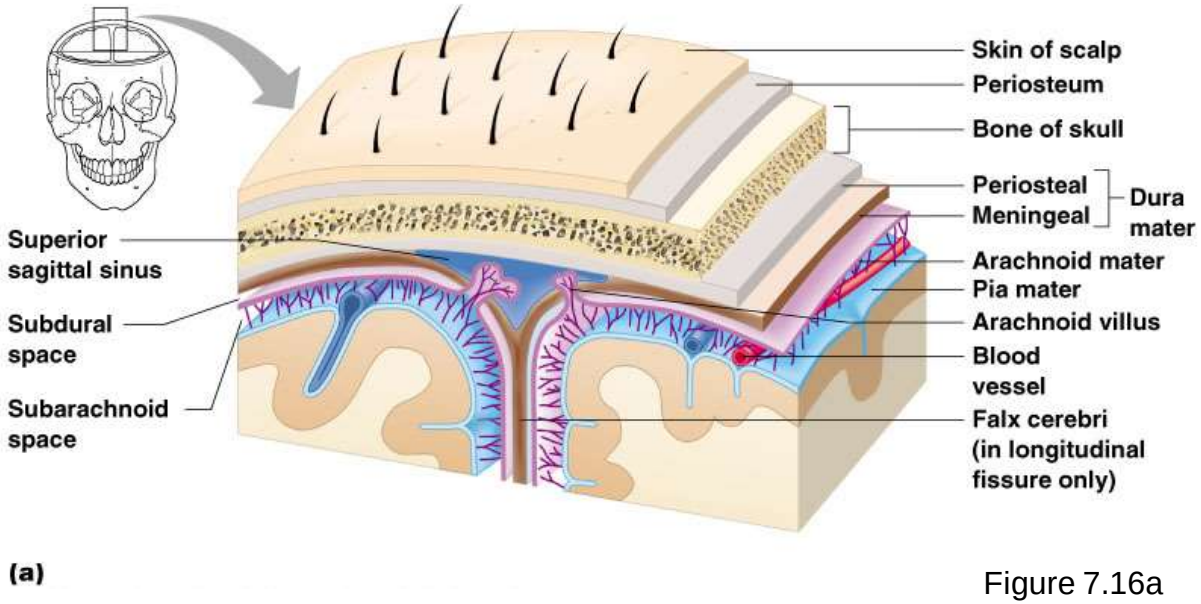

Figure 7.16a

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Meninges

- Dura mater
  - Double-layered external covering
    - Periosteum – attached to surface of the skull
    - Meningeal layer – outer covering of the brain
  - Folds inward in several areas

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Meninges

- Arachnoid layer
  - Middle layer
  - Web-like
- Pia mater
  - Internal layer
  - Clings to the surface of the brain

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Cerebrospinal Fluid

- Similar to blood plasma composition
- Formed by the choroid plexus
- Forms a watery cushion to protect the brain
- Circulated in arachnoid space, ventricles, and central canal of the spinal cord

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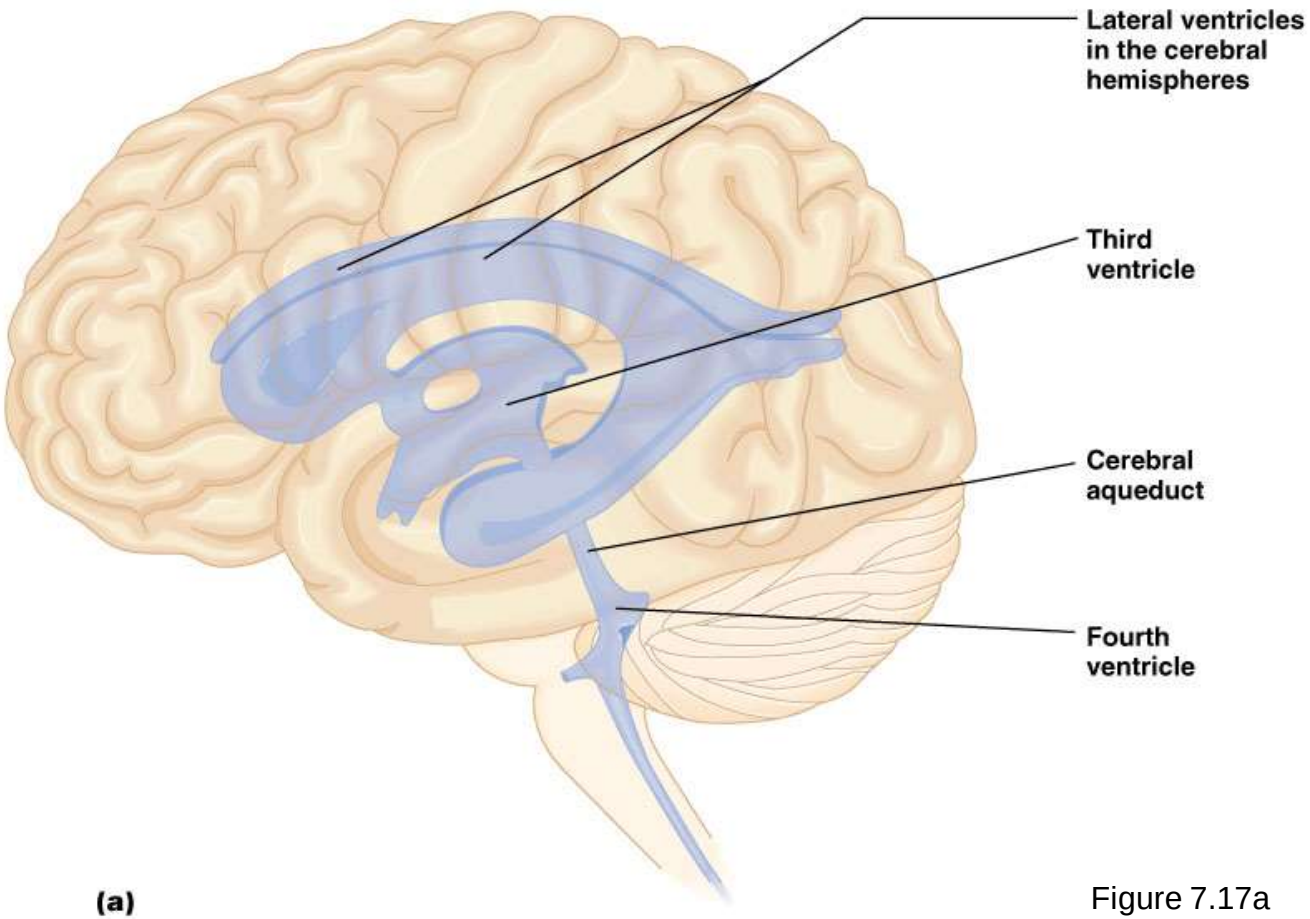
Ventricles and Location of the Cerebrospinal Fluid



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Ventricles and Location of the Cerebrospinal Fluid

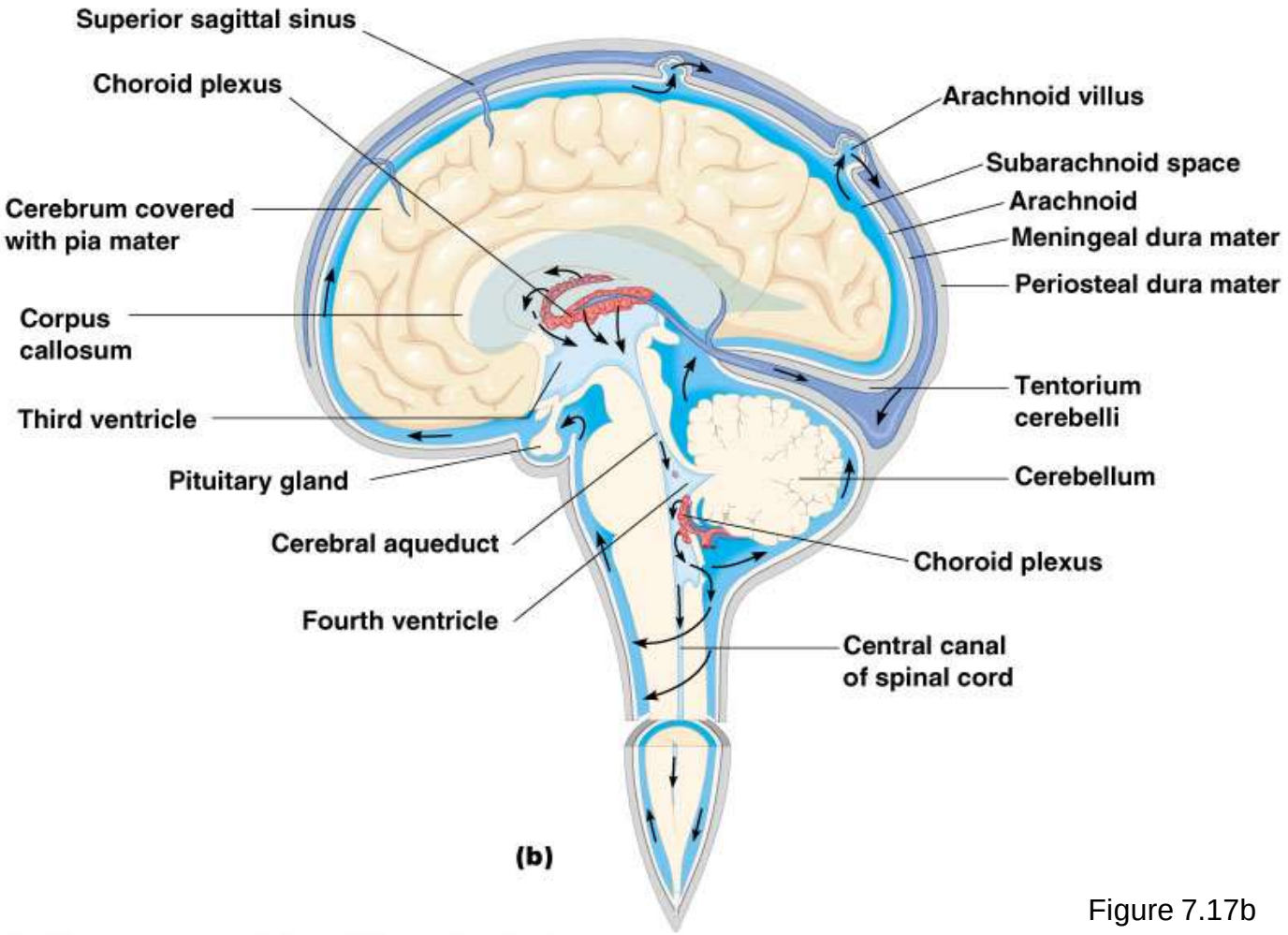

Figure 7.17b

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Blood Brain Barrier

- Includes the least permeable capillaries of the body
- Excludes many potentially harmful substances
- Useless against some substances
  - Fats and fat soluble molecules
  - Respiratory gases
  - Alcohol
  - Nicotine
  - Anesthesia

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Traumatic Brain Injuries

- Concussion
  - Slight brain injury
  - No permanent brain damage
- Contusion
  - Nervous tissue destruction occurs
  - Nervous tissue does not regenerate
- Cerebral edema
  - Swelling from the inflammatory response
  - May compress and kill brain tissue

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Cerebrovascular Accident (CVA)

- Commonly called a stroke
- The result of a ruptured blood vessel supplying a region of the brain
- Brain tissue supplied with oxygen from that blood source dies
- Loss of some functions or death may result

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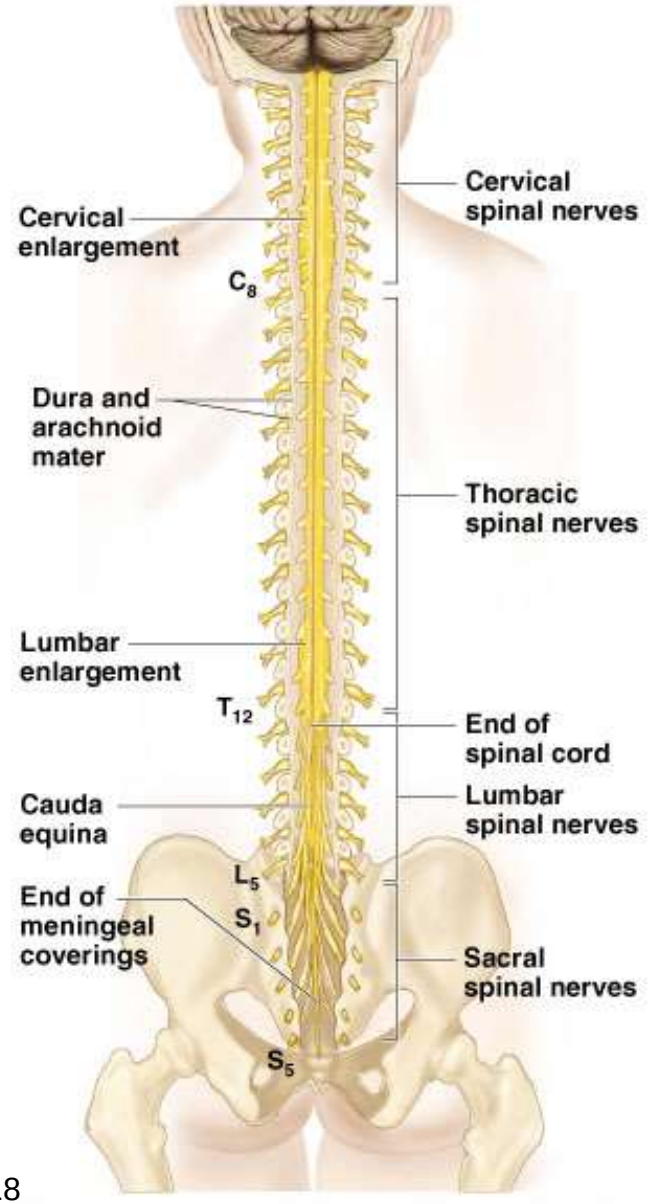
Spinal Cord

- Extends from the medulla oblongata to the region of T12
- Below T12 is the cauda equina (a collection of spinal nerves)
- Enlargements occur in the cervical and lumbar regions



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Alzheimer's Disease

- Progressive degenerative brain disease
- Mostly seen in the elderly, but may begin in middle age
- Structural changes in the brain include abnormal protein deposits and twisted fibers within neurons
- Victims experience memory loss, irritability, confusion and ultimately, hallucinations and death

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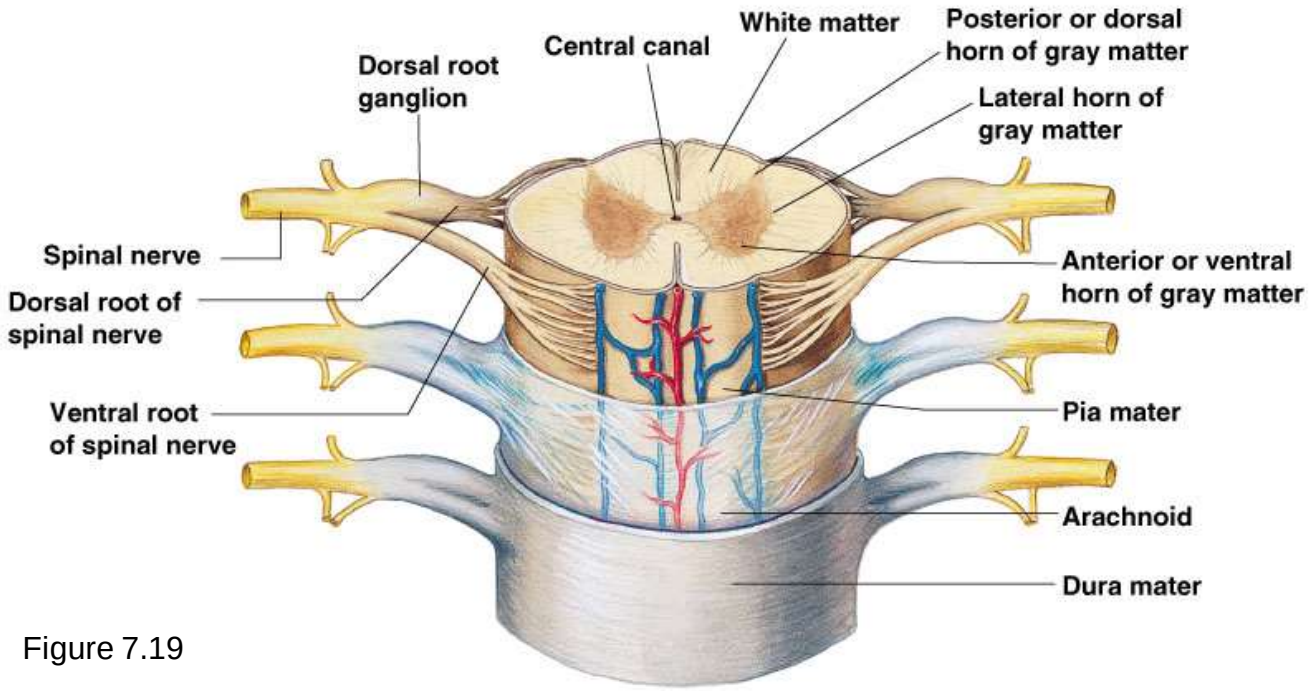
Spinal Cord Anatomy

- Exterior white mater – conduction tracts



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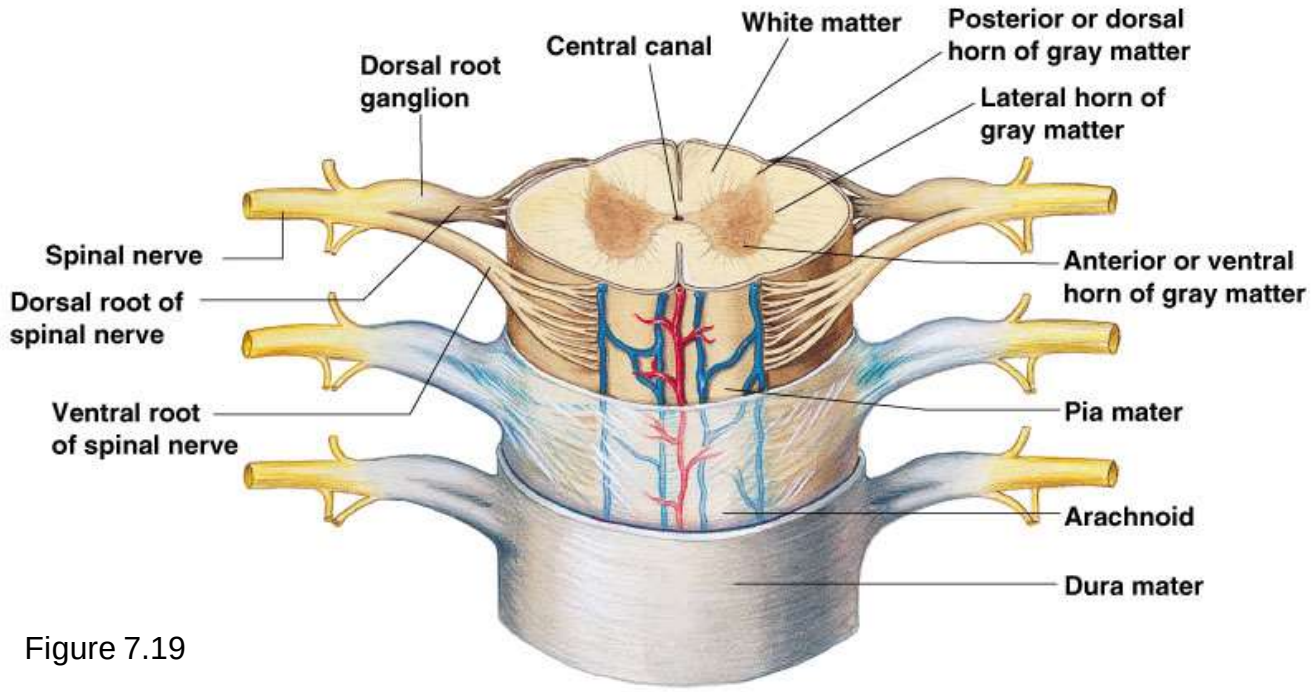
Spinal Cord Anatomy

- Internal gray matter - mostly cell bodies
  - Dorsal (posterior) horns
  - Anterior (ventral) horns



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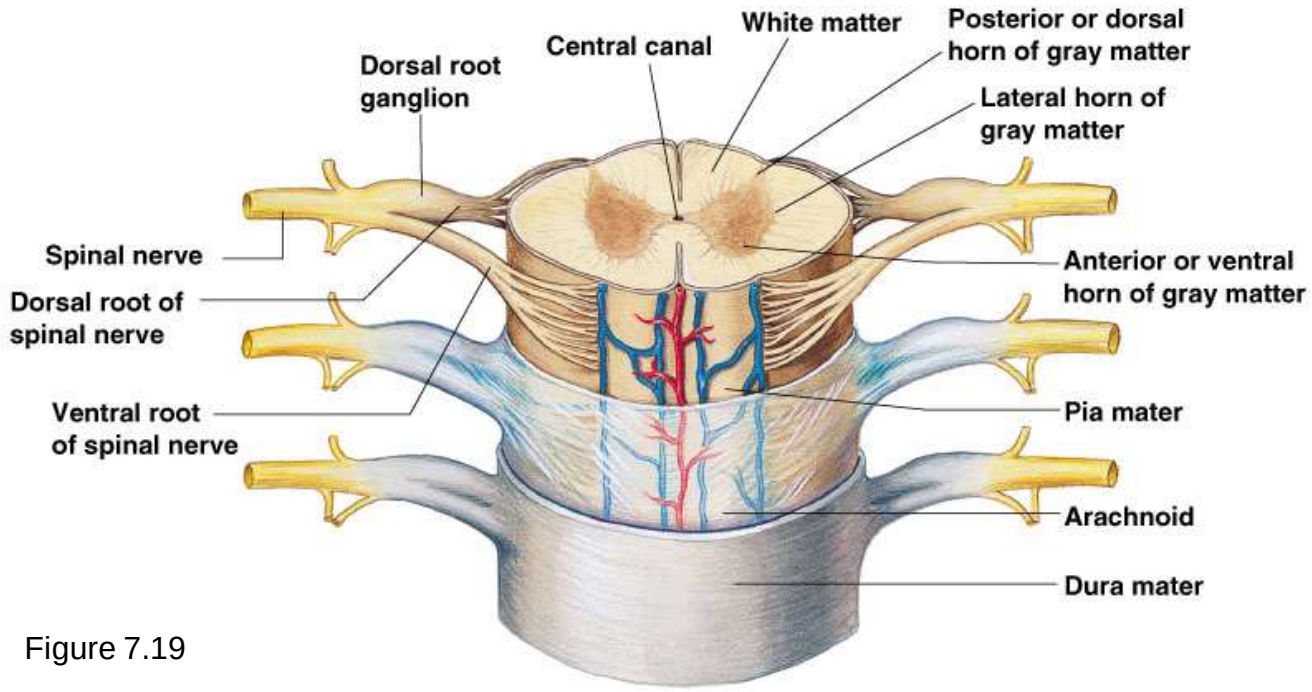
Spinal Cord Anatomy

- Central canal filled with cerebrospinal fluid



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Spinal Cord Anatomy

- Meninges cover the spinal cord
- Nerves leave at the level of each vertebrae
  - Dorsal root
    - Associated with the dorsal root ganglia – collections of cell bodies outside the central nervous system
  - Ventral root

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Peripheral Nervous System

- Nerves and ganglia outside the central nervous system
- Nerve = bundle of neuron fibers
- Neuron fibers are bundled by connective tissue

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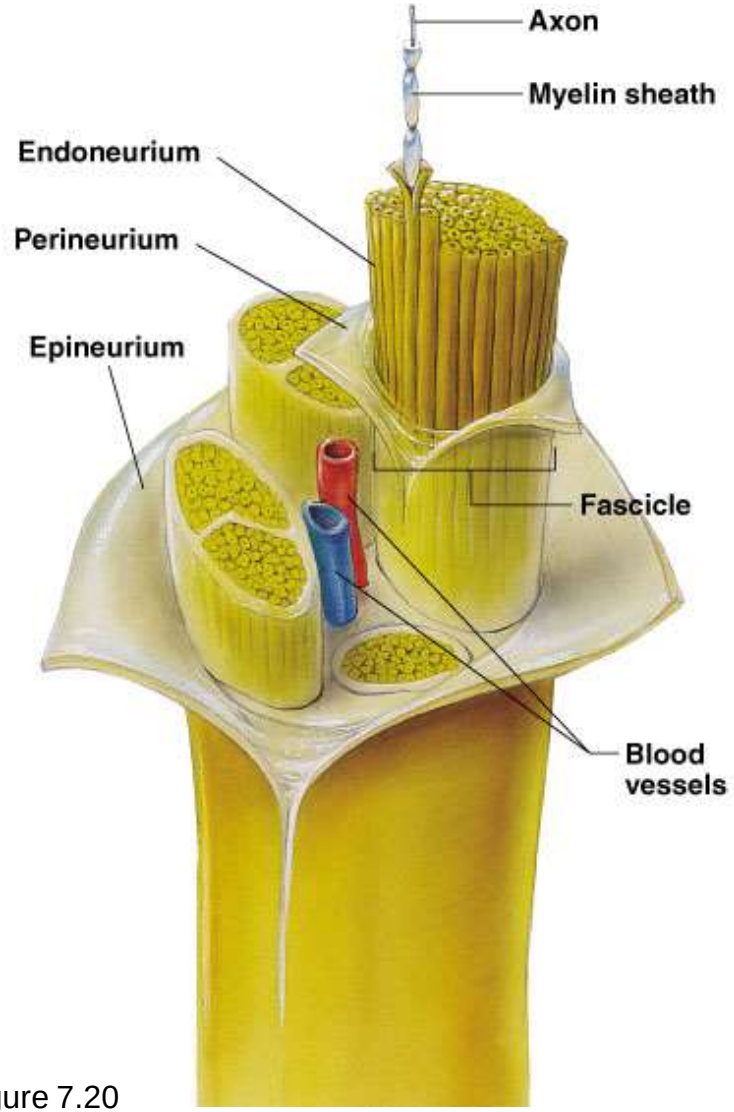
Structure of a Nerve

- Endoneurium surrounds each fiber
- Groups of fibers are bound into fascicles by perineurium
- Fascicles are bound together by epineurium



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Classification of Nerves

- Mixed nerves – both sensory and motor fibers
- Afferent (sensory) nerves – carry impulses toward the CNS
- Efferent (motor) nerves – carry impulses away from the CNS

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Spinal Nerves

- There is a pair of spinal nerves at the level of each vertebrae for a total of 31 pairs

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Spinal Nerves

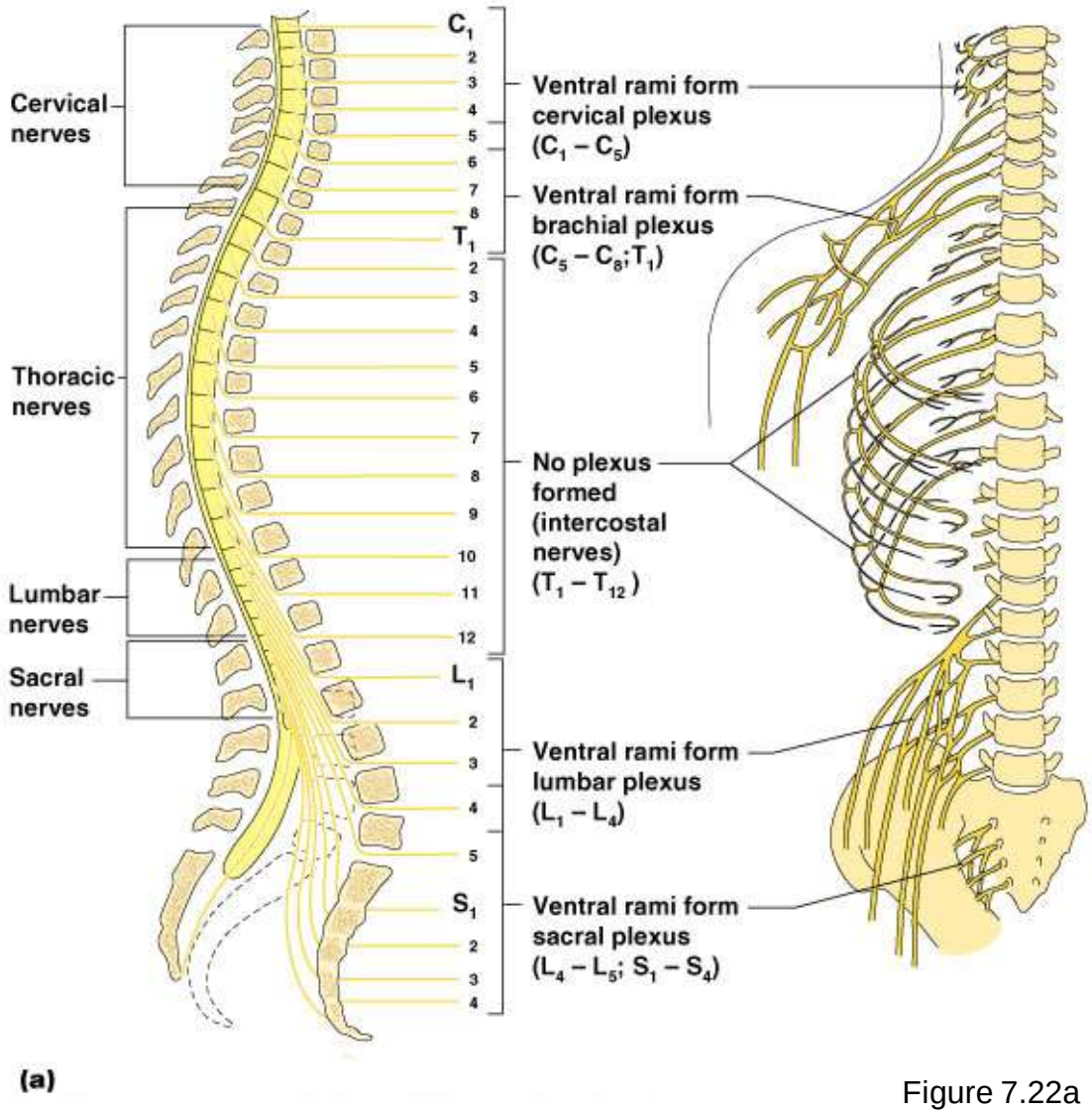

Figure 7.22a

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Autonomic Nervous System

- The involuntary branch of the nervous system
- Consists of only motor nerves
- Divided into two divisions
  - Sympathetic division
  - Parasympathetic division

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Comparison of Somatic and Autonomic Nervous Systems

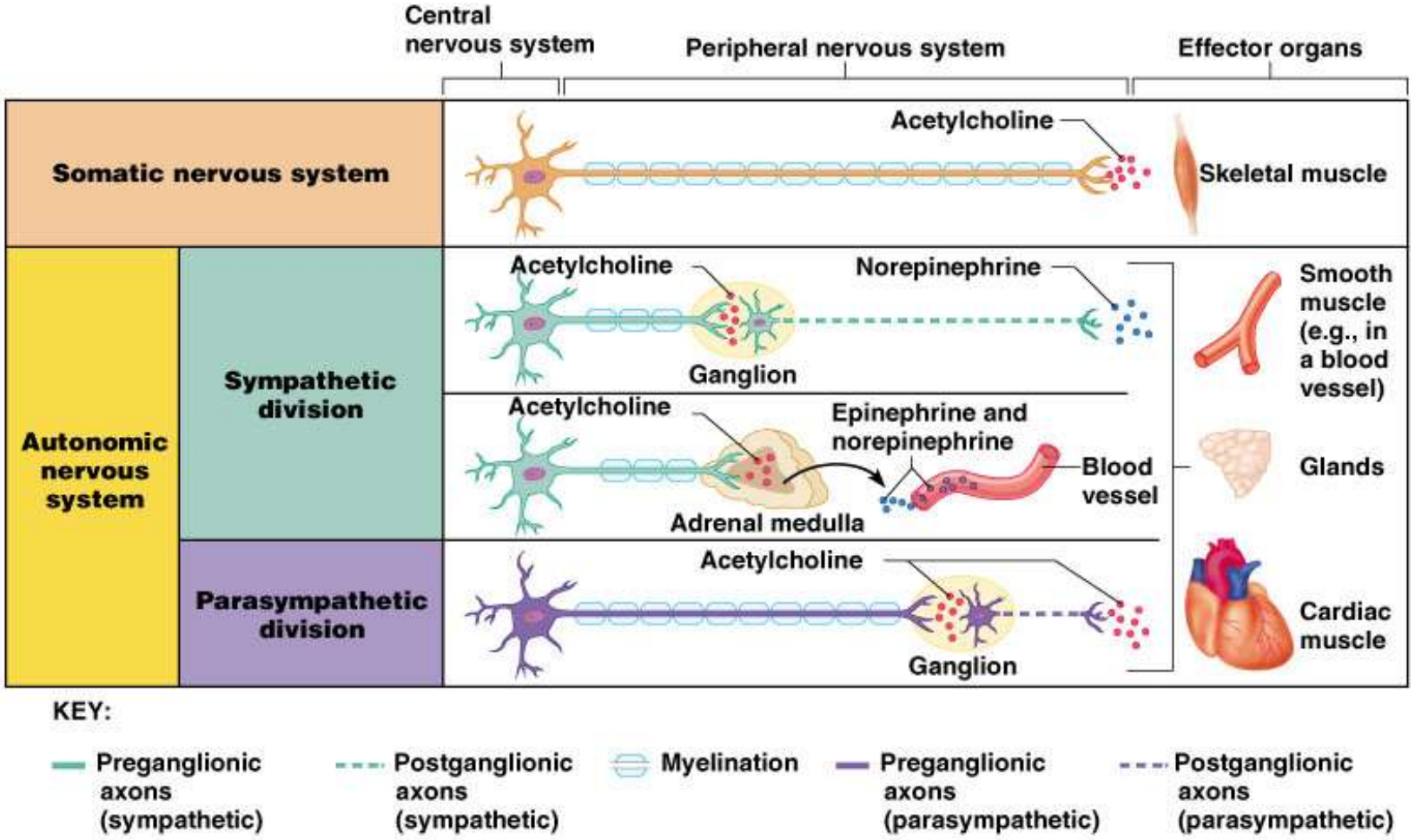

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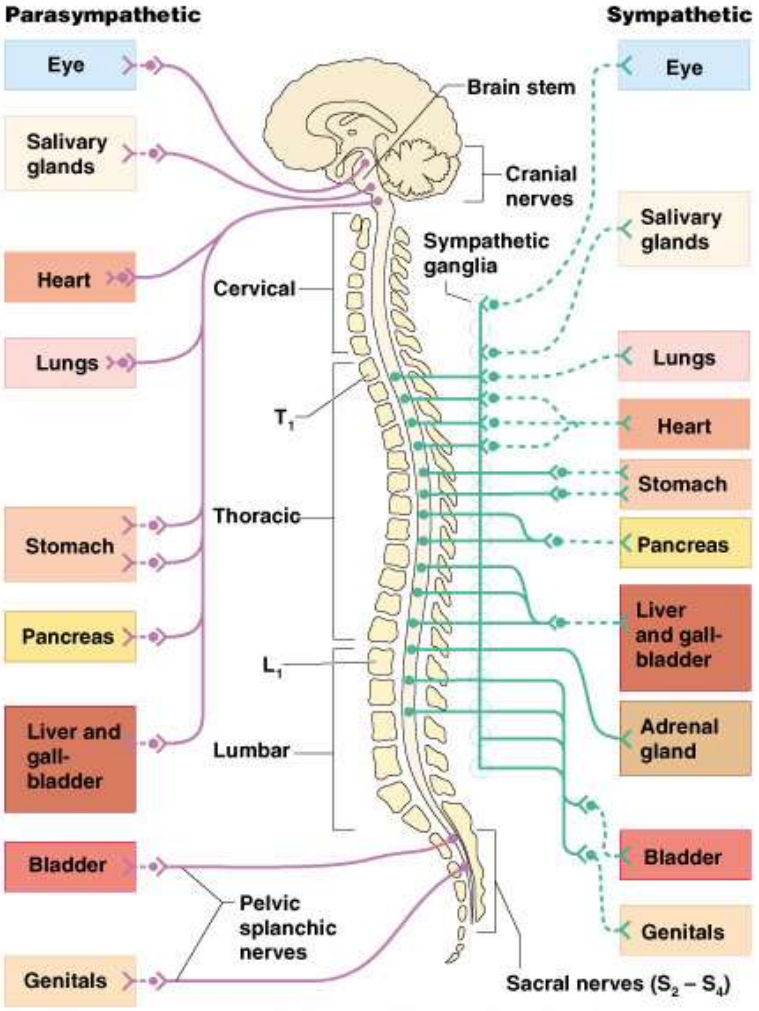
Figure 7.24

Anatomy of the Autonomic Nervous System



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Autonomic Functioning

- Sympathetic – “fight-or-flight”
  - Response to unusual stimulus
  - Takes over to increase activities
  - Remember as the “E” division = exercise, excitement, emergency, and embarrassment

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Autonomic Functioning

- Parasympathetic – housekeeping activites
  - Conserves energy
  - Maintains daily necessary body functions
  - Remember as the “D” division - digestion, defecation, and diuresis

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Development Aspects of the Nervous System

- The nervous system is formed during the first month of embryonic development
- Any maternal infection can have extremely harmful effects
- The hypothalamus is one of the last areas of the brain to develop

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Development Aspects of the Nervous System

- No more neurons are formed after birth, but growth and maturation continues for several years (new evidence!)
- The brain reaches maximum weight as a young adult
- However, we can always grow dendrites!

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Pune District Education Association's Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre.

Nuclear Magnetic Resonance Spectroscopy

Presented by : Dr. Vijaya U. Barge
(Vice Principal & Professor)

Learning Objectives :

- Nuclear magnetic resonance (NMR) spectroscopy is a very important spectrosc opio method that provides crucial information relating to chemical structure of molecules.
- This chapter deals with proton NMR, a technique giving information about the type and number of magnetically distinct protons in a molecule.
- All aspects of this highly versatile technique have been covered includinginstru mentation details.Sample handling, solvent selection and use of internal standa rds have beendiscussed in detail.
- Chemical environment of the protons determines their chemical shifts. Various aspects affecting the chemical shifts of protons have been highlighted The conc ept of multiplicity of the peaks has been extensively discussed. It is an importa nt facet of proton NMR, and its interpretation provides extremely valuable stru ctural information.The origin and concept of spin-spin coupling has been given in the chapter and interpretation has been given taking relevant examples of pr

Contents

- Principle
- Chemical shift
- Reference standard
- Factors affecting chemical shift
- Spin-spin coupling
- Coupling constant
- Chemical and magnetic equivalence
- Solvents used
- Instrumentation
- Applications

Public

Principle

1. **The spinning nucleus -** Nucleus of the hydrogen atom behaves as a spinning bar magnet, because it possesses both electric charge and magnetic spin . Spinning charged body will generate a magnetic field .

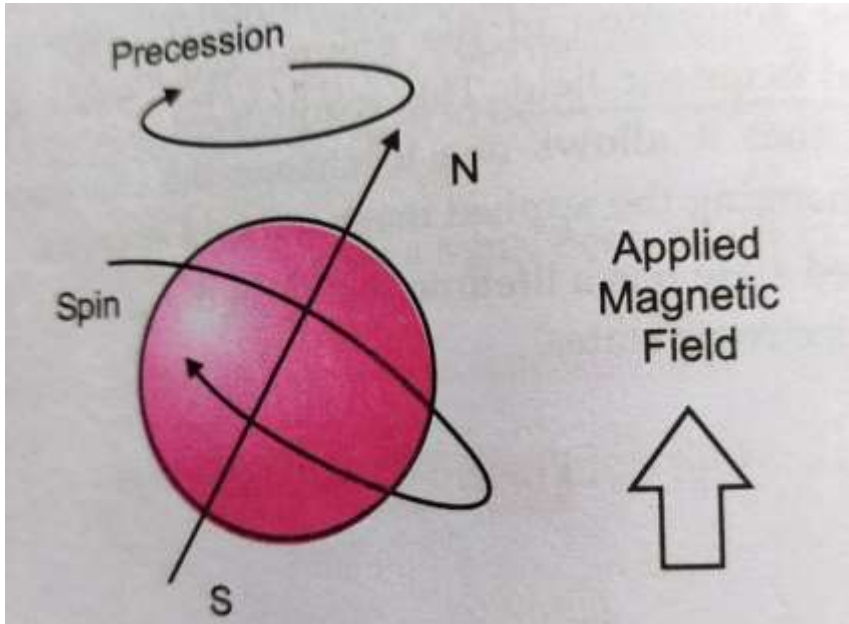


Fig. 6.1: The spinning nucleus

2 . **The effect of an external magnetic field** – Proton will respond to influence of an external magnetic field and will tend to align itself with that field . Proton can adopt two orientations – aligned (parallel) with the field or opposed(antiparallel) with the field

Principle

3 . **Precessional motion** – Consider the behavior of a spinning top as describing its spinning motion , in which the spinning axis of the top moves slowly around the vertical. This precessional motion, and the top is said to be precessing around the vertical axis . The precession arises from the interaction of spin gyroscopic motion . As the proton is a spinning magnet , it will , precess around the axis of an applied external magnetic field in aligned with the field or opposed to the field.

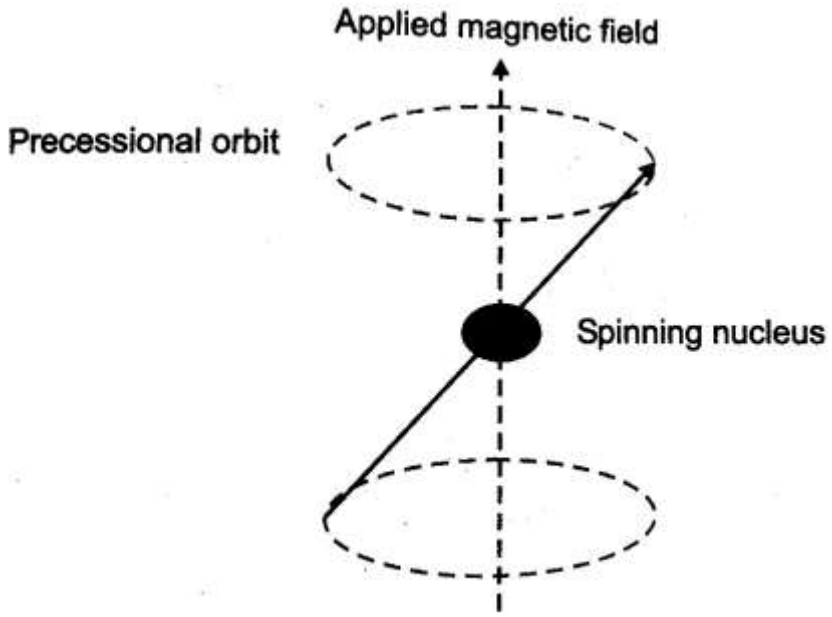


Fig. 6.2: Precessional motion of nuclei

Public

Principle

4 .**Precessional frequency** - The precessional frequency of the nucleus is directly proportional to the strength of an external field and depends on the nature of the nuclear magnet . Magnetic nuclei of different atoms have different characteristic precessional frequency .

According to Larmor precession theory,

$\omega = \gamma B_0$

Where ω = Larmor precession frequency

But $\omega = 2\pi v$

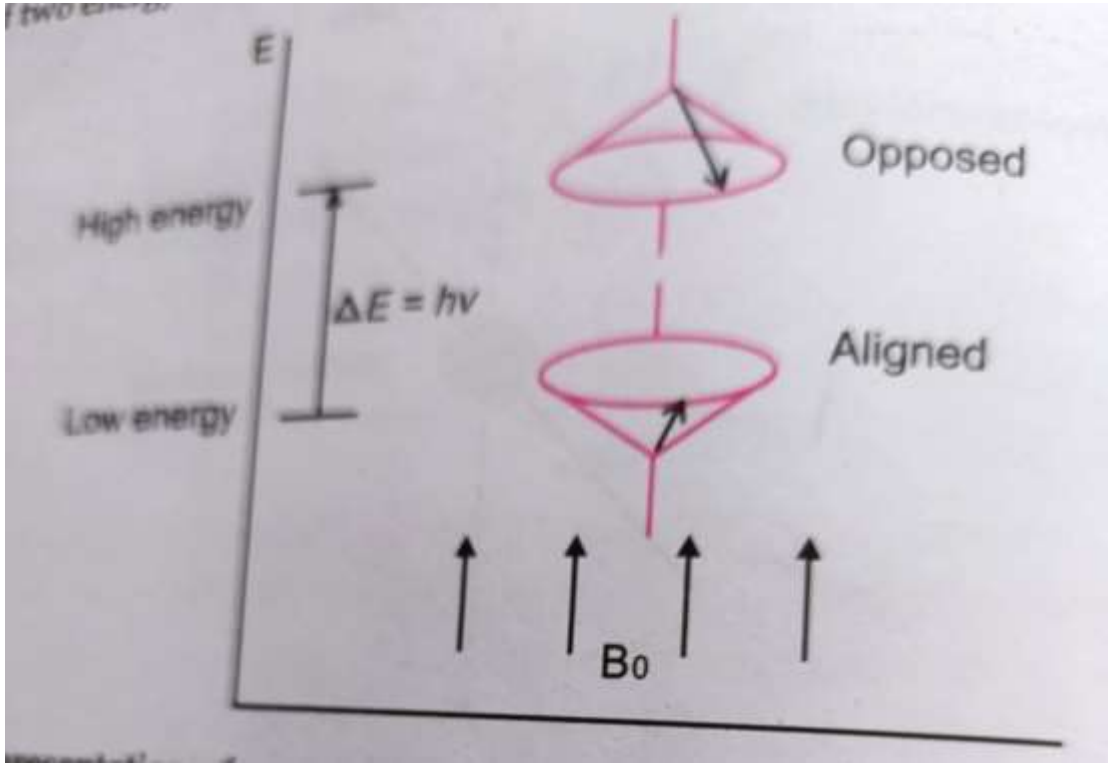
$2\pi v = \gamma B_0$

$v \alpha B_0$

Public

Principle

5 . **Energy transition** - If a proton is precessing in the aligned orientation , it can absorb energy and pass into the opposed orientation , subsequently it can lose this extra energy and relax back into the aligned position . The transition from one energy state to the other is called flipping of the proton . The transition of two energy states can be brought by the radiofrequency wave region .



Public

Chemical shift

When proton is placed in a magnetic field , its electrons are caused to circulate , so they produce induced magnetic field.

- If induced field opposes(diamagnetic)the applied field , then proton is said to be shielded . This shifts the absorption up field.
- If induced field parallel(paramagnetic) to applied field , then proton feels higher field strength and such proton is said to be deshielded . This shifts the absorption down field.

$$\delta = \frac{\text{Frequency of sample} - \text{Frequency of reference}}{\text{Frequency of reference}} \times 10^{6} \text{ ppm}$$

$$\tau = 10 - \delta$$

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Factors affecting chemical shift

1. **Electronegativity** - As the electronegativity of the functional group is increased , the protons come to resonate at a higher delta values .
2. **Vander Waal's deshielding** - In a rigid molecule , a proton to occupy a sterically hindered position . Electron cloud of a hindering group will tend to repel the electron cloud surrounding the proton by electrostatic repulsion . The proton will be deshielded and appear at higher delta values that would be predicted in the absence of the effect .
3. **Anisotropic effect** - In this case pi electrons circulate under the influence of the applied field and can lead shifts to higher frequency (down field shift or paramagnetic shift) or to lower frequency (up field shift or diamagnetic shift) . In addition , the effects are paramagnetic in certain direction around the pi clouds , and diamagnetic in others , so that these effects are described as anisotropic.
4. **Hydrogen bonding** - A hydrogen atom exhibiting property of hydrogen bonding in a compound absorbs at a lower field . The hydrogen bonded proton being attached to a highly electronegative atom will have smaller electron density around it . Deshielded in nature , the field felt by such a proton will be more and hence resonance downfield . The downfield shift depends upon the strength of hydrogen bonding.

Public

Reference standard

In TMS , silicon pushes electrons into the methyl groups of tetra methyl silane by a positive inductive effect and this powerful shielding effect means TMS protons come to resonance at low frequency . It is referred due to following reasons –

i. TMS contains 12 p all are magnetically equivalent .

ii. It is chemically inert .

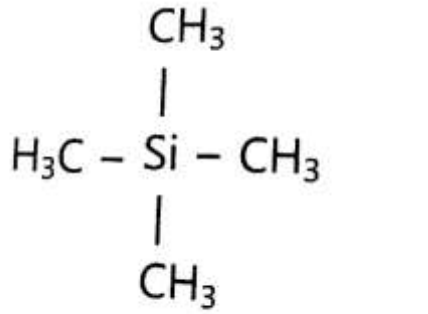
iii. It is miscible with a large range of solvents .

iv. It is highly volatile .

v. It can be easily removed to get back the sample .

vi. It does not take part in intermolecular association with the sample .

vii. Its resonance position is far away from absorption due to protons in most organ ic molecules .



Tetramethylsilane (TMS)

Public

Spin – spin coupling

- **Singlet** - The signal for OH proton appears as a singlet because of exchangeable protons . Exchange of OH protons among alcohol molecules is catalyzed by acid , base and other impurities commonly found in alcohols . The frequency of exchange is high and hence such protons are unable to couple with protons on neighboring atoms . Hence OH protons give singlet .

Pascal's triangle

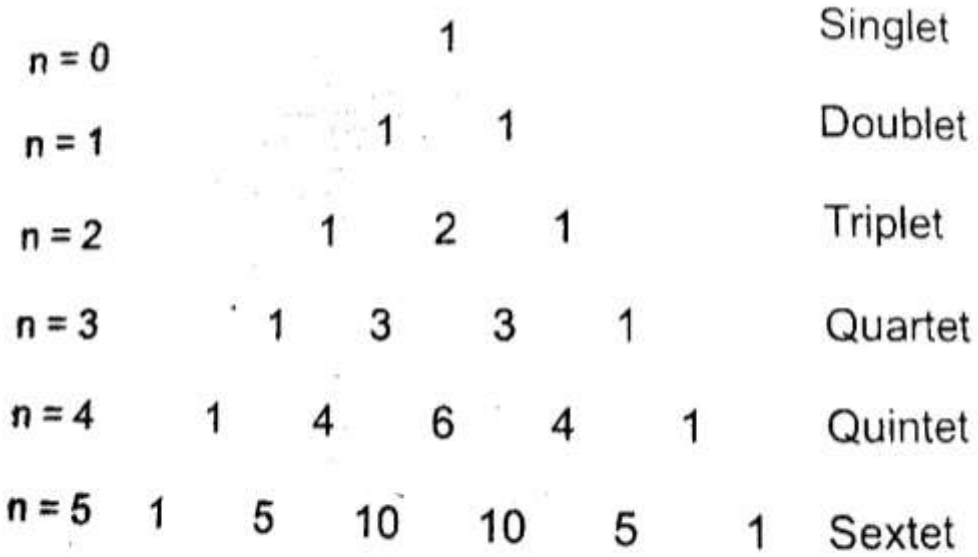


Fig. 1.7: Pascals Triangle

Public

Spin – spin coupling

- **Doublet -** In dibromoethane , signal of the protons in CH_2 group splits as a doublet due to the presence of one proton on neighboring carbon atom . When placed in an applied electric field the adjacent proton can be aligned with or against B_0 . Since the absorbing protons feel two different magnetic fields , they absorb two different frequencies in the NMR spectrum , thus splitting absorption signal into a doublet .

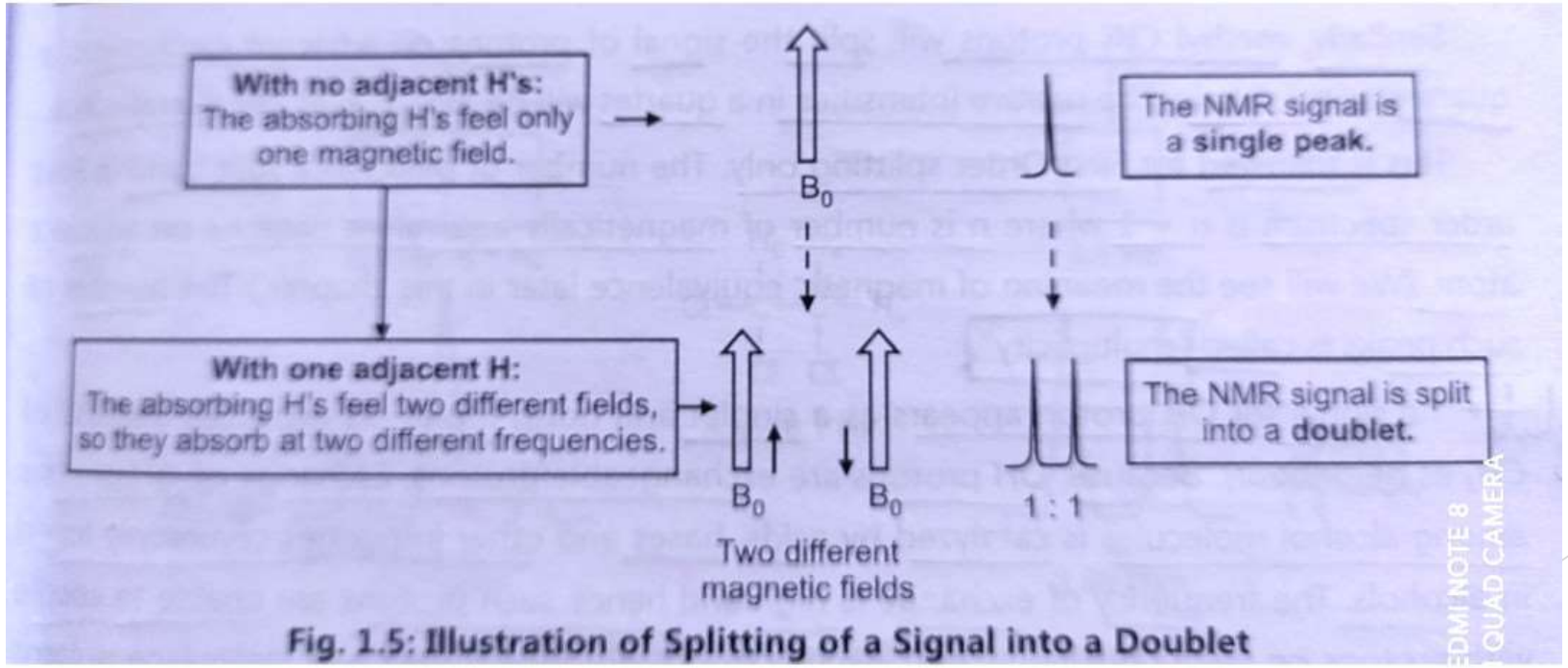


Fig. 1.5: Illustration of Splitting of a Signal into a Doublet

Public

Spin – spin coupling

- **Triplet -** In ethanol , CH_2 has 4 possible spin states . In CH 3 both the spins are aligned against the external magnetic field . The signal will shift up field . But OH protons has both the spins aligned with external magnetic field . Thus sign al will shift down field . The effect of methylene protons spin state will be tw ice that of methyl protons and hydroxyl protons . Thus the area under the pea k of triplet will be twice that of other two .

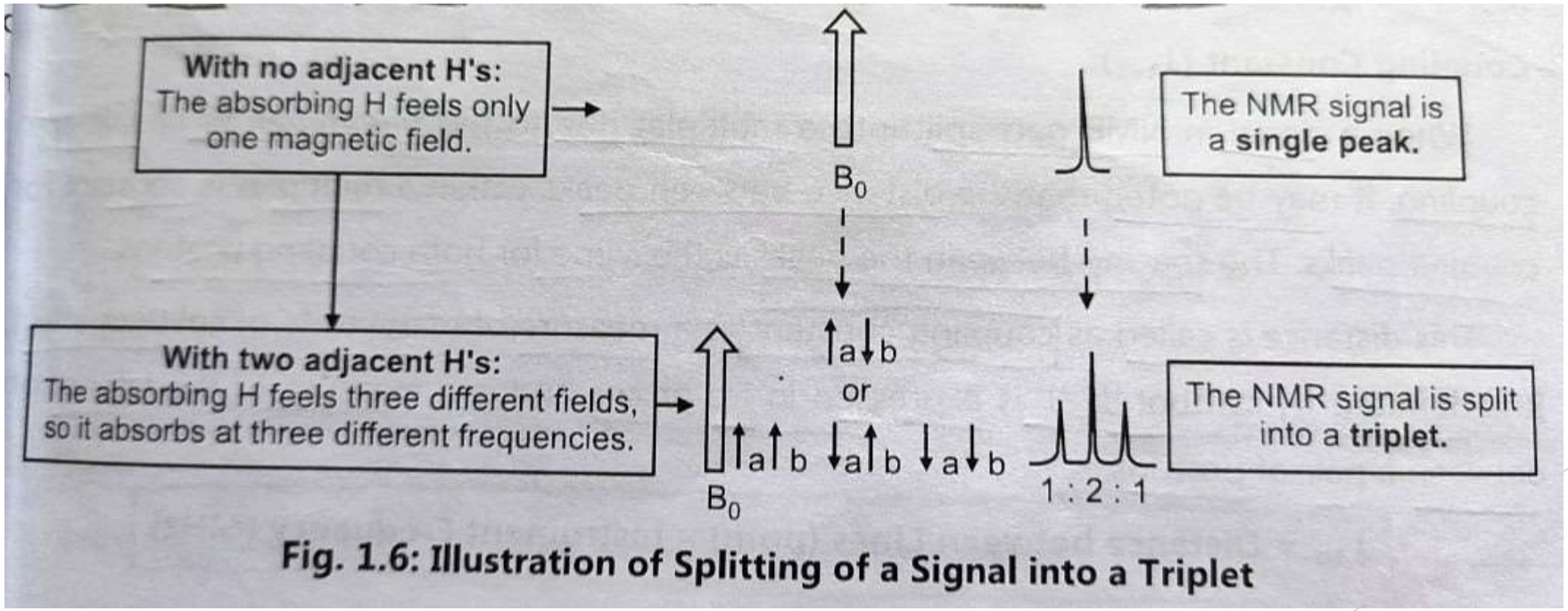


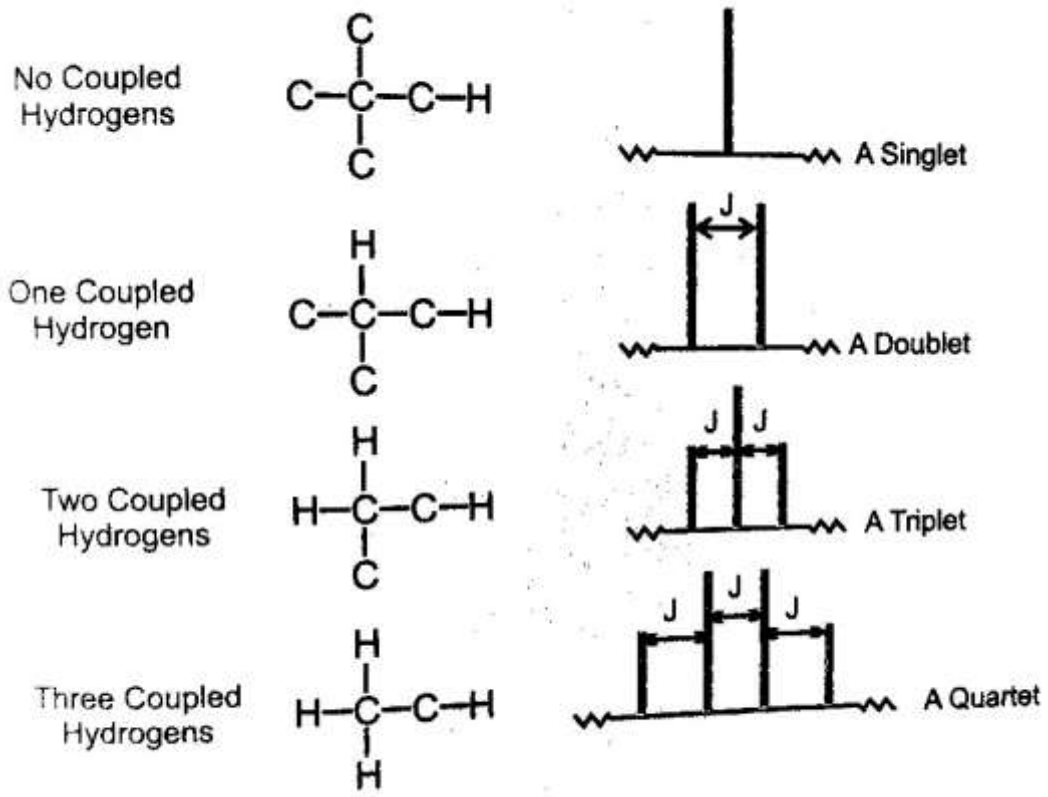
Fig. 1.6: Illustration of Splitting of a Signal into a Triplet

Public

Coupling constant

The distance between two adjacent peaks is constant and is called coupling constant . It is denoted by letter J . It is measured in Hz or in cps . The value of coupling constant is independent of the external field .

J = Distance between lines (ppm) x Instrument frequency (Hz)



Public

Chemical and magnetic equivalence

Chemical equivalence - All the protons found in chemically identical environments in a molecule have same chemical shift value . These are chemically equivalent protons . If two or more nuclei are equivalent by symmetry they are said to be chemical equivalents .

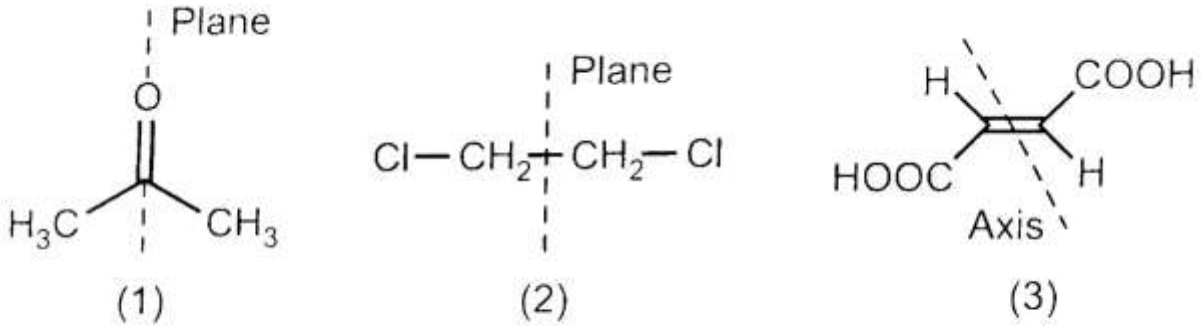
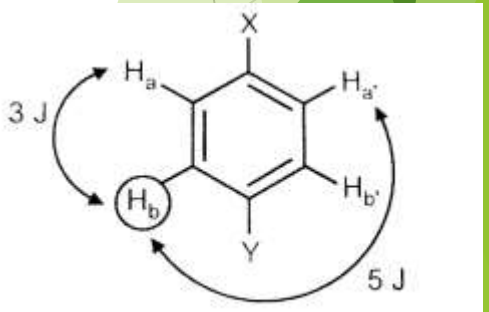


Fig. 1.9: Chemical Equivalence due to Plane or Axis of Symmetry

Magnetic equivalence - When chemically equivalent protons do not split each other they are said to be magnetically equivalent . For protons to be magnetically equivalent they have to satisfy two conditions –

1. They should have same chemical shift .
2. They must have equal coupling constant .



para-Disubstituted benzene

Public

Solvents used

- A substance free from protons should be used as a solvent . It should not give absorption of its own in the spectrum . The solvent must be capable of dissolv ing at least 10% of the substance under investigation . Following solvents are commonly used –

1. Carbon tetrachloride
2. Carbon disulphide
3. Deuterochloride
4. Hexachloroacetone

Public

Instrumentation

- **Magnet** – It is used to supply principle part of field . The magnet in most commercial NMR spectrometers may be either electromagnet or permanent magnet . The magnet used must have a very high degree of field homogeneity between the pole pieces if high resolution work is needed .
- **Magnetic field sweep** – An alteration over a small range in the applied field may be made by making use of a pair of coils located parallel to the magnet . These coils super impose on the main field of the magnet the additional field required to bring the total resonance condition . By varying a direct current through these coils the effective field can be changed by a few hundred milligauss without any loss in field homogeneity .
- **Radio frequency source** –The signal from a radio frequency oscillator is fed into a pair of coils mounted at right angles to the path of the field . A plane polarized beam of radiation has been obtained .
- **Sample holder** – A glass tube is used of diameter 5 mm . Its capacity is 0.4 ml . The sample must be in the liquid state for high resolution spectra . It is contained in a tube which is spun by means of air turbine .

Public

Instrumentation

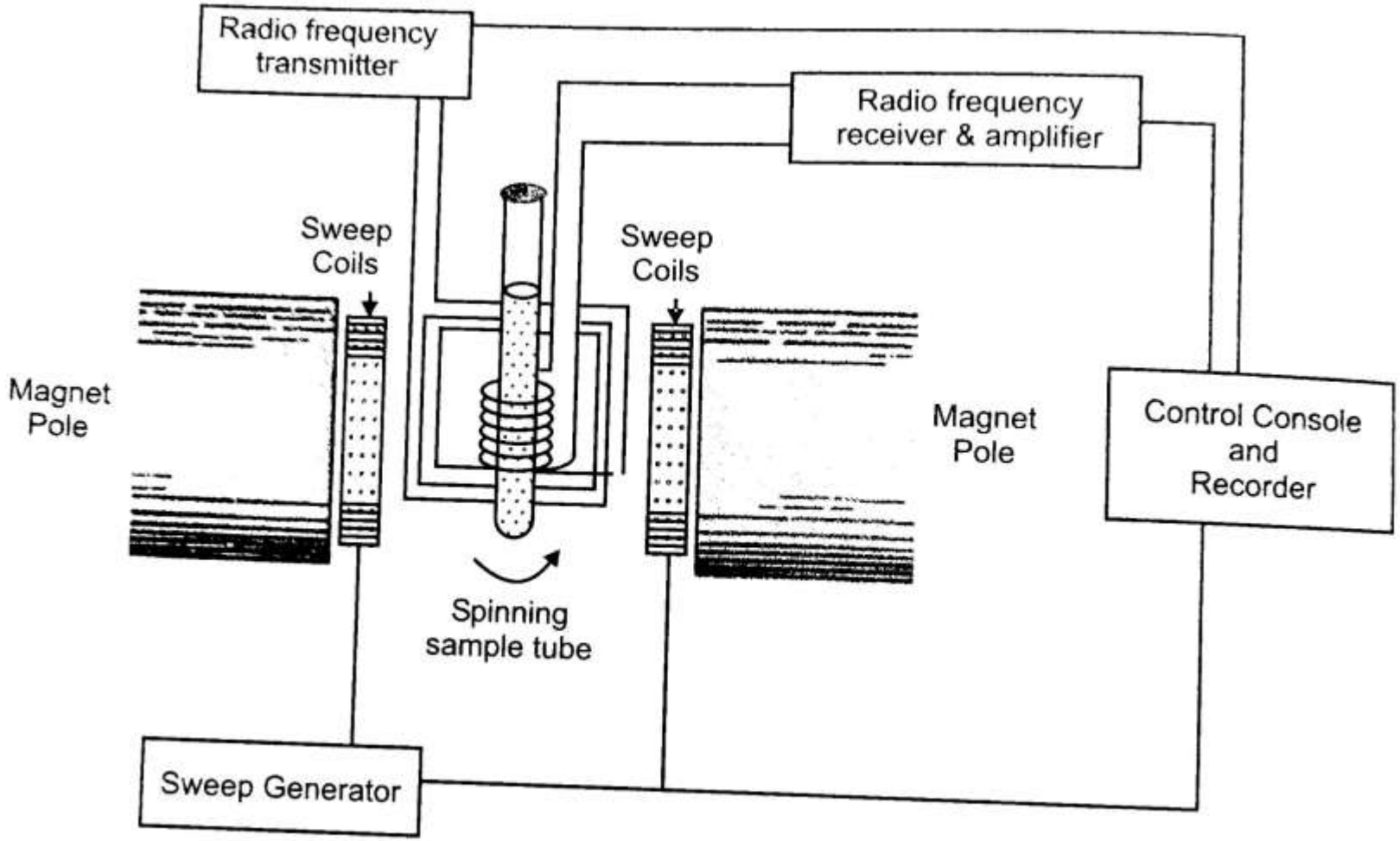


Fig. 6.16: Instrumentation of NMR

Public

Instrumentation

- **Sample probe** –It is a device that holds the sample tube in a fixed position in the field . It is provided with a air driven turbine for rotating the sample alon g its longitudinal axis at several hundred RPM . This rotation minimizes the eff ects of inhomogeneity in the field . It helps in obtaining sharper lines and bett er resolution . Probe may be either a single coil or a turn coil system.
- **The receiver** –The resonance signal is detected by one of the two methods . I n single coil instrument a Wheatstone bridge is employed . The applied signal is balanced against the received signal . The absorption signal is recorded as a n out of balance emf which may be amplified and recorded . In double coil tra nsmitter and receiver coils are set at right angles to each other about the sam ple .
- **Signal detector and recording system -** The receiver coil directs the radio fr equency signal produced by the resonating nuclei . The electrical signal gener ated into the coil must be amplified before it can be recorded . Resonance lin e intensity is proportional to the number of nuclei responsible for the signal . The area under the signal is a direct measure of the intensity . It is determine d in a cumulative manner by an electronic integrator .

Public

Applications

1. **Structure elucidation –** NMR spectroscopy gives information about types of protons , environment of protons , number of protons .
2. **Assay of components –** Single component or multicomponent without separation of compounds can be quantitatively estimated . Specific peak for each component is identified . The peak area given by integral value is found by using standard and sample .
3. **Detection of aromaticity –** Protons attached to heterocyclic compounds whose pi electrons follow huckels rule . Protons are deshielded due to circulating ring current of pi electrons . As a result of this the signals for the aromatic protons appear at a very low field . From this , the aromatic character of the compound under investigation can be predicted .
4. **Distinction between cis – trans isomerism –** The concerned protons have different values of chemical shift and coupling constant .

Public

Applications

5. Hydrogen **bonding** – Proton signal is shifted towards low field in case of hydrogen bonding . This reveals that hydrogen bond formation results in decrease in the electron shielding of the proton . So it can be used to study hydrogen bonding in organic compounds .

6. **Materials science** – It can be used to investigate new materials of great technological importance such as glasses , ceramics , polymers , semiconductors . It can be used to investigate reaction taking place in catalytic surfaces .

7. **Food chemistry** – It can be used in verification of wine aging . It is also used in identification of oil fatty constituents .

8. **Clinical applications** – It is used in the localization and characterization of metabolites in biological fluids . So it can be utilized in the diagnosis of many diseases .

Public

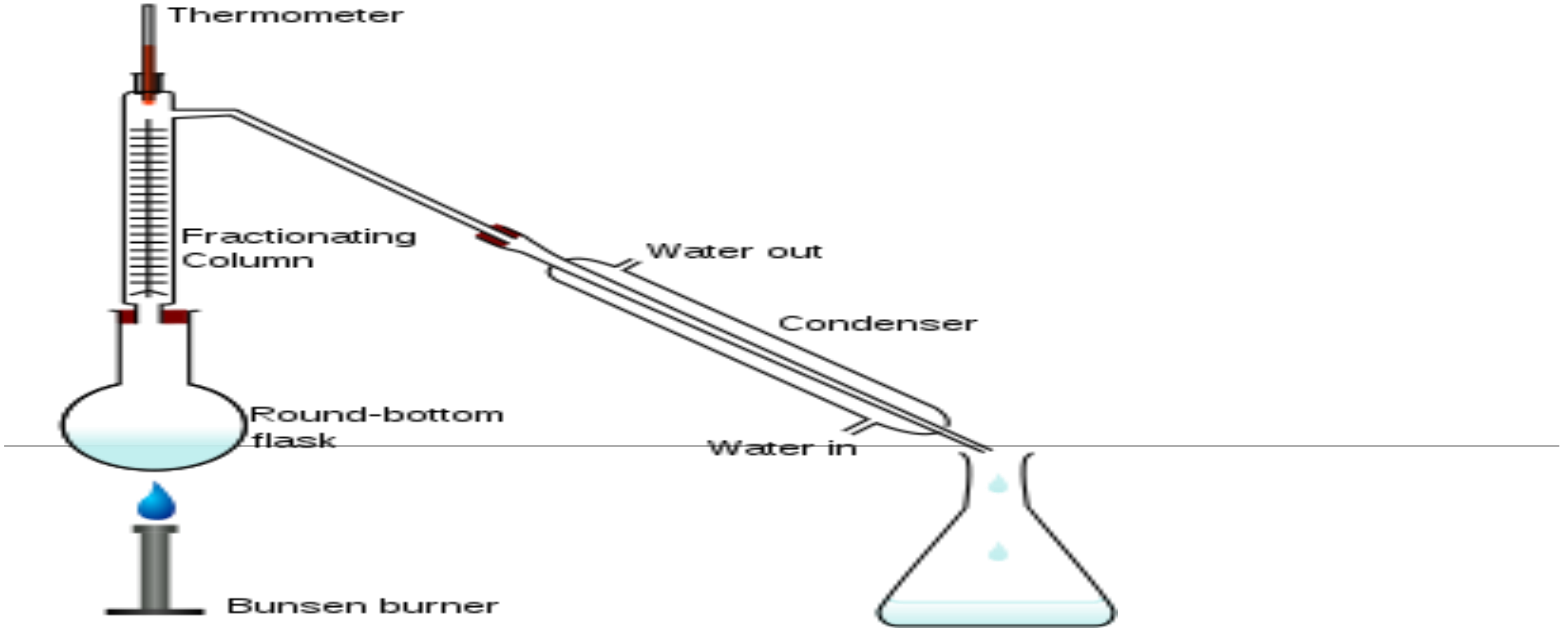
Reference

- Dr. Shashikant D. Bharhate , Dr.MD. Rageeb MD. Usman , Poonam A. Salunke , Shital S. Patil , Mordern Pharmaceutical Analytical Techniques , Edition 2019 , S.Vikas and Company Medical Publishers
- Dr. Mrinalini C. Damle , Dr.Vandana T. Gawande , Pharmaceutical Analysis – IV , First Edition , Nirali Prakshan

Public

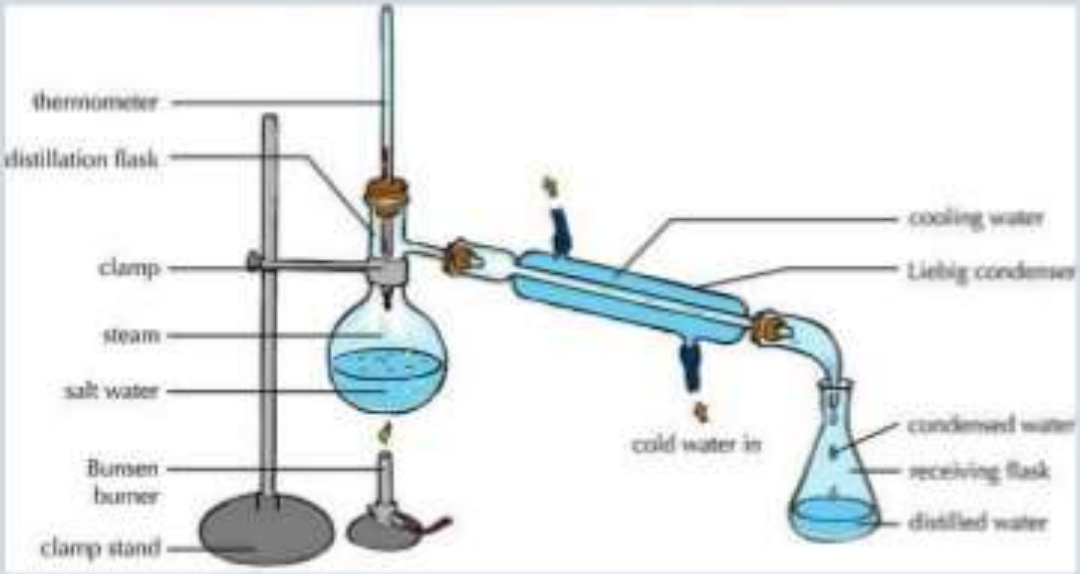
Public

Fractional Distillation



DISTILLATION

Distillation is the process of separating the components or substances from a liquid mixture by selective boiling and condensation.



FRACTIONAL DISTILLATION

Fractional distillation is a process in which vaporization of liquid mixture gives rise to a mixture of constituents from which the desired component is separated in pure form.

This method is also known as rectification, because a part of the vapour is condensed and returned as a liquid. This method is used to separate miscible volatile liquids, whose boiling points are close, by means of a fractionating column.

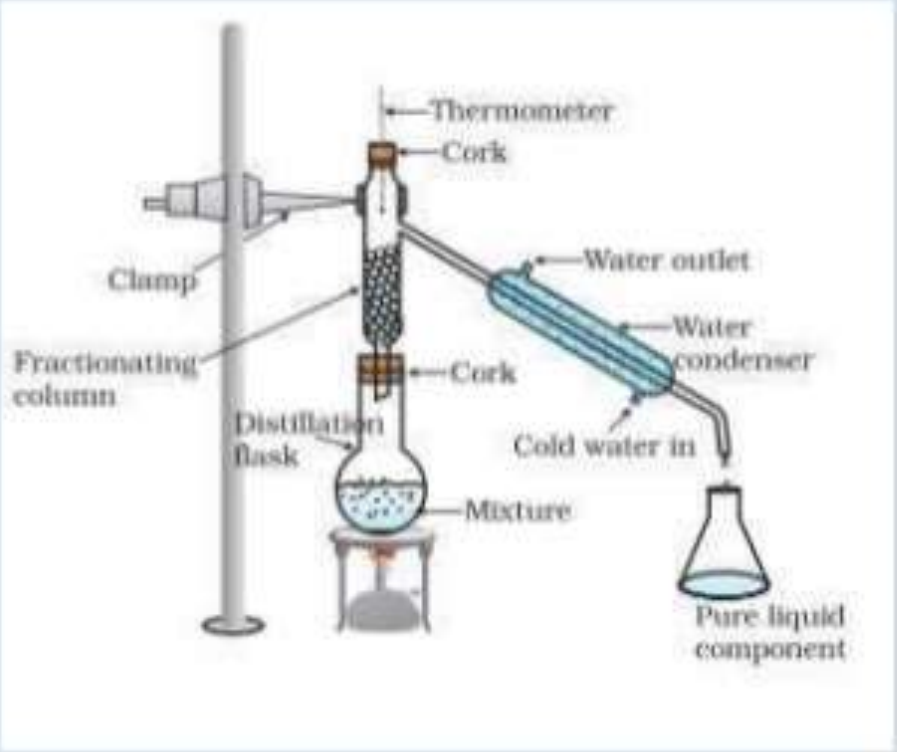


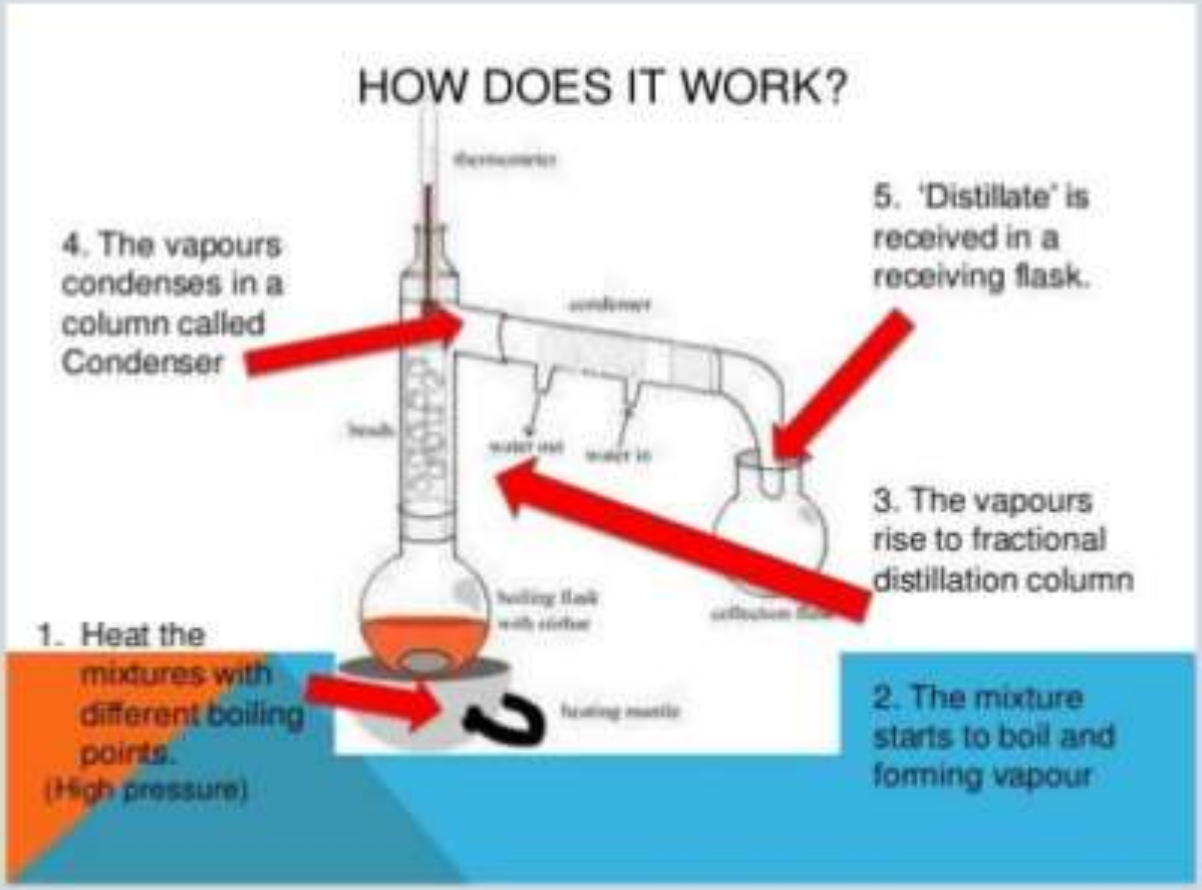
Fig : Fractional Distillation

PRINCIPLE

From the operational point of view, fractional distillation is a mass transfer process involving counter-current diffusion of the components at each equilibrium stage.

When a liquid mixture is distilled, the partial condensation of the vapour is allowed to occur in a fractionating column. In the column, ascending vapour from the still is allowed to come in contact with the condensing vapour returning to the still. This results in enrichment of the vapour with the more volatile component. By condensing the vapour and reheating the liquid repeatedly, equilibrium between liquid and vapour is set up at each stage, which ultimately results in the separation of a more volatile component

HOW DOES IT WORK?



APPLICATIONS

Fractional Distillation is used for :

- Separation of miscible liquids such as acetone and water, chloroform and benzene.
- Preparation of alcohol which is one of the most important pharmaceutical substance.
- Separation of non-volatile substance from volatile substance, for eg. Preparation of distilled water for injection.
- Purification of many organic compounds, such as ethers, amide, etc.

FRACTIONAL vs SIMPLE DISTILLATION

- In Simple distillation, vapour is directly passed through the condenser whereas in fractional distillation the vapour must pass through a fractionating column in which partial condensation of vapour is allowed to occur.

- In simple distillation, condensate is collected directly into the receiver, while in fractional distillation, condensation takes place in the fractionating column, so that a part of the condensing vapour returns to the still.

ADVANTAGES

- It is extremely easy to use.
- Readily purifies complex mixtures.
- Separates liquids with smaller boiling point separation.

DISADVANTAGES

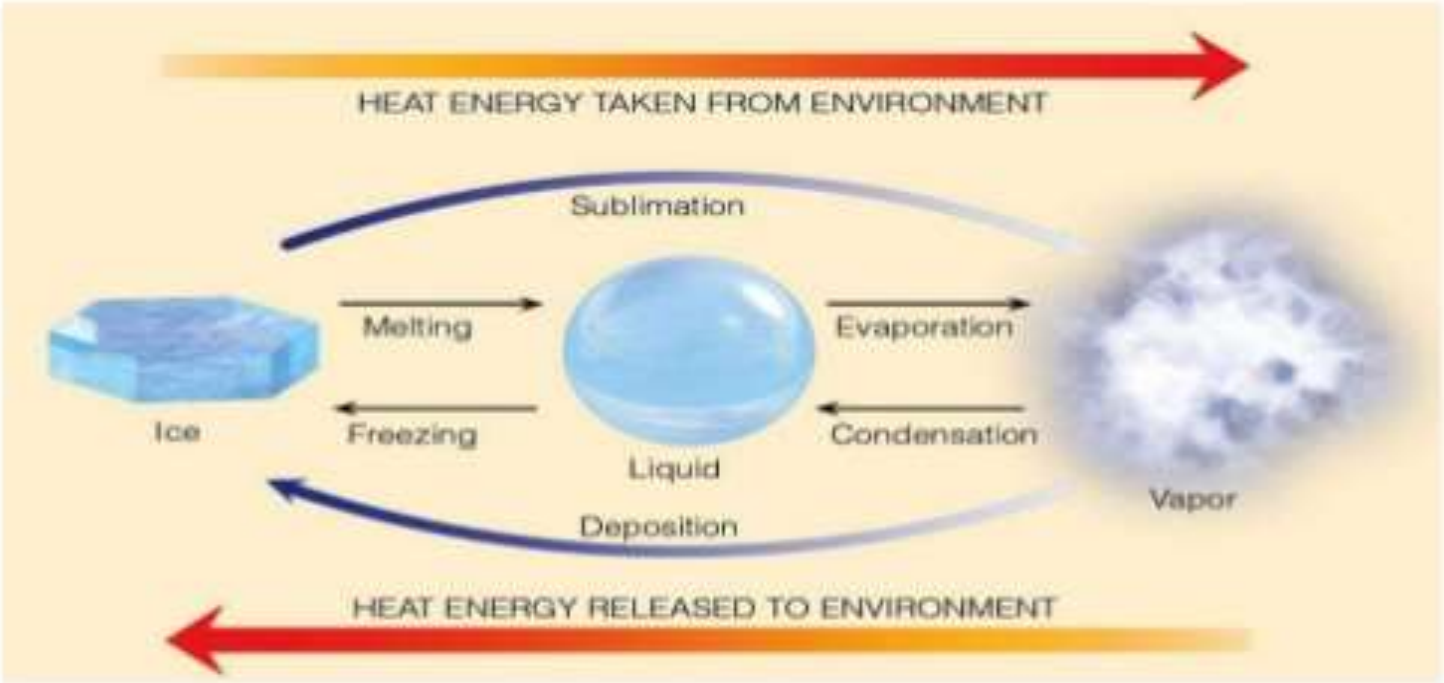
- It cannot be used to separate miscible liquids, which form azeotropic mixtures.
- Consumes more energy.
- The setup is costly and complicated.
- Takes longer time for liquids to distill.

FRACTIONAL LIBERATION

- A mixture of alkaloid salts in aqueous solution, when treated with aliquots of alkali gives first the weakest base in the free state followed by base liberation in ascending order of basicity.
- If the mixture is shaken with organic solvent after each addition of aliquot of a alkali, a fractional series of bases shall be obtained.

What is Sublimation

Sublimation is the process where a solid changes from a solid to a vapor without passing through the liquid state.



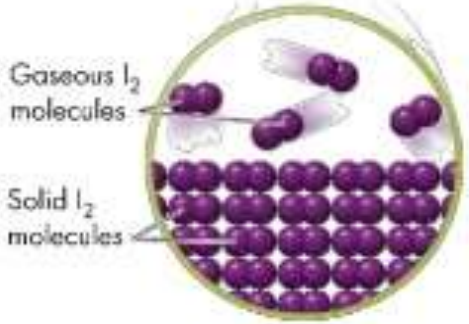
How substance undergo sublimation?

Sublimation is an endothermic phase transition that occurs at temperatures and pressures below a substance's triple point in its phase diagram.

In thermodynamics, the triple point of a substance is the temperature and pressure at which the three phases (gas, liquid, and solid) coexist in thermodynamic equilibrium. A phase transition in which a solid is converted to a gas, without passing though an intermediate liquid phase.

Iodine is an example of a substance that undergoes sublimation.

- The outer test tube contains solid iodine that is being gently heated.
- The inner test tube contains liquid water and ice.
- The iodine crystals at the bottom of the outer test tube change directly to iodine vapor on heating.
- When the vapor reaches the cool surface of the inner test tube, it goes directly from the gaseous to the solid state.



Advantages of sublimation

1- The main advantage of sublimation is for purification process.

2- The minimum amount of product is loss.

3- Solvents are not used.

4- Most traces of any solvent in compound are effectively eliminated.

5- when the substance weighs less than 100mg the best method for purification is sublimation.

What is derivatization?

- What is GC Derivatization?
- Derivatization is the process of chemically modifying a compound to produce a new compound which has properties that are suitable for analysis using a GC.

Derivatization is a technique used in chemistry which converts a chemical compound into a product (the reaction's **derivate**) of similar chemical structure, called a derivative.

Generally, a specific functional group of the compound participates in the derivatization reaction and transforms the educt to a derivate of deviating reactivity, solubility, boiling point, melting point, aggregate state, or chemical composition. Resulting new chemical properties can be used for quantification or separation of the educt.

Why Derivatize:

- ✓ To permit analysis of compounds not directly amenable to analysis due to, for example, inadequate volatility or stability.
- ✓ Improve chromatographic behavior or detectability.
- ✓Many compounds do not produce a usable chromatograph (i.e.multiple peaks, or one big blob), or the sample of interest goes undetected. As a result it may be necessary to derivatize the compound before GC analysis is done.
- ✓Derivatization is a useful tool allowing the use of GC and GC/MS to be done on samples that would otherwise not be possible in various areas of chemistry such as medical, forensic, and environmental.

What Does Derivatization Accomplish?

- ✓ Increases volatility (i.e. sugars):
- ✓ Eliminates the presence of polar OH, NH, & SH groups
- ✓ Derivatization targets O,S, N and P functional groups (with hydrogens available.
- ✓ Increases detectability, I.e. steroids/ cholesterol
- ✓Increases stability.
- ✓ Enhances sensitivity for ECD (Electron Capture Detection). The introduction of ECD detectable groups, such as halogenated acyl groups, allows detection of previously undetectable compounds.

TECHNIQUES OF DERIVATISATION

- SILYLATION
- ACYLATION
- PERFLOURO-ACYLATION
- ALKYLATION
- ESTERIFICATION
- CONDENSATION
- CYCLISATION

Silylation

- Silylation produces silyl derivatives which are more volatile, less stable, and more thermally stable.
- Replaces active hydrogens with a TMS (trimethylsilyl group).
- Silylation occurs through nucleophilic attack (SN2). The better the leaving group, the better the siliylation.
- Silylation reagents will react with water and alcohols first. Care must be taken to ensure that both sample and solvents are dry.
- Solvents should be as pure as possible. This will eliminate excessive peaks. Try using as little solvent as possible as this will prevent a large solvent peak.

Acylation

- Acylation reduces the polarity of amino, hydroxyl, and thiol groups and adds halogenated functionalities for ECD. In comparison to silylating reagents, the acylating reagents target highly polar, multifunctional compounds, such as carbohydrates and amino acids.
- Acyl derivatives are formed with acyl anhydrides, acyl halides, and activated acyl amide reagents.
- The anhydrides and acyl halides form acid by-products which must be removed before GC analysis.

perflouro-Acylation

- This group increases the mol.wt of the sample relative to the analogous hydrocarbon.
- Best method to increase the retention time.
- Eg.
  - N-Triflouro acetic anhydride
  - Direct acylation with Triflouro acetic anhydride in triflouro acetic acid followed by methylation with diazomethane in methanol.

Alkylation

- ***Alkylation reduces molecular polarity by replacing active hydrogens*** *with an alkyl group. These reagents are used to modify compounds with acidic hydrogens, such as carboxylic acids and phenols. These reagents make esters, ethers, alkyl amines and alkyl amides.*
- *Reagents containing fluorinated benzoyl groups can be used for ECD.*
- The principal reaction employed for preparation of these derivatives is nucleophilic displacement.
- Alkylation is used to modify compounds with acidic hydrogens, such as carboxylic acids and phenols.

esterification

Esterification:

Esterification is used to prepare derivatives of carboxyl group.

The conversion of the carboxyl group to ester increases volatality by decreasing hydrogen bonding.

Ex:- Analgesics, prostaglandins, aminoacids, & anti-inflammatory agents.

Derivatization by esterification can be carried out by using Fischer esterification procedure in which strongly acidic conditions are present.

Condensation:

If ketone or aldehyde is present in a sample, it is frequently derivatized to prevent hydrogen bonding due to enolization & helps in resolution from an interfering substance.

The most commonly used reagent is methoxylamine to protect enolizable ketogroups in steroids by formation of methoximes.

Cyclization:

Cyclization is performed on compounds containing two functional groups in close proximity so that 5 or 6 membered Heterocyclic rings can be formed.

applications

Qualitative analysis:

- ✓Retention time data should be useful for identification of mixtures.
- ✓Comparing the retention time of the sample as well as the standard.
- ✓Checking the purity of a compound: compar the standard and sample.
- ✓Additional peaks are obtained.....impurities are present....compound is not pure.

Quantitative analysis:

- Direct comparison method:
  - -comparing the area of the peak, peak height, width of peak.
- Calibration curves:
  - -standards of varying concentration are used determine peak areas .
- Internal standard method:
  - -A known concentration of the internal standard is added separately to the standard solution
  - -The peak area ratio of sample and internal standard....unknown concentration is easily determined .

Fractional Crystallization

- Fractional crystallization is a method of refining substances based on differences in solubility

- If a mixture of two or more substances in solution is allowed to crystallize, for example by allowing the temperature of the solution to decrease, the precipitate will contain more of the least soluble substance.

Fractional Crystallization

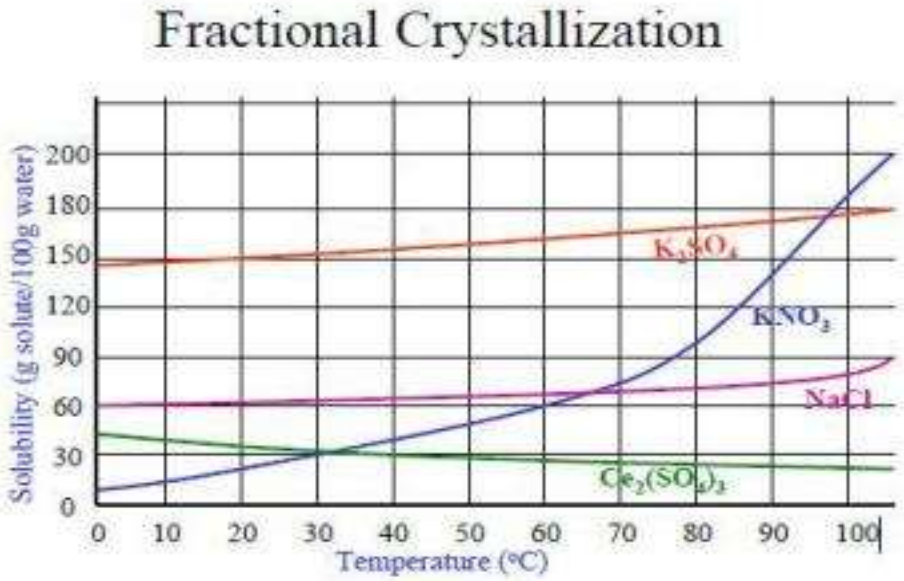
- Is it possible to separate mixtures of different ionic compounds by dissolving them in water and adjusting the temperature of the solution so that one compound crystallizes out and the other does not?

- YES!! You are given a mixture containing 80 g of $K_2SO_{4(s)}$ and 80 g of $KNO_{3(s)}$ and are asked to produce as much pure KNO_3 as possible. How do you do it?

Fractional Crystallization

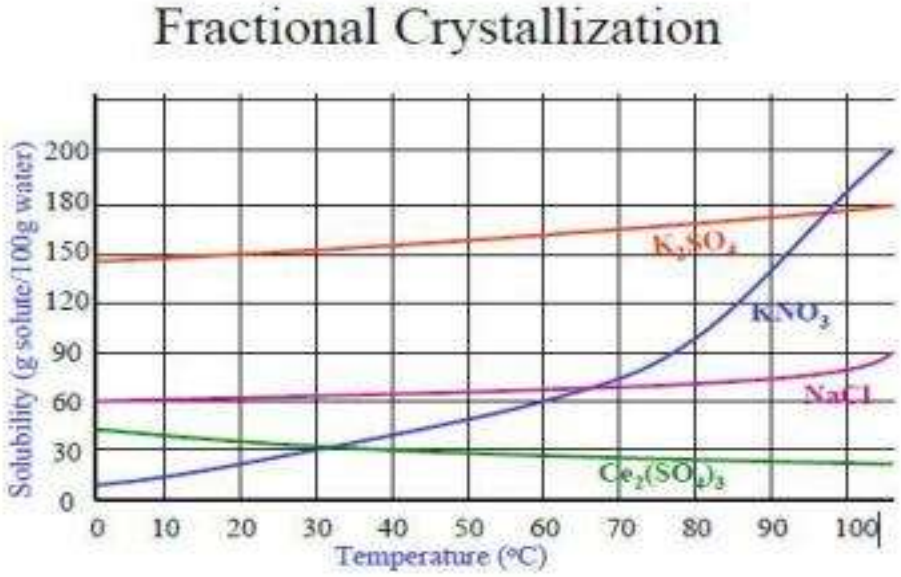
- Step 1: Dissolve salts in 100 g distilled water at 80°C.

- By looking at the solubility curves, you will see that 100 g of KNO_3 and 170 g of K_2SO_4 will dissolve at this temperature.
- Thus, everything will dissolve.



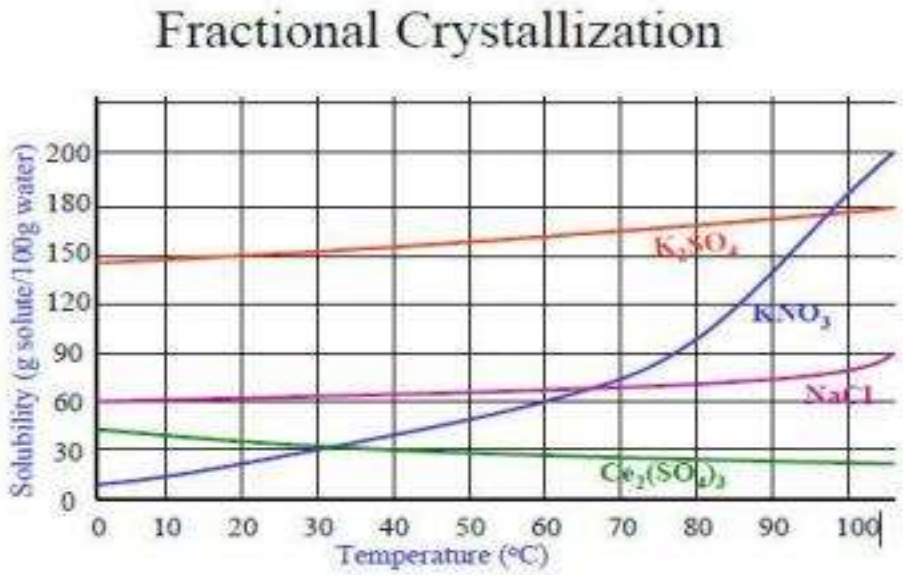
Fractional Crystallization

- Step 2: Cool the solution to 0°C.
- K_2SO_4
  - At 0°C about 150 g of K_2SO_4 is soluble in 100 g of water.
  - We only dissolved 80 g of K_2SO_4 so NONE of it has precipitated out.



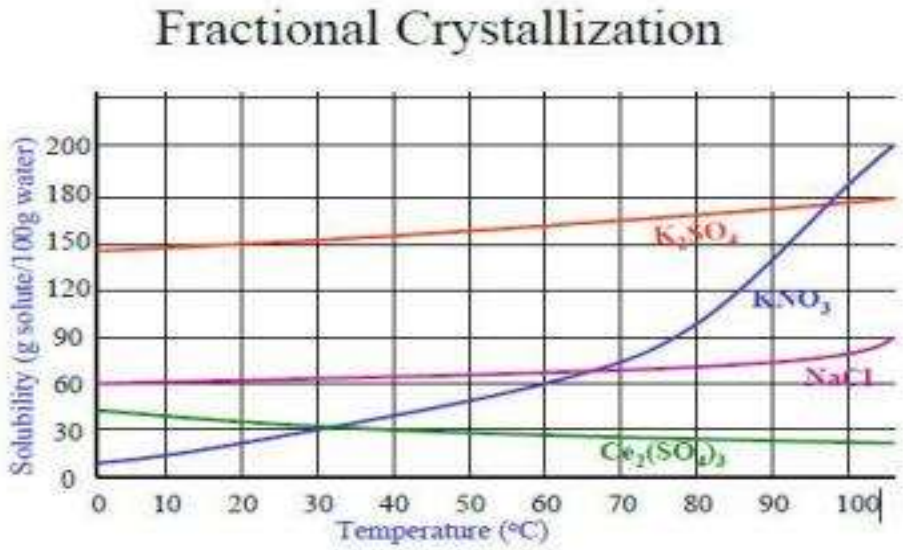
Fractional Crystallization

- KNO_3
  - At 0°C only 12 g of KNO_3 can remain in 100 g of water.
  - We dissolved 80 g of KNO_3 so (80g-12g=68g) of pure KNO_3 precipitated from the solution.



Fractional Crystallization

- A steeper solubility curve indicates that a greater mass of precipitate will form during a reduction of temperature.
- Conversely, a steeper solubility curve indicates that a greater mass of solid will dissolved during an increase of temperature.



DEFINITION

Centrifugation is a process used to separate or concentrate materials suspended in a liquid medium. The theoretical basis of this technique is the effect of gravity on particles (including macromolecules) in suspension. Two particles of different masses will settle in a tube at different rates in response to gravity.

INTRODUCTION

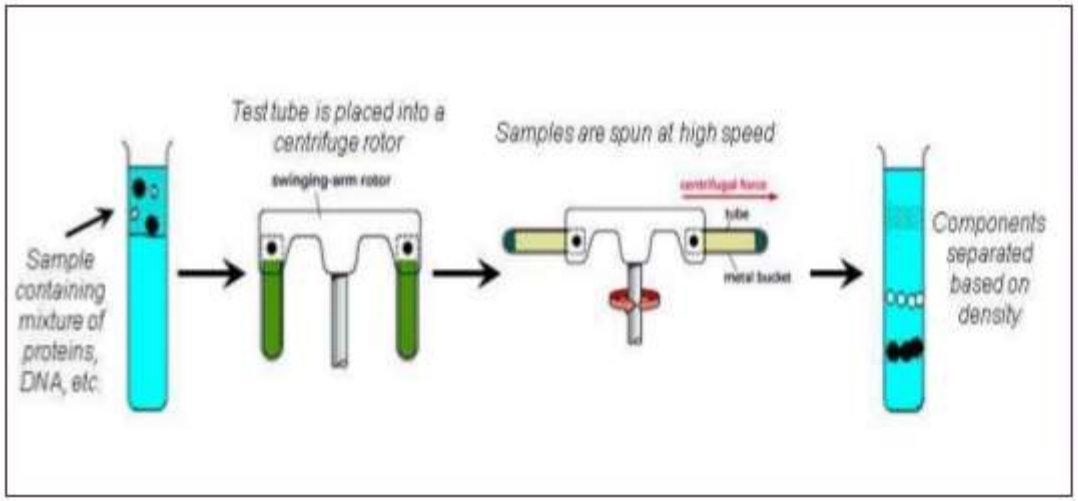
- **Centrifugation** is a process which involves the use of the centrifugal force for the sedimentation of heterogeneous mixtures with a centrifuge, used in industry and in laboratory settings.
- This process is used to separate two immiscible liquids.
- More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis.

- **Centrifugation** is a process which involves the use of the centrifugal force for the sedimentation of heterogeneous mixtures with a centrifuge, used in industry and in laboratory settings.
- This process is used to separate two immiscible liquids.
- More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis.

PRINCIPLE

- A centrifuge is a device for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed.
- In a solution, particles whose density is higher than that of the solvent **sink (sediment), and** particles that are lighter than it **float to the** top.
- The greater the difference in density, the faster they move. If there is no difference in density (isopyknic conditions), the particles stay steady.

- To take advantage of even tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful "**centrifugal force**" provided by a centrifuge.



CENTRIFUGE

- A **centrifuge** is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong force perpendicular to the axis of spin (outward).
- The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances and particles to move outward in the radial direction.
- At the same time, objects that are less dense are displaced and move to the center. In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low-density substances rise to the top.

INSTRUMENTATION

- It consist of two components, an electric motor to spin the sample and a rotor to hold tubes.

LOW SPEED CENTRIFUGE

HIGH SPEED CENTRIFUGE

ULTRA CENTRIFUGE

LOW SPEED CENTRIFUGE

- Most laboratories have a standard low-speed centrifuge used for routine sedimentation of heavy particles.
- The low speed centrifuge has a maximum speed of 4000-5000rpm.
- These instruments usually operate at room temperatures with no means of temperature control.
- Two types of rotors are used in it, fixed angle and swinging bucket.
- It is used for sedimentation of red blood cells until the particles are tightly packed into a pellet and supernatant is separated by decantation.

HIGH SPEED CENTRIFUGE

- High speed centrifuges are used in more sophisticated biochemical applications, higher speeds and temperature control of the rotor chamber are essential.
- The operator of this instrument can carefully control speed and temperature which is required for sensitive biological samples.
- Three types of rotors are available for high speed centrifugation-fixed angle, swinging bucket, vertical rotors

ULTRA CENTRIFUGE

- It is the most sophisticated instrument
- Intense heat is generated due to high speed thus the spinning chambers must be refrigerated and kept at high vacuum.
- It is used for both preparative work and analytical work.

1 Froth Flotation – Fundamental Principles

Froth flotation is a highly versatile method for physically separating particles based on differences in the ability of air bubbles to selectively adhere to specific mineral surfaces in a mineral/water slurry. The particles with attached air bubbles are then carried to the surface and removed, while the particles that remain completely wetted stay in the liquid phase. Froth flotation can be adapted to a broad range of mineral separations, as it is possible to use chemical treatments to selectively alter mineral surfaces so that they have the necessary properties for the separation. It is currently in use for many diverse applications, with a few examples being: separating sulfide minerals from silica gangue (and from other sulfide minerals); separating potassium chloride (sylvite) from sodium chloride (halite); separating coal from ash-forming minerals; removing silicate minerals from iron ores; separating phosphate minerals from silicates; and even non-mineral applications such as de-inking recycled newsprint. It is particularly useful for processing fine-grained ores that are not amenable to conventional gravity concentration.

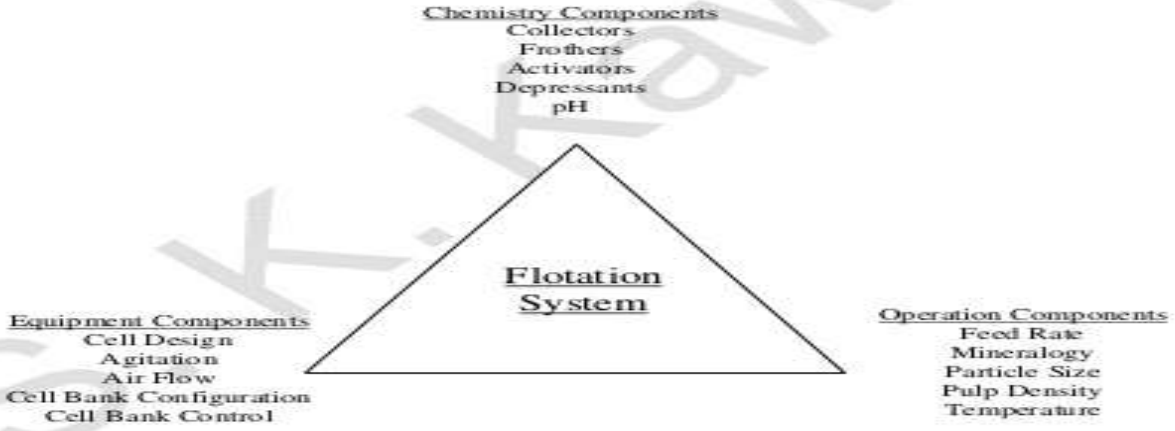


Figure 1: The flotation system includes many interrelated components, and changes in one area will produce compensating effects in other areas (Klimpel, 1995)

Froth flotation is a good example of an engineering "system", in that the various important parameters are highly inter-related, as shown in Figure 1. It is therefore important to take all of

1.2 Hydrophobicity/hydrophilicity

The basis of froth flotation is the difference in wettabilities of different minerals. Particles range from those that are easily wettable by water (hydrophilic) to those that are water-repellent (hydrophobic). If a mixture of hydrophobic and hydrophilic particles are suspended in water, and air is bubbled through the suspension, then the hydrophobic particles will tend to attach to the air bubbles and float to the surface, as shown in Figure 3. The froth layer that forms on the surface will then be heavily loaded with they hydrophobic mineral, and can be removed as a separated product. The hydrophilic particles will have much less tendency to attach to air bubbles, and so it will remain in suspension and be flushed away (Whelan and Brown, 1956).

Particles can either be naturally hydrophobic, or the hydrophobicity can be induced by chemical treatments. Naturally hydrophobic materials include hydrocarbons, and non-polar solids such as elemental sulfur. Coal is a good example of a material that is typically naturally hydrophobic, because it is mostly composed of hydrocarbons. Chemical treatments to render a surface hydrophobic are essentially methods for selectively coating a particle surface with a monolayer of non-polar oil.

1.3 Particle/Bubble Contact

Once the particles are rendered hydrophobic, they must be brought in contact with gas bubbles so that the bubbles can attach to the surface. If the bubbles and surfaces never come in contact, then no flotation can occur. Contact between particles and bubbles can be accomplished in a flotation cell such as the one shown schematically in Figure 5.

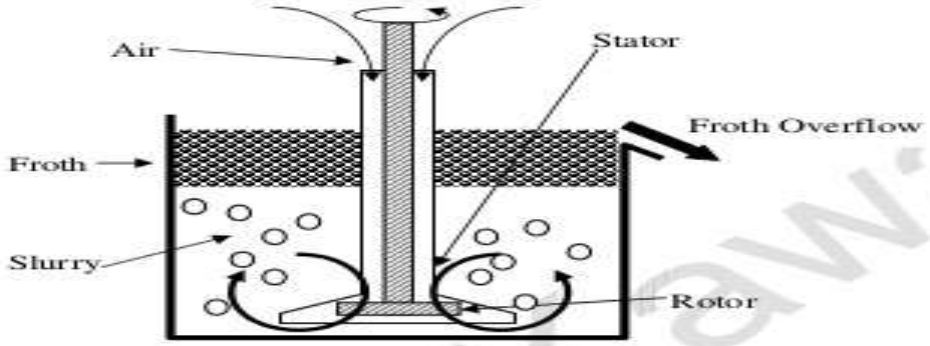


Figure 5: Simplified schematic of a conventional flotation cell. The rotor draws slurry through the stator and expels it to the sides, creating a suction that draws air down the shaft of the stator. The air is then dispersed as bubbles through the slurry, and comes in contact with particles in the slurry that is drawn through the stator.

Particle/bubble collision is affected by the relative sizes of the particles. If the bubbles are large relative to the particles, then fluid flowing around the bubbles can sweep the particles past without coming in contact. It is therefore best if the bubble diameter is comparable to the particle diameter in order to ensure good particle/bubble contact.

1.4 Collection in the Froth Layer

Once a particle and bubble have come in contact, the bubble must be large enough for its buoyancy to lift the particle to the surface. This is obviously easier if the particles are low-density (as is the case for coal) than if they are high-density (such as lead sulfide). The particle and bubble must remain attached while they move up into the froth layer at the top of the cell. The froth layer must persist long enough to either flow over the discharge lip of the cell by

gravity, or to be removed by mechanical froth scrapers. If the froth is insufficiently stable, the bubbles will break and drop the hydrophobic particles back into the slurry prematurely. However, the froth should not be so stable as to become persistent foam, as a foam is difficult to convey and pump through the plant.

The surface area of the bubbles in the froth is also important. Since particles are carried into the froth by attachment to bubble surfaces, increasing amounts of bubble surface area allows a more rapid flotation rate of particles. At the same time, increased surface area also carries more water into the froth as the film between the bubbles. Since fine particles that are not attached to air bubbles will be unselectively carried into the froth along with the water (entrainment), excessive amounts of water in the froth can result in significant contamination of the product with gangue minerals.

1.5 Reagents

The properties of raw mineral mixtures suspended in plain water are rarely suitable for froth flotation. Chemicals are needed both to control the relative hydrophobicities of the particles, and to maintain the proper froth characteristics. There are therefore many different reagents involved in the froth flotation process, with the selection of reagents depending on the specific mineral mixtures being treated.

1.5.1 Collectors

Collectors are reagents that are used to selectively adsorb onto the surfaces of particles. They form a monolayer on the particle surface that essentially makes a thin film of non-polar hydrophobic hydrocarbons. The collectors greatly increase the contact angle so that bubbles will adhere to the surface. Selection of the correct collector is critical for an effective separation by froth flotation. Collectors can be generally classed depending on their ionic charge: they can be nonionic, anionic, or cationic, as shown in Figure 6. The nonionic collectors are simple hydrocarbon oils, while the anionic and cationic collectors consist of a polar part that selectively attaches to the mineral surfaces, and a non-polar part that projects out into the solution and makes the surface hydrophobic. Collectors can either chemically bond to the mineral surface (chemisorption), or be held on the surface by physical forces (physical adsorption).

1.5.1.1 Chemisorption

In chemisorption, ions or molecules from solution undergo a chemical reaction with the surface, becoming irreversibly bonded. This permanently changes the nature of the surface. Chemisorption of collectors is highly selective, as the chemical bonds are specific to particular atoms.

1.6.2 Flotation Columns

Flotation columns provide a means for improving the effectiveness of froth flotation (Eberts, 1986). A column essentially performs as if it were a multistage flotation circuit arranged vertically (Degner and Sabey, 1985), with slurry flowing downward while the air bubbles travel upward, producing a countercurrent flow. The first flotation machine design to use a countercurrent flow of slurry and air was developed by Town and Flynn in 1919. It was not until the work of Boutin and Tremblay in the early 1960's that a new generation of countercurrent columns was developed that ultimately became industrially successful (Rubinstein, 1995).

A typical flotation column is shown in Figure 13. The basic principle of column flotation is the use of countercurrent flow of air bubbles and solid particles. This is achieved by injecting air at the base of the column, and feed near the midpoint. The particles then sink through a rising swarm of air bubbles.

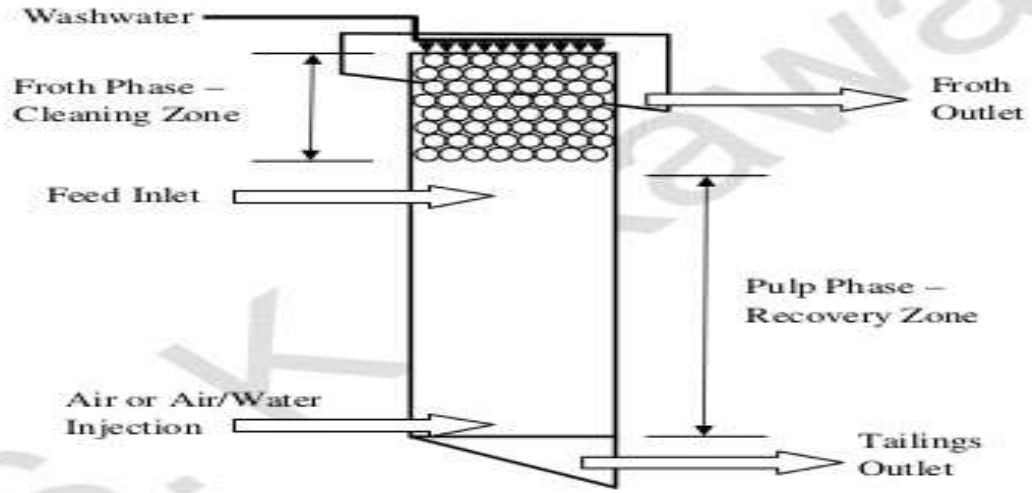


Figure 13: Schematic of a flotation column

Countercurrent flow is accentuated in most columns by the addition of washwater at the top of the column, which forces all of the water which entered with the feed downward, to the tailings outlet. This flow pattern is in direct contrast to that found in conventional cells, where both the air and the solid particles are driven in the same direction. The result is that columns provide improved hydrodynamic conditions for flotation, and thus produce a cleaner product while

maintaining high recovery and low power consumption. The performance differences between columns and conventional cells may best be described in terms of the following factors: collection zone size, particle/bubble contact efficiency, and fines entrainment (Kawatra and Eisele, 1987).

The collection zone is the volume where particle/bubble contact occurs, and it differs greatly in size between column and conventional flotation. In conventional cells, contact occurs primarily in the region surrounding the mechanical impeller. The remainder of the cell acts mainly as a storage volume for material which has not yet been through the collection zone. This creates a bottleneck which keeps the flotation rate down. In contrast, flotation columns have a collection zone which fills the entire volume of the machine, so that there are more opportunities for particle/bubble collisions. The reduced level of turbulence needed to achieve a good rate of recovery in columns also reduces the tendency of coarse particles to be torn away from the bubbles which they attach to, and therefore columns are more effective for floating coarser particles (Kawatra and Eisele, 2001).

Columns exhibit higher particle/bubble contact efficiency than conventional machines, due to the particles colliding with the bubbles head-on. As a result, the energy intensity needed to promote contact is less, and so power consumption is reduced.

A second beneficial effect in certain types of flotation columns is the reduction of bubble diameter (Yoon and Luttrell, 1986). As bubble diameter is reduced, the flotation rate of both the coarser and finer particles is improved. The effects producing the improvement are illustrated in Figure 14. Coarse particles can attach to more than one bubble if the bubbles are small, and therefore the chances of the particle being torn loose and sinking again is reduced. For fine particles, the probability of collision with the bubble is improved if the bubble is small, as then the hydrodynamic forces tending to sweep the particle away from a collision are reduced. The reduction of bubble diameter has the added benefit of increasing the available bubble surface area for the same amount of injected air. It is therefore desirable to produce bubbles as fine as possible.

The entrainment of fine waste material in the froth product is a serious failing of conventional flotation machines. It results from the need for a certain amount of water to be carried into the froth as the film surrounding the air bubbles. As a result, fine suspended particles are swept into the froth with this water, even though they are not physically attached to the air bubbles. In most column flotation machines, the entrainment problem is addressed through the use of washwater, as shown in Figure 15. Where the conventional cell must allow a certain amount of feed water to enter the froth, the washwater in the column cell displaces this feed water to the tailings, thus preventing entrained contaminants from reaching the froth. The only drawback to the use of froth washing is that the demand for clean water is increased, which may cause water problems in some situations.

NUCLEIC ACID

By

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THE NUCLEIC ACIDS

HISTORIC RESUME
Friedrich Miescher in 1869

- Isolated what he called **nuclein** from the nuclei of pus cells
- Nuclein was shown to have acidic properties, hence it became called **nucleic acid**

NUCLEIC ACID

- Nucleic acid are polymers that consist of nucleotide residues.
- Located in nuclei of cell
- Hereditary determinants of living organisms
- Elemental composition – carbon, hydrogen, oxygen, nitrogen and phosphorus

TYPES OF NUCLEIC ACID

- Deoxyribonucleic acid (DNA)
- Ribonucleic acid (RNA)

The distribution of nucleic acids in the eukaryotic cell

- DNA is found in the nucleus
  - with small amounts in mitochondria and chloroplasts
- RNA is found throughout the cell

NUCLEIC ACID STRUCTURE

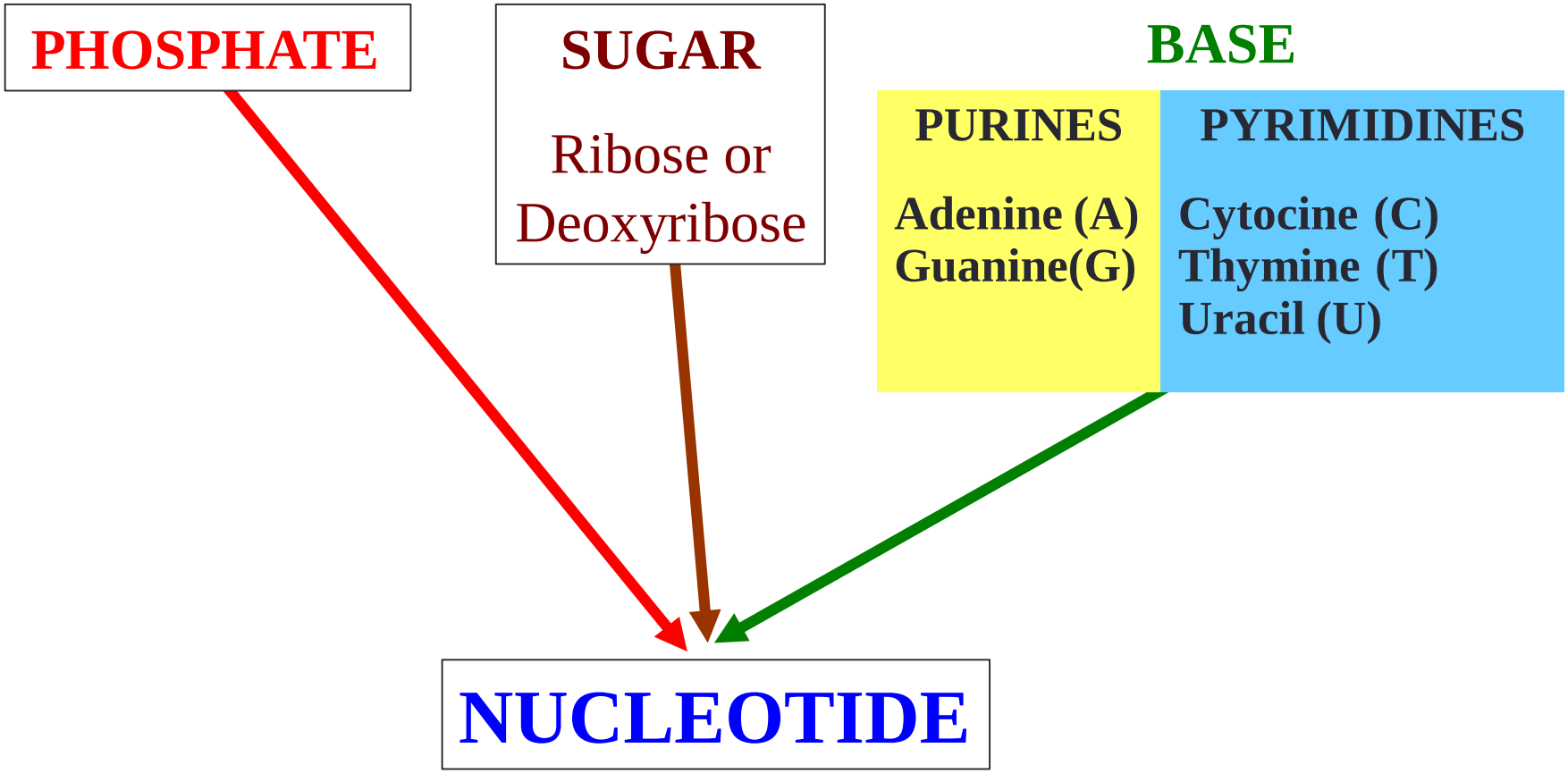
- Nucleic acids are **polynucleotides**

- Their building blocks are **nucleotides**

NUCLEOTIDES

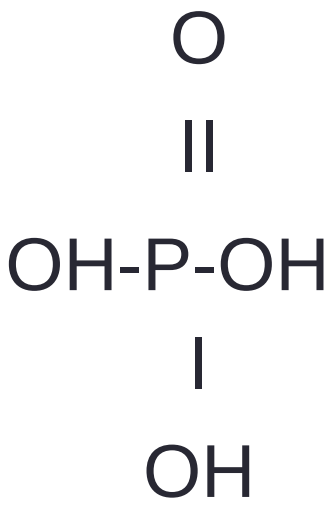
- Energy rich compounds that drive metabolic process in cell
- Serve as chemical signals, key links in cellular systems that respond to hormones and other extracellular stimuli
- Structural component of no of enzyme cofactor and metabollic intermediate
- Each nucleotide is formed by 3 units – PHOSPHATE, SUGAR, NITROGENOUS BASE

NUCLEOTIDE STRUCTURE



Phosphoric acid

- Molecular formula H3PO4
- Contains 3 monovalent hydroxyl group and a divalent oxygen atom
- All linked to pentavalent phosphorous atom



Sugar

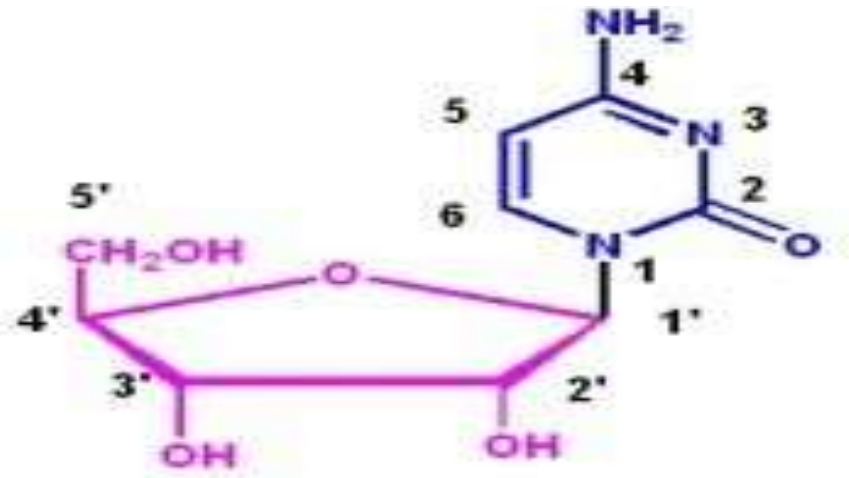
- 5 carbon keto sugar or pentose
- One possess d2 deoxyribose and other contain d ribose
- Both sugar are prsent in furanose form and beta configuration
- Pentose sugar form esters with phosphoric acid and is called phosphodiester bond

Nitrogenous base

- 2 types of nitrogenous base
- PURIN AND PYRIMIDINES DERIVATIVES
- This base is linked to sugar moiety by same carbon used in sugar-sugar bond
- PURINS are – ADENINE AND GUANINE
- PYRIMIDINE DERIVATIVES are – URACIL,THYMINE AND CYTOSINE

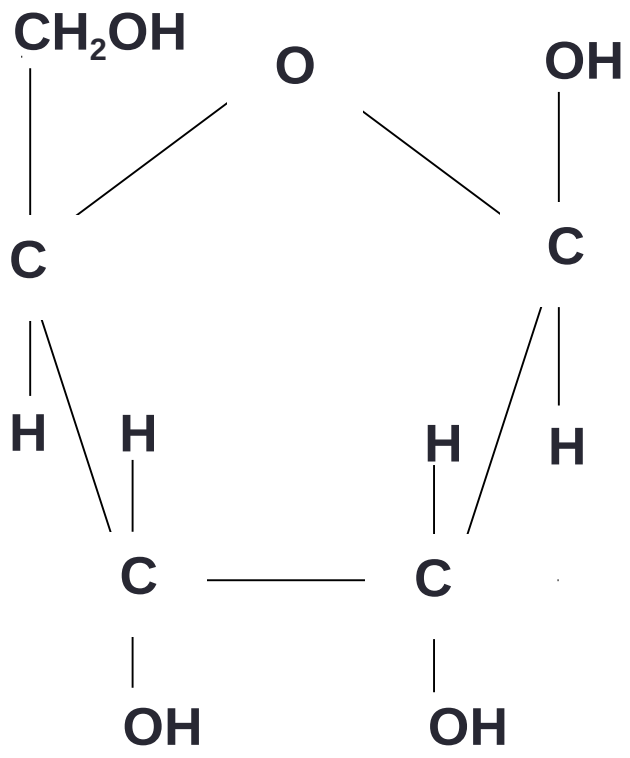
NUCLEOSIDES

- When ribose or 2-deoxyribose is combined with purine or pyramidine base Nucleoside is formed

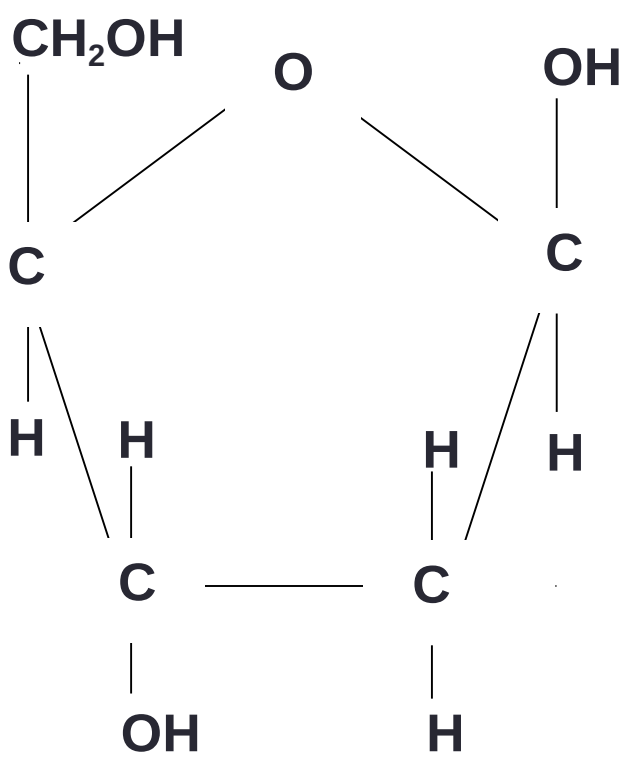


A nucleoside:
β-glycosidic bond between D-ribose and cytosine nitrogenouos base

RIBOSE

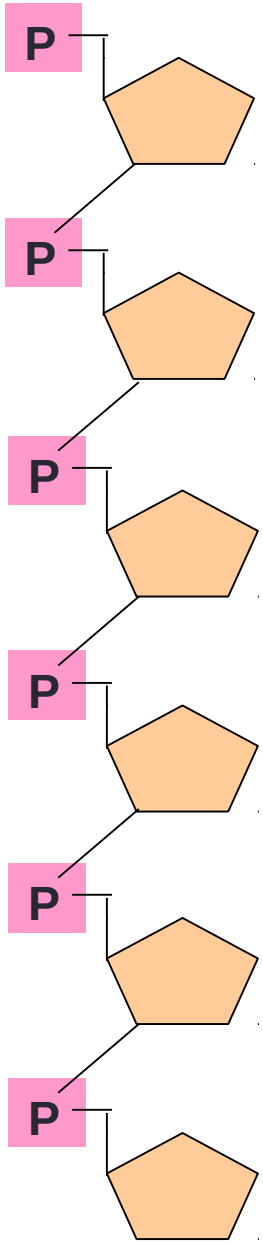


DEOXYRIBOSE



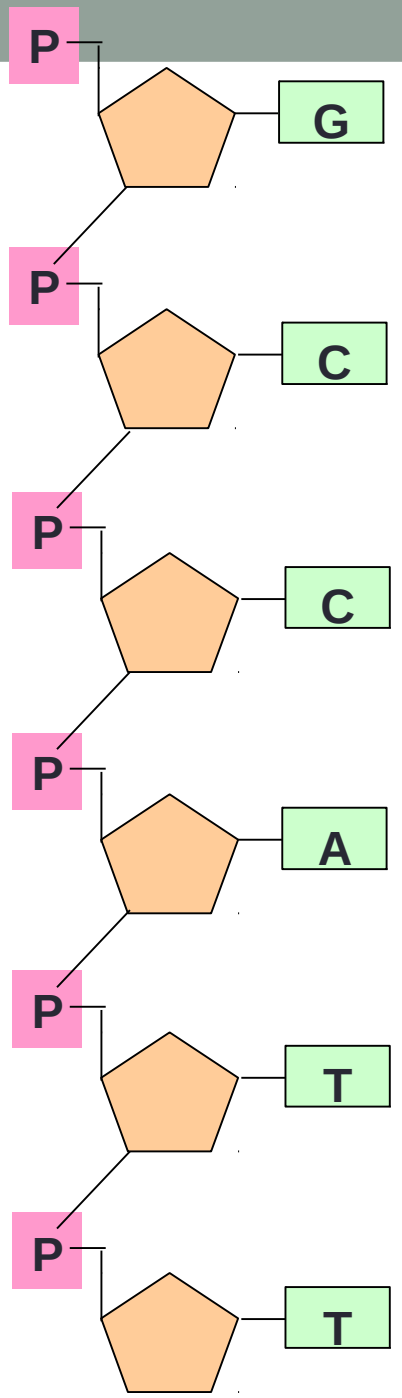
THE SUGAR-PHOSPHATE BACKBONE

- The nucleotides are all orientated in the same direction
- The phosphate group joins the 3rd Carbon of one sugar to the 5th Carbon of the next in line.



ADDING IN THE BASES

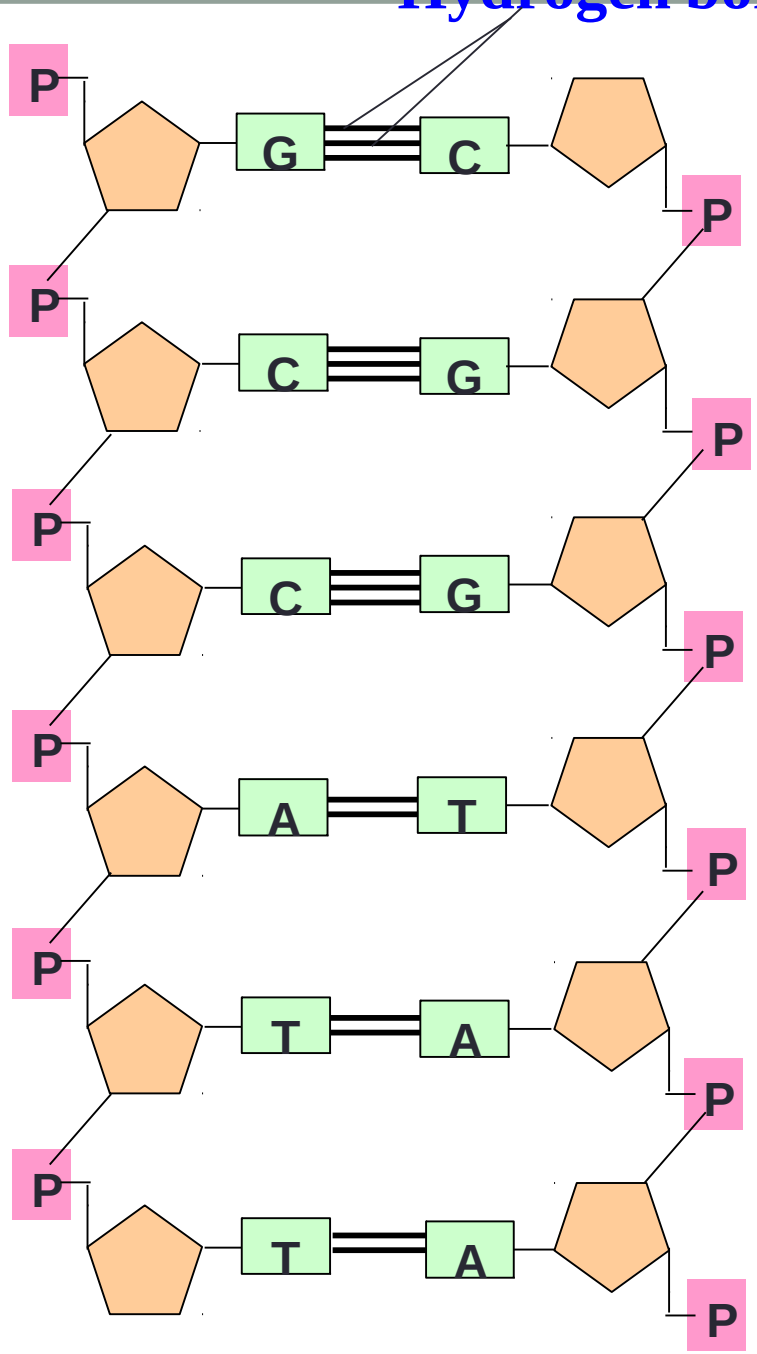
- The bases are attached to the 1st Carbon
- Their order is important
  It determines the genetic information of the molecule



DEOXY RIBONUCLEIC ACID

- Every living organism contain **DNA**
- **MATERIAL OF INHERITENCE**
- Discovered in 1960
- By **FRANKILIN, WATSON AND CRICK**
- Through series of experiments and concluded that DNA is the genetic material present in nucleus of cell
- Human DNA contains 3 million deoxyribonucleotide residues and contain 25000 genes
- Genes are stretch of DNA that carries codes of protein production

DNA IS MADE OF TWO STRANDS OF POLYNUCLEOTIDE



DNA IS MADE OF TWO STRANDS OF POLYNUCLEOTIDE

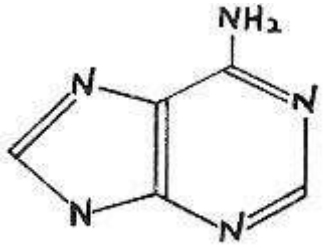
- The sister strands of the DNA molecule run in opposite directions (**antiparallel**)
- They are joined by the bases
- Each base is paired with a specific partner:

  A is always paired with **T**

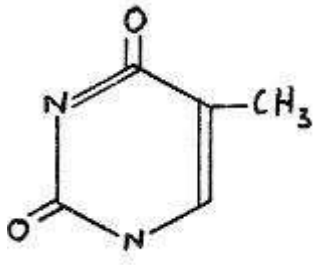
  G is always paired with **C**

  Ie,,,, each Purine is paired with Pyrimidine
- Thus the sister strands are **complementary** but **not** identical
- The bases are joined by **hydrogen bonds**

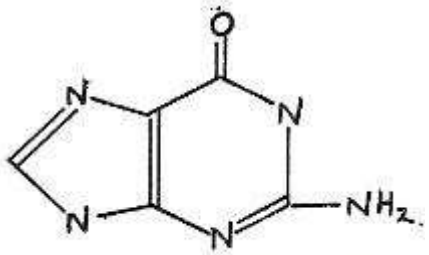
Purines & Pyrimidines



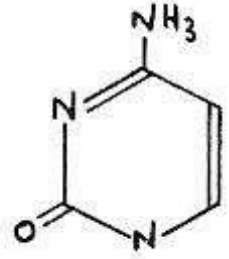
Adenine



Thymine

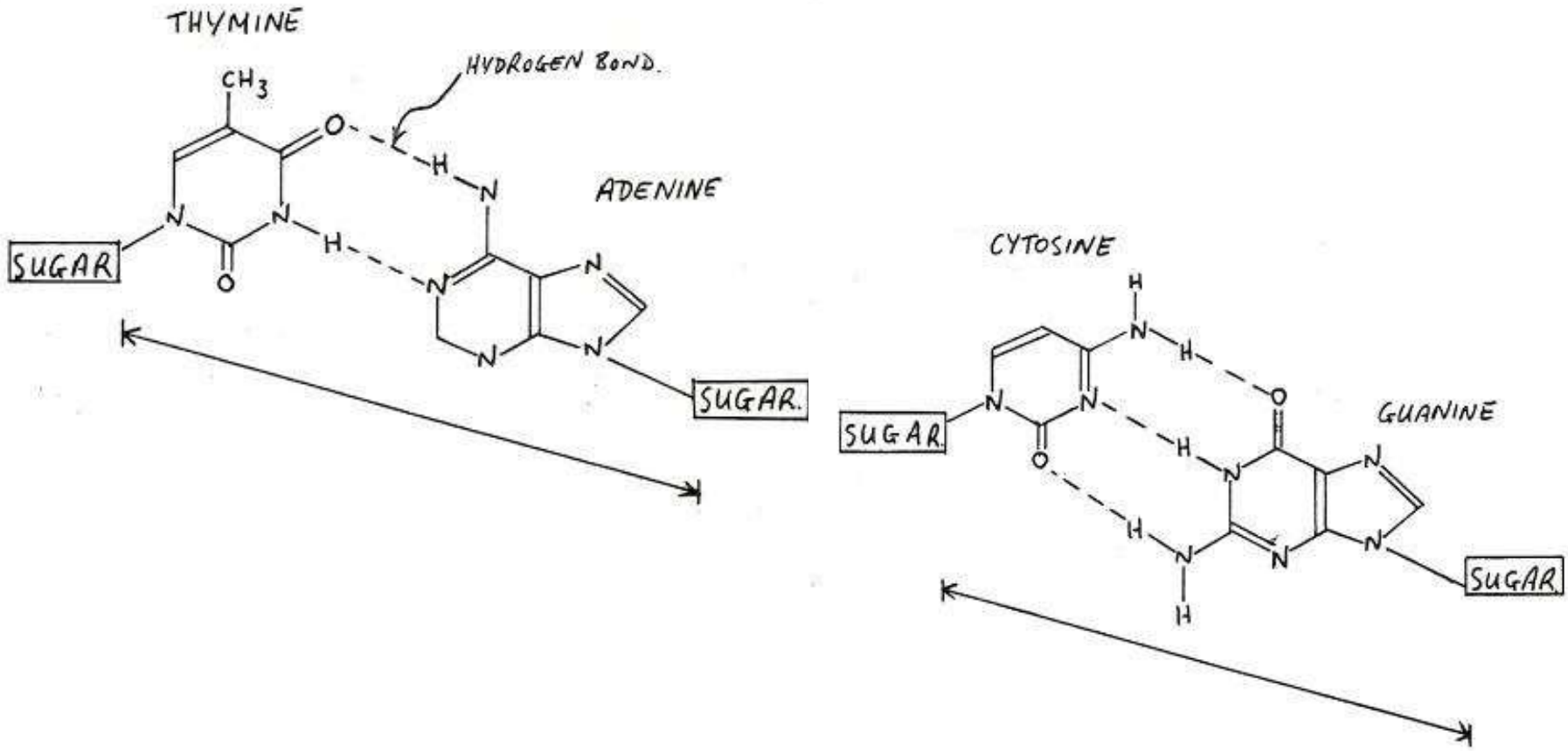


Guanine

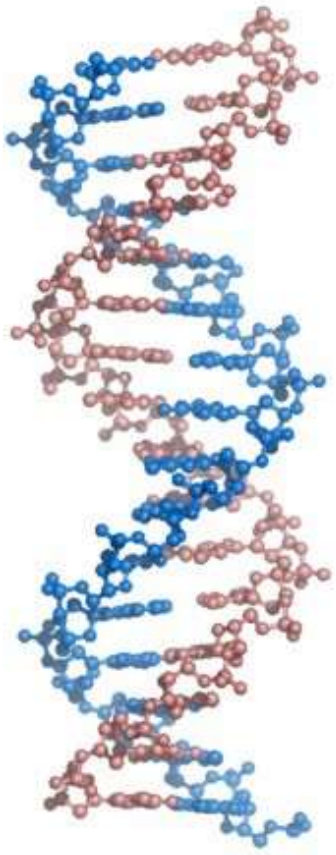


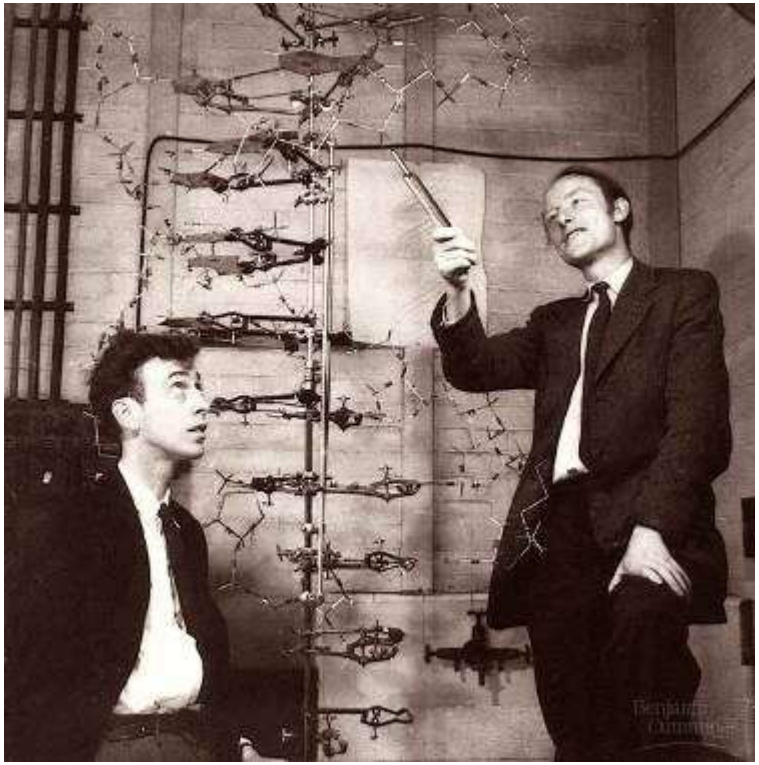
Cytosine

Watson & Crick Base pairing



The Double Helix (1953)





STRUCTURE OF DNA

- Structure of DNA can be understood in terms of 3 levels of structure

1. **PRIMARY STRUCTURE** ; refers to the sequence of its nucleotide residue

2. **SECONDARY STRUCTURE**; it pertains to the helix formed by two DNA strands

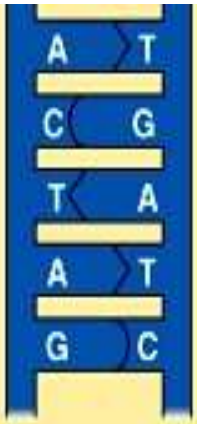
3. **TERTIARY STRUCTURE**: refers to the 3 dimensional shape. Arises from supercoiling where double helix is being twisted into compact shape

DNA REPLICATION

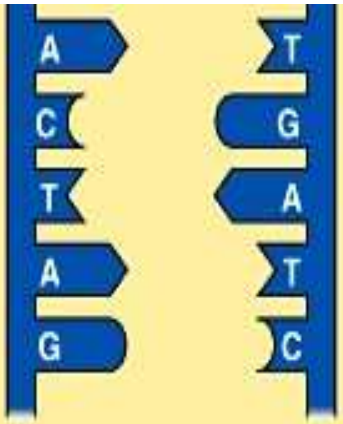
- DNA carries all information necessary for making protein required by living organisms
- To pass information stored in DNA to new generation of cell DNA replication must take place
- This information is carried in primary structure of dna
- To pass the information stored in DNA to a new generation of cells, DNA replication must take place
- When DNA is replicated, each strand of the doublehelix serves as a template for the manufacture of a new strand of DNA
- In each of the daughter *DNA* strands, one strand from the parent DNA is present

Contd…

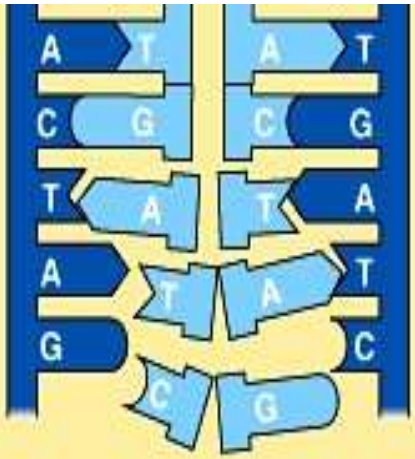
- This is called semiconservative replication
- The production of new DNA is carried out by enzymes called **DNA** polymerases
- DNA polymerase catalyzes ***the addition of deoxyribonucleotide residues*** to a growing DNA strand



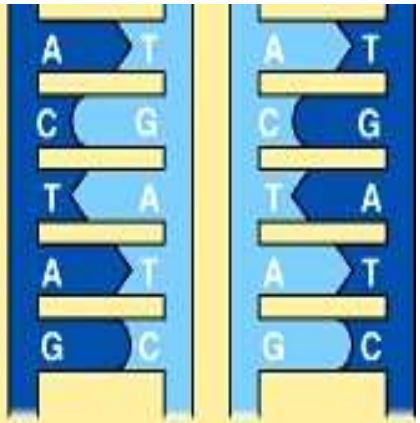
The parent molecule has two complementary strands of DNA. Each base is paired by hydrogen bonding with its specific partner, A with T and G with C.



(b) The first step in replication is separation of the two DNA strands.



(c) Each parental strand now serves as a template that determines the order of nucleotides along a new complementary strand.



(d) The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each "daughter" DNA molecule consists of one parental strand and one new strand.

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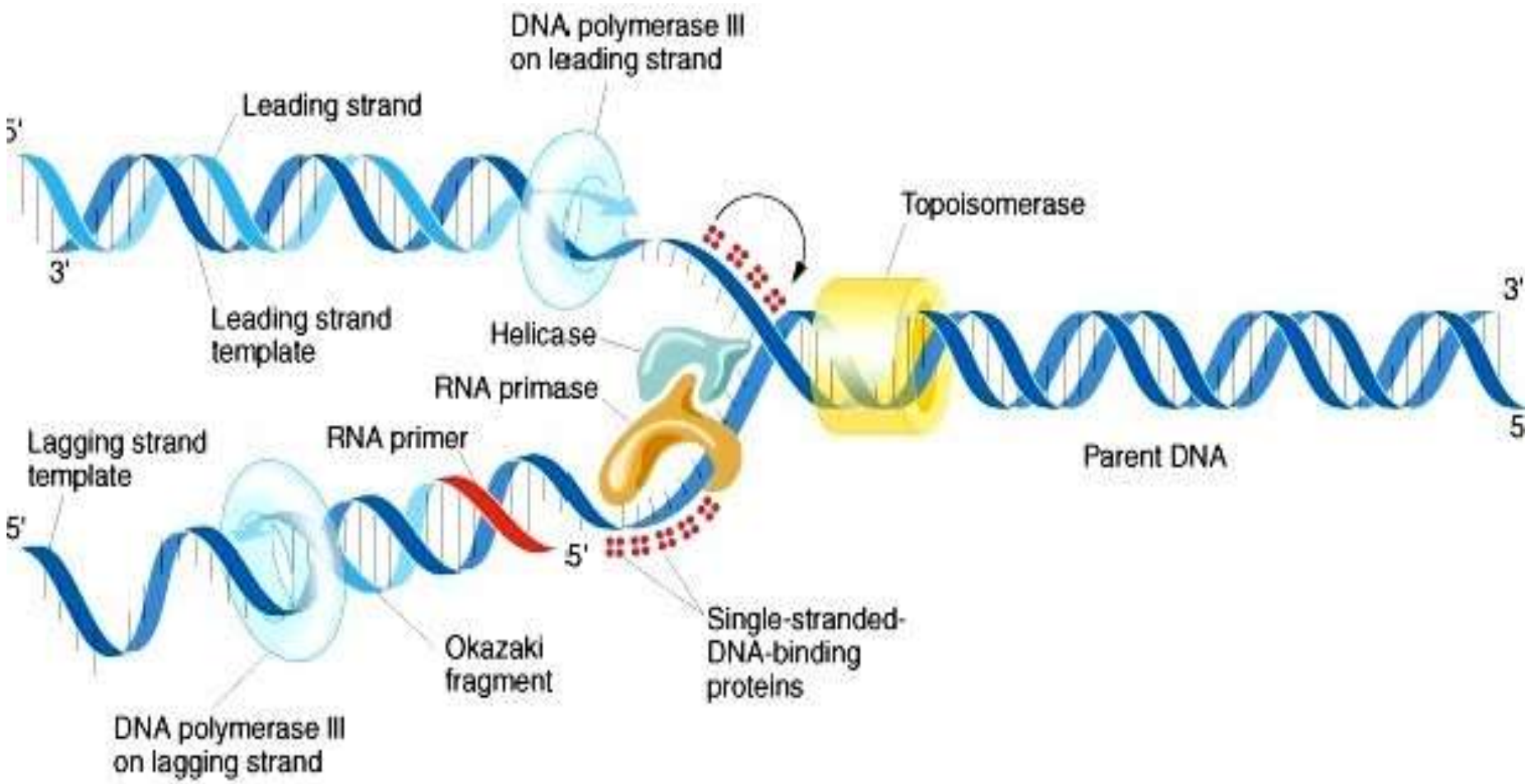
Detailed image of TRANSLATION



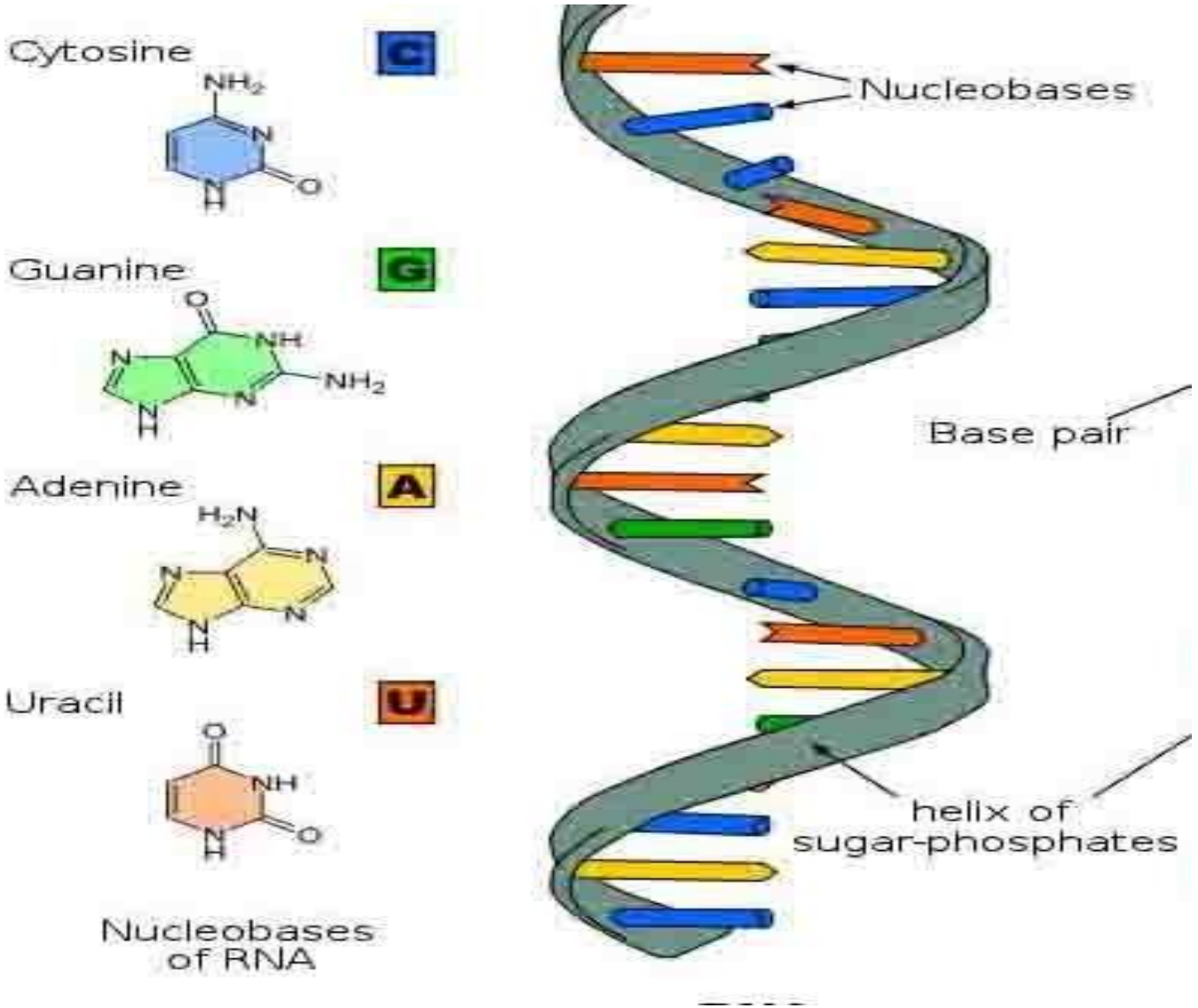
RNA

- Ribonucleic acid (RNA), like DNA, is a long, unbranched macromolecule consisting of nucleotides joined by $3' \rightarrow 5'$ phosphodiester bonds
- The number of ribonucleotides in RNA ranges from as few as 75 to many thousands

STRUCURE O RNA

- RNA structure is similar to DNA
- both are sugar-phosphate polymers and both have
- nitrogen-containing bases attached
- but there are3 differences between them

- 1) RNA contains a different monosaccharide residue
- 2) RNA contains the bases A, G, C, and **U** instead of A, G, C, and **T**
- 3) RNA exists as a single strand instead of a double strand

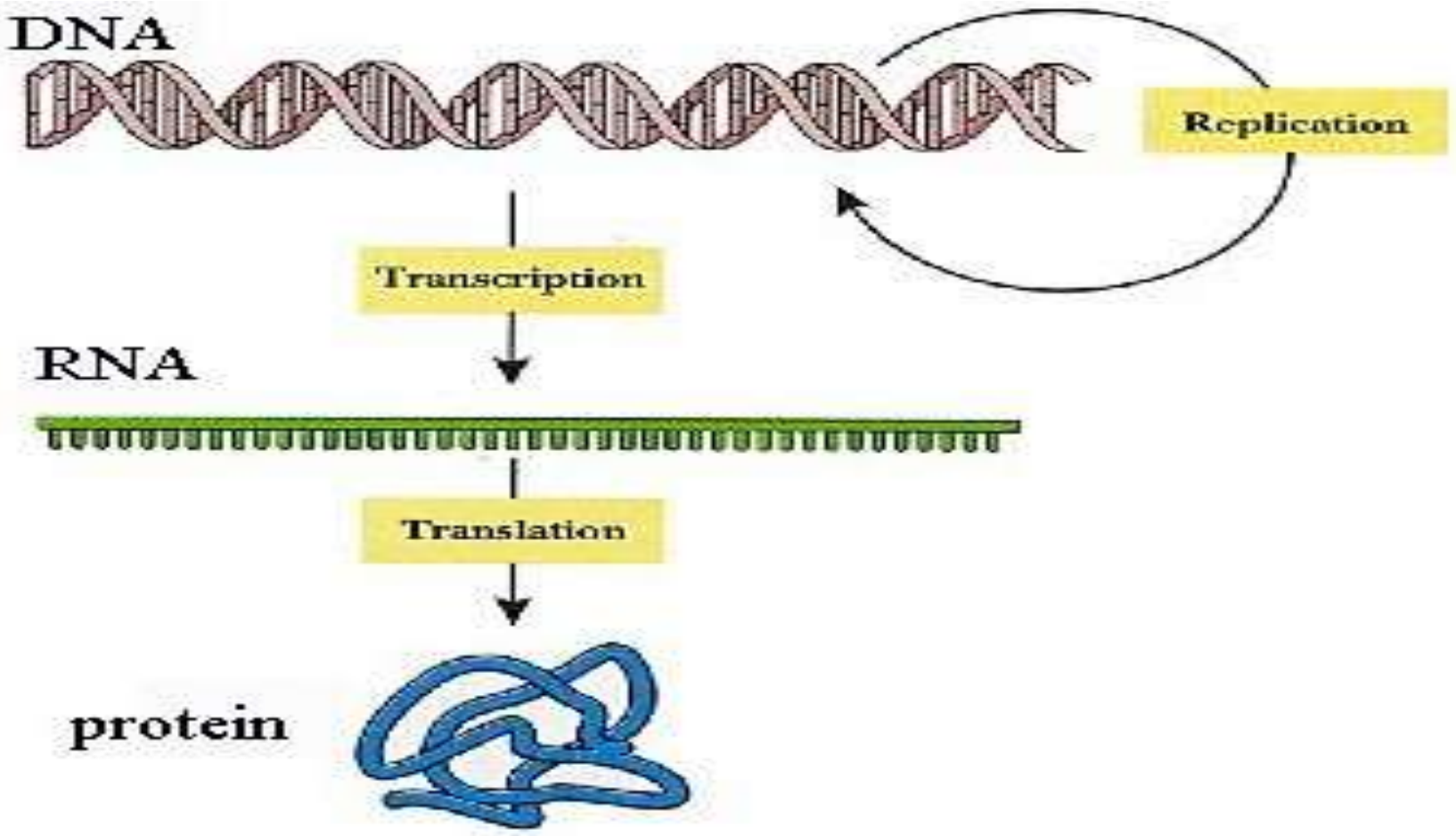

Cytosine
C
Guanine
G
Adenine
A
Uracil
U
Nucleobases of RNA
Nucleobases
Base pair
helix of sugar-phosphates

TYPES OF RNA AND FUNCTIONS

Type	Abbreviation	Function(s)
Messenger RNA	mRNA	Transfers genetic information from genes to ribosomes to synthesize proteins.
Heterogeneous nuclear RNA	hnRNA	Serves as precursor for mRNA and other RNAs
Transfer RNA	tRNA	Transfers amino acid to mRNA for protein synthesis.
Ribosomal RNA	rRNA	Provides structural framework for ribosomes
Small nuclear RNA	snRNA	Involved in mRNA processing
Small nucleolar RNA	snoRNA	Plays a key role in processing of rRNA molecules
Small cytoplasmic RNA	scRNA	Involved in selection of proteins for export.
Transfer messenger RNA	tmRNA	Mostly present in Bacteria. Adds short peptide tags to proteins to facilitate the degradation of incorrectly synthesized proteins.

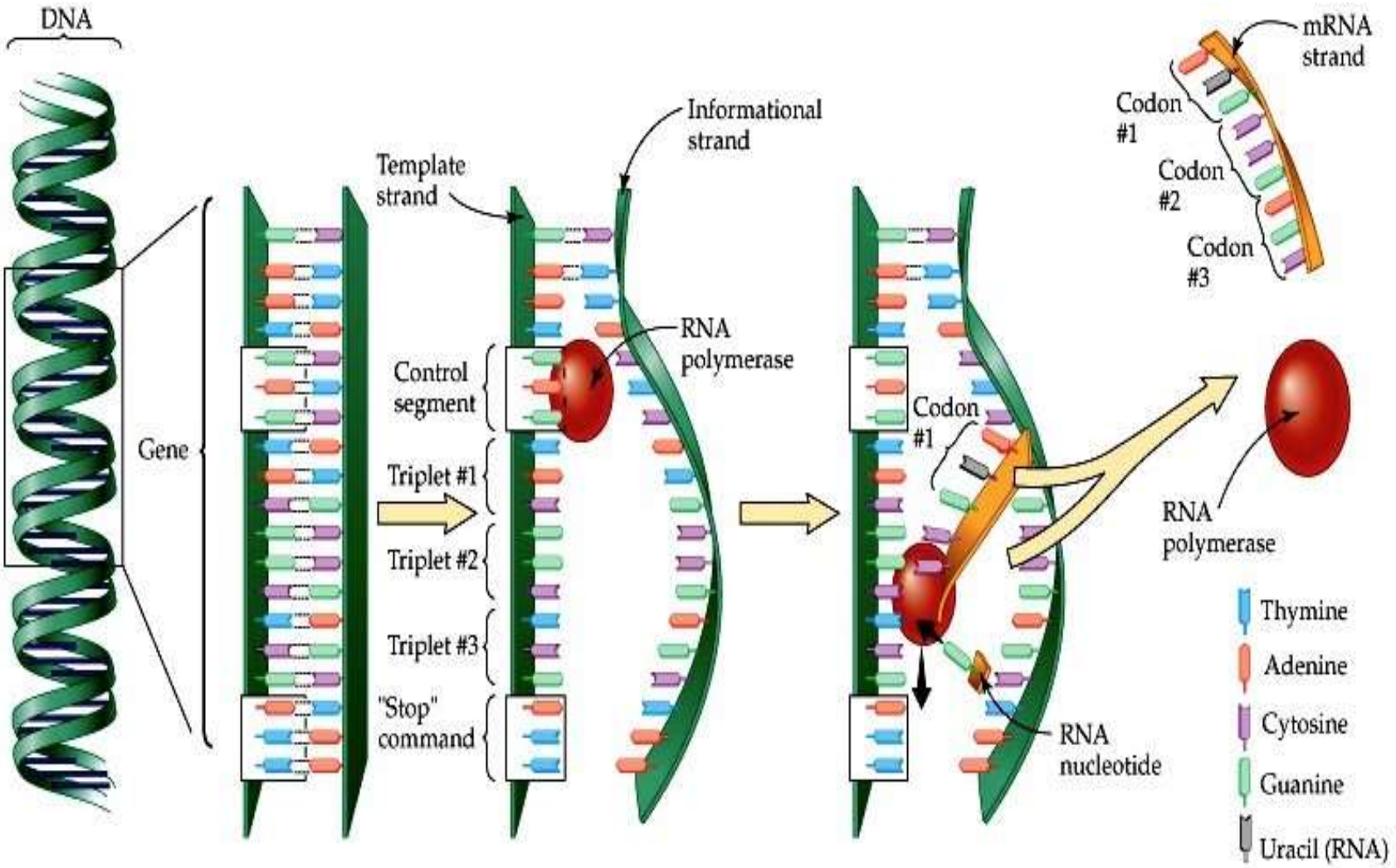
NUCLEIC ACID ND PROEIN SYNTHESIS

- The following three processes are involved In duplication, transfer, and use of genetic information
- ***1) Replication****:* The process by which a replica, or identical copy, of DNA is made when a cell divides
- ***2) Transcription***: The process by which the genetic messages contained in DNA are read and copied
- ***3) Translation*****:** The process by which the geneticmessages carried by RNA are decoded and used to build proteins


DNA
Replication
Transcription
RNA
Translation
protein

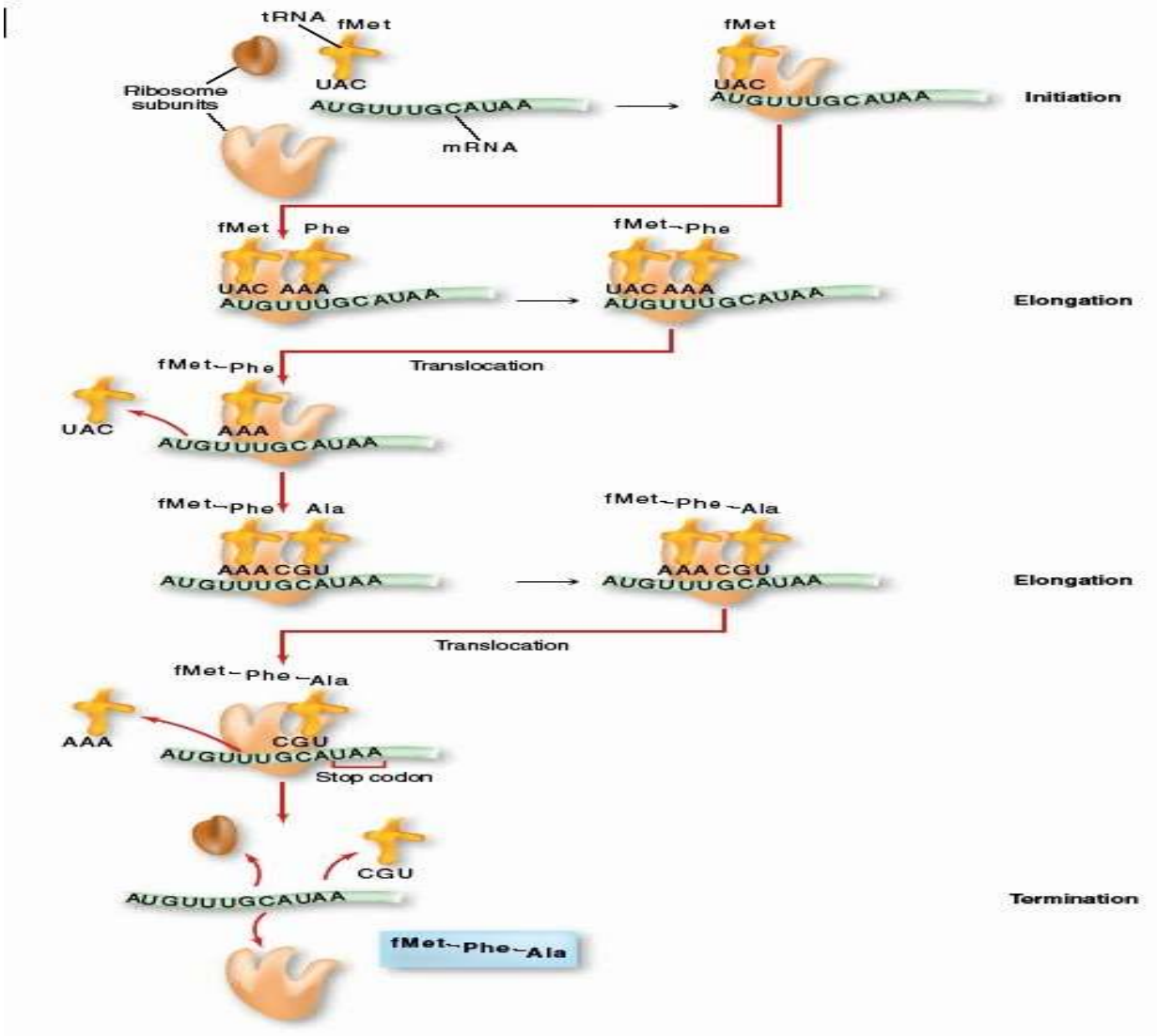
TRANSCRIPTION

- The first step in using the information stored in DNA to produce proteins is transcription - using DNA as a template to make RNA
- Controlled by interactions of promoter and enhancers
- Several different types of rnas are produced including mRna, tRna and rRna
- TRANSCRIPTION INVOLVES 4 STEPS
- **INITIATION**
- **ELONGATION**
- **TERMINATION**
- **PROCESSING**


DNA
Gene
Template strand
Informational strand
Control segment
Triplet #1
Triplet #2
Triplet #3
"Stop" command
RNA polymerase
Codon #1
RNA nucleotide
mRNA strand
Codon #1
Codon #2
Codon #3
RNA polymerase
Thymine
Adenine
Cytosine
Guanine
Uracil (RNA)

Translation: Protein Synthesis

- The synthesis of proteins occur at ribosomes, which are outside the nucleus and within the cytoplasm of cells
- The mRNA connects with the ribosome, and the amino acids attached to *transfer RNA* are delivered one by one
- **Protein synthesis, or translation, takes place in *three* steps:**
- **1. Initiation** - a ribosome, mRNA, and tRNA come Together to form a complex
- **2. Elongation** - amino acids are joined to the growing polypeptide chain
- **3.** Termination - the protein has been synthesized and the ribosome-mRNA-tRNA complex dissociates


tRNA
fMet
Ribosome subunits
UAC
AUGUUUGCAUAA
mRNA
fMet
UAC
AUGUUUGCAUAA
Initiation
fMet
Phe
UAC AAA
AUGUUUGCAUAA
fMet–Phe
UAC AAA
AUGUUUGCAUAA
Elongation
Translocation
fMet–Phe
UAC
AAA
AUGUUUGCAUAA
fMet–Phe
Ala
AAA CGU
AUGUUUGCAUAA
fMet–Phe–Ala
AAA CGU
AUGUUUGCAUAA
Elongation
Translocation
fMet–Phe–Ala
AAA
CGU
AUGUUUGCAUAA
Stop codon
CGU
AUGUUUGCAUAA
Termination
fMet–Phe–Ala

GENETIC CODE

- The ribonucleotide sequence in a mRNA chain is like a coded sentence that specifies the order in which amino acid residues should be joined to form a protein
- Each word, or **codon** in the mRNA sentence is a series of ***three*** ribonucleotides that code for specific amino acid
- For example, the series uracil-uracil-guanine (UUG) on an mRNA chain is a codon directing incorporation of the amino acid leucine into a growing protein chain

CONTROL OF GENE EXPRESSION

- The DNA of each living thing contains thousands of genes
- These genes are not continually **expressed** (*read to make proteins*), because the production of unneeded proteins would be an inefficient use of resources
- Control of gene expression prevents the manufacture of unwanted/unneeded proteins

GENE REGULATION

- Label for the cellular process that control the rate and manner of gene expression
- Mechanisms of gene regulation include;
- 1. regulating the rate of transcription
- 2. regulating the processing of RNA molecules
- 3. regulating the stability of mrna molecule
- 4. regulating the rate of translation

MUTATION

- Any permanent change in the primary structure of (sequence of nucleotide residues in) DNA is called **mutation**
- Mutations might involve the switching of one base pair for another or the addition or deletion of base pairs
- Errors in replication and exposure to mutagens (mutation causing agents, including x rays, UV radiation, nuclear radiation, and chemicals) are the common causes of mutations
- When the mutations are in the sex cells (sperm or ovarian cells), the mutation can be inherited - **genetic diseases**

RECOMBINANT DNA

- **Recombinant DNA** contains two or more DNA segments not found together in nature
- made by cutting a gene out of one organism and recombining it into the genetic machinery of another organism
- The protein encoded by the inserted gene is then synthesized by the target organism
- Insulin has been made this way

THANK YOU

Learning outcome

- After completion of this unit student should be able to

1. Discuss scope and types of nutraceuticals products available in the market
2. Know health benefits and role of Nutraceuticals in different ailments
3. Understand Herb-Drug and Herb-Food Interactions

Nutraceuticals

- ✓ The term ‘Nutraceutical' was derived from two words i.e. 'nutrition' and 'pharmaceutical'
- ✓ It was coined in 1989 by Dr. Stephen De Felice, USA
- ✓ It was originally defined as a food (or part of the food) that provides medical or health benefits, including the prevention and/or treatment of a disease

"Let food be your medicine and medicine be your food".

-Hippocrates (2000 year ago)

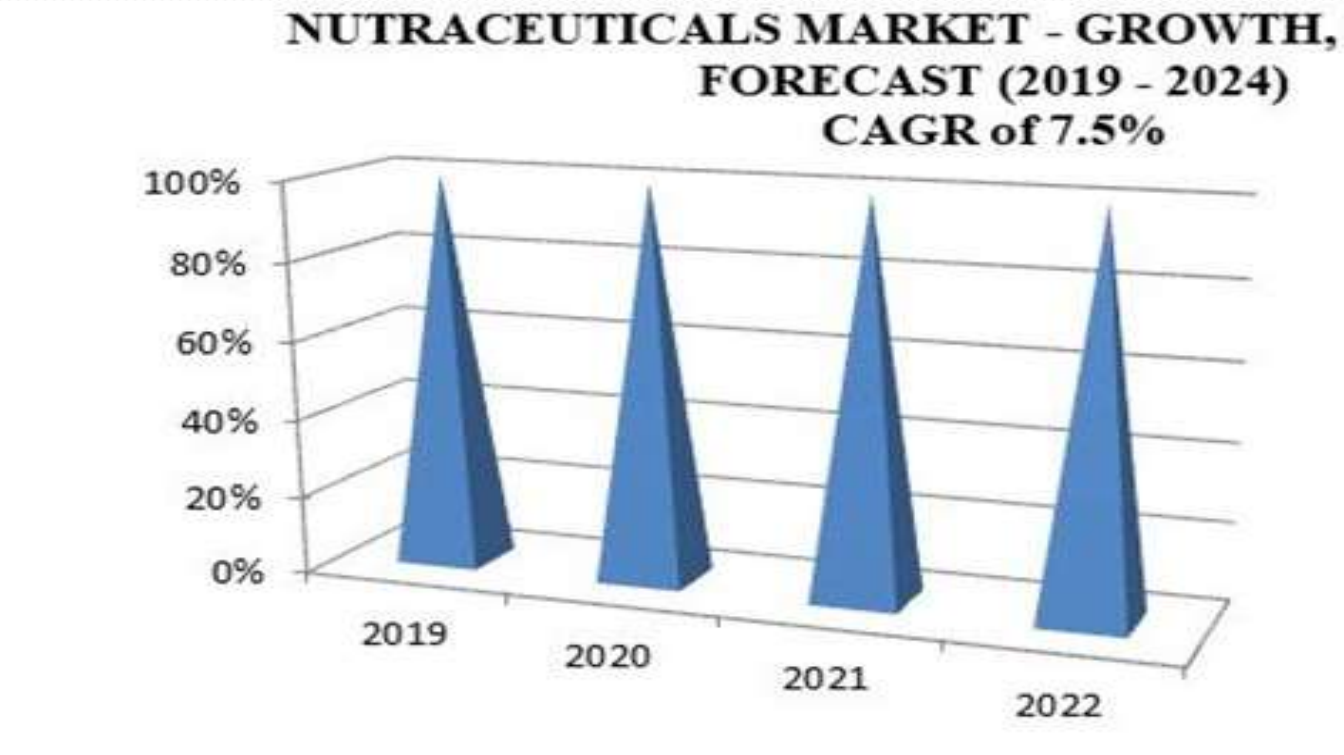
- Nutraceuticals are a group of products that are more than food but less than pharmaceuticals.
- Unfortunately there is still no internationally accepted definition of these products therefore their judgement varies country by country.
- In most countries nutraceuticals are taken as part of dietary supplements.
- Frequency of nutraceuticals use is 50%–70% in developed countries population and this number is increasing by the age.

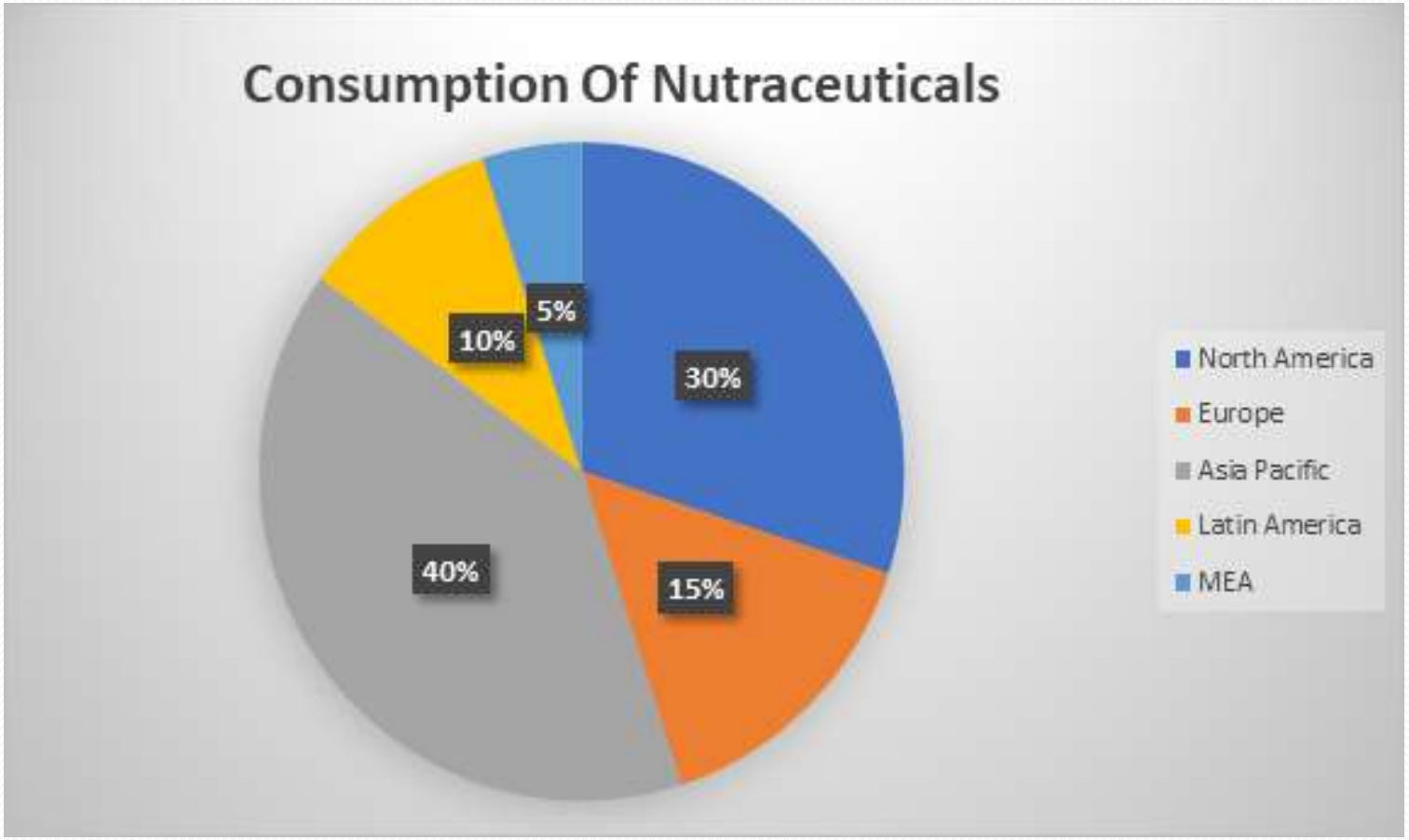
NUTRACEUTICALS vs PHARMACEUTICALS

NUTRACEUTICALS	PHARMACEUTICALS
• Works on root causes and take longer time for recovery	• Relatively more instant in effect
• Do not have side effects	• For severe illnesses have side effects
• Focuses on prevention & wellness	• Focuses on illness & treatment
• No prescription needed for buying	• Sold only on prescription except OTC
• Needs no approval of FDA	• Needs approval of FDA

Global Market Overview

- Predicted to record a revenue of USD 671.30 billion by 2024

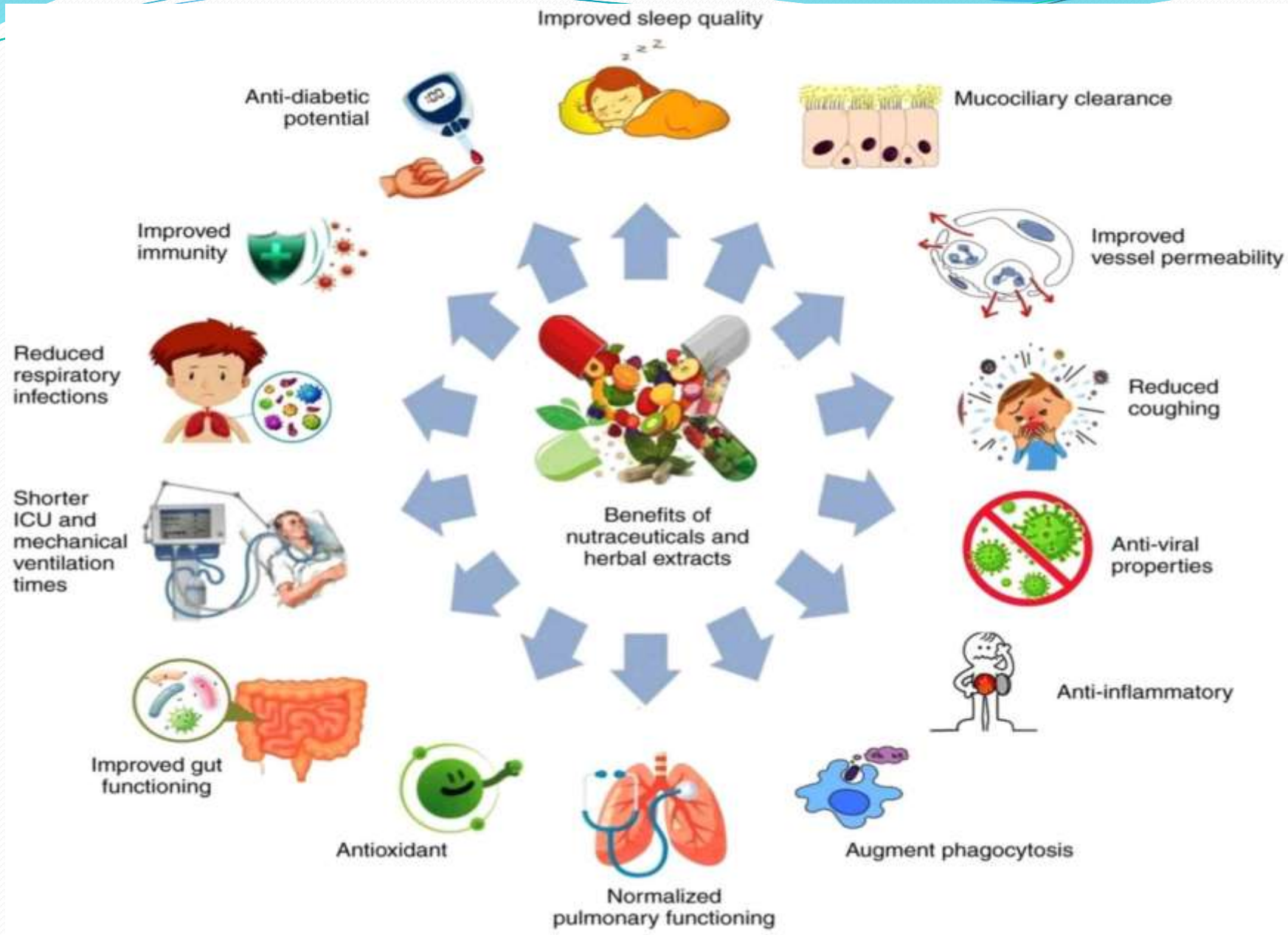




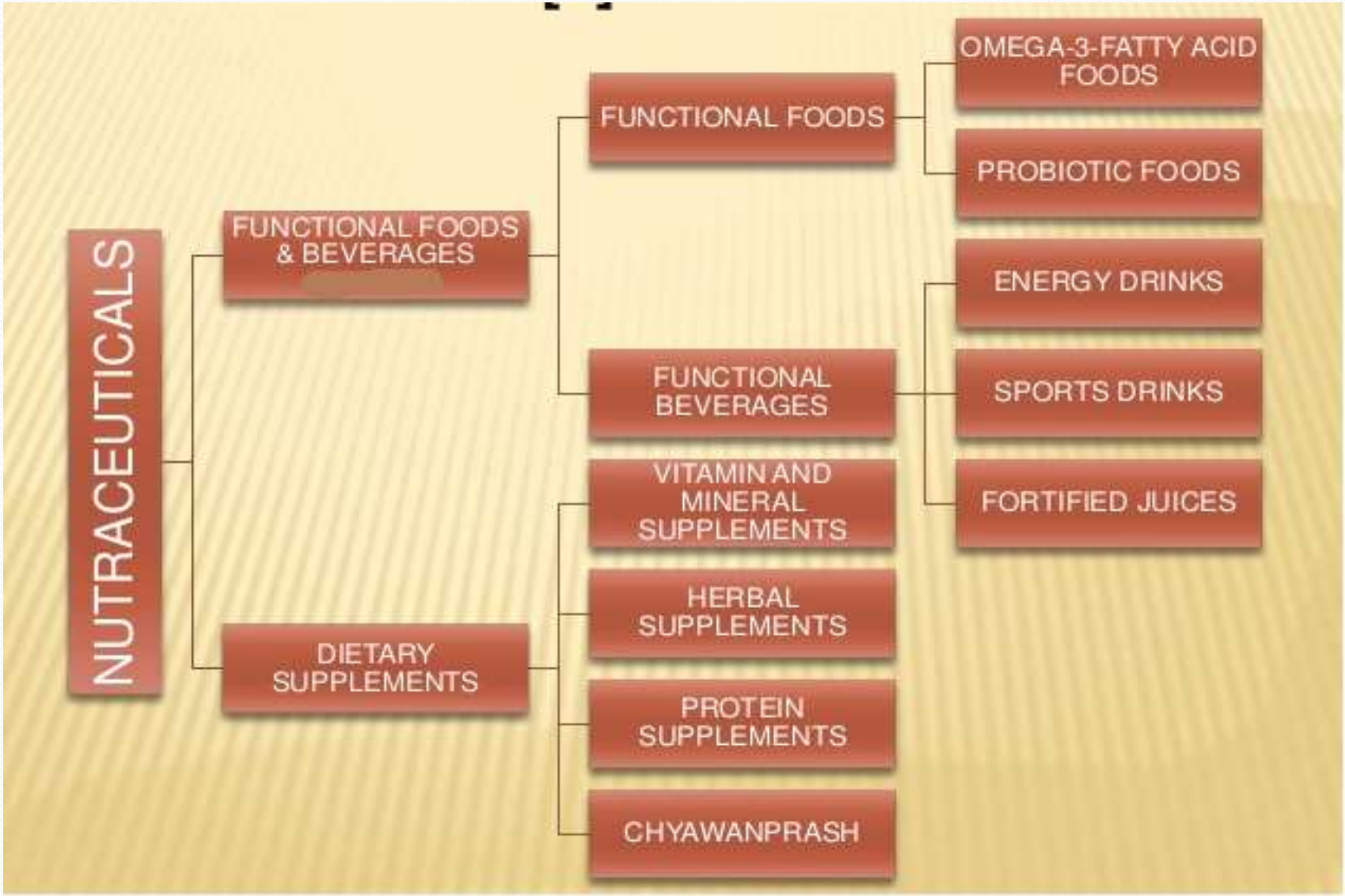
*MEA-Middle east countries Bahrain, Cyprus, Egypt, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Oman, Palestine, Qatar, Saudi Arabia etc.

- Developing countries have high prevalence of non communicable diseases like cancer, diabetes, cardiovascular ailments therefore demand of nutraceutical is expected to rise in these nations.
- Gaining global importance and have become a part of daily diet due to increased risk of diseases due to change in life style and people consciously adapting preventive health care measures.
- Nutraceuticals have become an opportunity for economic growth of many developing countries which have a rich source of medicinal herbs and traditional knowledge like India, China and South america.

RANBAXY
LABORATORIES LIMITED
Abbott
Piramal
DR. REDDY'S
ZANDU
Ayurveda Products
Pfizer
Dabur
Amul
The Taste of India
PARRYS
gsk
Zydus
Wellness
Heinz
Amway
HERBALIFE
Baidyanath
Yakult


Improved sleep quality
Anti-diabetic potential
Mucociliary clearance
Improved immunity
Improved vessel permeability
Reduced respiratory infections
Reduced coughing
Shorter ICU and mechanical ventilation times
Benefits of nutraceuticals and herbal extracts
Anti-viral properties
Anti-inflammatory
Improved gut functioning
Antioxidant
Augment phagocytosis
Normalized pulmonary functioning

Classification



Role of Nutraceuticals in Diabetes

- Diabetes mellitus is characterized by abnormally high levels of blood glucose, either due to insufficient insulin production, or due to its ineffectiveness.
- The most common forms of diabetes are type 1 diabetes (5%), an autoimmune disorder, and type 2 diabetes (95%), which is associated with obesity.
- Gestational diabetes occurs in pregnancy.
- Globally the total number of people with diabetes is projected to raise very high.
- Docosahexaenoic acid modulates insulin resistance and is also vital for neurovisual development.
- This is especially important in women with gestational diabetes mellitus which foster the recommendation for essential fatty acids during pregnancy.

- Lipoic acid is a universal antioxidant, now used in Germany for the treatment of diabetic neuropathy.
- It is possible that lipoic acid may be more effective as a long-term dietary supplement aimed at the prophylactic protection of diabetics from complications.
- Dietary fibers from psyllium have been used extensively both as pharmacological supplements, food ingredients, in processed food to aid weight reduction, for glucose control in diabetic patients and to reduce lipid levels in hyperlipidemia.
- Good magnesium status reduces diabetes risk and improves insulin sensitivity.

- Chromium, calcium and vitamin D appear to promote insulin sensitivity and improve glycemic control in some diabetics; extracts of bitter melon and of cinnamon have the potential to treat and possibly prevent diabetes.
- However it has been suggested that Nutraceuticals with meaningful doses of combinations may substantially prevent and presumably could be marketed legally.

Role in Cardio Vascular Diseases

- Worldwide, the burdens of chronic diseases like cardiovascular diseases, cancers, diabetes and obesity is rapidly increasing.
- In 2001, chronic diseases contributed approximately 59% of the 56.5 million total reported deaths in the world and 46% of the global burden of disease.
- Cardiovascular diseases (CVD) is the name for the group of disorders of the heart and blood vessels and include hypertension (high blood pressure), coronary heart disease (heart attack), cerebrovascular disease (stroke), heart failure, peripheral vascular disease etc.

- Majority of the CVD are preventable and controllable.
- It was reported that low intake of fruits and vegetables is associated with a high mortality in cardiovascular disease.
- Many research studies have identified a protective role for a diet rich in fruits and vegetables against CVD.
- Nutraceuticals in the form of antioxidants, dietary fibers, omega-3 polyunsaturated fatty acids , vitamins, and minerals are recommended together with physical exercise for prevention and treatment of CVD.

- It has been demonstrated that the molecules like polyphenols present in grapes and in wine alter cellular metabolism and signalling, which is consistent with reducing arterial disease.
- Flavonoids are widely distributed in onion, cruciferous vegetables, black grapes, red wine, grapefruits, apples, cherries and berries.
- Flavanoids in plants available as flavones (apigenin in chamomile); flavanones (hesperidins - citrus fruits; silybin- milk thistle) flavonols (tea: quercetin, kaempferol and rutin grapefruit; ginkgo flavonglycosides - ginkgo) play a major role in curing the cardiovascular diseases.

- Flavonoids block the angiotensin-converting enzyme (ACE) that raises blood pressure; by blocking the "suicide" enzyme cyclooxygenase that breaks down prostaglandins, they prevent platelet stickiness and hence platelet aggregation.
- Flavonoids also protect the vascular system and strengthen the tiny capillaries that carry oxygen and essential nutrients to all cells.

Role of Nutraceuticals in Cancer

- Cancer is a growing health problem around the world particularly with the steady rise in life expectancy, increasing urbanization and the subsequent changes in environmental conditions and lifestyle.
- The transformation of a normal cell into a cancerous cells requires stages of initiation, progression, and promotion by altering specific genes.
- The predominant forms of cancer, as determined by population and epidemiological studies, are those of the lung and bronchus, breast, colorectal and prostate.

- These cancers are prevalent in the western parts of the world, while their incidence is much lower in Asian countries
- A well-balanced diet that includes more of vegetables and fruits with less fat/meat intake is a staple of many Asian countries
- So, many hypotheses highlight that diet and environment greatly influence cellular function and health.
- It has also been estimated that one-third of all cancers deaths are preventable by life-style changes including appropriate nutrition

- Botanicals have a long history of use in the treatment of cancer. Many cancer chemotherapeutics drugs are derived from plants including the alkaloids of the Vinca species (vincristine and vinblastine) and the Pacific yew *Taxus brevifolia (Taxol).*
- There is evidence that foods, relatively low in simple carbohydrates with moderate amounts of high-quality protein, fiber, and fat (especially fats of the omega-3 fatty acid series) are beneficial for cancer patients.

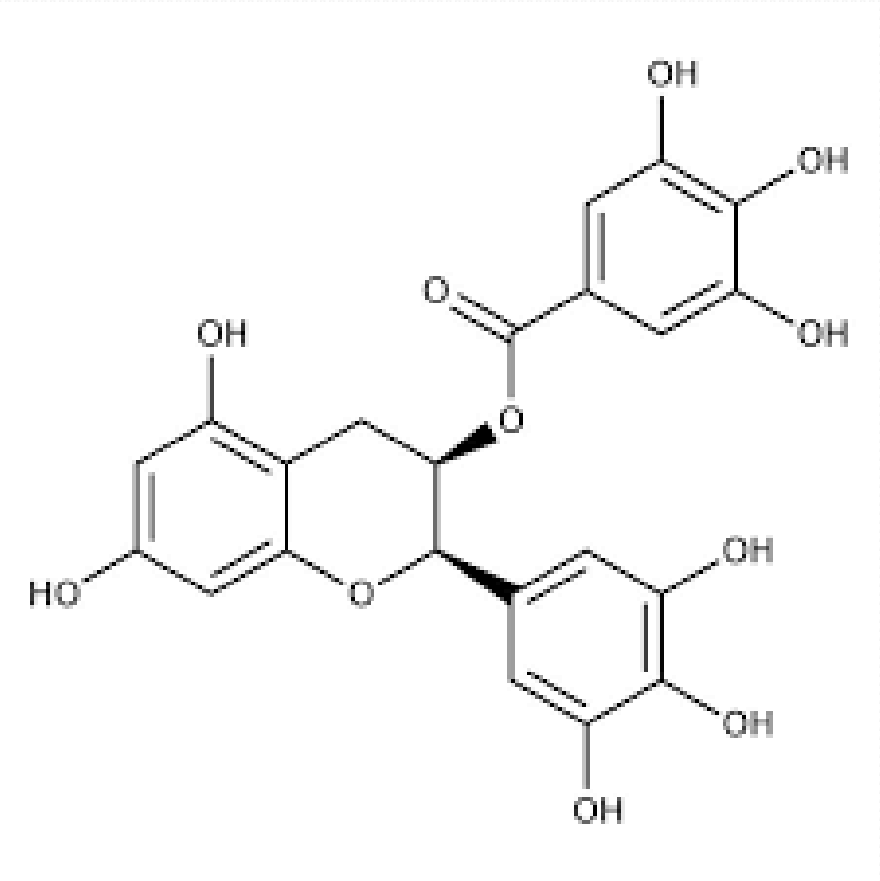
Phytopharmaceuticals for Cancer

- *Polyphenols*
- Polyphenols are plant secondary metabolites that contain one or more hydroxyl group attached to a benzene ring in their structure.
- More than 8000 different polyphenols found in food (wine, tea, coffee, cocoa, vegetables and cereals) are present in the human diet.
- In this context, the groups of phenolic acids, flavonoid, stilbenes and curcuminoids are the most important
- They have capacity for blocking initiation of carcinogenic process and to suppress cancer progression.

Epigallocatechin-3-gallate

- EGCG (epigallocatechin-3-gallate) is the major catechin found in green tea (*Camellia sinensis).*
- The frequent consumption of green tea in Asian countries has been related to several health benefits and it is recognized as the most effective cancer-preventive beverage.
- The antitumor properties of EGCG has been recognized on multiple cancer cell lines, including less common tumors such as anaplastic thyroid carcinoma and malignant mesothelioma [cancer of the thin tissue (mesothelium) that lines the lung, chest wall, and abdomen].

Epigallocatechin-3-gallate

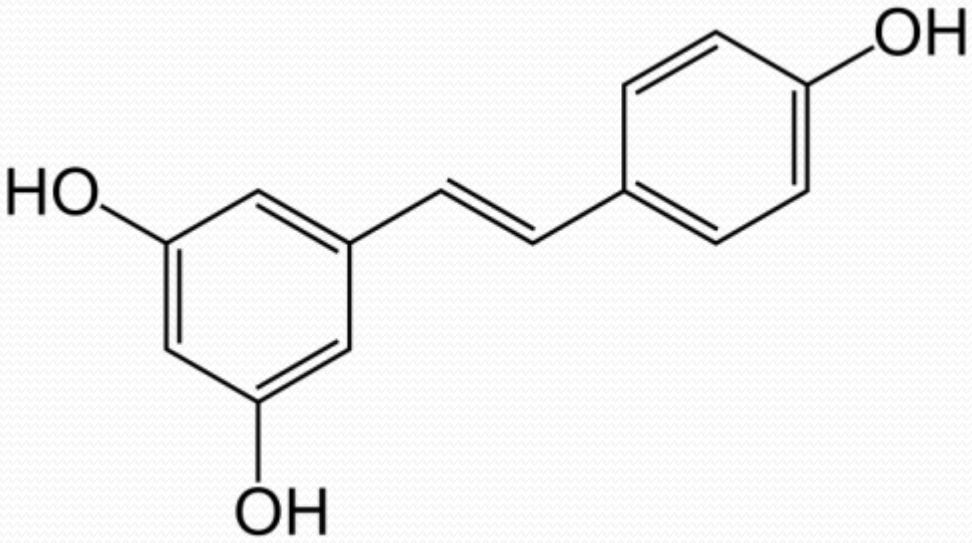


- The wide range of antitumor effects exerted by epigallocatechin-3-gallate suggests that this compound is a potential tool for cancer prevention therapy, both alone and in combination with antitumor drugs or other phytochemicals.

Resveratrol

- Resveratrol is the most important stilbene related to cancer.
- It possesses a natural anti-proliferative activity due to its role as a phytoalexins (plant antibiotic). It is believed to have also multiple bioactivities including anti-cancer, anti-carcinogenesis and anti-inflammatory effects.
- The mechanisms by which Resveratrol might produce these effects are not completely understood, but the main molecular mechanism seems to be the activation of sirtuin proteins (Signaling protein)

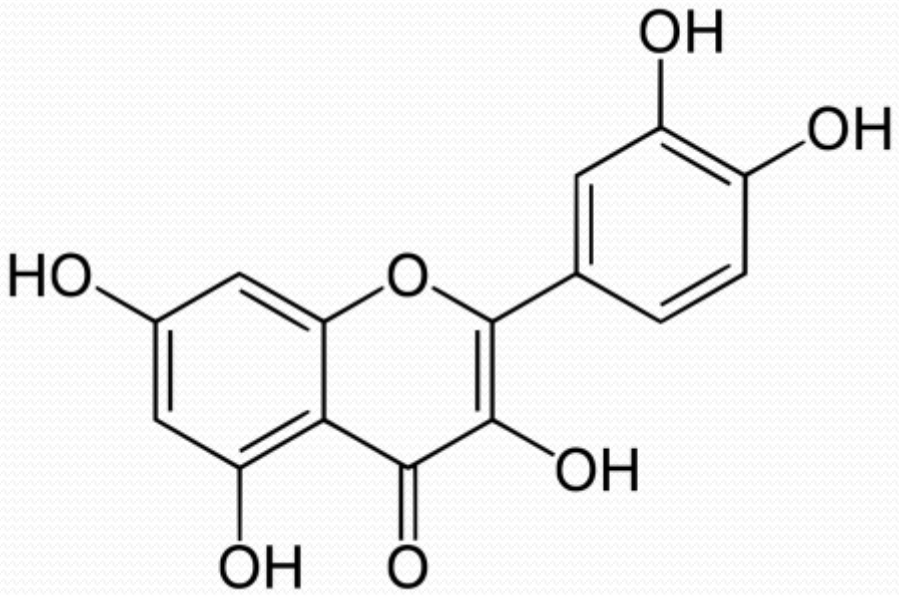
Resveratrol



Quercetin

- Quercetin, a representative member of the flavonoid class, is a plant-derived compound obtained from various fruits and vegetables that can reach levels in the human diet as high as 16-25 mg/day.
- The effects of quercetin are considered to be related to the induction of cell apoptosis through multiple mechanisms.
- In vivo studies of the anticancer effects of quercetin have demonstrated that oral administration *can* prevent induced carcinogenesis, particularly in the colon.

Quercetin



- **Vitamins and Minerals in Cancer Management**
- The role of vitamins A, C, E and trace elements like selenium has been suggested to prevent cancer in several independent studies.
- Supplementation with micronutrients as adjuvant in cancer patients may prove to be helpful.
- Ascorbate (vitamic C) is an essential nutrient in the human diet, but is also widely used as a medicinal product, and has long been held as a remedy for various diseases

- Studies focusing on the mechanism of ascorbate toxicity reported the induction of apoptosis through cell cycle arrest, activation of the apoptosis factors and interference with iron uptake in cells.
- However, ascorbate is known to act as an electron donor in redox reactions, and a body of evidence supports the idea that oxidative stress plays major role in the mechanism of ascorbate toxicity in tumor cells.

Role of Nutraceuticals in Inflammatory Bowel Disease

- Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are a group of idiopathic, chronic and relapsing inflammatory disorders of the gastrointestinal tract, whose incidence and prevalence has been increasing in the last decades.
- Phenolic compounds represent the most widely distributed plant secondary metabolites and several studies have investigated their effects on intestinal inflammation, either as pure molecules or as plant extracts, in humans.

- IBS is a functional bowl disorder that features with abdominal pain or discomfort and defecation disrupt.
- Comparing with other G.I. disorders, the special part of IBS is that it is commonly associates with psychological conditions like depression, anxiety.
- It is reported that around 60% of IBS patients have major psychological problems.
- *Andrographis paniculata* is a plant growing mainly in south and southeastern Asia.

- Boswellic acids are active components of *Boswellia serrata*.
- More than 12 different boswellic acids have been identified so far .
- They have been demonstrated anti-inflammatory activity by inhibition of leukotriene synthesis and prostaglandin synthesis.
- Another study evaluated a mixture of several herbal medicines (*Boswellia carterii, Zingiber offcinale, and Achillea millefolium*) with positive effects in patients with IBS

- Curcumin is the principal natural curcuminoid found in *Curcuma longa*, which is commonly used as a spice, food preservative and a coloring agent in foods.
- Its anti-inflammatory mechanism works mainly through the suppression of the inflammatory pathway, representing a safe and promising agent for treatment of IBD.

ALFALFA

- Alfalfa (*Medicago sativa* L.) F- fabaceae is a legume that has been cultivated for about 2000 years and mainly used in animal nutrition due to its high protein and fiber contents.
- Although primarily used in animal feed, alfalfa has been introduced recently into human diet due to its richness in easily assimilated proteins, minerals, vitamins and essential amino acids.
- Furthermore, as a nutritious food, the herb has a mild flavor and works well in salads, and soups.
- In Europe, it is mainly consumed as sprouts, which are added to salads and sandwiches either raw or cooked

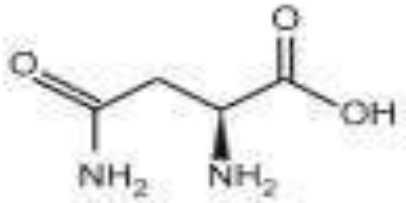
Also called **lucerne**, is a perennial flowering plant in the legume family Fabaceae.

- Alfalfa is usually available as a nutritional supplement (capsules, tablets, powder) consisting of plant protein isolate or powdered aerial part, as well as in the form of infusions from leaves and seeds.
- Due to its rich content in proteins, vitamins, minerals, isoflavones and other substances with estrogenic activity, anti-inflammatory properties and antioxidant activity (carotens, chlorophyll), alfalfa can be used as an efficient functional ingredient in the dietary prevention and treatment of several metabolic conditions.

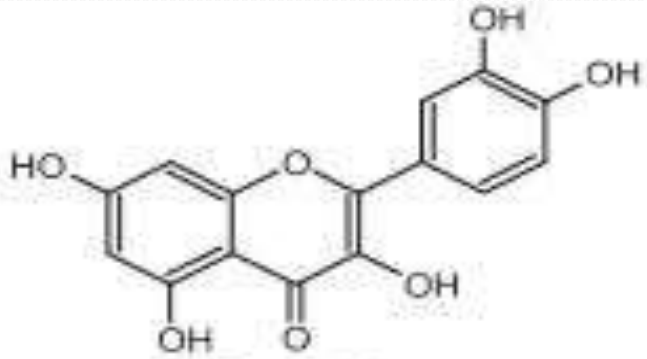
- Leaves of alfalfa, in particular, have significant potential for human use due to their nutritive value and their high yields of protein per hectare.
- Based on their solubility, leaf proteins of alfalfa are classified into two categories.

  1. The insoluble green fraction is associated with lipids, chlorophyll and caretenoids and it consists mainly of chloroplastidial lamellar proteins.

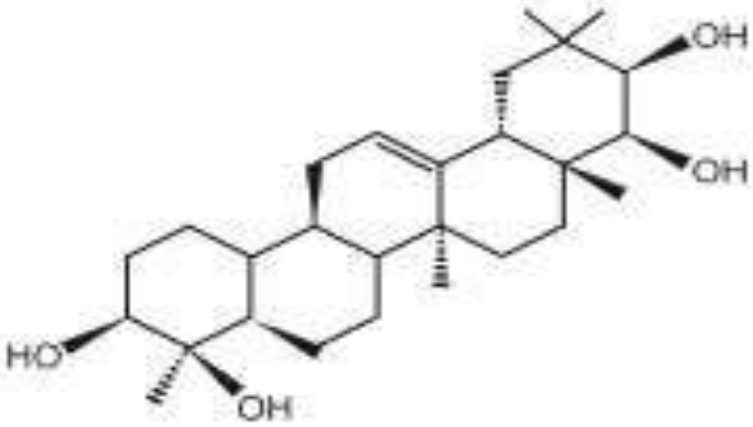
  2. The white fraction (soluble), has 65% RuBisCO (Ribulose-1,5-diphosphate carboxylase /oxygenase), which is the protein considered most widespread in the world.



Asparagin



Quercetin



Soyasapogenol A

Chemical constituents of Alfalfa

- Both fractions have a good balance in their essential amino acid composition according to FAO (Food and Agriculture Organization) and the WHO (World Health Organization) recommendations.
- Their high functionality make alfalfa proteins an important source for both food and pharmaceutical industries.
- Alfalfa proteins have great functional properties and therefore it has many applications in the pharmaceutical sector.

Health benefits of alfalfa

Alfalfa is used to treat various disorders such as kidney , bladder and prostate disorders, treating menopausal symptoms, high BP, asthma, rheumatoid arthritis and stomach upset.

- Alfalfa roots and leaves have shown antibacterial activity against *Corynebacterium michiganense, Corynebacterium insidiosum and Agrobacterium tumefaciens*. It also possesses antifungal and nematicidal activity.
- It is used to treat kidney stones, fever, gravel, dysuria and to relieve fluid retention and swelling.
- Alfalfa decreases intestinal absorption and increase fecal excretion of cholesterol without changing levels of HDL. It is antiatherosclerotic agent. It reduces accumulation of cholesterol in liver. It also reduces serum cholesterol, low-density lipoprotein (LDL) cholesterol and apolipoprotein B.
- It possess estrogenic activity and used in treatment of hormone-related cancers.
- It exerts antidopaminergic action and also shows immunomodulatory action.
- Polysaccharides from alfalfa show anti HIV activity.

- It exhibits antioxidant and cerebroprotective effects.
- It potentiates the action of aminopyrine N-demethylase, a liver drug metabolizing enzyme.
- It helps to maintain function of digestive system, nutrition balance in human body, and prevents osteoporosis and aging.
- It has been found to reduce basal plasma glucose concentrations, also potentiates insulin secretion and action, hence it is used to treat diabetes.
- Alfalfa can be used in treatment of peptic ulcers, polycystic ovaries, as a tonic after blood loss, anemia, urinary and bowel problems, scurvy, secondary hypothyroidism and Herpes simplex viral infection.
- It is beneficial in arthritis, cardiovascular disorders, convalescence, debility, in protein allergies and GI upset.

CHICORY

- **Common chicory** (*Cichorium intybus*) is a somewhat woody, perennial herbaceous plant of family Asteraceae, usually with bright blue flowers, rarely white or pink.

Chemical Constituents-

- Chicory Root- Inulin (68%), Sucrose (14%)
  Cellulose (5%), Protein (6%)

  Extract showed presence of volatile oil, alkaloids, fatty acids, triterpenoids, flavonoids, tannins and saponins.

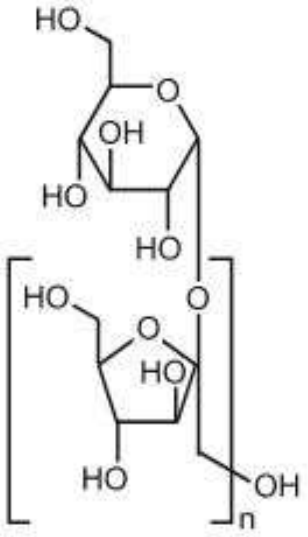
- Chicory seeds- amino acids like arginine, histidine, isoleucine, leucine, cysteine, tyrosine, threonine, valine, serine, glutamic acid, glycine, alanine.

  Minerals- Ca, Mg, Na, Fe, Zn,

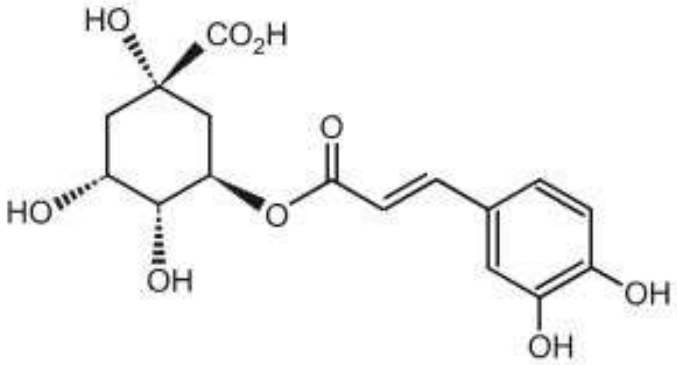
Leaves, flowers, shoots contains inulin, fructose, choline, resin, chicoric acid (dicaffeoyl tartaric acid), esculetin, esculin (esculetin-6-glucoside), cichoriin, umbelliferone, scopoletin and 6,7-dihydrocoumarin, sesquiterpene lactones and their glycosides.

Chicory plant shows presence of sesquiterpene lactones mainly lactucin, lactucopicrin, 8-desoxy lactucin, guaianolide glycosides, including chicoroisides B and C, sonchuside C etc.

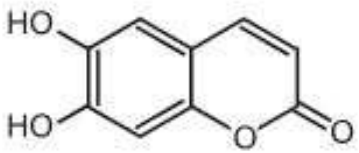
Caffeic acid derivatives like chiroric acid, chlorogenic acid, isochlorogenic acid, dicaffeoyl tartaric acid are also present in plant.



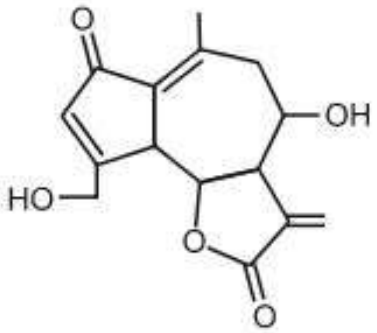
Inulin



Chlorogenic acid



Esculetin



Lactucin

Health benefits of Chicory

- Traditionally chicory is used for the treatment of diarrhoea, prostate and other reproductive organ disorders, pulmonary disease and cough, cancer, hangover, for purification of biliary tract, liver diseases, as spasm, GIT disorders, in sore throat, hemorrhoids, tuberculosis, abdominal cramps, deafness, rashes and as laxative etc.
- Chicory exhibit hepatoprotective effects by suppressing the oxidative degradation of DNA in the tissue debris.
- It helps to reduce blood glucose, total cholesterol (TC) and triglycerides (TG) levels.
- Being inhibitor of COX-2 protein expression chicory acts as anti-inflammatory agent. It also has analgesic and sedative effects.

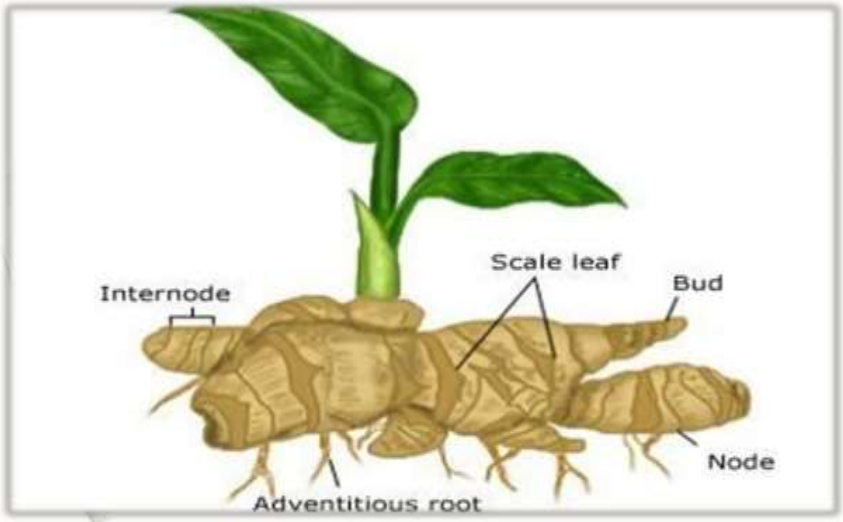
- It acts as antioxidant, immunomodulatory agent and has potent wound healing activity.
- It has gastroprotective effect and maintains good health of gastrointestinal tract.
- Chicory inhibits oedema and leukocyte infiltration in pancreatitis tissue. Hence, acts as pancreas protective agent.
- By inhibiting protein tyrosine phosphatase 1B (PTP1B) it exhibits effect on energy homoeostasis.
- It produces vasorelaxation and inhibits platelet aggregation hence acts as cardioprotective agent.
- Chicory inhibits mast cell-mediated immediate-type allergic reactions.
- It can be used to treat prostate cancer, breast cancer, leukemia and colon cancer etc. It is potent antiproliferative and cytotoxic agent.
- It has antimicrobial effects against *Escherichia coli, Staphylococcus aureus, Bacillus thuringiensis, Bacillus subtilis,* and *Salmonella typhi.* It also possesses antifungal effects.
- Chicory has anti-obesity effect and improves lipid metabolism.
- It has anthelmintic and anti-protozoal effects.

GINGER

Synonyms: Adrak, African ginger, Amomum zingiber, Ardraka, Black ginger, Cochin ginger.

Biological Name: It consists of fresh and dried rhizomes of *Zingiber officinale* Roscoe, belonging to Zingiberaceae.

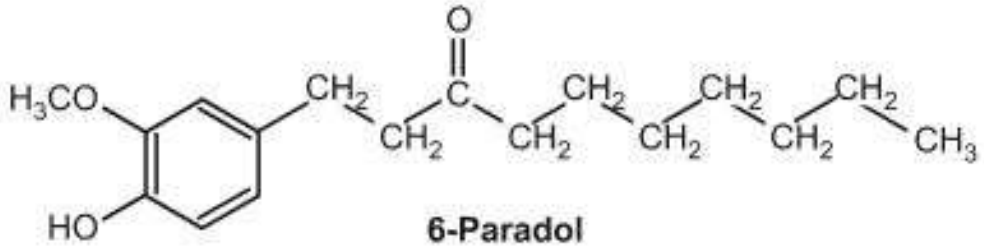
Geographical Source: Tropics of the south and south-eastern Aasia. Ginger is cultivated in China, Nepal, US, India, Bangladesh, Taiwan, Jamaica, Nigeria. India is the largest producer of ginger.



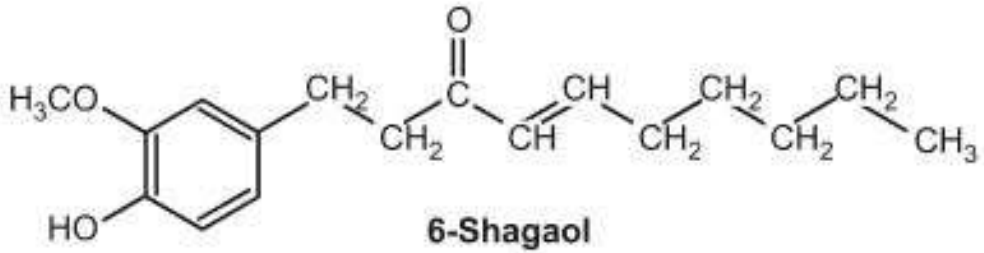
Chemical Constituents: Ginger consists of volatile and non-volatile compounds. The volatile oil of ginger is a mixture of over 24 constituents, mainly monoterpenes like phellandrene, carnphene, cineolc, citral, and borneol and sesquiterpenes like zingiberene, α-curcumene, β-bisabolene, (-) β-sesquiphellandrene and (E, E)-α-farnesene etc. Zingiberol is main aromatic principle of ginger. Other constituents of volatile oil includes α-pinene, camphene, 6-methyl-5-hepten-2-one, myrcene, α-and β-phellandrene, limonene, 1,8-cineole, linalool, borneol, α-terpineol, citronellol, neral, geraniol, geranial, bornyl acetate, 2 undecanone, citronellyl acetate, α-copaene and geranyl acetate etc.

The main pungent non-volatile principle of ginger is gingerols, a phenolic compound. Other non-volatile principle of ginger are 6-, 8- and 10-gingerol,

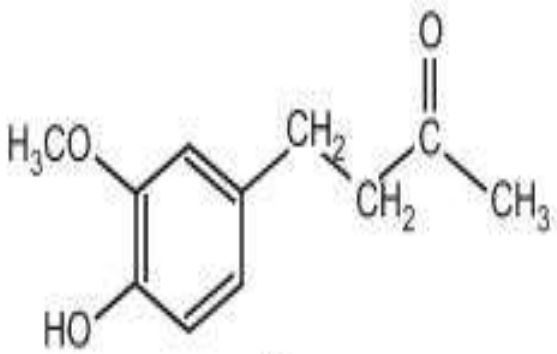
6-Gingerol



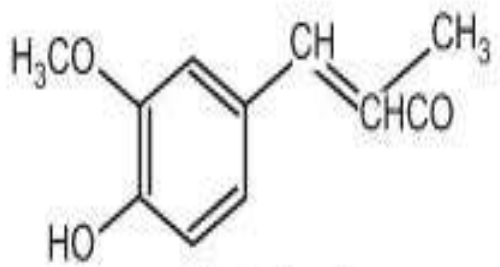
6-Paradol



6-Shagaol



Zingerone



Dehydrozingerone

Health benefits of Ginger

- Traditionally ginger is used to treat indigestion, tastelessness, loss of appetite, flatulence, intestinal, biliary colic, nausea, vomiting, allergic reactions, acute and chronic cough, common cold, fever, allergic rhinitis, sinusitis, acute and chronic bronchitis, respiratory troubles, pain, headache, backache, muscular pain, toothache and swelling gums etc.
- It prevents morning sickness and motion sickness.
- It has antimicrobial activities against various bacterias such as *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus cereus, Klebsiella pneumoniae and Pseudomonas aeruginosa*. Also possesses antifungal activity against two strains of Candida albicans (PTCC 5027 and ATCC 10231).
- Ginger is used to treat type I diabetes by increasing insulin levels and decreasing fasting glucose levels. It also improves insulin sensitivity and impaired insulin signaling.
- It also decreases serum cholesterol, serum triglyceride and blood pressure.

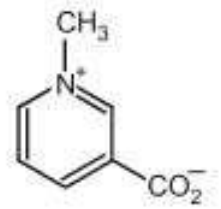
- It has Larvicidal activity against round worm *Angiostrongylus cantonensisa* which causes eosinophilic meningitis. Larvicidal activity is also reported against Anisakis *simplex Adese aegypti* and *Culex quinquefasciatus*.
- Ginger can be used to treat colorectal, gastric, ovarian, liver, skin, breast and prostate cancers. It can act by different mechanisms such as inhibition of proliferation, induction of apoptosis, and suppression of NFκB and heme oxygenase (HO) expression. It also inhibits angiogenesis.
- Ginger can inhibit platelet aggregation.
- Ginger has antiserotoninergic and 5-HT_3 receptor antagonism effects hence can be used to treat nausea, vomiting or morning sickness during pregnancy.
- It has anti-inflammatory and analgesic effects as reduces prostaglandin synthesis and inhibits NFκB, TNF-α, COX-2 expression. It also has analgesic and antipyretic activity.
- Antioxidant properties of ginger improve body's defence mechanism. It scavenges reactive oxygen species (ROS), free radicals, peroxides and prevent oxidative stress.

FENUGREEK

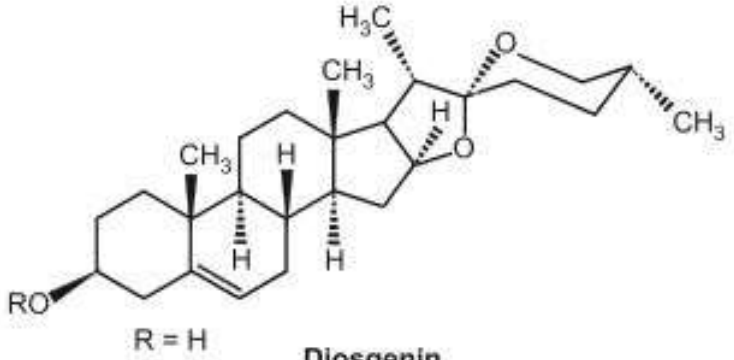
- Synonyms- Methi, Metha
- Biological Source- It consist of herb of *Trigonella foenum gracum L.* family- Fabaceae
- Geographical source- Asia, Europe, Africa, America and some parts of Australia. Rajasthan is major fenugreek producing state.
- Description- Aromatic, 30-60cm tall, annual herb, roots bear nodule, leaflets are 2 -2.5 cm long, oblong, flowers are 1 to 2, sessile, white to yellowish in color, pods are long containing 10-29 seeds, solid, hard, pebble like, yellowish brown or light brown in color, bitter taste

Chemical Constituents

- It contains steroids like diosgenin and sapogenins. Other Steroidal sapinogens present in fenugreek are yamogenin, smilagenin, sarsasapogenin, tigogenin, neotigogenin, gitogenin, neogitogenin, yuccagenin and saponaretin etc.
- Fenugreek consists of saponins such as graicunins, fenugreen B, fenugreekine, trigofoenosides A-G.
- Alkaloides: Major alkaloid is Trigonelline. Other alkaloids are Trimethylamine, Neurin, Choline, Gentianine, Carpaine and Betain.
- Seeds contains flavonoids like vitexin, tricin, naringenin, quercetin, tricin -O-β-d-glucopyranoside. Other phenolic compounds are scopoletin, coumarins, p-coumaric acid, phytolexins, chlorogenic acid etc.
- Stolone furanone is volatile principle present in fenugreek.
- It contains amino acids such as Isoleucine, 4-Hydroxyisoleucine, Histidine, Leucine, Lysine, L-tryptophan, Argenine.
- The seed contain minerals like calcium, phosphorus, iron, sodium and potassium. Seeds also contain vitamins like carotene, thiamine, riboflavin, nicotinic acid and folic acid etc.

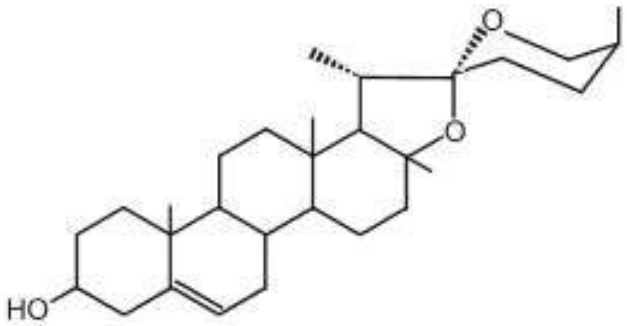


Trigonelline

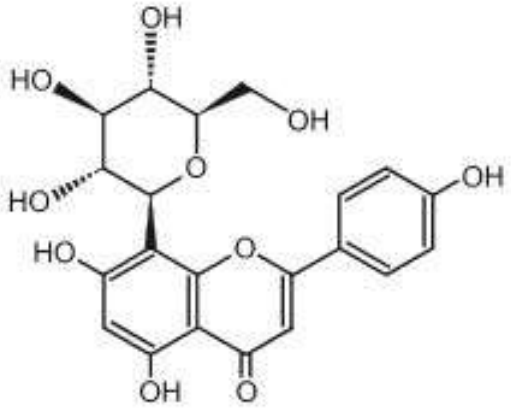


R = H

Diosgenin



Yamogenin



Vitexin

Chemical constituents of Fenugreek

Health benefits of Fenugreek

- It acts as preservative and flavouring agent. Traditionally fresh fenugreek leaves are used to treat indigestion, flatulence and a sluggish liver. Leaves are good hair growth promoter and treat dandruff.
- Traditionally, it can be used in digestive problems such as loss of appetite, upset stomach, constipation, gastritis, painful menstruation, menopause, polycystic ovary syndrome, arthritis, poor thyroid function and obesity.
- It prevents atherosclerosis and reduces levels of cholesterol and triglycerides.
- Seeds are used to treat sore throat. Fenugreek seeds increase the flow of milk. It has soothing effect on skin so used to treat eczema or skin disorders. It is applied as a warm poultice in muscle aches and gout pain. Seeds are used to remove kidney stones.
- Due to presence of Galactomannan and amino acids, fenugreek shows antidiabetic activity. It stimulates pancreatic β-cell, delays gastric emptying and inhibits of glucose transport. It also increases insulin secretion and insulin receptors. Hence, it reduces levels of glucose.
- Fenugreek acts as antihyperlipidemic agent. It increases faecal bile acid and cholesterol excretion due to presence of saponin which can form large micelles. Fibres in fenugreek seeds reduces the rate of hepatic cholesterol synthesis.

- Fenugreek inhibits mammary hyperplasia, hence use to prevent breast cancer. Flavonoids present in fenugreek induce apoptosis in cancer cells, so it can be used in treatment of lung cancer, colon cancer, prostate cancer, stomach cancer, brain tumor, head and neck squamous carcinoma and cervical cancer etc. It also stimulates action of macrophages.
- Fenugreek seeds have anti-secretary action, reduce mucosal injury and have effects on mucosal glycoproteins. So they can act as antiulcer agent.
- Fenugreek has hydroxyl radicals (OH) scavenging activity and inhibit hydrogen peroxide-induced lipid peroxidation. This antioxidant effects are due to presence of polyphenolic compounds.
- It exhibits antiinflammatory, analgesic and antipyretic effects.
- Fenugreek regulates hyperthyroidism. It has Anthelmintic and immunomodulatory activity.

- It possesses antifertility activity. It reduces sperm concentration, reduces plasma androgen and increases concentration of progesterone in females.
- It is diaphoretic, decongestant, relieves cold, bronchial complaints, influenza, asthma, constipation, sinusitis, pneumonia, sore throat, hay fever and is blood purifier.
- Fenugreek can be used in treatment of kidney disorders, beriberi disease, mouth ulcers, boils, bronchitis, cellulitis, tuberculosis, chronic coughs, chapped lips, baldness, Parkinson's disease, and heart burn.

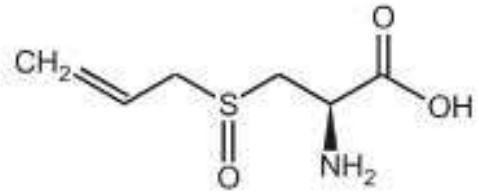
GARLIC

- Synonym- Lahsun, Lashan, Lasuna
- Biological Source- It consists of bulb of *Allium sativum*

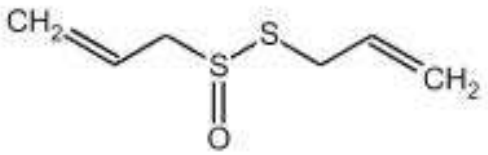
  Family- Liliaceae
- Geographical source- cultivated all over the world, central Asia, southern Europe, USA and India
- Description- It is bulbous perennial herb similar to onion. Underground bulbs which are round composed of smaller bulblets known as cloves. Bulb and cloves are covered with whitish or pinkish membranous coat. Cloves are held together by basal plate. Stem is erect and flowering with pink or purple flower. Leaves are sword shaped

Chemical Constituent

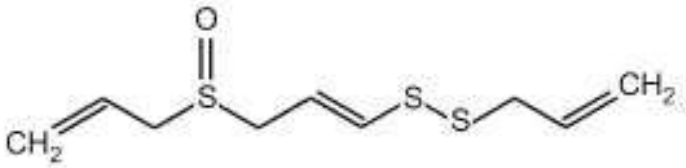
- Glutamyl cysteine – Sulphur containing compound, hydrolyzed to form Alliin
- When garlic crushed Alliin reacts with enzyme Allinase to form Allicin.
- Chemically Allicin is Di-allyl thiosulphinate.
- Allicin has strong odour.
- Allicin is highly unstable and get oxidised to allyl sulphide, ajoene.
- Garlic also shows presence of magnesium, zinc,selenium, vitamin A and volatile oil.



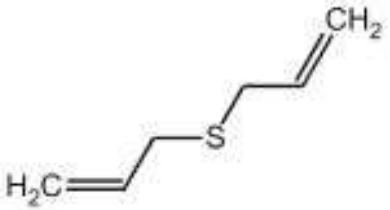
Alliin



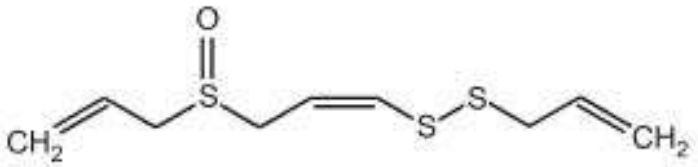
Allicin



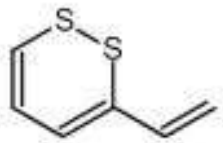
(E)-ajoene



Allyl sulfide



(Z)-ajoene



1,2-vinyldithiin

Health benefits of Garlic

- Cardiovascular system

  High Blood pressure, high cholesterol, angina pectoris, coronary heart disease.

  Improves blood circulation, acts as blood thinner.
- Anticancer agent-

  Acts as anticancer by arresting cell cycle of cancer cell prevents cancer like colon cancer, stomach cancer, prostate cancer, breast cancer, bladder cancer and lung cancer.
- It has diuretic action beneficial in the treatment of edema, urinary infections.
- Respiratory system- Asthma, chronic bronchitis, cold, cough, Breathing difficulty, TB.

- Garlic prevents earache and ear infection.
- It is antibacterial, antifungal.
- Effective against organism causing cholera, dysentry.
- Antiviral effective against herpes simplex, HIV and human rotavirus.
- It has good wound healing activity.
- It acts as immunomodulatory
- Used to treat GI disorders
- It possess hepatoprotective action

HONEY

- Synonym – Madhu, Madvika.
- Biological Source- it is produced from the nectar of flowers by *Apis mellifera* family- Apidae
- Honey bees convert nectar of flower by processes called regurgitation and evaporation using enzyme present in the gut.

Properties-

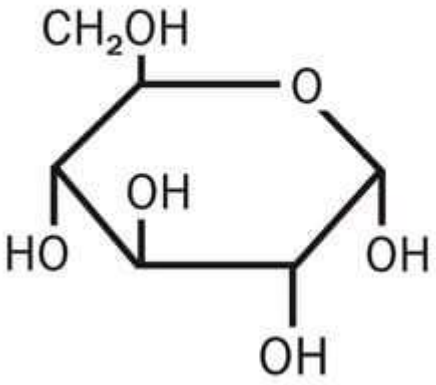
- ✓ Honey is viscous hygroscopic liquid. Colour differs depending on age, botanical source and storage.
- ✓ It crystallizes upon storage.

Composition-

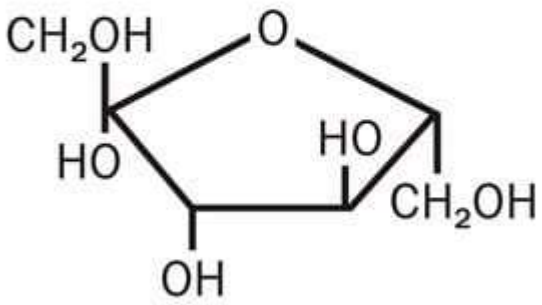
- ✓ It is a mixture of sugar and water.
- ✓ It consist of fructose, glucose and small amount of disaccharides, trisaccharides and oligosaccharides and water.
- ✓ It also contains essential and non essential amino acids
- ✓ Vitamin like B_2, B_4, B_5 , B_6, B_{11} and Vitamin C

- Minerals such as calcium, iron, zinc, potassium, magnesium, phosphorus, selenium, chromium and manganese are present in honey.
- Honey possesses particular flavour and antimicrobial property because of presence of organic acids like gluconic acid, butyric, acetic, formic, lactic, succinic, maleic, citric, oxalic and pyroglutamic acid.
- Honey shows presence of flavonoids such as apigenin, pinocembrin, kaempferol, quercetin, galangin, hesperetin etc.

Glucose



Fructose



Health benefits of Honey

- Traditionally used for eyes, good vision, for bleeding disorders.
- It relieves constipation, stomach pain.
- Honey is used in the treatment of gastrointestinal infections such as gastritis, duodenitis and gastric ulceration. It inhibits attachment of bacteria to gut cell by forming coat around bacteria.

- Honey acts as anti-inflammatory agent by reducing the activities of cyclooxygenase-1 and cyclooxygenase-2. It also decreases concentrations of prostaglandin E2, prostaglandin F2α, and thromboxane B2.
- Due to presence of phenolic compounds honey acts as potential antioxidant.
- Honey stimulates tissue regeneration, reduces inflammation hence it possesses potential wound healing effects.

Amla

- Synonym-

  Amlaki, Indian gooseberry.

- Biological Source-

  It consist of fresh as well as dried fruits of *Emblica officinalis* F.- Euphorbiaceae

- Geographical source-

  India, Pakistan, Bangladesh, Srilanka, Southern china and malaysia

- Description-

  Medium sized tree, leaves are feathery, oblong. Fruits are Spherical or globular

Chemical Constituents

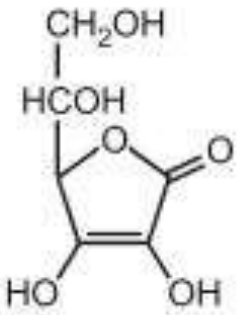
Leaves of amla plant contain gallic acid, chebulic acid, ellagic acid, chebulinic acid, chebulagic acid, amlic acid. Alkaloids like phyllantine and phyllantidine are presnt.

Seeds shows presence of fixed oil, phosphatides and a small quantity of essential oil. Different fatty acids present in seed are linolenic acid (8.78%), linoleic (44%), oleic (28.40%), steric (2.15%), palmitic (2.99%) and miristic acid (0.95%) etc.

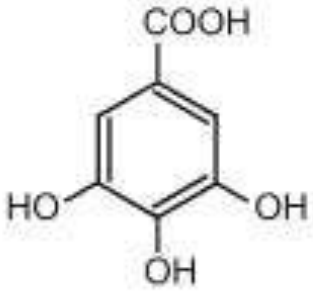
Barks are rich in leukodelphinidin, tannin and proanthocyanidin.

Amla plant roots contains ellagic acid and lupeol. Fruit pulp contains gallic acid 1.32%, tannin, gum 13.75%; albumin 13.08%; crude cellulose 17.08%; mineral matter 4.12% and moisture 3.83%.

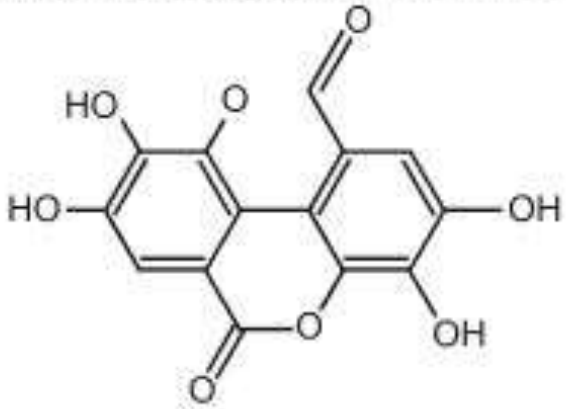
Amla consists of chromium, zinc, copper, manganese etc. It is rich source of Vitamin C.



Vitamin C



Gallic acid



Ellagic acid

Chemical constituents of Amla

Health Benefits of Amla

- Traditionally amla is used as rejuvenative herbs to build lost vitality and vigor. It is tonic for heart and brain. It is useful in conditions like fever, hemorrhage, diarrhoea and dysentery, fever, constipation, eye diseases, dental problems and rheumatism.
- Amla balances tridoshas. It is used for weight loss, source of vitamin C, antiaging agent, skin disorders and burning sensation etc.
- It acts as anabolic, antibacterial, anti-inflammatory, analgesic, hair growth promoter, and immunomodulator.
- Amla shows antifungal activity against *Fusarium equiseti* and *Candida albicans.*
- It acts as insecticidal or cytotoxic agent.
- Amla contains polyphenolic compounds. So it acts as potent inhibitor of lipid peroxide formation and scavenger of hydroxyl, nitric oxide and superoxide radicals. Hence, it exhibits antioxidant effects.
- Due to antioxidant nature amla inhibits the production of advanced glycosylated proteins formed due to oxidative stress. It reduces lipid peroxidation, improve albumin and adiponectin levels. Hence, it is used to control diabetes.
- It can prevent hyperthyroidism and gastric ulcers.
- It possesses larvicidal, insecticidal and mosquitocidal activity.
- It shows immunomodulatory effects by inhibiting apoptosis, DNA fragmentation and restoring IL-2 and γ-IFN production.

- It exhibited hepatoprotective effects by reducing elevated levels of collagen-hydroxyproline and inhibiting the induction of fibrosis.
- It reduces gastric motility and inhibits PGE2-induced enteropooling thereby acts as antidiarrhoeal agent.
- It is used as antitussive agent by exhibiting antiphlogistic, antispasmolytic, antioxidant and mucus secretary property in airways.
- Amla reduces serum cholesterol, TG, phospholipid and LDL levels. Hence, it is antihyperlipidemic agent.
- Amla induces apoptosis and inhibit cell cycle regulating enzymes so it can be used in cancer treatment. Antiproliferative effects are also observed due to polyphenolic compounds present in amla.
- Amla possesses cardioprotective, radioprotective and antidepressant effects.
- Amla also inhibit HIV-Reverse Transcriptase (HIV-RT).
- Amla possesses antimutagenic effects and also shows wound healing activity by enhancing cellular proliferation at the wound site.

GINSENG

Synonyms: American ginseng, Asiatic ginseng, Chinese ginseng, Korean ginseng, Tartar root, Western ginseng.

Biological Source: It consists of dried roots of plant Panax ginseng (Renshen, Chinese ginseng) belongs to family Araliaceae. Other species consists of *Panax quinquefolium* (*Xiyangshen*, American ginseng), *Panax notoginseng* (*Sanqi*) and *Panax japonicas* (Japanese ginseng).

Geographical Source: Ginseng is native to China, Korea and Russia.

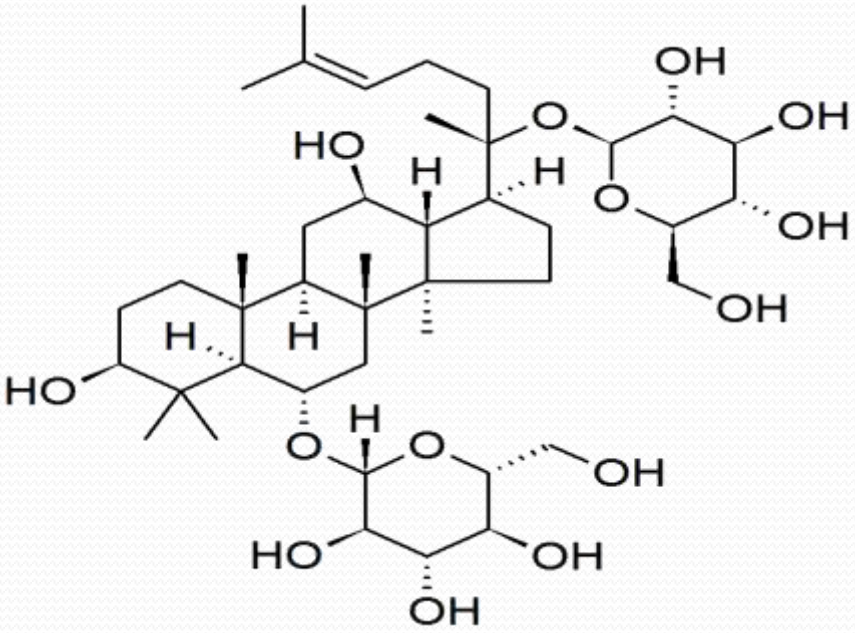
Description: Ginseng is deciduous perennial plant. There are two forms, red and white ginseng. It grows well in shade. The leaves are five-fingered, compound with five pointed and toothed leaflets. Plant bears tiny white or greenish-yellow flowers form single, ball-like cluster, red berries, and a yellowish-brown root. Thick roots show human-like shape.

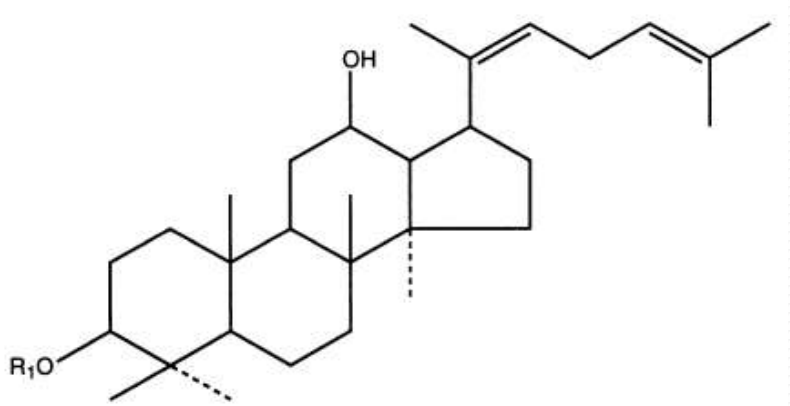
Chemical constituents

- Mixture of several saponin glycosides, belonging to triterpenoid group
- Ginsenosides
- Panaxosides
- Chikusetsusaponin
- Ginsenoside contain aglycone dammarol while panaxoside have oleanolic acid as aglycone
- 13 different gensenosides have been identified
- Panaxosides give oleanolic acid, panaxadiol and panaxatriol on decomposition

- Ginsenoside

Panaxoside





Health benefits of Ginseng

- Ginseng is used to treat infertility, liver disease, amnesia, colds, menopause, as adaptogen and a restorative tonic, aphrodisiac and erectile dysfunction.
- It can improve stamina, decrease fatigue, antistress agent, and improve concentration.
- Ginseng has various therapeutic activities such as in anaemia, antidiabetic agent, antipyretic, anticancer, antiasthamaytic, antiaging agent.

- It is used in gastritis, neurasthenia, bleeding disorders, loss of appetite, vomiting, colitis, dysentery, insomnia, neuralgia, rheumatism, dizziness, headache, convulsions, disorders of pregnancy, hot flashes due to menopause.
- Ginseng use to treat diabetes. It improves glucose homeostasis, insulin sensitivity, glycated hemoglobin, serum lipid, amino-terminal propeptide concentration, and physical activity.
- Ginseng acts as antimicrobial, antifungal, insecticidal and antifeedant agent.

- It improves sperm count and motility; hence use to treat male infertility.
- Saponins of Ginseng have cytotoxic effects. It inhibits cancer cell proliferation, adhesion and invasion of tumor cells, induces apoptosis and also has anti-angiogenesis activity. Hence, ginseng acts as anticancer agent.
- Panax ginseng acts as antiinflammatory agent by inhibiting cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and nuclear factor kappa B. It also possesses radioprotective effect, antioxidant and immunomodulatory effects.
- It can be used as anti-neurodegenerative agent. It affects neurological functions by interacting with the cholinergic and serotoninergic neurotransmitter systems and improves cognitive performance. Hence, ginseng is used in treatment of Alzheimer's diseases and Parkinson's diseases. By decreasing the levels of apoptotic proteins including p-Akt, Bax, and Bcl-2 it produces beneficiary effects on Parkinson's disease.

- Ginseng is effective against ischemic cerebral damage by promoting clearance of extracellular glutamate, increasing the expression of non-selective cation channels, acid-sensing ion channel, and decreasing the levels of apoptotic proteins such as cytochrome c (CytoC), apoptosis inducing factor (AIF), and caspase-3. It also inhibits synthesis of COX-2 and iNOS.
- Due to antioxidant effects and calcium modulation, ginseng is used to treat various cardiac disorders such as cardiotoxicity, cardiac hypertrophy, myocardial infarction after ischemia and reperfusion, atherosclerosis, angina pectoris and heart failure etc. It inhibits apoptosis in cardiomyocytes by modulating Bcl-2 and caspase-3. It can acts as cardioprotective agent by modulating ion channels and signal transduction, improving lipid profiles, regulating blood pressure, and reducing platelet adhesion.

Ashwagandha

Synonyms: Asvagandha, Winter cherry, Asgandh, Gandhrapatri.

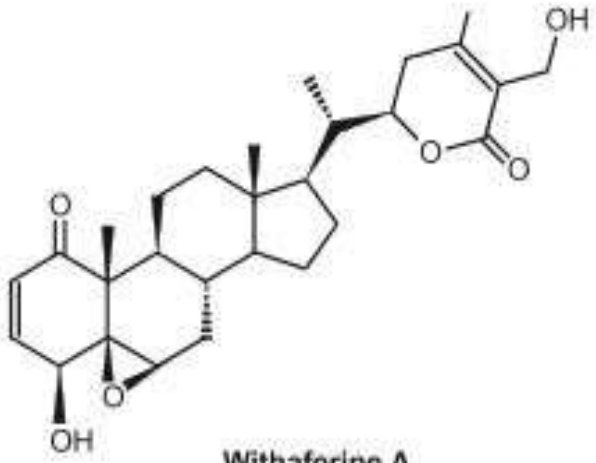
Biological Name: Dried roots of *Withania somnifera Dunal* belonging to the family Solanaceae.

Geographical Source: In India, Sri Lanka, Afghanistan, Baluchistan, Sind, parts of Africa and in the Mediterranean regions, the Canaries and Cape of Good Hop etc.

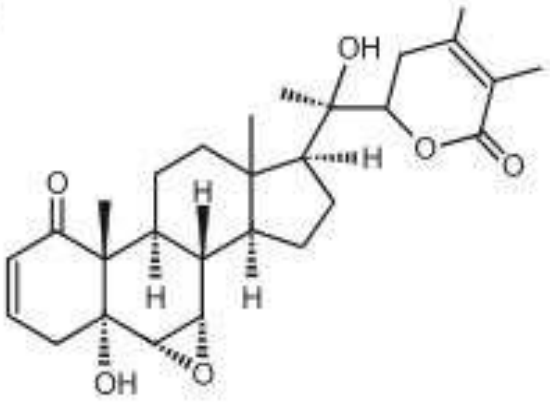
Description: Ashvagandha is an evergreen, erect, branching shrub, 30-150 cm in height and has minute star shaped hairs. Plant has simple, ovate, glabrous alternate leaves with sub-acute apex and entire margins. Plant shows axillary, umbellate cymes type Inflorescence. Flowers are greenish or lurid yellow, Monoecious, small about 1 cm long; Fruits (berries) are smooth, orange-red when mature, globose enclosed in the inflated and membranous calyx, and are 5 angled. Seeds are yellow, reniform.

Chemical Constituents

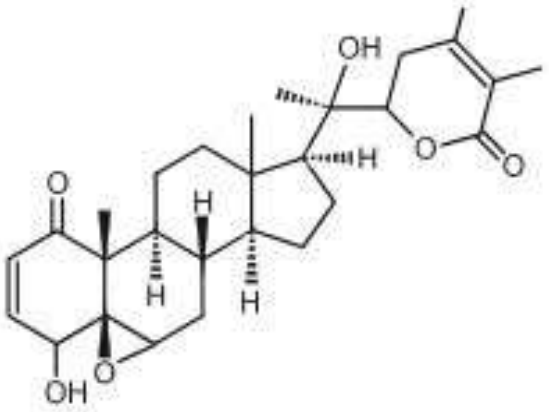
Plant contains more than 13 alkaloids, 138 withanolides, and several sitoindosides in aerial parts, roots and berries of Withania species. The major chemical constituents are withanolides (0.001 to 0.5% dry weight), Withaferin A, ashwagandhanolide etc. It consists of steroidal compounds including ergostane-type steroidal lactones, withaferin A, withanolides A-Y, withasomniferin A, withasomidienone, withasomniferols A-C, withanone, Plant also contains acylsteryl glucosides, starch, hantreacotane, ducitol, and a variety of amino acids and high amount of iron.



Withaferine A



Withanolide A



Withanolide D

Health benefits of Ashwagandha

- Due to presence of saponins it act as potent anti-arthritic and anti-inflammatory agent.
- Withaferin A shows antibiotic activity against Gram-positive bacteria, acid-fast and aerobic bacilli, and pathogenic fungi.
- It is potent anticancer and chemoprotective agent.
- Ashvagandha roots acts as hepatoprotictive agent by influencing the levels of lipid peroxidation.
- It has immunosuppressive effect on human B and T lymphocytes. It also has immunomodulatory effects.
- Withania reduces serum cholesterol, triglycerides and lipoprotein levels.
- Ashvagandha improve attention and impulse control in children with ADHD. Effective in patients with bipolar disorder.
- It possesses protective effects against various CNS disorders like epilepsy, anxiety, depression, catalepsy, Alzheimer's disease and sleep disorders.

- It exhibits muscle relaxant and antispasmodic effects on intestinal, uterine, bronchial, tracheal and blood vascular muscles.
- It is used to treat disorders such as osteoarthritis, OCD, parkinson's disease, fibromyalgia and TB etc.
- Active principles of withania increase superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Thus, due to this, antioxidant property has anti-stress, antiaging and cognition-facilitating effects. Antiaging effects include improvement of Hb, RBC and hair melanin etc.
- Ashvagandha protect oxidative damage to pancreatic islet cells by its free radical scavenging activity. Hence, it can act as antihyperglycaemic agent.
- It activates chemotactic activity of macrophages and enhances production of interleukin-1 (IL-1) and tumour necrosis factor (TNF). Hence, it can regulate immune responses.
- It also exhibits cardioprotective effects.
- Traditionally it is used as sedative, hypnotic, narcotic, galactagouge, blood purifier, general tonic, diuretic aphrodisiac, and uterine tonic. It is implemented in treatment of rheumatoid arthritis, lumbago, painful swellings, leucoderma, asthma, amnesia, anxiety disorders, ulcers, scabies etc.

Patenting and Regulatory requirements of natural products

Patent: A patent is a monopoly right, which is granted to a patentee for a limited period of time during which he is given the exclusive right to hinder anyone else from using her invention without consent.

A patent can be granted for an invention which may be related to any process or product. The word “Invention “has been defined under the Patents Act 1970 as amended from time to time.

“An invention means a new product or process involving an inventive step and capable of industrial application. A patent gives its owner the right to exclude other from making,using,selling and importing an invention for a limited period of time, usually 20 years. The patent rights are granted in exchange for an enabling public disclosure of the invention.

There are four different patent types:

Utility patent. This is what most people think of when they think about a patent. ...

Provisional patent. ...

Design patent. ...

Plant patent.

•**Utility patent.-** This is what most people think of when they think about a patent. It's a long, technical document that teaches the public how to use a new machine, process, or system. The kinds of inventions protected by utility patents are defined by Congress. New technologies like genetic engineering and internet-delivered software are challenging the boundaries of what kinds of inventions can receive utility patent protection.

•**Provisional patent.-** United States law allows inventors to file a less formal document that proves the inventor was in possession of the invention and had adequately figured out how to make the invention work. Once that is on file, the invention is patent pending. If, however, the inventor fails to file a formal utility patent within a year from filing the provisional patent, he or she will lose this filing date. Any public disclosures made relying on that provisional patent application will now count as public disclosures to the United States Patent and Trademark Office (USPTO).

•**Design patent.-** This patent offer protection for an ornamental design on a useful item. The shape of a bottle or the design of a shoe, for example, can be protected by a design patent. The document itself is almost entirely made of pictures or drawings of the design on the useful item. Design patents are notoriously difficult to search simply because there are very few words used in a design patent. In recent years, software companies have used design patents to protect elements of user interfaces and even the shape of touchscreen devices.

•Plant patent.

Just what it sounds like, a plant patent protects new kinds of plants produced by cuttings or other nonsexual means. Plant patents generally do not cover genetically modified organisms and focus more on conventional horticulture.

•Intellectual property rights (IPRs): These are rights to make, use, and sell a new product or technology that are granted, usually for a period of 17- 20 years, solely to the inventor or the corporation which files a claim on the inventor's behalf. IPRs are meant to reward innovators, inventors and researchers.

•Under intellectual property law, owners are granted certain exclusive rights to a variety of intangible assets, such as musical, literary, and artistic works, discoveries and inventions, words, phrases, symbols, and designs.

•Common types of intellectual property include copyrights, trademarks, patents, industrial design rights and trade secrets in some jurisdictions. Among various kinds of IPRs patents and trademarks are more important to pharmaceutical industries.

Farmers Rights:

Farmers may have little or no understanding of the scientific basis of genetic diversity, but they certainly understand its paramount importance to agriculture, and the need for promoting variability in agricultural practices. The autonomy that every farmer exercises in selecting, saving and maintaining seed for re-sowing has been fundamental for the agronomic transformation of plant species into crops, and their further selection. Farmers' rights and intellectual property rights:

Farmers' rights and intellectual property rights: The basic principle underlying IPR on plant varieties is the recognition of human innovation in developing a new plant variety through selection, with or without recombination, which is novel and distinct from the pre-existing varieties.

International Regime for Protection of Plant Varieties

TRIPS Agreement Article 27 of TRIPS Agreement requires patent protection to be made available for both process and products, in all fields of technology.

UPOV (Union for Protection of New Varieties of Plant)

The UPOV Convention) have recognised the protection of the plant breeders' rights. UPOV 1961 is the first international treaty regarding intellectual property protection to plants. UPOV 1961 provides that breeder may be granted only one type of protection either through a special title of protection or a patent. Thus it bans dual protection for one and the same botanical genus or species.

Plant breeders Rights (PBR)

These are also known as plant variety rights (PVR). These are the rights granted to the breeder of a new variety of plant that give the breeder exclusive control over the propagating material and harvested material of a new variety for a number of years. With these rights, the breeder can choose to become the exclusive rights, a variety must be new, distinct, uniform and stable. PBRs allow a plant breeder to exclude others from the production, processing, stocking, distribution, marketing, sale, export and import of propagating material of a protected variety for a specified number of years. It also allows the breeder to license such rights to others, and to receive royalties generated from the authorized use of the propagating material.

Biopiracy

When researchers use traditional knowledge without permission, or exploits the cultures they're drawing from – it's called biopiracy. Biopiracy happens when researchers or research organisations take biological resources without official sanction, largely from less affluent countries or marginalised people. Biopiracy is not limited to drug development. It also occurs in agricultural and industrial contexts. Indian products such as the neem tree, tamarind, turmeric, and Darjeeling tea have all been patented by foreign firms for different lucrative purposes.

The term biopiracy was coined by Pat Mooney, to describe a practice in which indigenous knowledge of nature, originating with indigenous peoples, is used by others for profit, without authorization or compensation to the indigenous people themselves. For example, when bioprospectors draw on indigenous knowledge of medicinal plants which is later patented by medical companies without recognizing the fact that the knowledge is not new or invented by the patenter, this deprives the indigenous community of their potential rights to the commercial product derived from the technology that they themselves had developed.

Bioprospecting:

It is the process of discovery and commercialization of new products based on biological resources. Despite indigenous knowledge being intuitively helpful, bioprospecting has only recently begun to incorporate such knowledge in focusing screening efforts for bioactive compounds. During 1981-2010, one third of all small molecule new chemical entities approved by the U.S. Food and Drug Administration (FDA) were either natural products or compounds derived from natural products. Despite indigenous knowledge being intuitively helpful, bioprospecting has only recently begun to incorporate such knowledge in focusing screening efforts for bioactive compounds. Bioprospecting may involve biopiracy, the exploitative appropriation of indigenous forms of knowledge by commercial actors, and can include the patenting of already widely used natural resources, such as plant varieties, by commercial entities.

Traditional knowledge (TK):

The most practical method of protection is the prevention of unauthorized use by third parties beyond the traditional circle. This form of protection focuses on the use of any indigenous knowledge as technical, ecological, scientific, medical or cultural by a traditional community.

A report by WHO says that about 80% of World's population is depending on the traditional knowledge on the ancient methods for curing disease.

For example, the Neem and its uses can be registered under traditional knowledge by indigenous people of India for its medical uses which includes first aid, cosmetic nature and for curing inflammation and redness caused by any medical issue. This is based on herbal plant used for medical purpose and it is called "arogyapaacha".

"Traditional knowledge refers to the knowledge, innovations and practices of indigenous and local communities around the world. Developed from experience gained over the centuries and adapted to the local culture and environment

traditional knowledge is transmitted orally from generation to generation. It tends to be collectively owned and takes the form of stories, songs, folklore, proverbs, cultural values, beliefs, rituals, community laws, local language, and agricultural practices, including the development of plant species and animal breeds. Traditional knowledge is mainly of a practical nature, particularly in such fields as agriculture, fisheries, health, horticulture, forestry and environmental management in general.

Case study of Neem:The

Neem tree (Azadirachta Indica) is a large tropical evergreen that can grow up to 30 meters tall and 2.5 meters in girth.The tree has been shown to be useful in many different areas including contraception, dental hygiene and pesticides, as well as being part of many traditional Indian medicines and cures.

The widespread growth of the Neem tree and its many practical uses has made the Neem tree very dear to the Indian people to whom it represents an integral part of their traditional and even religious heritage. Indian scientists have been researching the Neem tree as a natural pesticide since the 1920’s but Western awareness of its qualities wasn’t raised until 1959

.

German entomologist Heinrich Schmutterer witnessed a locust plague in the Sudan and noticed that the Neem trees were the only ones that had withstood the onslaught. He immediately started studying the Neem tree and his work in turn generated a great deal of western scientific interest in its pesticidal qualities.That the Neem tree could withstand locust infestations had been common knowledge among Indian farmers for centuries

\ Both the seeds and to a lesser extent the leaves contain the active substance azadirachtin, which is a powerful insecticide that is not harmful to human beings. Even before the discovery of the active substance in the later half of the 20th century, Neem seeds had been used by Indian farmers as a natural pesticide. The most common practice was to break up the seeds, soak them in water or alcohol, and then apply the resulting emulsion on their crops

.The first U.S. patent on a storage stable composition for Neem seed extract was issued in 1985 to inventor Robert O. Larsson..

W.R. Grace in partnership with The United States of America as represented by The Secretary of Agriculture jointly filed a Patent Application for the formulation with the EPO, who after a long drawn out examination process granted the applicants the patent in 1994.

The main claim of the patent had however been restricted by the EPO in relation to the patent granted in the U.S. The aim of the opponents was to revoke the patent, and more specifically on the grounds of prior use and TK so as to gain an important case law precedent in their battle against biopiracy.

The claim was rejected on the grounds of lacking novelty and the evidence upon which this decision was taken was the testimony of a witness who had worked with the process himself and who could verify its use among Indian farmers.

The decision of the Board of appeal to leave open whether prior art had been proven or not changed the whole focus of the case. In choosing the article as the closest prior art they relied on a scientific study published in a Western journal. Thereby the question of novelty and inventive step wasn’t truly judged on the grounds of TK.

The board shied away from dealing with the issue of prior use and decided the case on materials with which they were more comfortable. Another interesting aspect of the change is that the patentees who had declined an oral hearing provided no defence against the article on the grounds of inventive step and only a fleeting remark regarding the article in relation to novelty.

1990 • Multinational chemical corporation, W R Grace and Co (Neem-based bio-pesticides)

1994 • European Patent Office (EPO) granted a patent

1995 • Opposed By Vandana Shiva of the Research Foundation for Science, Technology and Ecology(RFSTE)

2000 • EPO revoked patent on the basis of lack of inventive step and novely.

(1993) Patent application by University of Mississippi Medical Center

(1995) USPTO granted patent for use of turmeric in wound healing

Opposed by India's Council of Scientific and Industrial Research(CSIR)

1997 Patent cancelled

Case study of Turmeric:

Turmeric is a tropical herb grown in east India. Turmeric powder is widely used in India as a medicine, a food ingredient and a dye. For instance, it is used as a blood purifier, in treating the common cold, and as an anti-parasitic for many skin infections. It is also used as an essential ingredient in cooking many Indian dishes.

In 1995, the United States awarded patent on turmeric to University of Mississippi medical center for wound healing property. The claimed subject matter was the use of "turmeric powder and its administration", both oral as well as topical, for wound healing.

The Indian Council for Scientific and Industrial Research (CSIR) had objected to the patent granted and provided documented evidences of the prior art to USPTO.

Though it was a well known fact that the use of turmeric was known in every household since ages in India, it was a herculean task to find published information on the use of turmeric powder through oral as well as topical route for wound healing. Due to extensive researches, 32 references were located in different languages namely Sanskrit, Urdu and Hindi. Therefore, the USPTO revoked the patent, stating that the claims made in the patent were obvious and anticipated, and agreeing that the use of turmeric was an old art of healing wounds. Therefore, the TK that belonged to India was safeguarded in Turmeric case.

Regulatory Issues in India Herbal Drug Regulation in India: Drug regulation is a public policy response to the demands of public health and the changing needs of pharmaceutical industry. Thus, the objective of regulatory control is a question of achieving a 'balance' between protecting and promoting public health and facilitating the industry vis-à-vis compliance with regulatory standards

The rationale behind this is to design a regulatory system where the choice of regulatory instruments not only match the imperatives/objectives of regulation, but also take into consideration the range and the intrinsic characteristics of each of the regulatory stakeholders.

Provision related to the manufacture and control of Ayurvedic, Sidha and Unani (ASU)
drugs has been prescribed in the Drugs and cosmetics acts 1940.
This act described the formation of drug Technical Advisory Board (DTAB), which consist of various nominated members and Drug Consultative committees (DCC).
DTAB is the highest constitutional decision making body on technical matters related to the drugs in the country. It is a part of central drug Standard Control Organisation (CDSCO) in the ministry of Health and family welfare.
The drug and cosmetic act provides the establishment of following agencies:

1. Advisory
2. Analytical
3. Executive

Administration of the act and rules

- **Advisory:**
  1. Drugs Technical advisory Board-DTAB
  2. Drugs Consultative committee-DCC
- **Analytical:**
  1. Central Drugs laboratory-CDL
  2. Drug Control Laboratory
  3. Government Analysts
  - **Executives:**
    1. Drug Inspector
    2. Licensing Authorities
    3. Controlling authorities

Drugs and cosmetics Act 1940: The Drugs and Cosmetics Act, 1940 is an Act of the Parliament of India which regulates the import, manufacture and distribution of drugs in India. The primary objective of the act is to ensure that the drugs and cosmetics sold in India are safe, effective and conform to state quality standards.

The related Drugs and Cosmetics Rules, 1945 contains provisions for classification of drugs under given schedules and there are guidelines for the storage, sale, display and prescription of each schedule.

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The term ‘drug’ is defined in the Drugs and Cosmetics Act. A comprehensive definition is given for the term drug in the Act. The Act says that “drug” includes— i) all medicines for internal or external use of human beings or animals and all substances intended to be used for or in the diagnosis, treatment, mitigation or prevention of any disease or disorder in human beings or animals, including preparations applied on human body for the purpose of repelling insects like mosquitoes; 123 Drugs and Cosmetics Acts and Rules

ii) such substances (other than food) intended to affect the structure or any function of human body or intended to be used for the destruction of (vermin) or insects which cause disease in human beings or animals, as may be specified from time to time by the Central Government.

iii) all substances intended for use as components of a drug including empty gelatin capsules;

and iv) such devices intended for internal or external use in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, as may be specified from time to time by the Central Government by notification in the Official Gazette

Provisions related to Ayurveda, Siddha and Unani drugs (ASU): •
The Drugs Technical Advisory Board (DTAB)
• The central Drugs laboratory (CDL) •
The Drugs consultative committee (DCC)

The Drugs Technical Advisory Board (DTAB):
(1) The Central Government shall, as soon as may be, constitute a Board (to be called the Drugs Technical Advisory Board) to advise the Central Government and the State Governments on technical matters arising out of the administration of this Act and to carry out the other functions assigned to it by this Act.

(2) The Board shall consist of the following members, namely:—
(i) the Director General of Health Services, ex officio, who shall be the Chairman;
(ii) the Drugs Controller, India, ex officio;
(iii) the Director of the Central Drugs Laboratory, Calcutta, ex officio;
(iv) the Director of the Central Research Institute, Kasauli, ex officio;

(v) the Director of the Indian Veterinary Research Institute, Izatnagar, ex officio;

(vi) the President of the Medical Council of India, ex officio;

(vii) the President of the Pharmacy Council of India, ex officio;

(viii) the Director of the Central Drug Research Institute, Lucknow, ex officio;

(ix) two persons to be nominated by the Central Government from among persons who are in charge of drugs control in the States;

(x) one person, to be elected by the Executive Committee of the Pharmacy Council of India, from among teachers in pharmacy or pharmaceutical chemistry or pharmacognosy on the staff of an Indian university or a college affiliated thereto;

(xi) one person, to be elected by the Executive Committee of the Medical Council of India, from among teachers in medicine or therapeutics on the staff of an Indian university or a college affiliated there to

The Central Drugs Laboratory (CDL): The Central Government shall, as soon as may be, establish a Central Drugs Laboratory under the control of a Director to be appointed by the Central Government, to carry out the functions entrusted to it by this Act or any rules made under this Chapter.

The Drugs Consultative Committee (DCC):
The Central Government may constitute an advisory committee to be called “the Drugs Consultative Committee” to advise the Central Government, the State Governments and the Drugs Technical Advisory Board on any matter ending to secure uniformity throughout [India] in the administration of this Act. The Drugs Consultative Committee shall consist of two representatives of the Central Government to be nominated by that Government and
one representative of each State Government to be nominated by the State Government concerned.
The Drugs Consultative Committee shall meet when required to do so by the Central Government and shall have power to regulate its own procedure.

Manufacture for sale of Ayurvedic, Siddha and Unani drugs:

(1) The Central Government shall, by notification in the Official Gazette and with effect from such date as may be specified therein, constitute a Board (to be called the[Ayurvedic, Siddha and Unani Drugs Technical Advisory Board]) to advise the Central Government and the State Governments on Technical matters arising out of this Chapter and to carry out the other functions assigned to it by this Chapter.

(2) The Board shall consist of the following members, namely:

(i) the Director General of Health Services ex officio;

(ii) the Drugs Controller, India, ex officio;

(iii) the principal officer dealing with Indian systems of medicine in the Ministry of Health, ex officio];

(iv) the Director of the Central Drugs Laboratory, Calcutta ex officio;

(v) one person holding the appointment of Government Analyst under section 33F, to be nominated by the Central Government;

(vi) one Pharmacognocist to be nominated by the Central Government; (vii) one Phyto-chemist to be nominated by the Central Government;

(3) The Central Government shall appoint a member of the Board as its Chairman.

(4) The nominated members of the Board shall hold office for three years but shall be eligible for renomination.

(5) The Board may, subject to the previous approval of the Central Government, make bye -laws fixing a quorum and regulating its own procedure and conduct of all business to be tran -sacted by it.

(6) The functions of the Board may be exercised notwithstanding any vacancy therein.

(7) The Central Government shall appoint a person to be Secretary of the Board and shall provide the Board with such clerical and other staff as the Central Government considers necessary

Pune District Education Association , Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre, Kharadi, Pune-411014

Presentation on

PHARMACEUTICAL ARROSOLS

PRESENTED BY :-

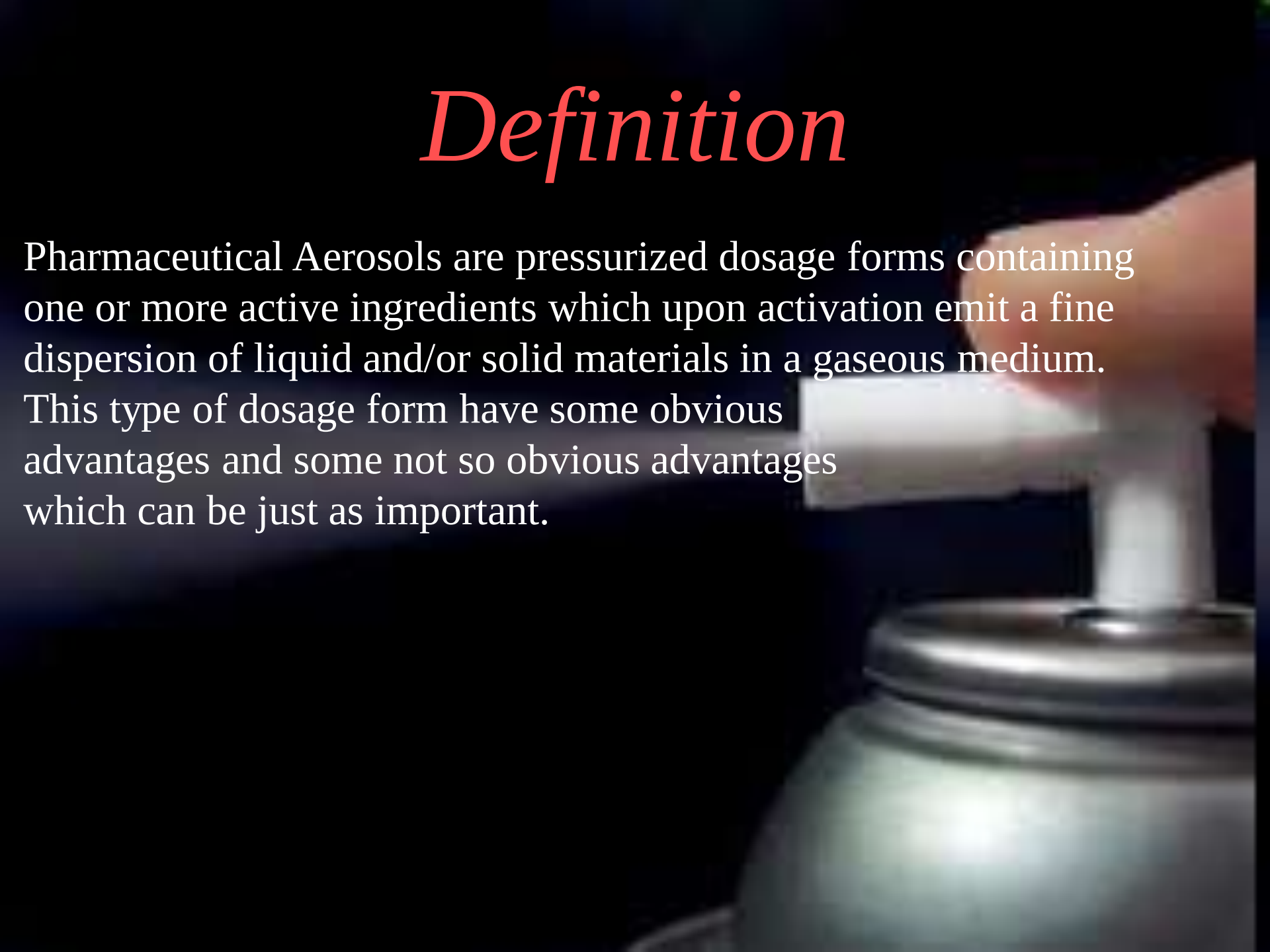
MRS. TRUSHA P. SHANGRAPAWAR

PHARMACEUTICAL

AEROSOLS

Definition

Pharmaceutical Aerosols are pressurized dosage forms containing one or more active ingredients which upon activation emit a fine dispersion of liquid and/or solid materials in a gaseous medium. This type of dosage form have some obvious advantages and some not so obvious advantages which can be just as important.



Advantages

A dose can be removed with out contamination of materials.

The medication can be delivered directly to the affected area in a desired form, such as spray, steam, quick breaking foam or stable foam.

Irritation produced by the mechanical application of topical medication is reduced or eliminated.

Ease of convenience of application.

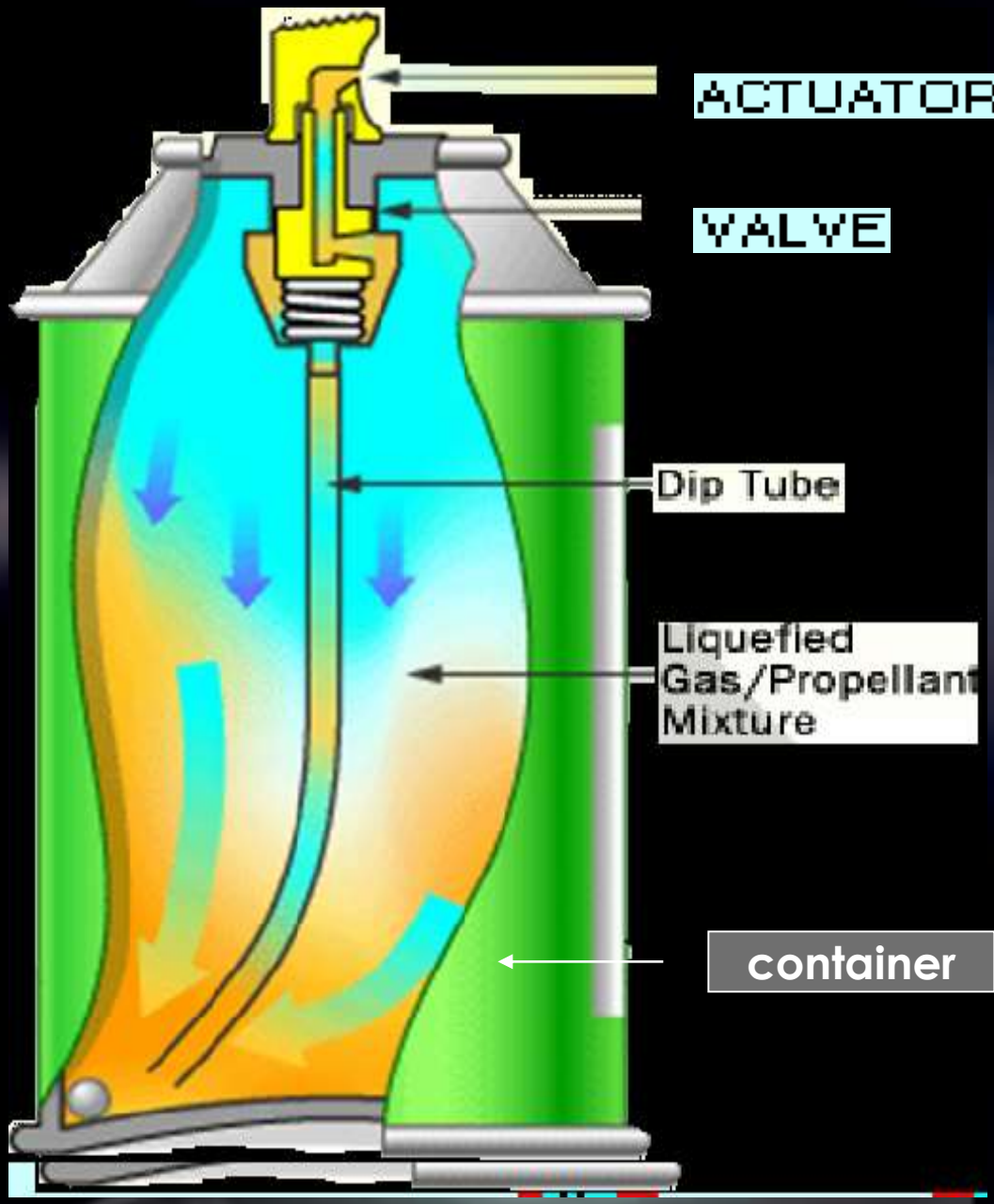
Application of medication in thin layer.

Components of aerosols

Container

Valve and actuator

Product concentrate



Propellant

PRODUCTION OF THE PRODUCT.

FOR ORAL AND INHALATION EG.

Fluorinated hydrocarbons

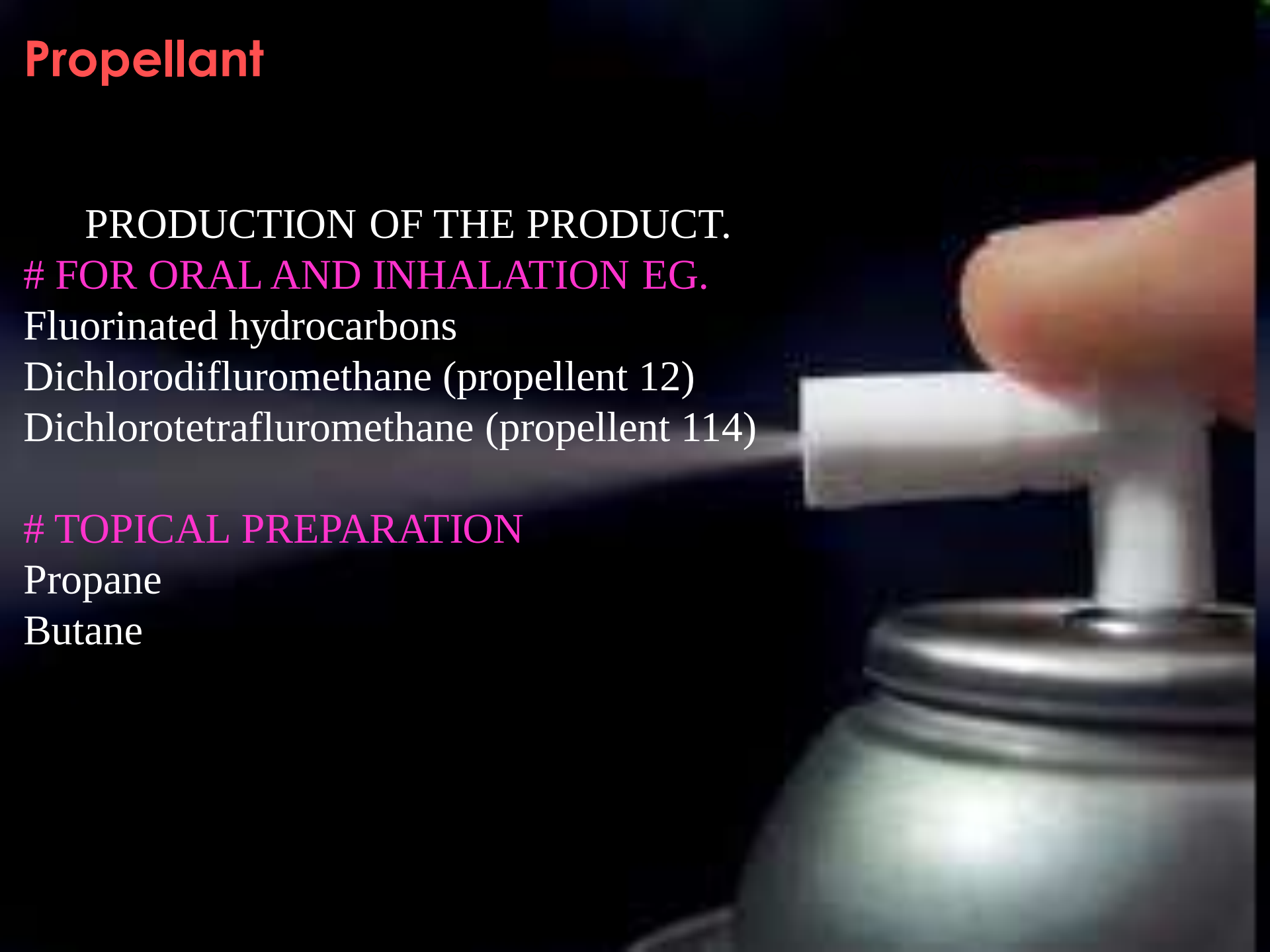
Dichlorodifluromethane (propellent 12)

Dichlorotetrafluromethane (propellent 114)

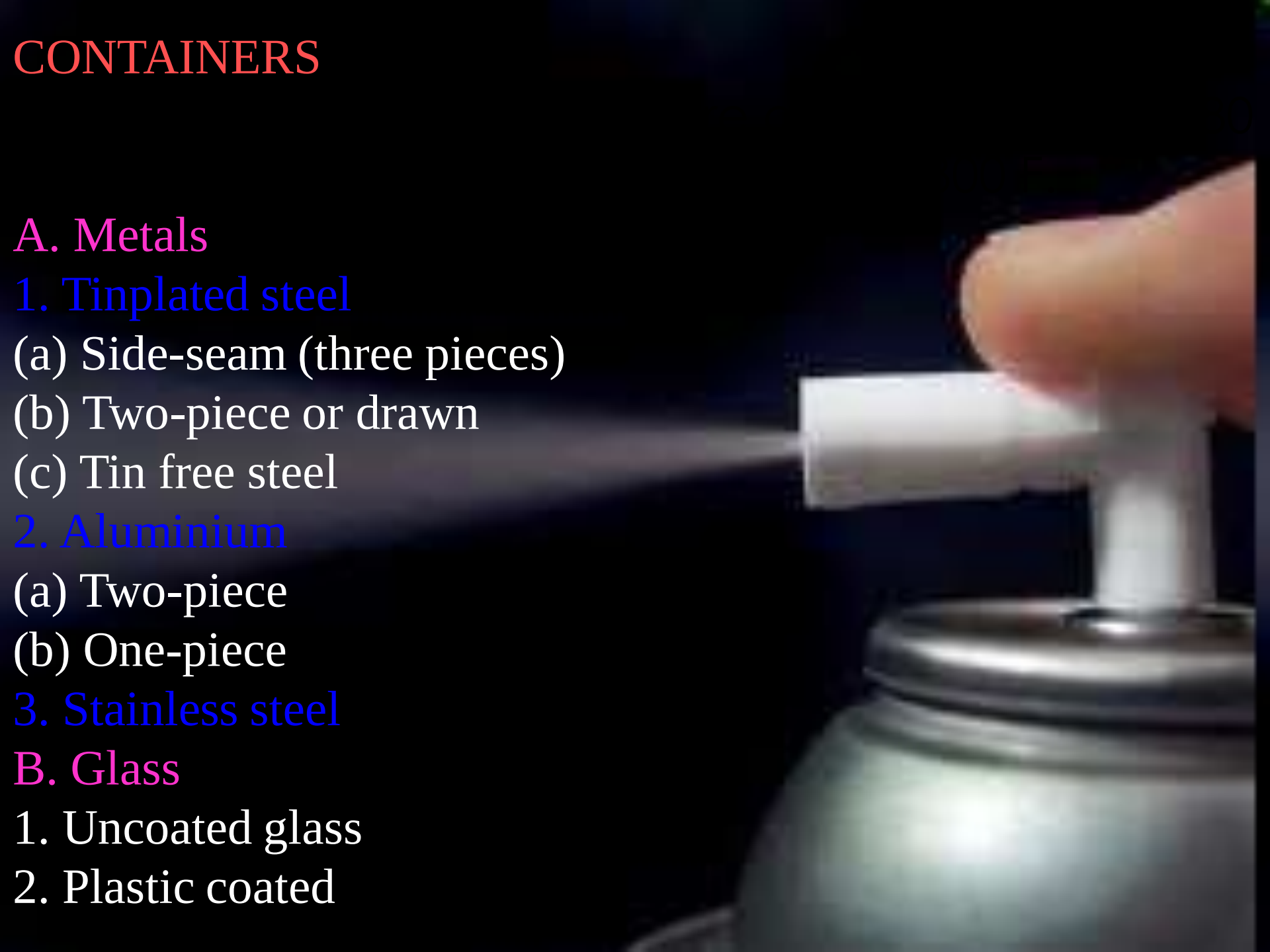
TOPICAL PREPARATION

Propane

Butane



CONTAINERS



A. Metals

1. Tinplated steel

(a) Side-seam (three pieces)

(b) Two-piece or drawn

(c) Tin free steel

2. Aluminium

(a) Two-piece

(b) One-piece

3. Stainless steel

B. Glass

1. Uncoated glass

2. Plastic coated

PHYSIOCHEMICAL PROPERTIES OF PROPELLANTS

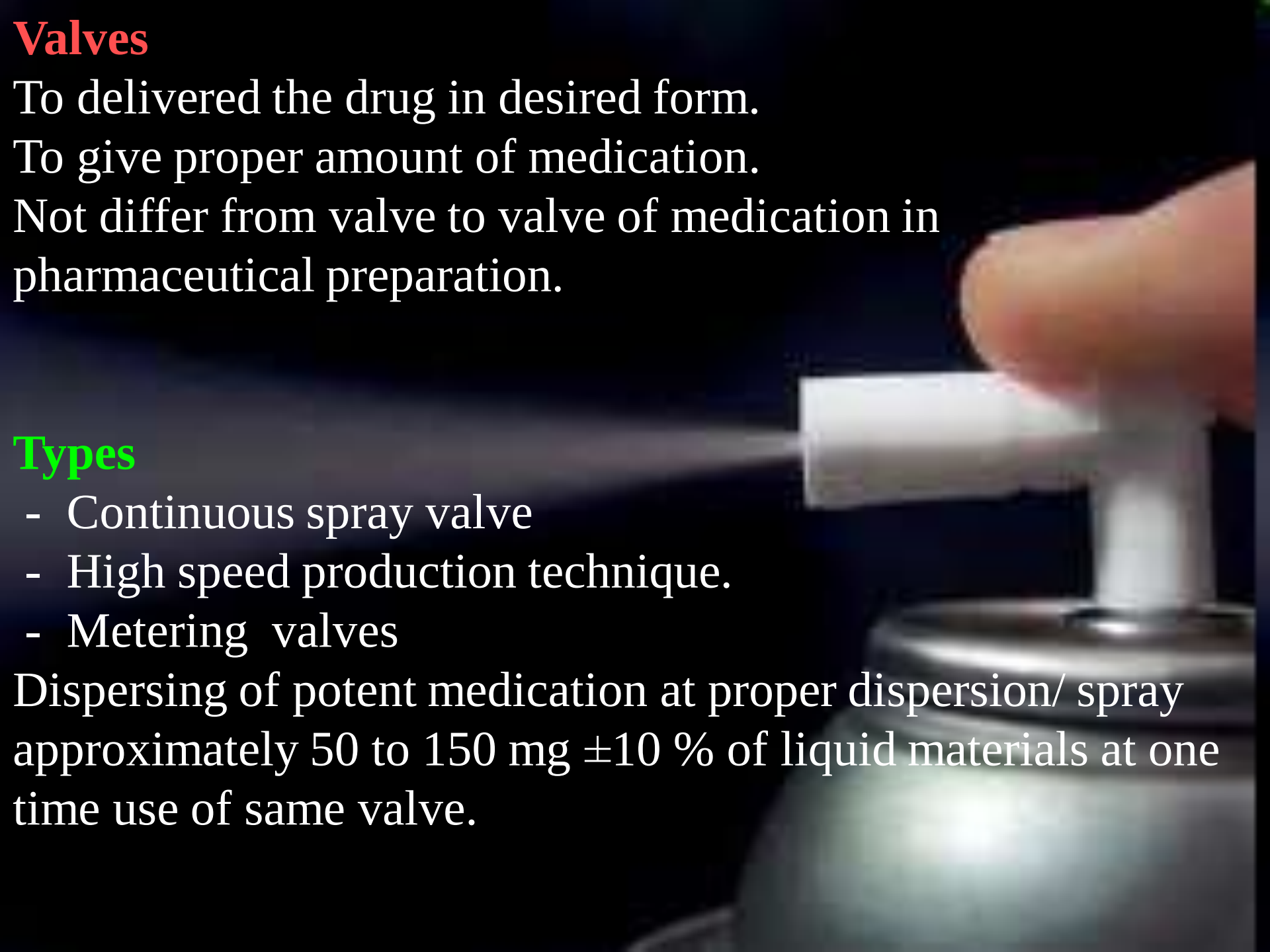
- Vapor pressure
- Boiling Points
- Liquid

Valves

To delivered the drug in desired form.

To give proper amount of medication.

Not differ from valve to valve of medication in pharmaceutical preparation.



Types

- Continuous spray valve
- High speed production technique.
- Metering valves

Dispersing of potent medication at proper dispersion/ spray approximately 50 to 150 mg ±10 % of liquid materials at one time use of same valve.

Valve components

- Ferrul or mount cap
- Valve body or housing
- Stem
- Gasket
- Spring
- Dip tube

Actuator

To ensure that aerosol product is delivered in the proper and desired form.

Different types of actuators

- Spray actuators
- Foam actuators
- Solid steam actuators
- Special actuators

FORMULATION OF PHARMACEUTICAL AEROSOLS

Contains two essential components

- Product concentrate
- Propellant

Product concentrate

Product concentrate contains ingredients or mixture of active ingredients and other such as solvents, antioxidants and surfactants.

Propellant

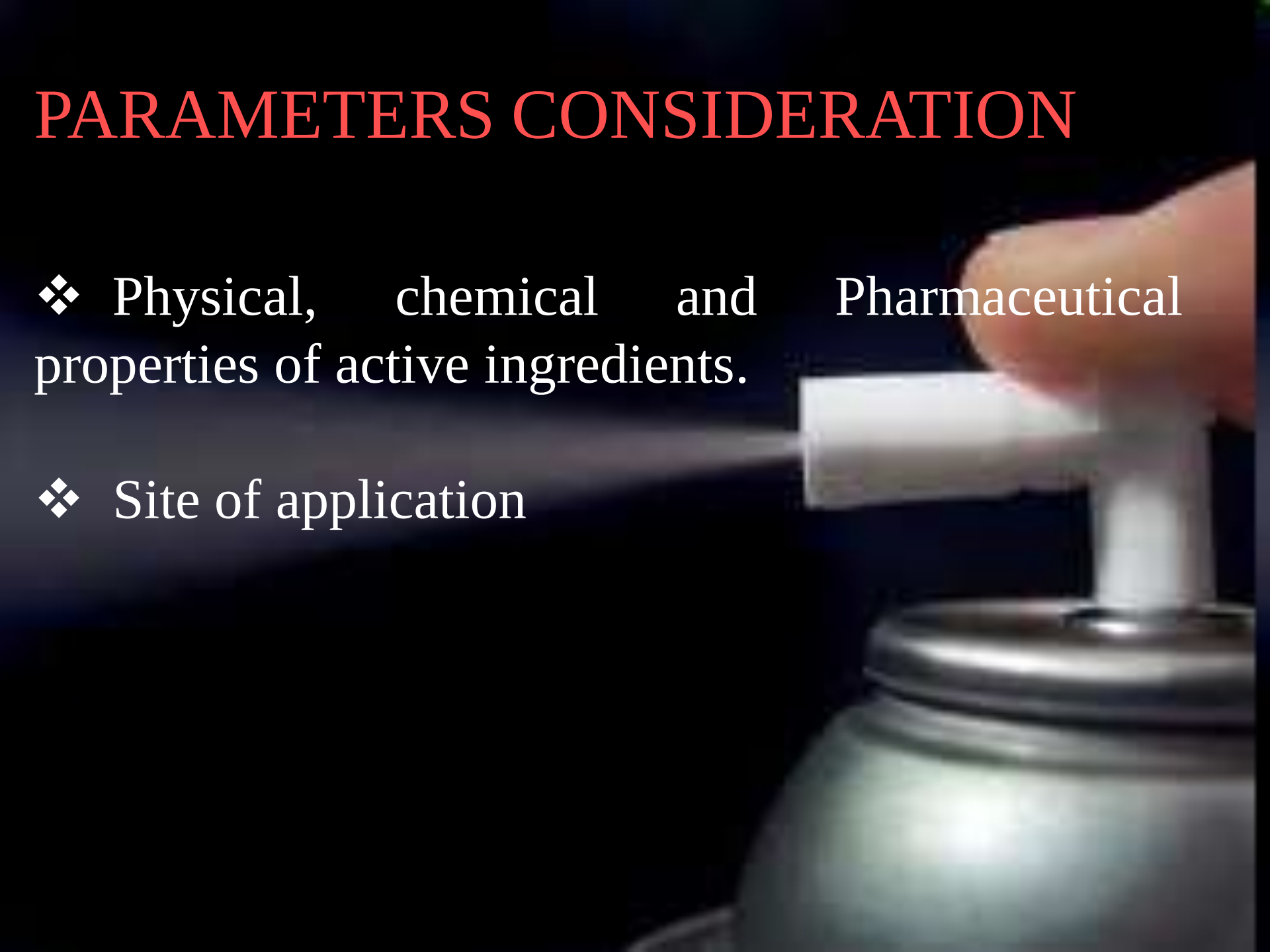
May be single or blend of various propellants

❖ Blends of propellant used in a p'ceutical formulation to achieve desired solubility characteristics or various surfactants are mixed to give the proper HLB value for emulsion system.

❖ To give the desired vapor pressure, solubility & particle size.

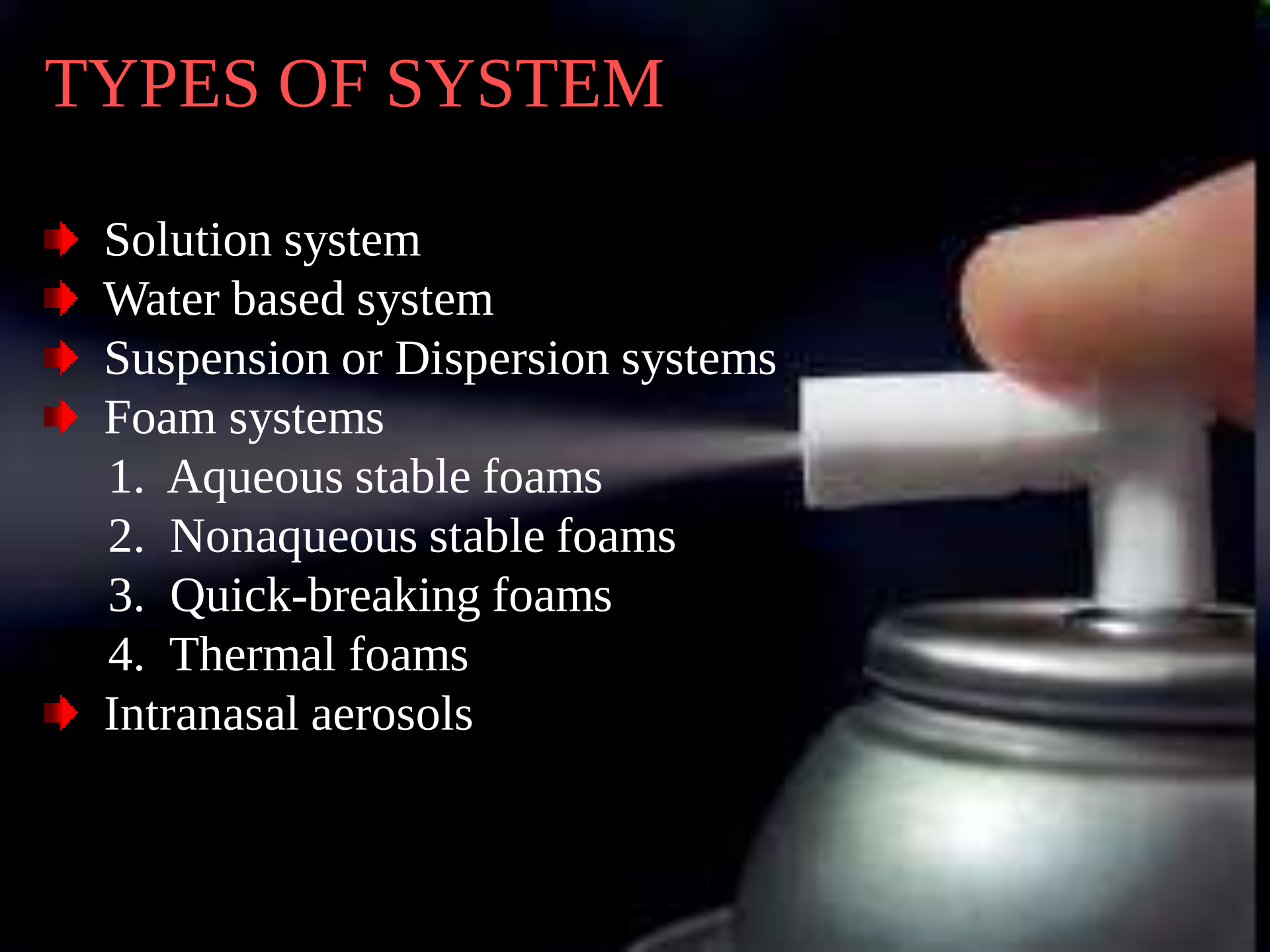
PARAMETERS CONSIDERATION

- Physical, chemical and Pharmaceutical properties of active ingredients.
- Site of application



TYPES OF SYSTEM

- Solution system
- Water based system
- Suspension or Dispersion systems
- Foam systems
  1. Aqueous stable foams
  2. Nonaqueous stable foams
  3. Quick-breaking foams
  4. Thermal foams
- Intranasal aerosols



THANKYOU!!

PHARMACODYNAMICS

Presented By
Mr. Krunal Kanase
HOD , Department of Pharmacology
PDEA's Shankarrao Ursal College of Pharmaceutical Sciences & Research Center, Kharadi.

- **Pharmacodynamics** is the study of the biochemical and physiological effects of drugs and their mechanisms of action.

- Simply stated, pharmacodynamics refers to the effects of a drug on the body.

Two state receptor model

- A receptor may exist in two interchangeable states, active **(*Rα*)** & inactive **(*Ri*)** which are in equilibrium.

- Binding of drug shift this equilibrium in either direction.

- ***Ligand***- any molecule which attaches selectively to particular receptors.

- ***Affinity -***The strength of the reversible interaction between a drug and its receptor, as measured by the *dissociation constant*, is defined as the affinity of one for the other.

- ***Intrinsic activity*** – capacity to induce a functional change in the receptor.

- ***Specificity -*** A drug that interacts with a single type of receptor that is expressed on only a limited number of differentiated cells will exhibit high specificity.

Agonists

- Drugs that bind to physiological receptors and mimic the regulatory effects of the endogenous signaling compounds are termed *agonists*.

- If the drug binds to the same *recognition site* as the endogenous agonist (the primary or orthosteric site on the receptor) the drug is said to be a ***primary agonist***.

- ***Allosteric* agonists** bind to a different region on the receptor referred to as an allosteric site.

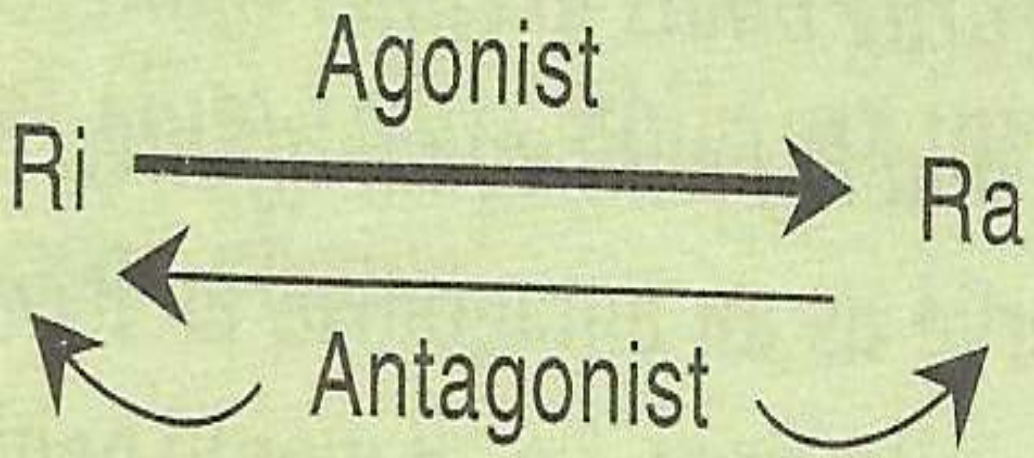


Fig 7.1 The Drug Action Viewed in Terms of Two-State Model of Receptor Activation.

Antagonist

- Drugs that block or reduce the action of an agonist are termed *antagonists*.
- Antagonism most commonly results from competition with an agonist for the same or overlapping site on the receptor (a *syntopic* interaction)

- **Physical antagonist** binds to the drug and prevents its absorption like charcoal binds to alkaloids and prevents their absorption.
- **Chemical antagonist** combines with a substance chemically like chelating agents binds with the metals.
- **Physiological antagonist** produces an action opposite to a substance but by binding to the different receptors e.g. adrenaline is a physiological antagonist of histamine because adrenaline causes bronchodilatation by binding to β2 receptors, which is opposite to bronchoconstriction caused by histamine through H1 receptors.
- **Pharmacological antagonists** produce no effect , shows no intrinsic activity.

- ***Partial agonists -***Agents that are only partly as effective as agonists regardless of the concentration employed.

- ***Inverse agonists -***Many receptors exhibit some constitutive activity in the absence of a regulatory ligand; drugs that stabilize such receptors in an inactive conformation are termed *inverse agonists* (produce effect opposite to that of agonist).

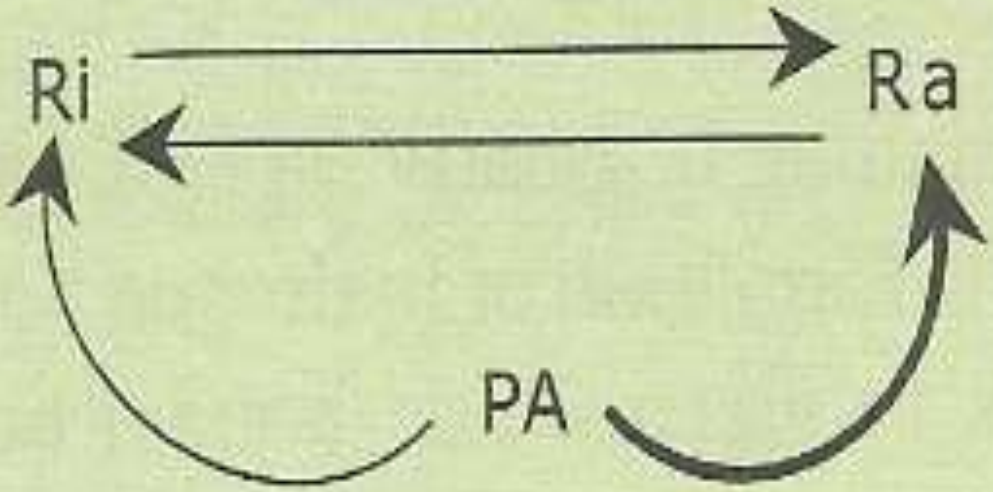


Fig 7.3

Partial Agonist (PA) has Slightly Higher Affinity for Ra (active state of the receptor), Although Lesser in Comparison to Agonist.

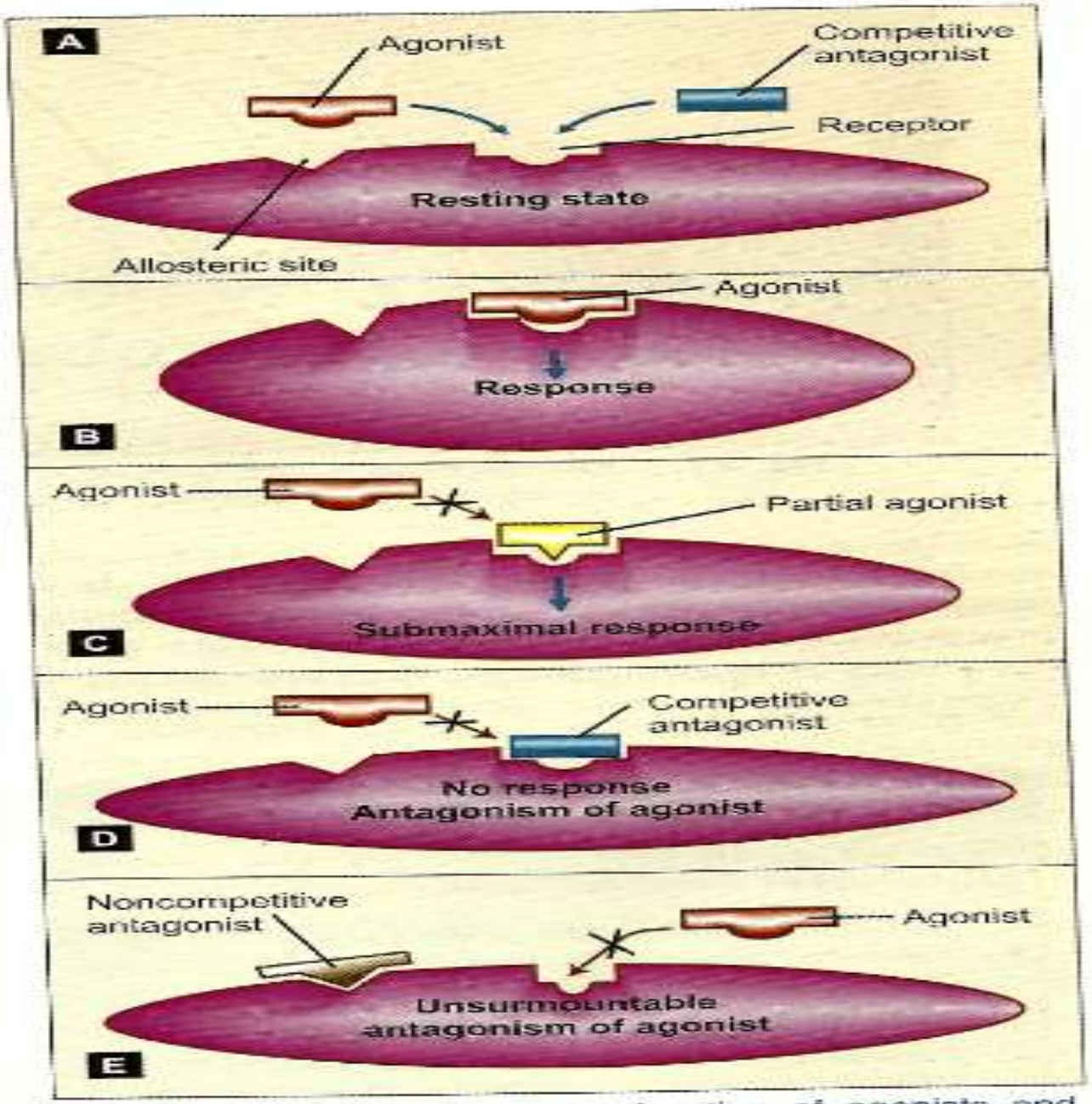


Fig. 4.16: Illustration of sites of action of agonists and antagonists (A), and the action of full agonist (B), partial agonist (C), competitive antagonist (D) and noncompetitive antagonist (E) on the receptor

Efficacy

- **A maximal effect (E_{max}) an agonist can produce.**

- **It can be measured with a graded dose-response curve only.**

POTENCY

- The amount of the drug needed to produce a given effect.
- potency is determined by the affinity of the receptor for the drug.
- The dose causing 50% from the maximal effect (EC50) can be obtained from graded dose-response curve.
- In quantal dose response curve, ED50, TD50 and LD50 are potency variables.

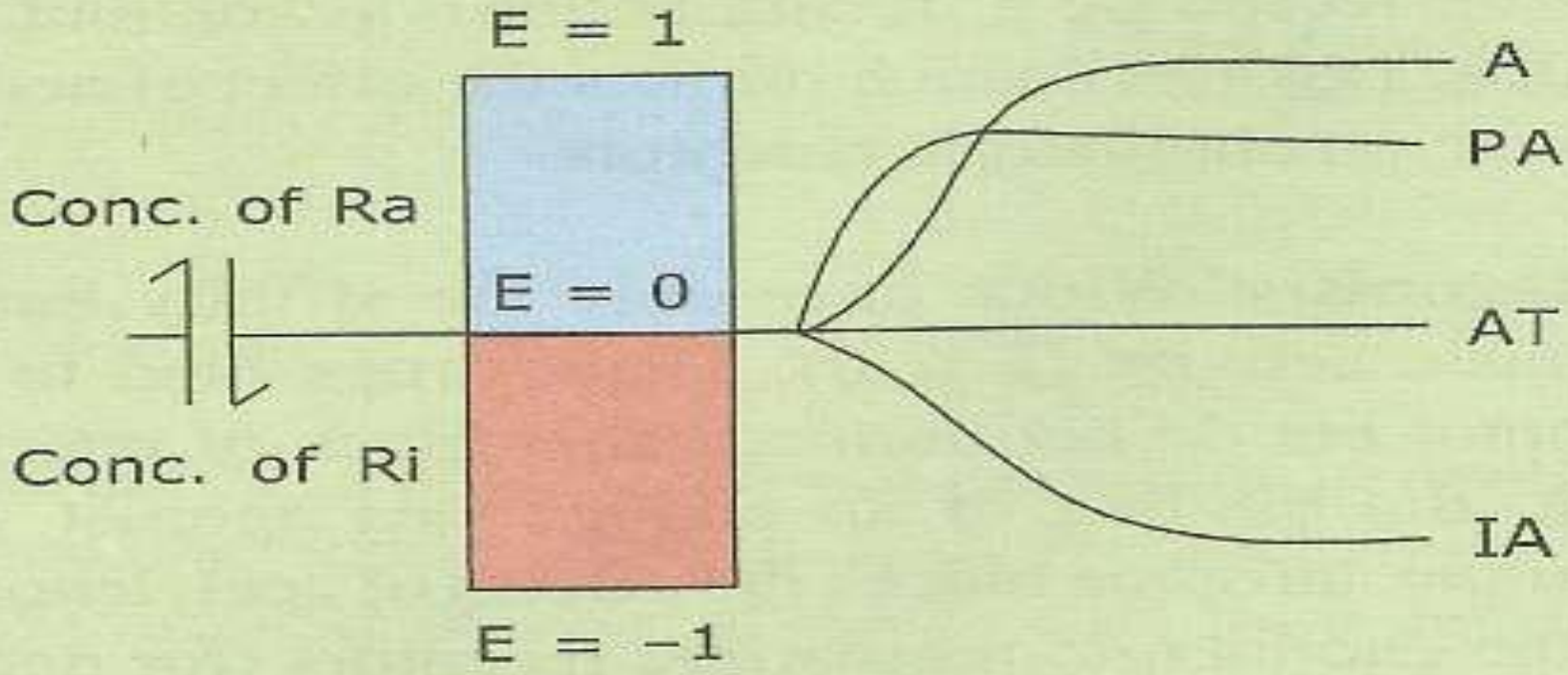
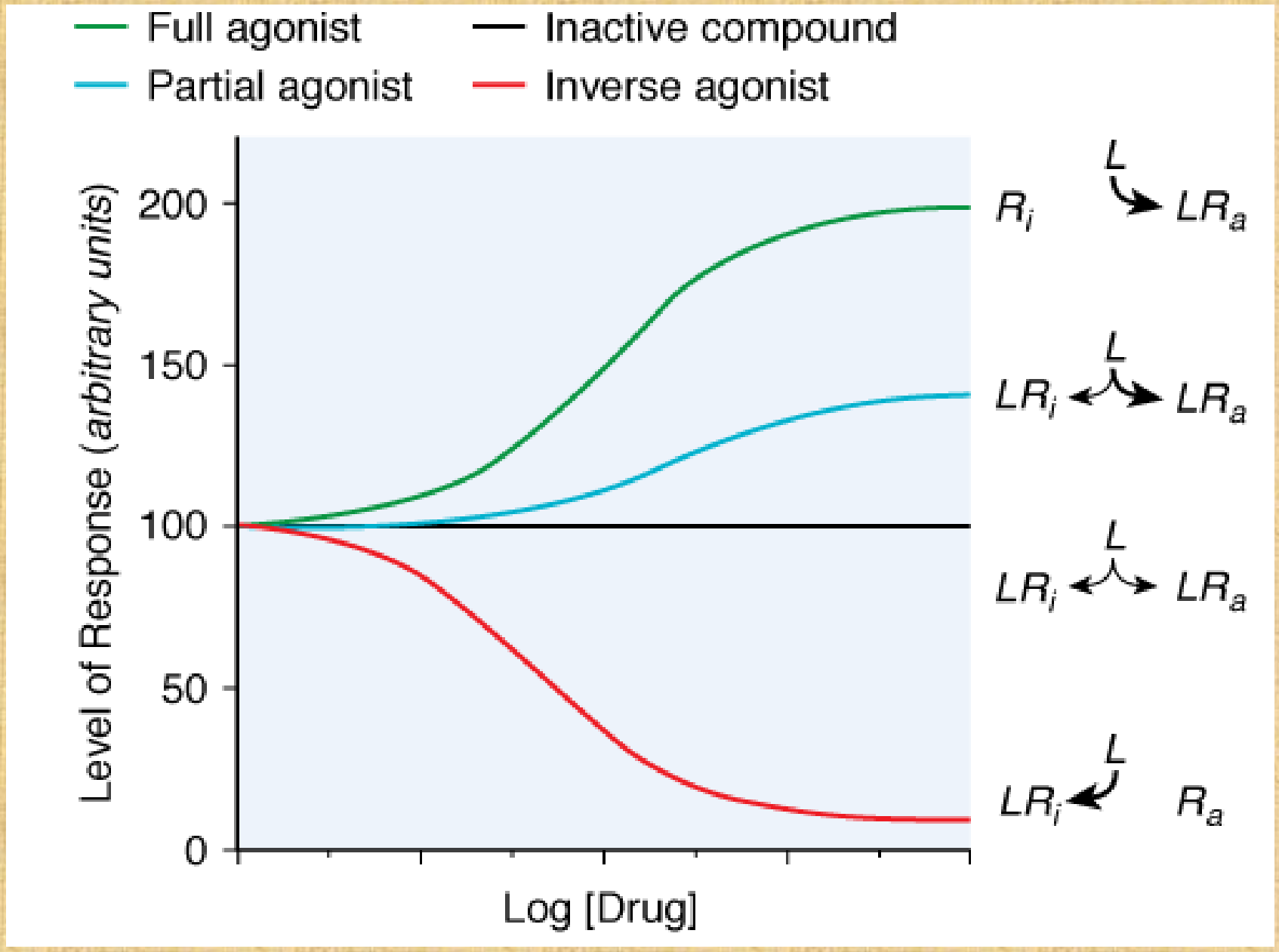
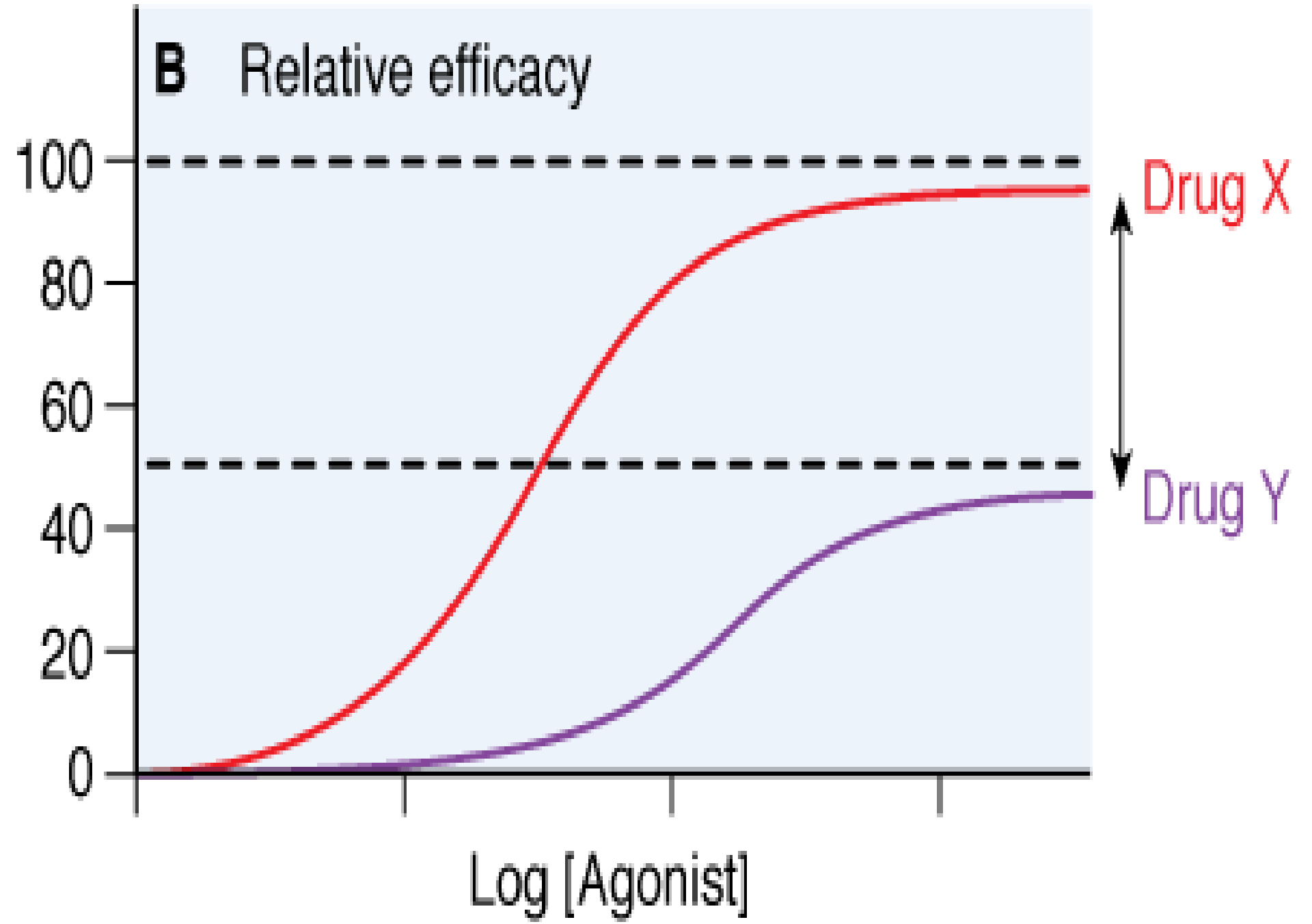
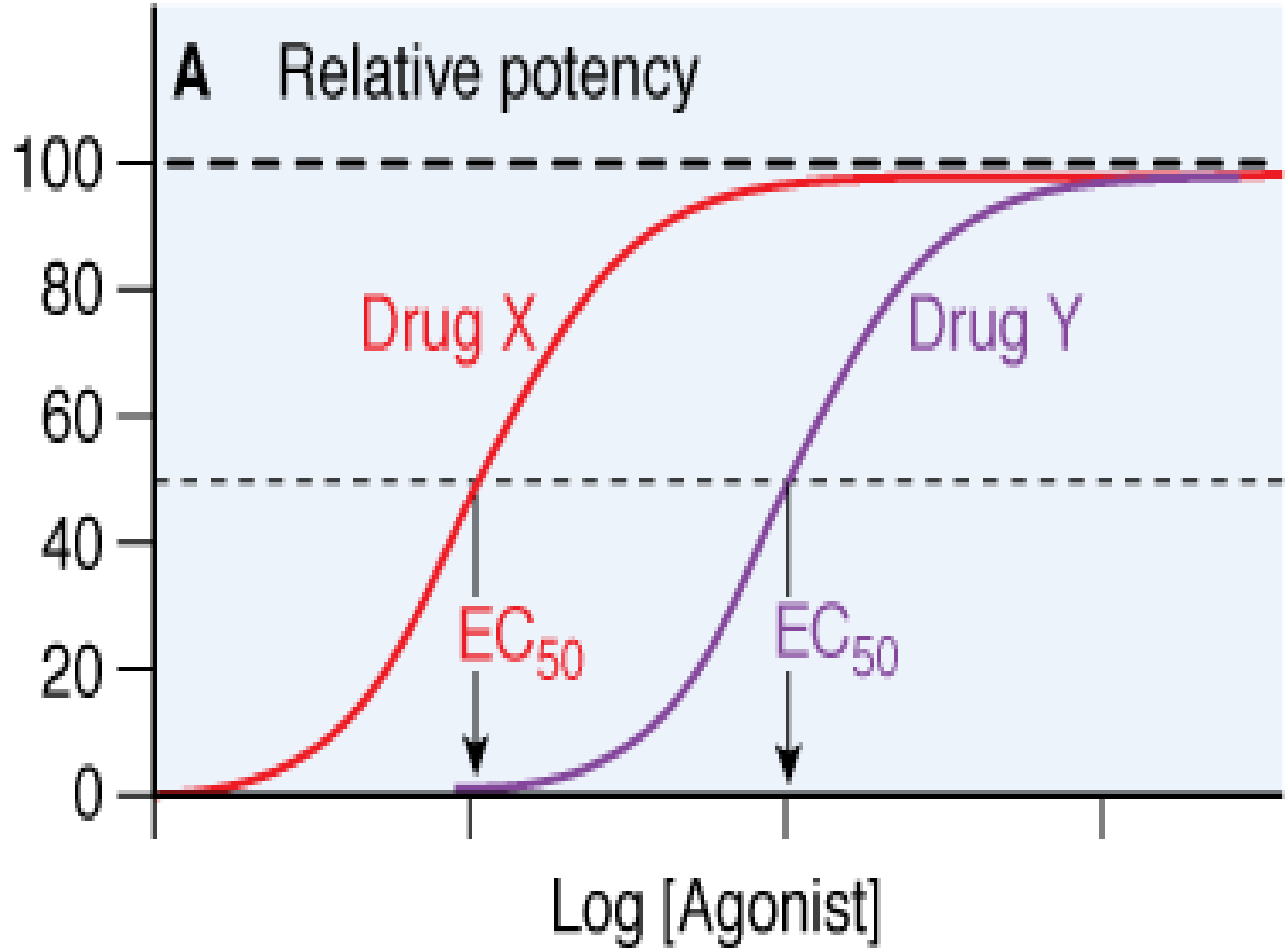


Fig 7.2 The Differential Influence of Agonist (A), Partial Agonist (PA), Antagonist (AT) and Inverse Agonist (IA) on Relative Distribution of Active (Ra) and Inactive (Ri) States of Receptors.


Full agonist
Partial agonist
Inactive compound
Inverse agonist
Level of Response (arbitrary units)
200
150
100
50
0
Log [Drug]
L
R_i
LR_a
L
LR_i
LR_a
L
LR_i
LR_a
L
LR_i
R_a


B Relative efficacy
100
80
60
40
20
0
Drug X
Drug Y
Log [Agonist]


A Relative potency
100
80
60
40
20
0
Drug X
Drug Y
EC50
EC50
Log [Agonist]

Repeated administration of a drug results in diminished effect "Tolerance".

- **Tachyphylaxis:** is a type of tolerance which occurs very rapidly.
- **Desensitization:** decreased response to the agonist after its repeated injection in small doses.

May be due to

1- Masking or internalization of the receptors.

2- Loss of receptors (down regulation)- decreased synthesis or increased destruction.

3- Exhaustion of mediators (depletion of catecholamine).

Competitive (equilibrium type)	Noncompetitive
1. Antagonist binds with the same receptor as the agonist	Binds to another site of receptor
2. Antagonist resembles chemically with the agonist	Does not resemble
3. Parallel rightward shift of agonist DRC	Flattening of agonist DRC
4. The same maximal response can be attained by increasing dose of agonist (surmountable antagonism)	Maximal response is suppressed (unsurmountable antagonism)
5. The antagonist appears to have inactivated a certain number of agonist molecules	The antagonist appears to have inactivated a certain number of receptors
6. Intensity of response depends on the concentration of both agonist and antagonist	Maximal response depends only on the concentration of antagonist
7. Examples: ACh—Atropine Morphine—Naloxone	Diazepam—Bicuculline

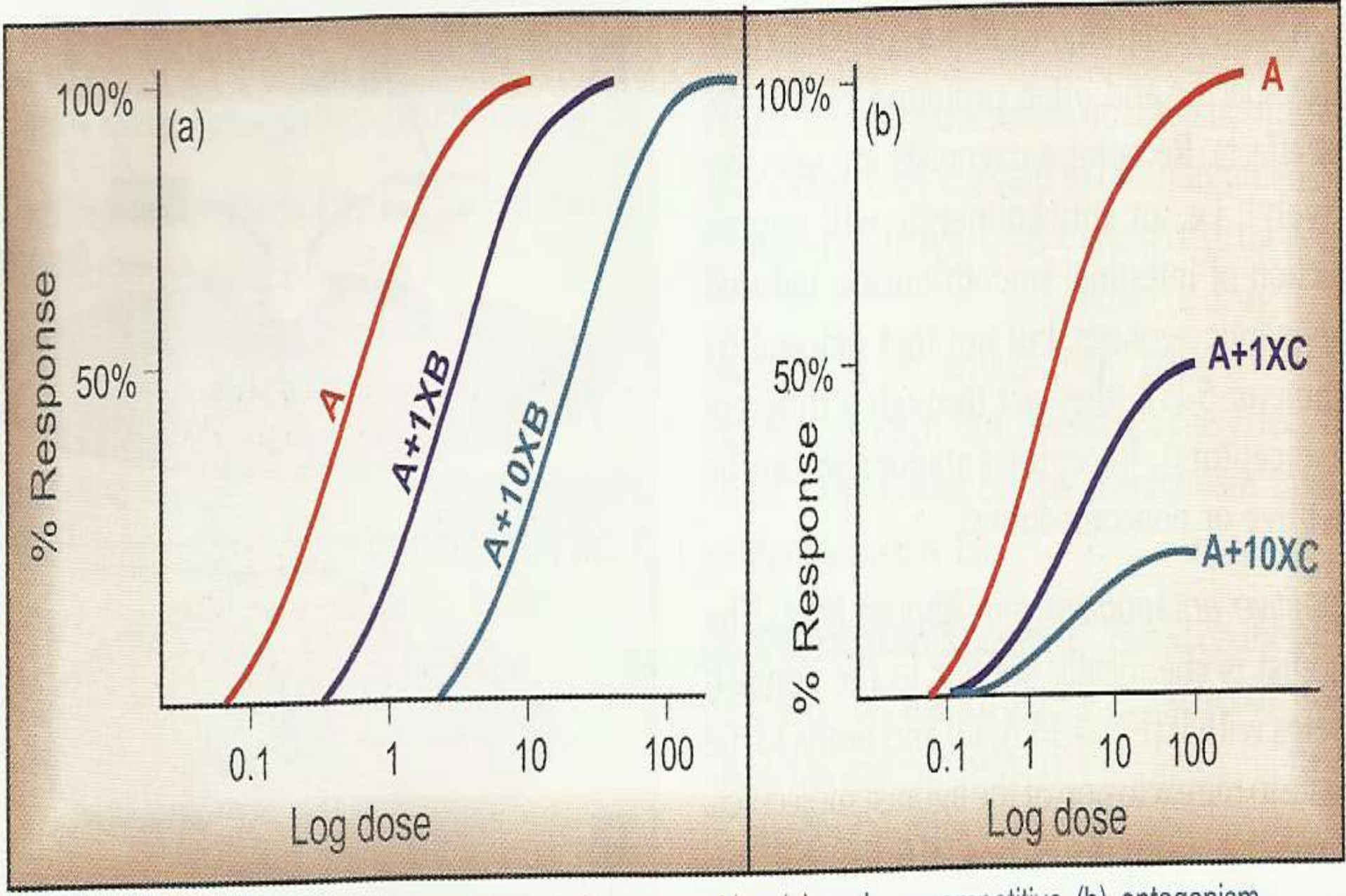


Fig. 4.17: Dose-response curves showing competitive (a) and noncompetitive (b) antagonism
A—agonist, B—competitive antagonist, C—noncompetitive antagonist

PHYSIOLOGICAL RECEPTORS

- GPCR
- Ion channels
- Transmembrane enzymes
- Transmembrane, non-enzymes
- Nuclear receptors
- Intracellular enzymes

Cellular Pathways Activated by Physiological Receptors

Signal Transduction Pathways

- Physiological receptors have at least two major functions, ligand binding and message propagation (i.e., signaling).
- Two functional domains within the receptor:

  -ligand-binding *domain* and

  -effector *domain*.
- The regulatory actions of a receptor may be exerted directly on its cellular target(s), on *effector protein(s),* or may be conveyed by intermediary cellular signaling molecules called ***transducers****.*

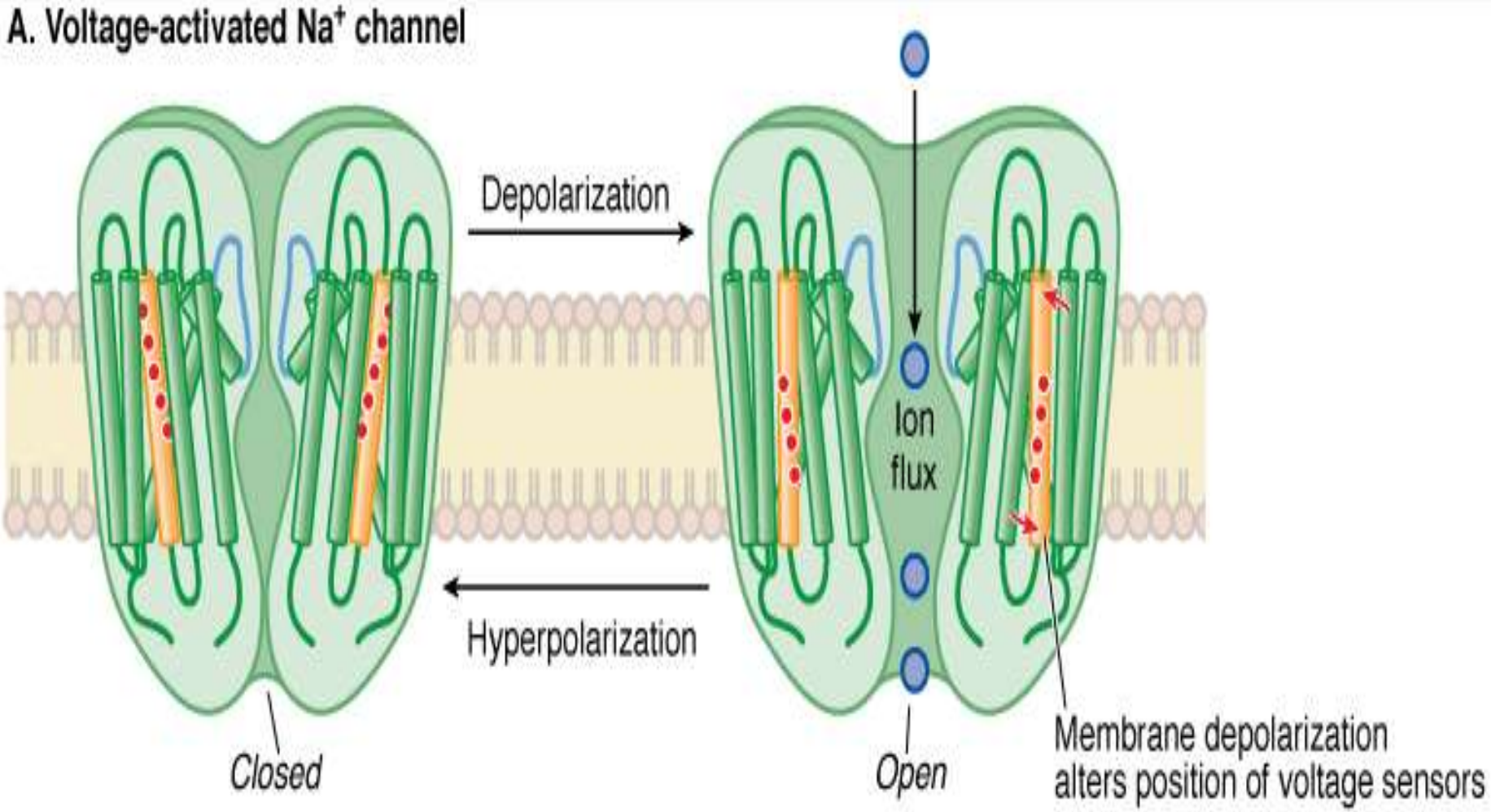
STRUCTURAL FAMILY	FUNCTIONAL FAMILY	PHYSIOLOGICAL LIGANDS	EFFECTORS AND TRANSDUCERS	EXAMPLE DRUGS
Ion channels	Ligand-gated	ACh (M_2), GABA, 5-HT	Na^+, Ca^{2+}, K^+, Cl^-	Nicotine, gabapentin
	Voltage-gated	None (activated by membrane depolarization)	Na^+, Ca^{2+}, K^+, other ions	Lidocaine, verapamil

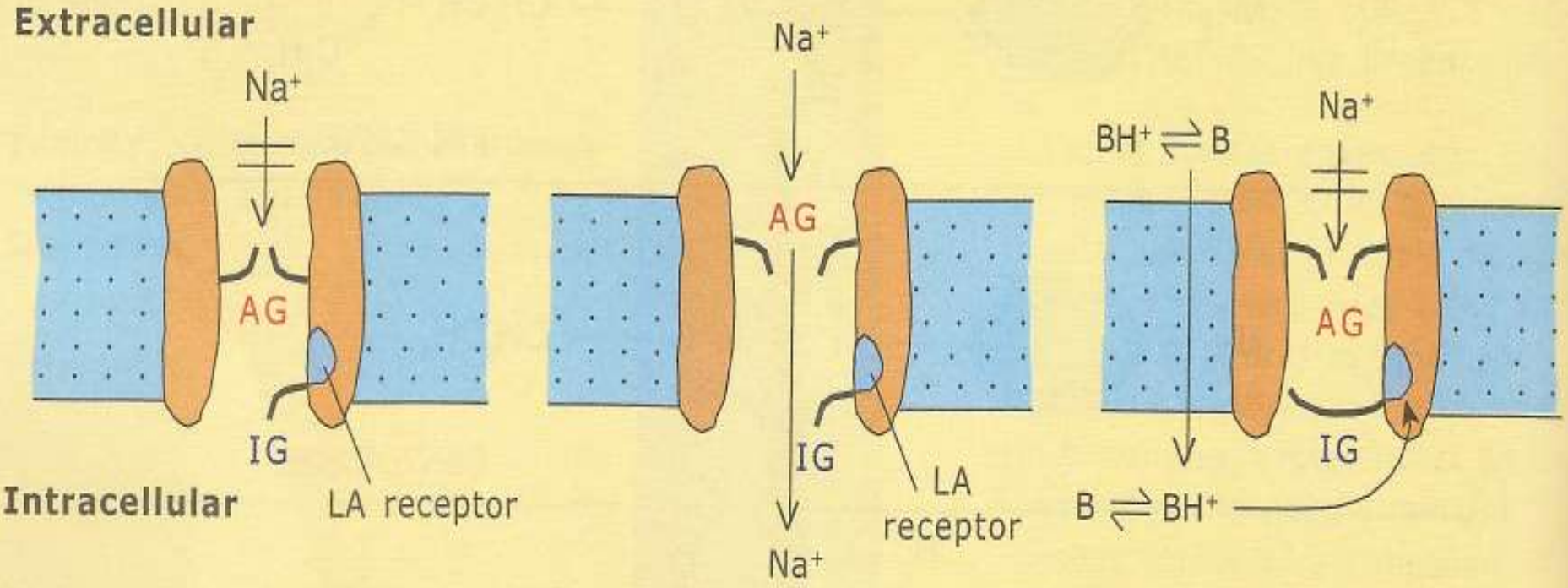
Receptors with intrinsic ion channel

- Ligand gated ion channels.
- No intervention of G-protein or second messenger.
- Response is fastest (in milliseconds).

e.g. $GABA_A$, $5HT_3$, NMDA

voltage-activated Na^+ channel with the pore in the open and closed state.





Resting or Closed State

The Activation Gate (AG) closed. The LA receptor is located in the trans-membrane pore of Na^+ channel.

Activated or Open State

Brief depolarisation opens AG and allows Na^+ permeation.

Inactivated or Blocked State

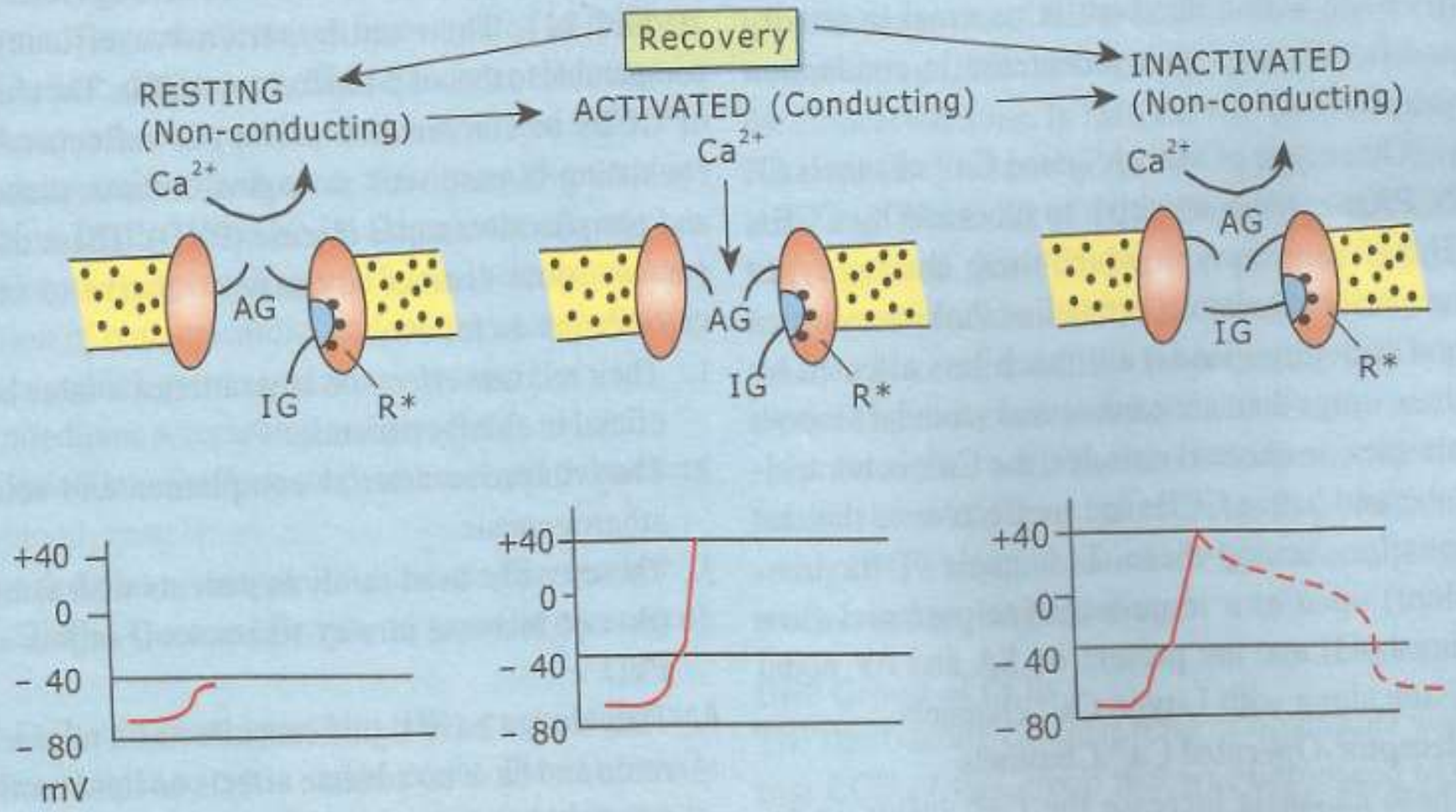
Ionised (BH^+) form of LA binds to LA receptor. IG closes, flow of Na^+ ceases.

Fig 15.2 A Model of Voltage-Gated Na^+ Channel Depicting the Site and Mechanism of Local Anaesthetic Action.

Table 19.1 Properties of Voltage-Sensitive Ca^{2+} Channels

Voltage-Sensitive Calcium Channels				
Properties	**L (long lasting)**	**T (transient)***	**N (neuronal)**	**P/Q** & R****
Activation threshold	High	Low	High	High
Inactivation rate	Slow	Fast	Medium	Slow
Location	Cardiac and smooth muscle, SA node and AV node	SA node Thalamic and other neurons	Neurons in CNS and Secretory glands	Cerebellar and Purkinje neurons
Function	Regulate excitation–contraction coupling; Regulate pacemaker activity; Regulate conduction velocity	Regulate pacemaker activity; Regulate T current and repetitive spikes in thalamic nuclei	Modulate transmitter release	Modulate transmitter release
Blockers	Verapamil Dihydropyridine group of CCBs, Cd^{2+}	Flunarizine Ethosuximide Ni^{2+}	Cd^{2+} Gabapentin	Toxin from funnel web spider

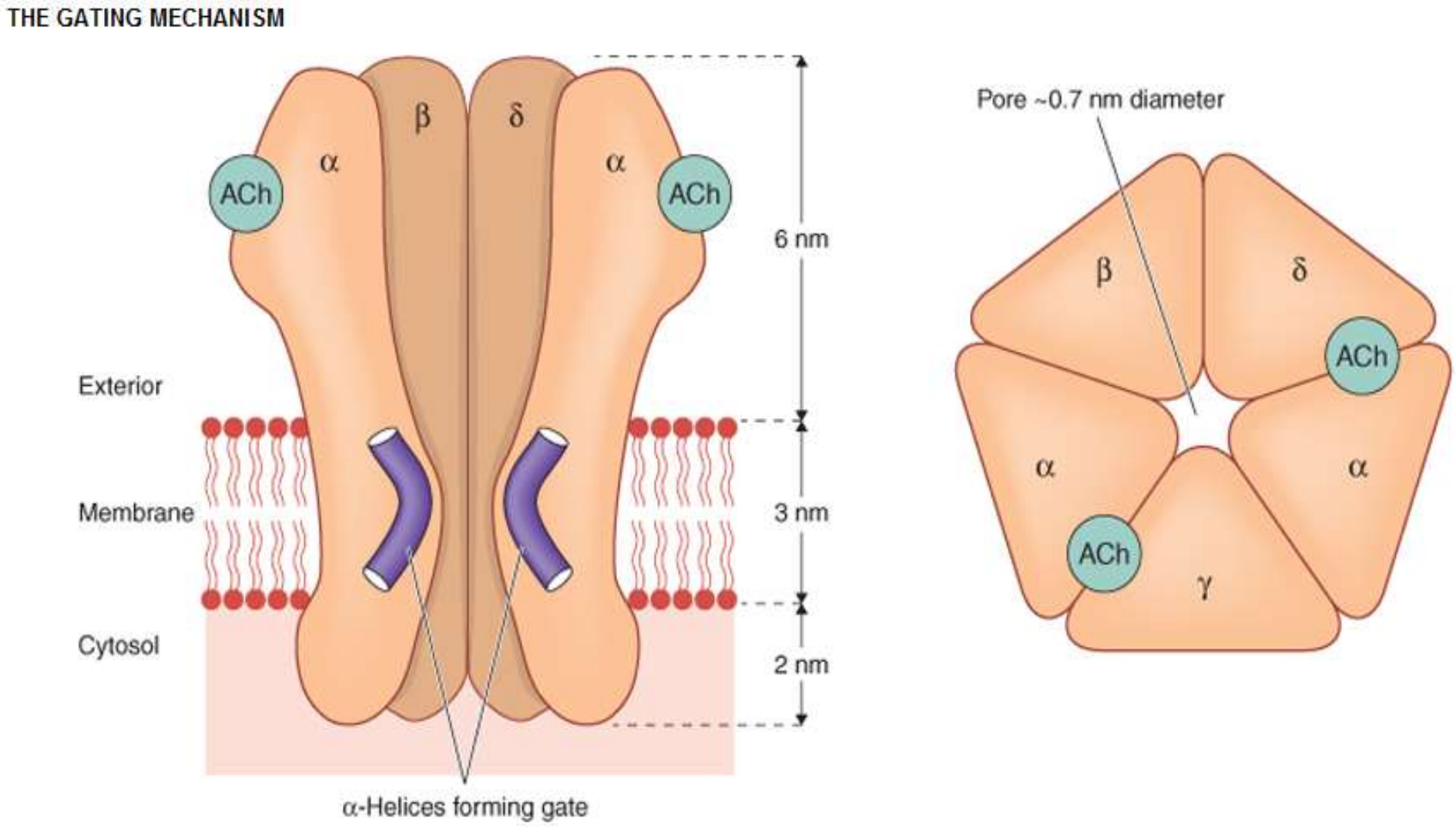
* T channels are resistant to be blocked by most of CCBs except for the drugs shown
** Q channels have properties similar to P channels. Not much is known about R channels. The neuronal pharmacology of CNS is modulated by N, P/Q and R channels. This underlines the normally observed weak effects of L channel blockers on CNS functions — The terminology of these channels sounds quite melodious. **L** stands for *long lasting*; **T** for *transient*; **N** for *neither* long lasting *nor* transient but *neuronal*; **O** somehow has been *omitted* but **P** and **Q** follow as simple English alphabets; Next is obviously **R**.



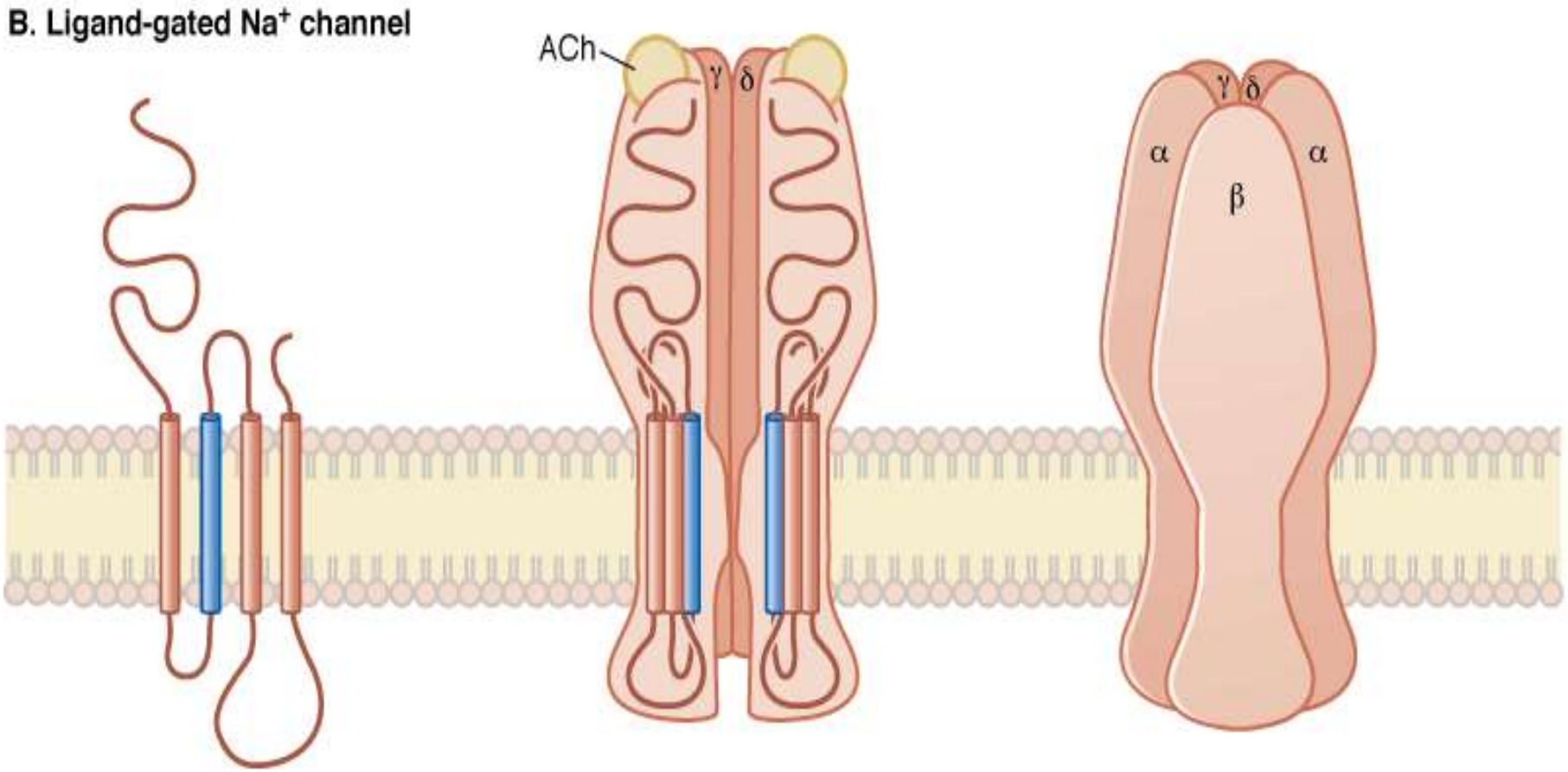
Key: AG—Activation gate; IG—Inactivation gate; R*—Receptor for CCBs with different binding sites. The part of AP during which most Ca^{2+} channels get completely or partly inactivated and not available for reactivation is shown by dashed line.

Fig 19.3 A Model of Voltage-Gated Ca^{2+} Channel Depicting the Site and Mechanism of CCBs Action

Structure of the nicotinic acetylcholine receptor (a typical ligand-gated ion channel. The five receptor subunits (α2, β, γ, δ) form a cluster surrounding a central transmembrane pore.



Ligand-gated nicotinic acetylcholine receptor expressed in the skeletal muscle neuromuscular junction. The pore is made up of five subunits, each with a large extracellular domain and four transmembrane helices. The helix that lines the pore is shown in blue.



Source: Brunton LL, Chabner BA, Knollmann BC: *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Edition:* www.accessmedicine.com
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PHYSIOLOGICAL RECEPTOR

STRUCTURAL FAMILY	FUNCTIONAL FAMILY	PHYSIOLOGICAL LIGANDS	EFFECTORS AND TRANSDUCERS	EXAMPLE DRUGS
GPCR	β Adrenergic receptors	NE, Epi, DA	G_s ; AC	Dobutamine
	Muscarinic cholinergic receptors	ACh	G_i and G_q; AC, ion channels, PLC	Atropine
	Eicosanoid receptors	Prostaglandins, leukotrienes, thromboxanes	G_s, G_i and G_q proteins	Misoprostol, montelukast

G Proteins

- **GPCRs** a family of heterotrimeric GTP-binding regulatory proteins termed **G proteins**.

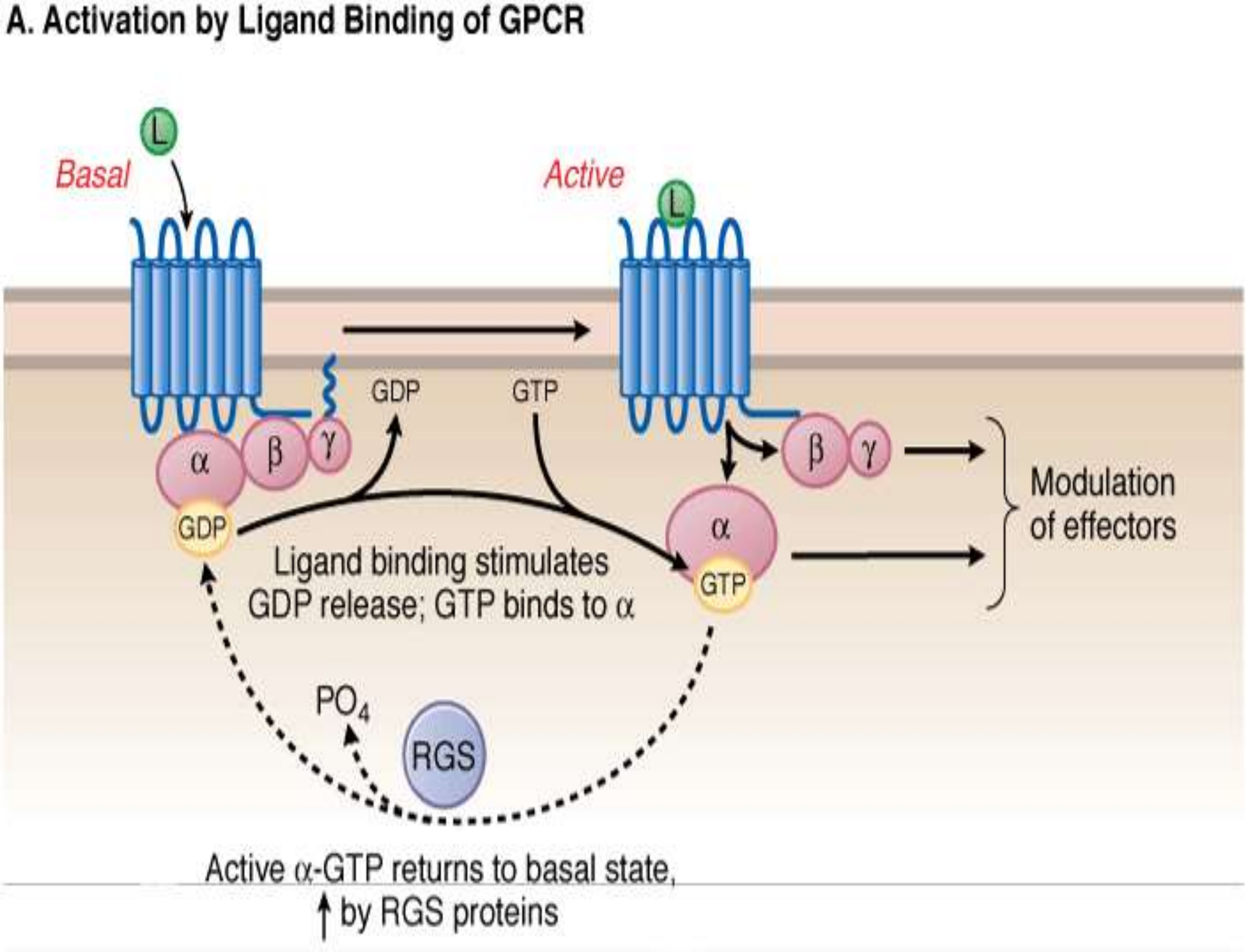
- **G proteins** are signal transducers that convey the information that agonist is bound to the receptor from the receptor to one or more effector proteins.

- The G protein heterotrimer is composed of a guanine nucleotide-binding **α subunit**, which confers specific recognition to both receptors and effectors, and an associated dimer of **β and ϒ subunits.**

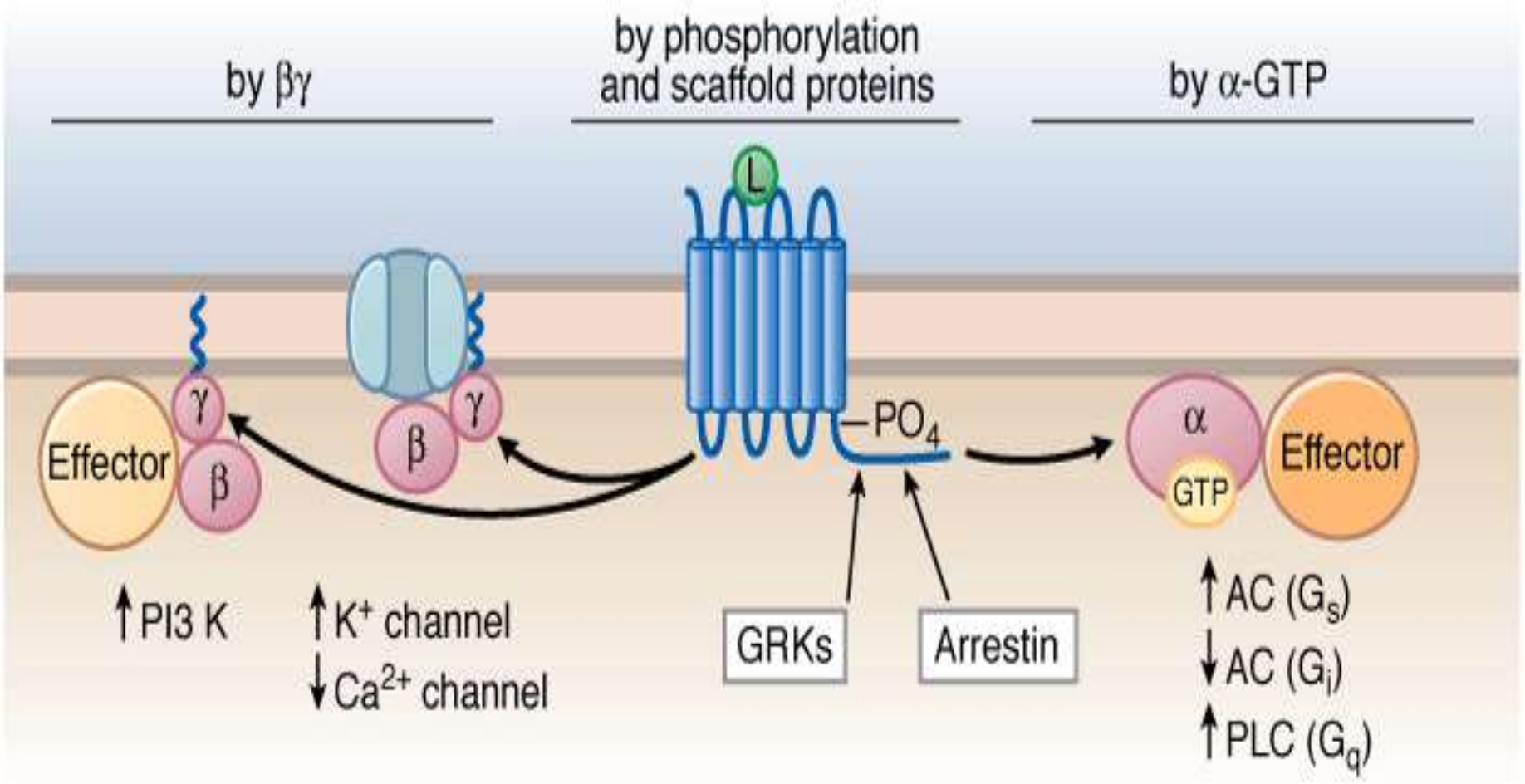
- G–protein-regulated effectors include enzymes such as
  -**adenylyl cyclase,**
  -phospholipase C,
  -cyclic GMP phosphodiesterase (PDE6),
  -membrane ion channels selective for Ca^{2+} and K^{+}.

G Protein–Coupled Receptors (GCPRs)

- GPCRs span the plasma membrane as a bundle of seven alfa-helices.
- Extracellular binding site for ligand.
- Cytosolic binding site for transducer G-protein.


A. Activation by Ligand Binding of GPCR
Basal
L
Active
L
GDP
GTP
α
β
γ
GDP
β
γ
α
GTP
Modulation of effectors
Ligand binding stimulates GDP release; GTP binds to α
PO4
RGS
Active α-GTP returns to basal state, by RGS proteins

B. Modulation of Effectors



Source: Brunton LL, Chabner BA, Knollmann BC: *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Edition:* www.accessmedicine.com

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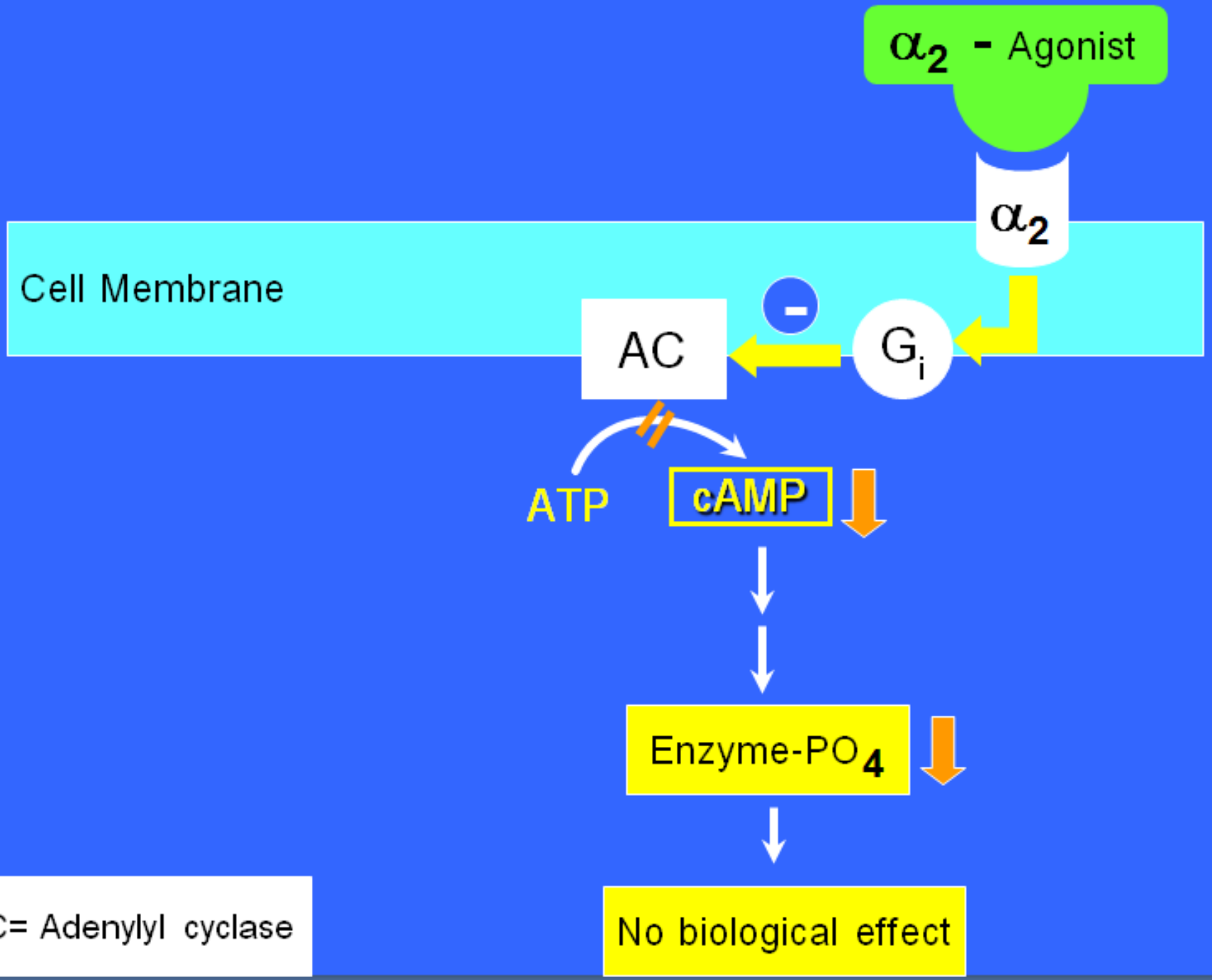
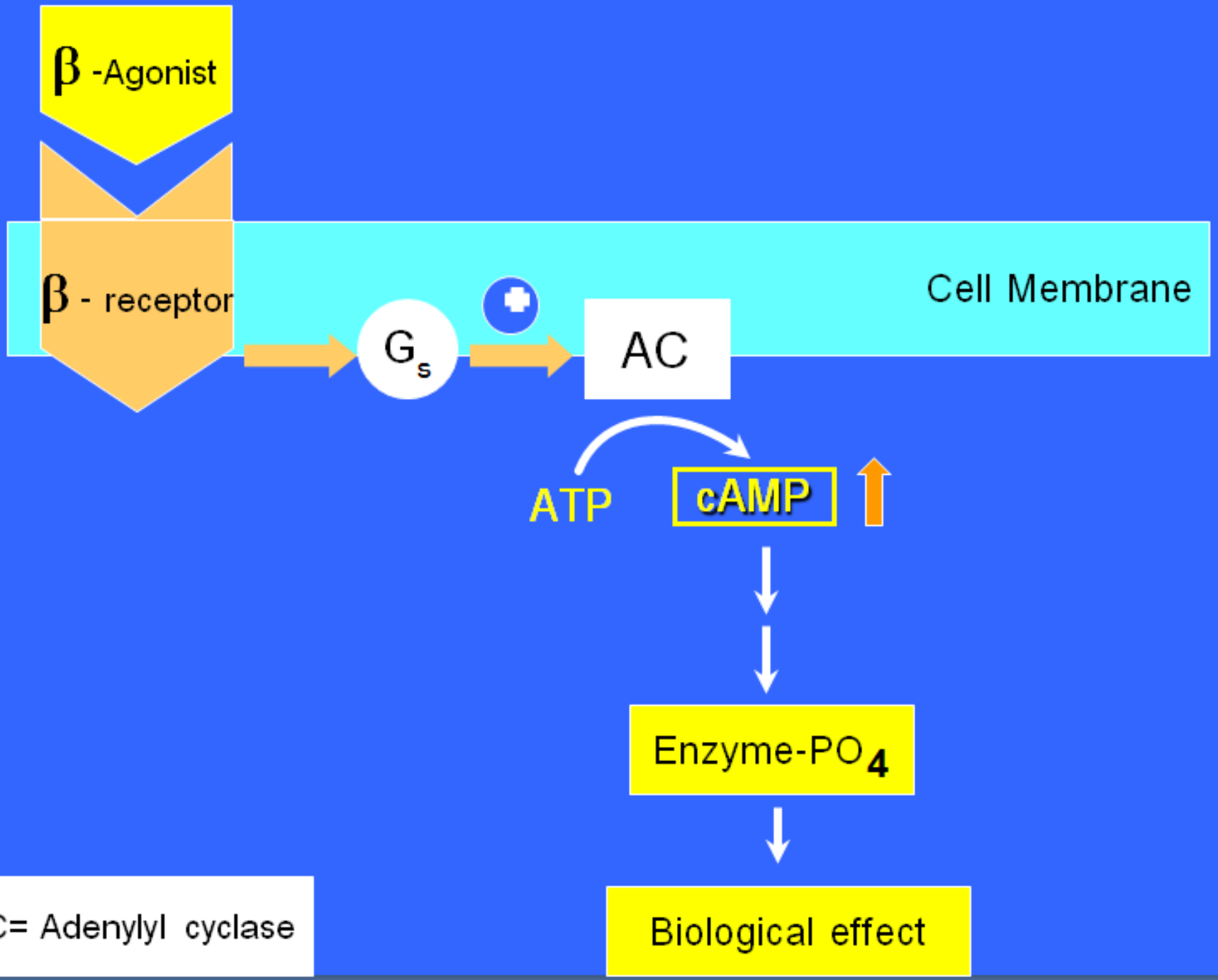
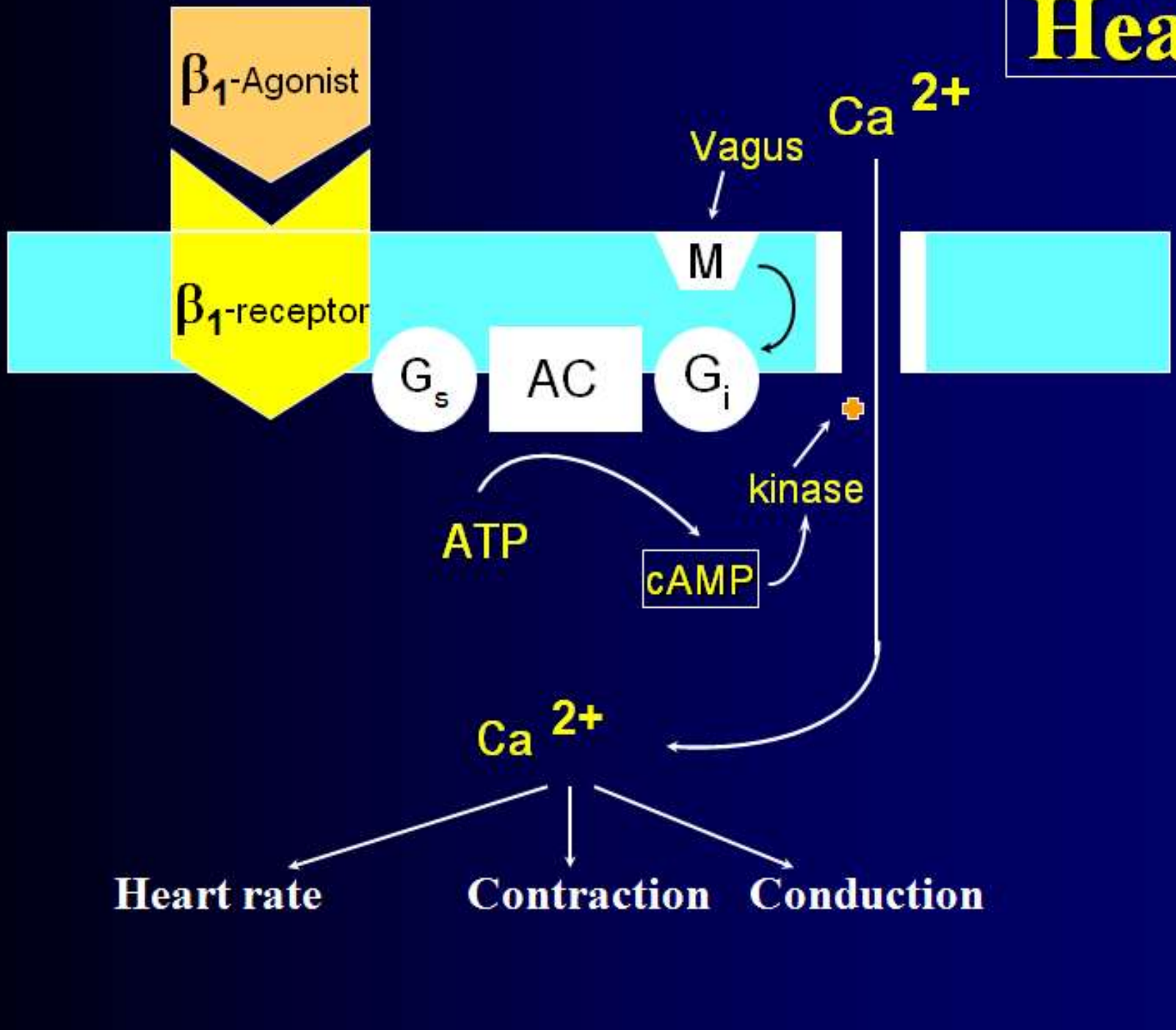
- The subunits fall into four families (G_s, G_i, G_q, and $G_{12/13}$).

- The **G_s** α-subunit uniformly activates adenylyl cyclase;
- the **G_i** α-subunit can inhibit certain isoforms of adenylyl cyclase;
- the **G_q** α-subunit activates all forms of phospholipase C;
- and the **$G_{12/13}$** α-subunits couple to guanine nucleotide exchange factors (GEFs), such as p115RhoGEF for the small GTP-binding proteins Rho and Rac.


α2 - Agonist
α2
Cell Membrane
AC
Gi
ATP
cAMP
Enzyme-PO4
No biological effect
AC= Adenylyl cyclase


β -Agonist
β - receptor
Cell Membrane
G_s
AC
ATP
cAMP
Enzyme-PO_4
Biological effect
AC= Adenylyl cyclase

Heart


β1-Agonist
β1-receptor
Vagus
Ca 2+
M
Gs
AC
Gi
kinase
ATP
cAMP
Ca 2+
Heart rate
Contraction
Conduction

Adenylyl cyclase-cAMP pathway

↑ cAMP (2nd messenger)

PK_A phosphorylation

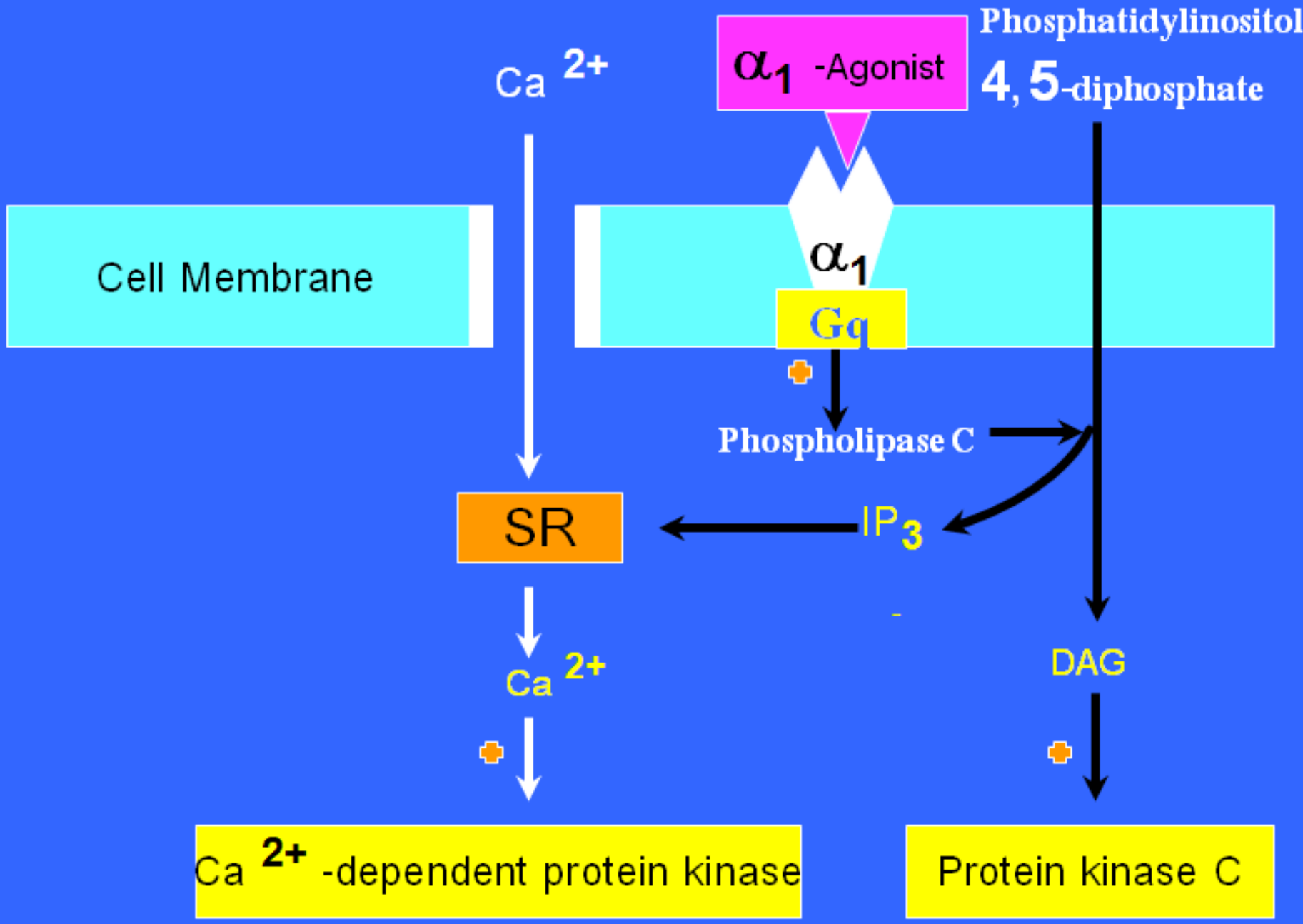
Various functions

-↑ heart contraction

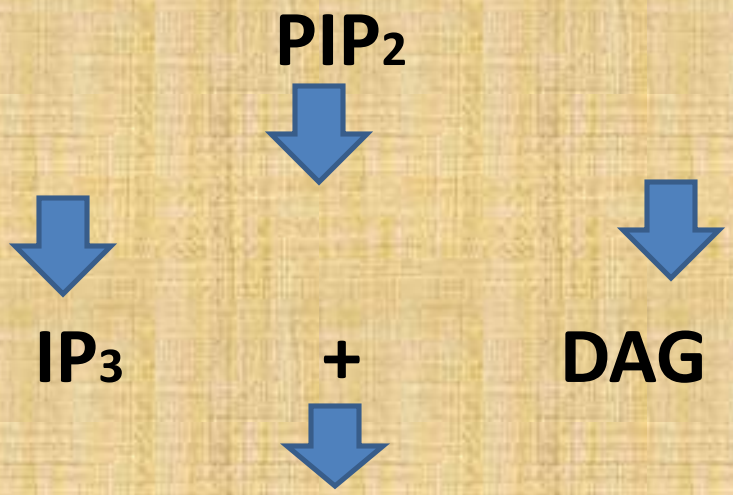
-Smooth mus relaxation

-Glycogenolysis

-lipolysis


Ca 2+
α1 -Agonist
Phosphatidylinositol
4, 5-diphosphate
Cell Membrane
α1
Gq
Phospholipase C
SR
IP3
Ca 2+
DAG
Ca 2+ -dependent protein kinase
Protein kinase C

Phospholipase C: IP_3-DAG pathway



Activation of **CCPK, MLCK, PKc**

-contraction

-neural excitation

-cell proliferation

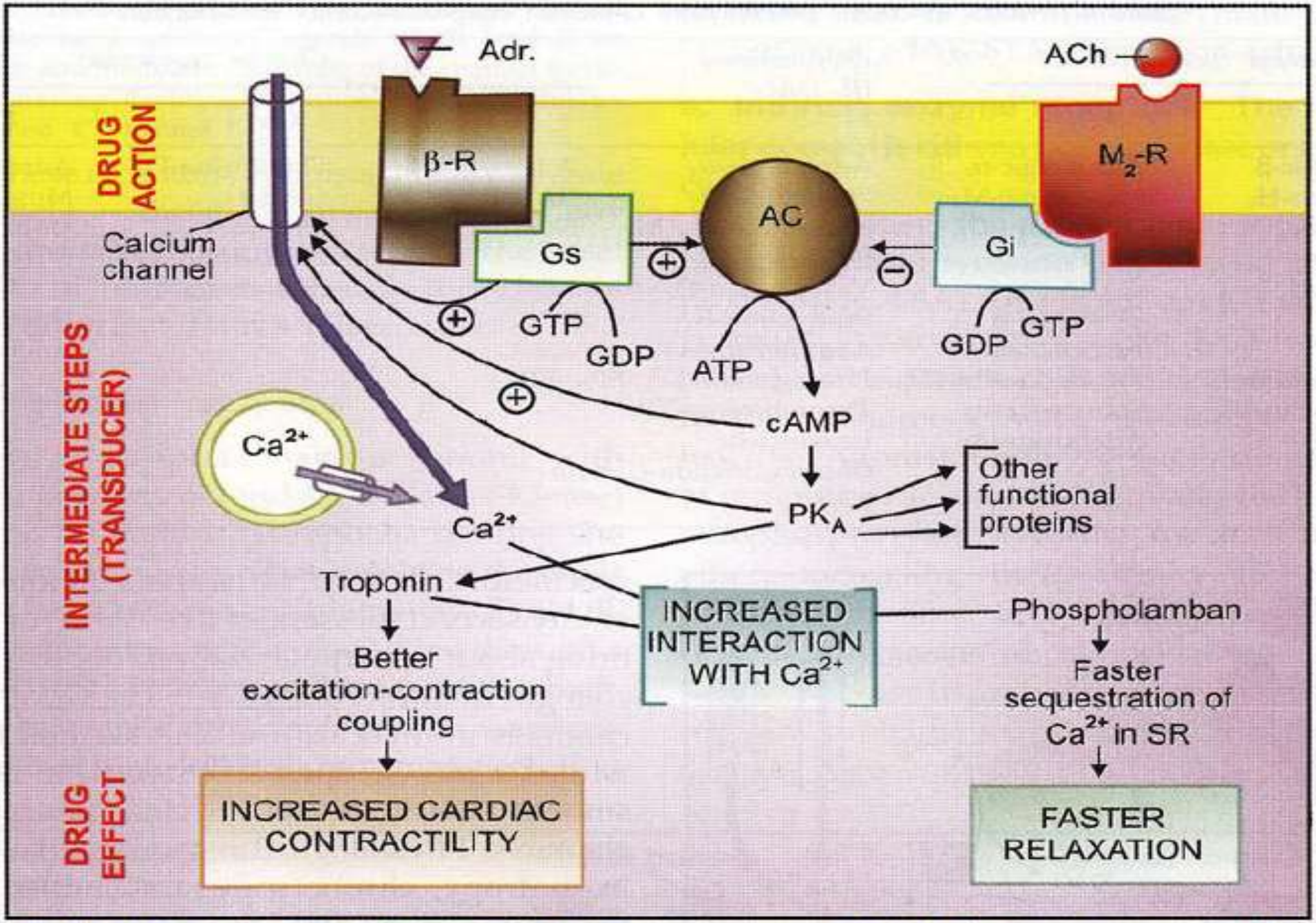


Fig. 4.6: The action-effect sequence of two G-protein coupled (β adrenergic and muscarinic M_2) receptor activation in myocardial cell

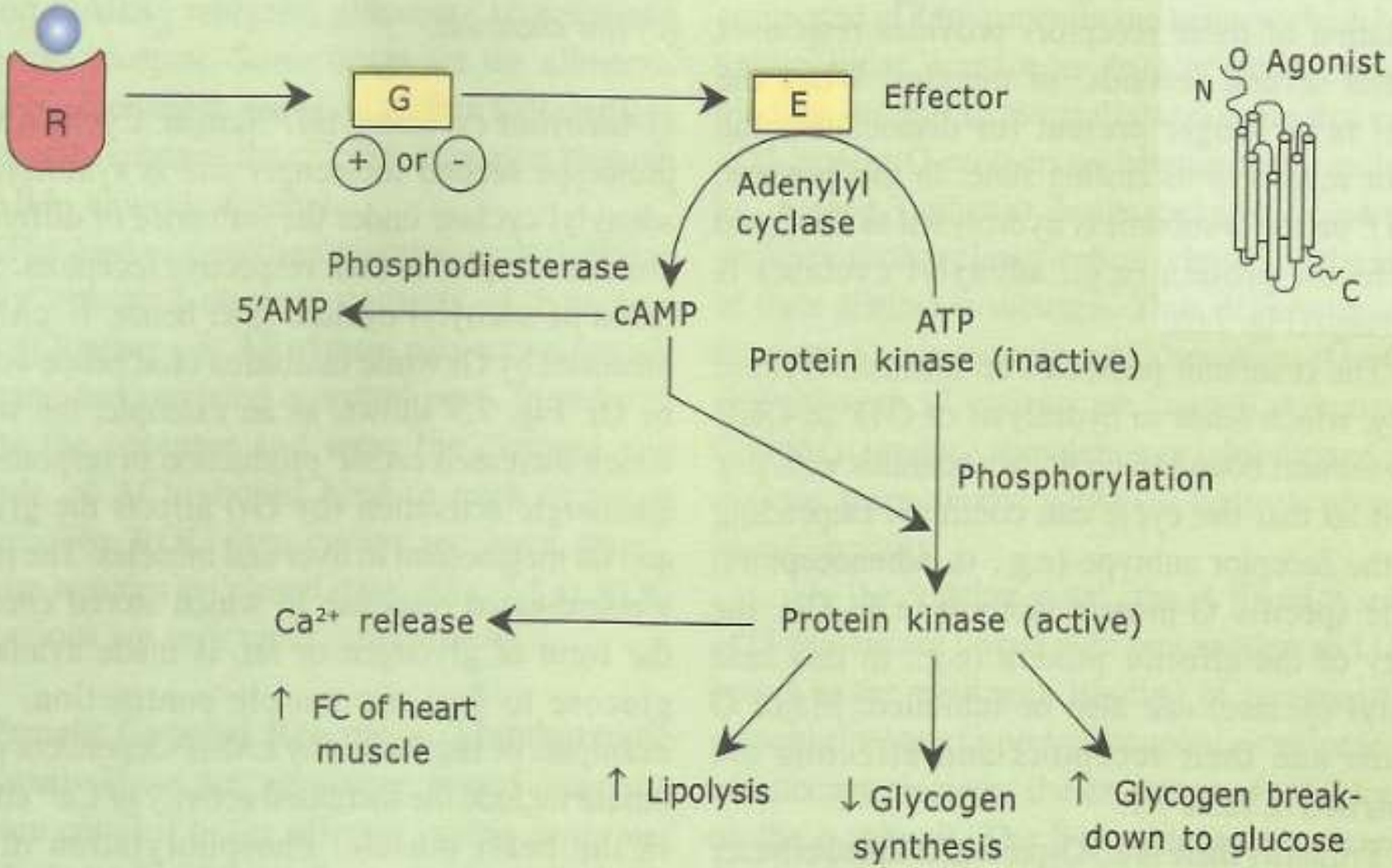


Fig 7.7 Regulation of Energy Metabolism and Contractility of the Heart by cAMP Dependent Protein Kinases. Inset: 7-transmembrane helical topology of G-protein coupled receptor (R). N terminal: Agonist binding domain; C terminal: G-protein coupling domain.

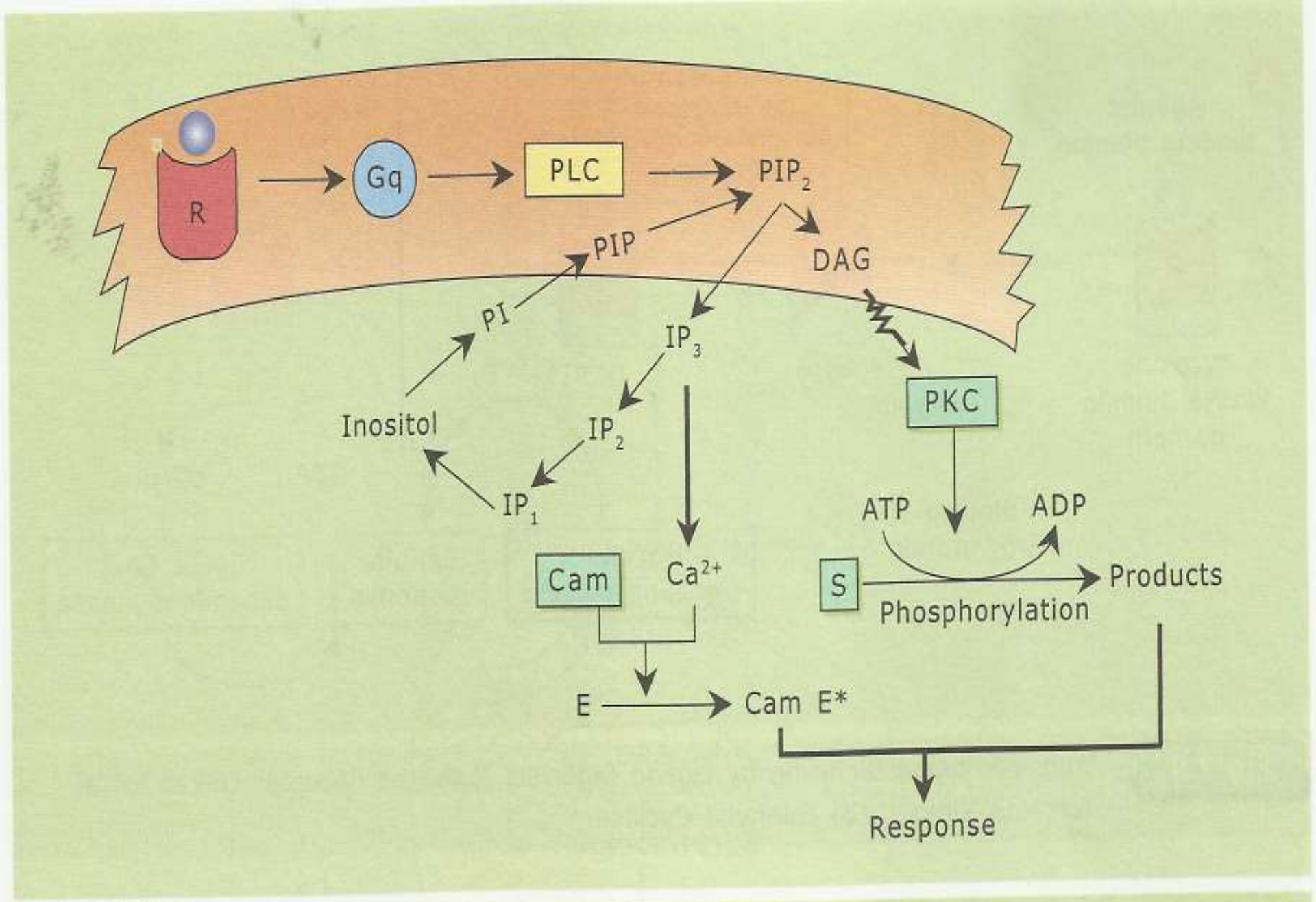


Fig 7.8 The Ca^{2+}-Phosphoinositide Signaling Pathway.

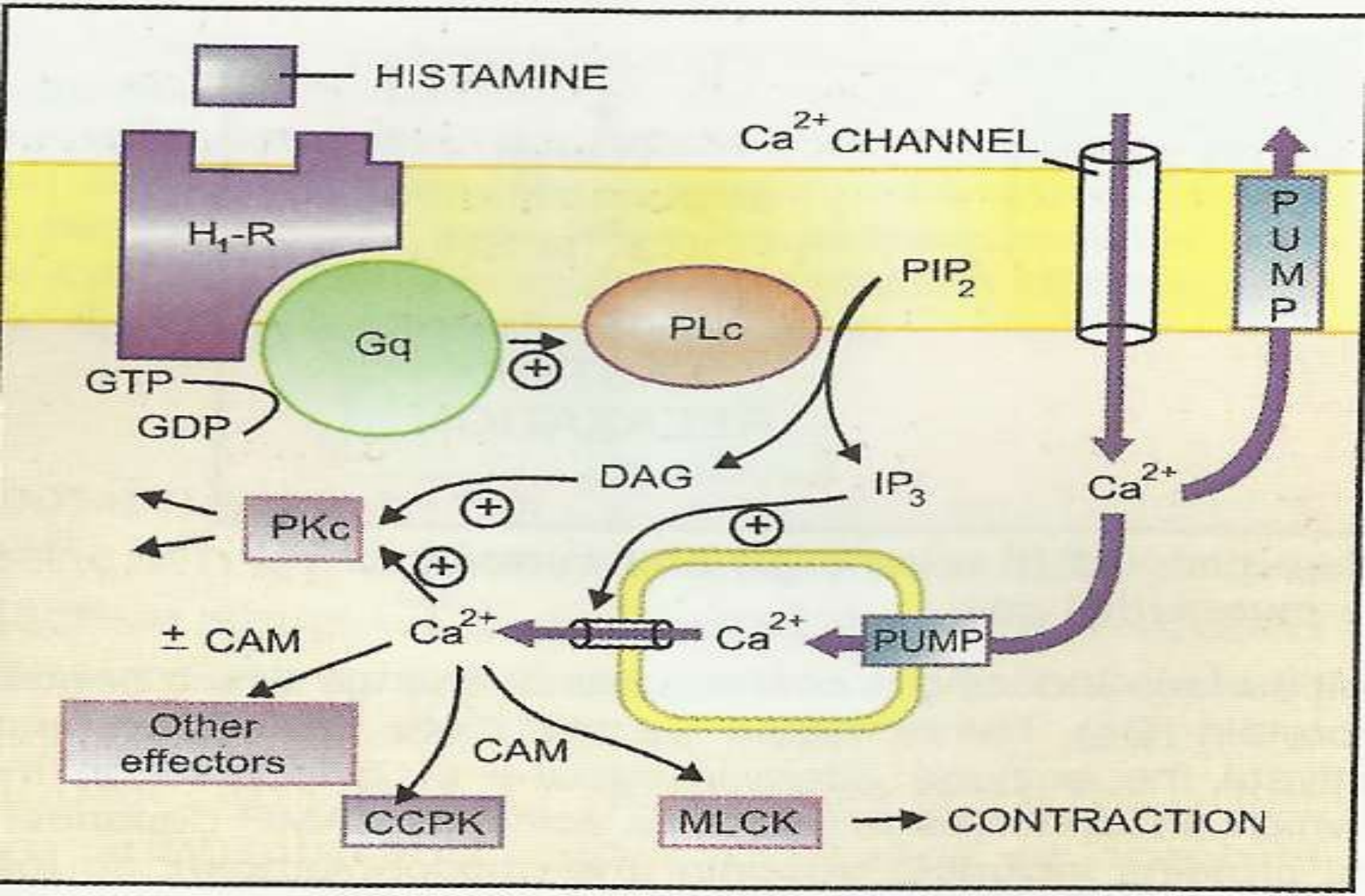


Fig. 4.7: The important steps of phospholipase C(PLc) pathway of response effectuation (in smooth muscle)

Table 4.1: Major functional pathways of G-protein coupled receptor transduction

Adenylyl cyclase: cAMP		*Phospholipase IP_3-DAG*	*Channel regulation*		
↑	↓		Ca^{2+}↑	Ca^{2+}↓	K^{+}↑
Adrenergic-β	Adrenergic-α_2	Adrenergic-α_1	Adrenergic-β_1	Dopamine-D2	Adrenergic-α_2
Histamine-H_2	Muscarinic-M_2	Histamine-H_1	(Heart,	$GABA_B$	Muscarinic-M_2
Dopamine-D1	Dopamine-D2	Muscarinic-M_1, M_3	Sk.muscle)	Opioid-κ	Dopamine-D2
Glucagon	5-HT_1	5-HT_2		Adenosine-A_1	5-HT_{1A}
FSH & LH	$GABA_B$	Vasopressin-Oxytocin		Somatostatin	$GABA_B$
ACTH	Opioid-μ, δ	Bradykinin-B_2			Opioid-μ, δ
TSH	Angiotensin-AT_1	Angiotensin-AT_1			Adenosine-A_1
Prostaglandin-EP_2	Prostaglandin-EP_3	Prostaglandin-FP, EP_1, EP_3			
Prostacyclin-IP	Somatostatin	Thromboxane-TP			
Adenosine-A_2	Adenosine-A_1	Leukotriene			
		Cholecystokinin-Gastrin			
		PAF			

RESENSITIZATION AND DOWN-REGULATION OF RECEPTOR

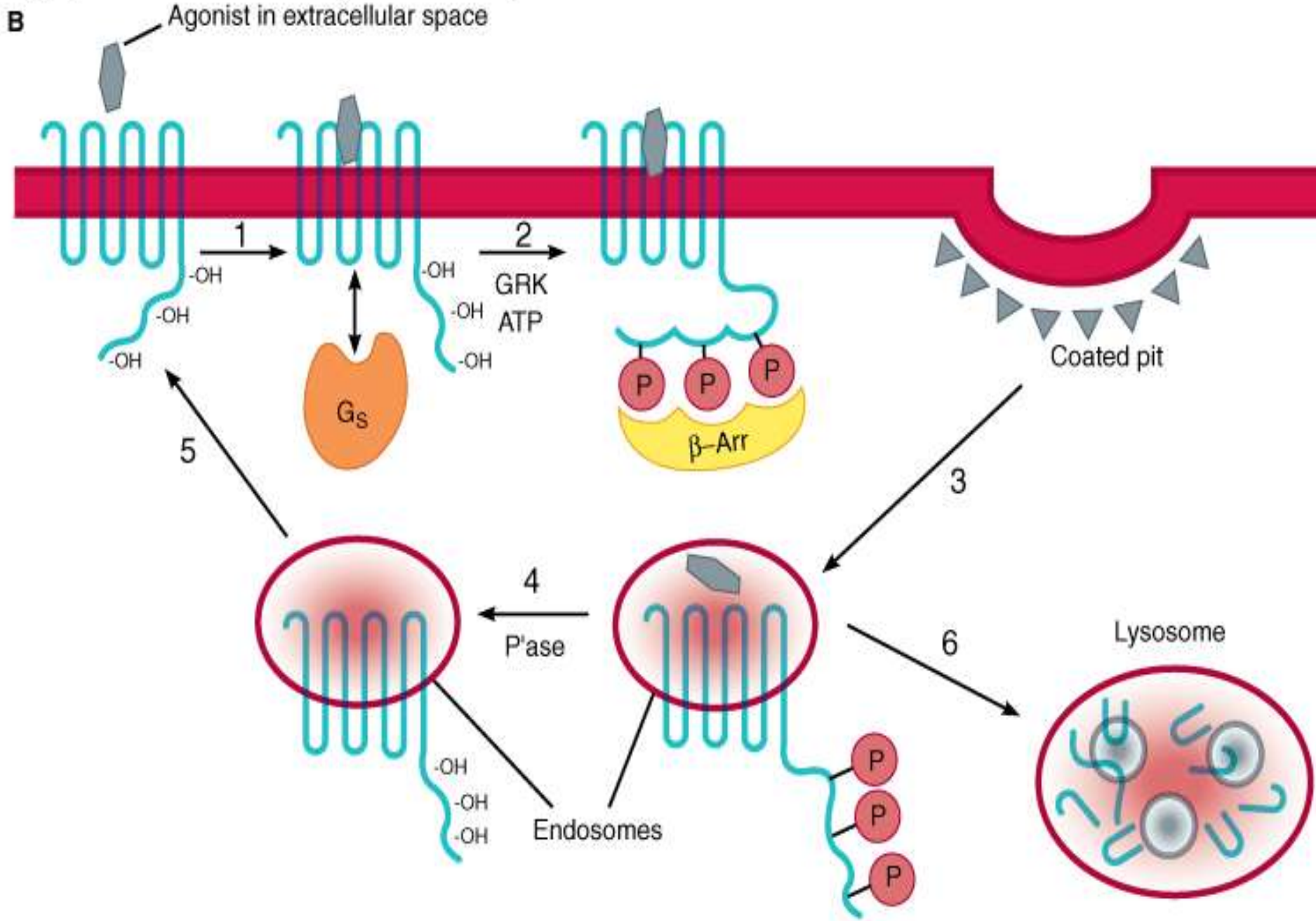
1. Agonist binding to receptors initiates signaling by promoting receptor interaction with G proteins (G_s) located in the cytoplasm.

2. Agonist-activated receptors are phosphorylated by a G protein-coupled receptor kinase (GRK), preventing receptor interaction with G_s and promoting binding of a different protein, - β arrestin (β-Arr), to the receptor.

3. The receptor- β arrestin complex binds to coated pits, promoting receptor internalization.

4. Dissociation of agonist from internalized receptors reduces - β Arr binding affinity, allowing dephosphorylation of receptors by a phosphatase.

5. Return of receptors to the plasma membrane result in the efficient resensitization of cellular responsiveness.
6. Repeated or prolonged exposure of cells to agonist favors the delivery of internalized receptors to lysosomes , promoting receptor down-regulation rather than resensitization.



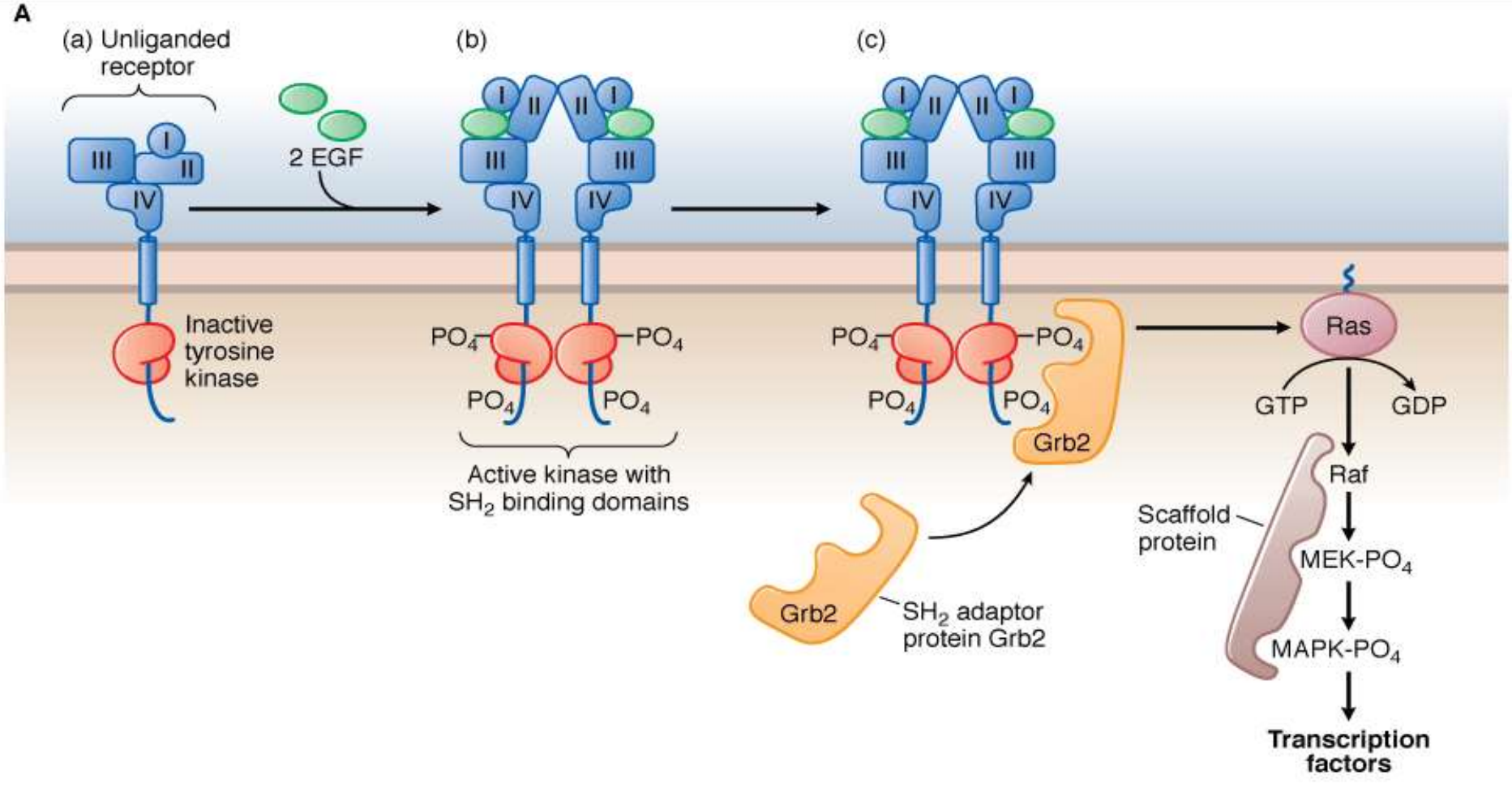
Source: Katzung BG, Masters SB, Trevor AJ: *Basic & Clinical Pharmacology, 11th Edition:* http://www.accessmedicine.com

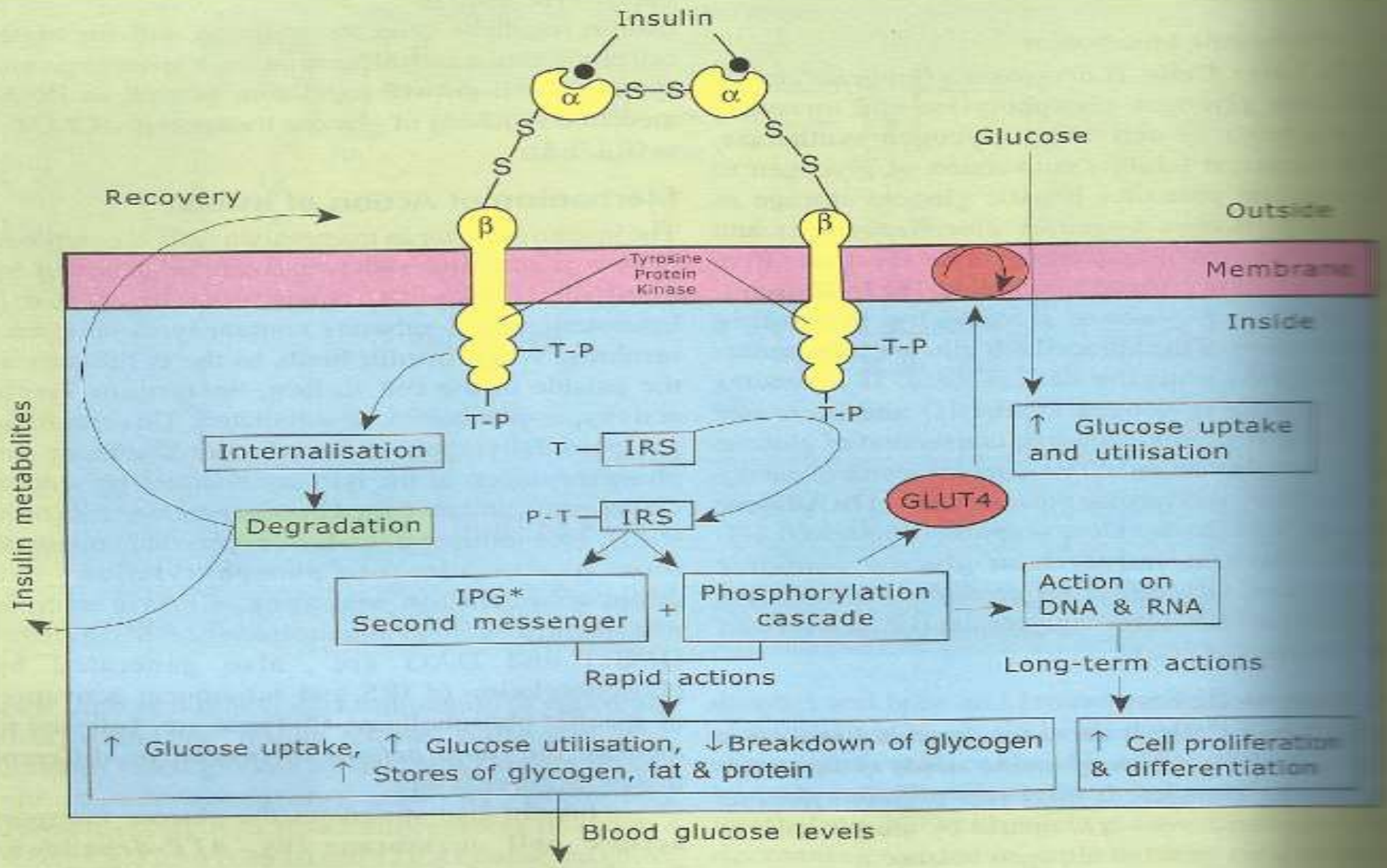
STRUCTURAL FAMILY	FUNCTIONAL FAMILY	PHYSIOLOGICAL LIGANDS	EFFECTORS AND TRANSDUCERS	EXAMPLE DRUGS
Transmembrane enzymes	Receptor tyrosine kinases	Insulin, PDGF, EGF, VEGF, growth factors	SH2 domain containing proteins	Herceptin, imatinib
	Membrane-bound GC Tyrosine phosphatases	Natriuretic peptides	Cyclic GMP	Neseritide

Enzyme linked receptors

- With intrinsic enzymatic activity.
- Without intrinsic enzymatic activity (but bind a JAK-STAT kinase on activation

receptor tyrosine kinase and a cytokine receptor. Activation of the EGF receptor. The extracellular structure of the unliganded receptor (a) contains four domains (I-IV), which rearrange significantly upon binding two EGF molecules. (b). The conformational changes lead to activation of the cytoplasmic tyrosine kinase domains and tyrosine phosphorylation of intracellular regions to form SH2 binding sites. (c). The adapter molecule Grb2 binds to the phosphoryated tyrosine residues and activates the Ras-MAP kinsase cascade





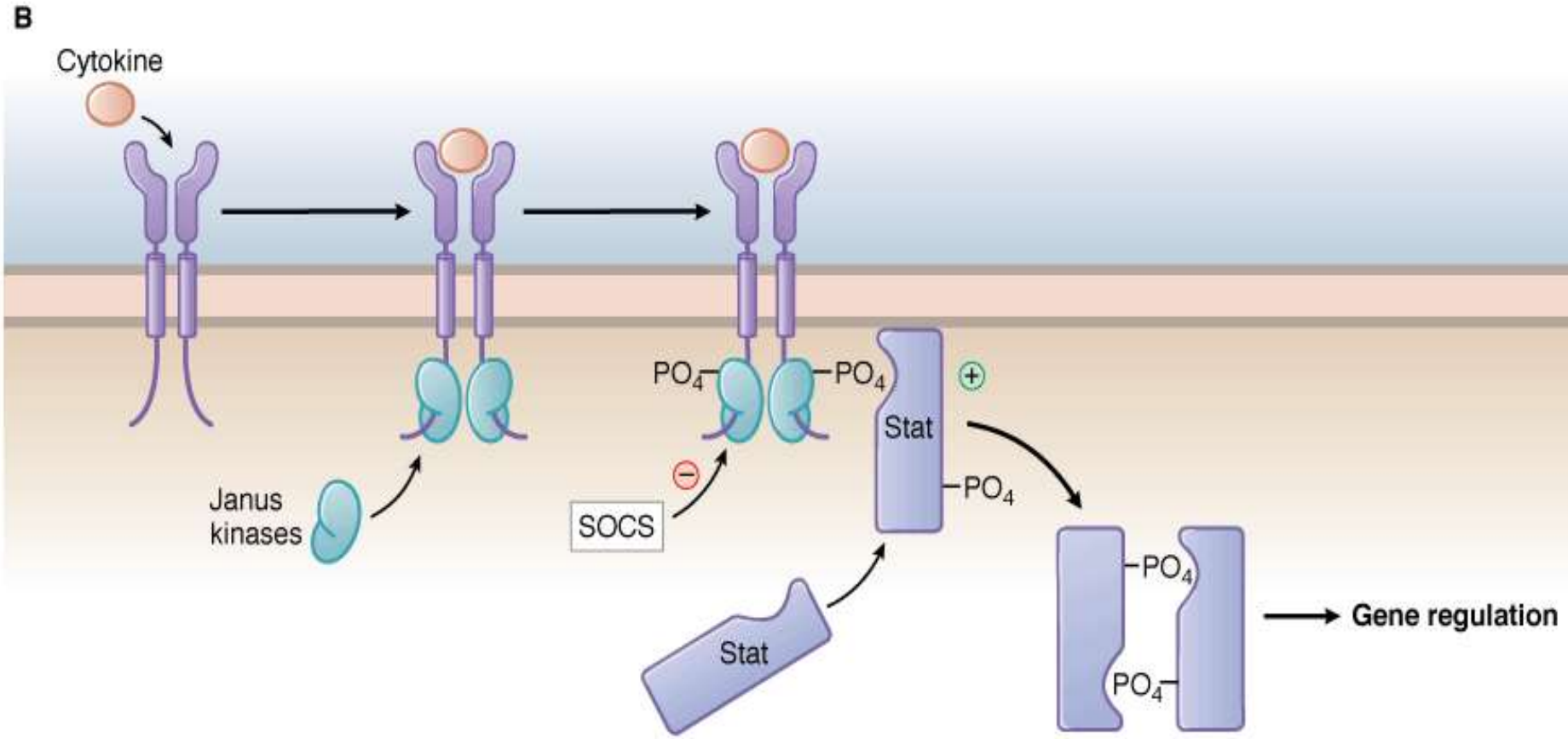
GLUT-4—Glucose transporter-4; IRS—Insulin receptor substrate; T—Tyrosine; P—Phosphate
*Inositolphosphoglycans (IPGs) are released outside of the cell but then transported back into the cell

Fig 47.3 Insulin Signaling Pathways

STRUCTURAL FAMILY	FUNCTIONAL FAMILY	PHYSIOLOGICAL LIGANDS	EFFECTORS AND TRANSDUCERS	EXAMPLE DRUGS
Transmembrane, non-enzymes	Cytokine receptors	Interleukins and other cytokines	Jak/STAT, soluble tyrosine kinases	

JAKs- STATS PATHWAY

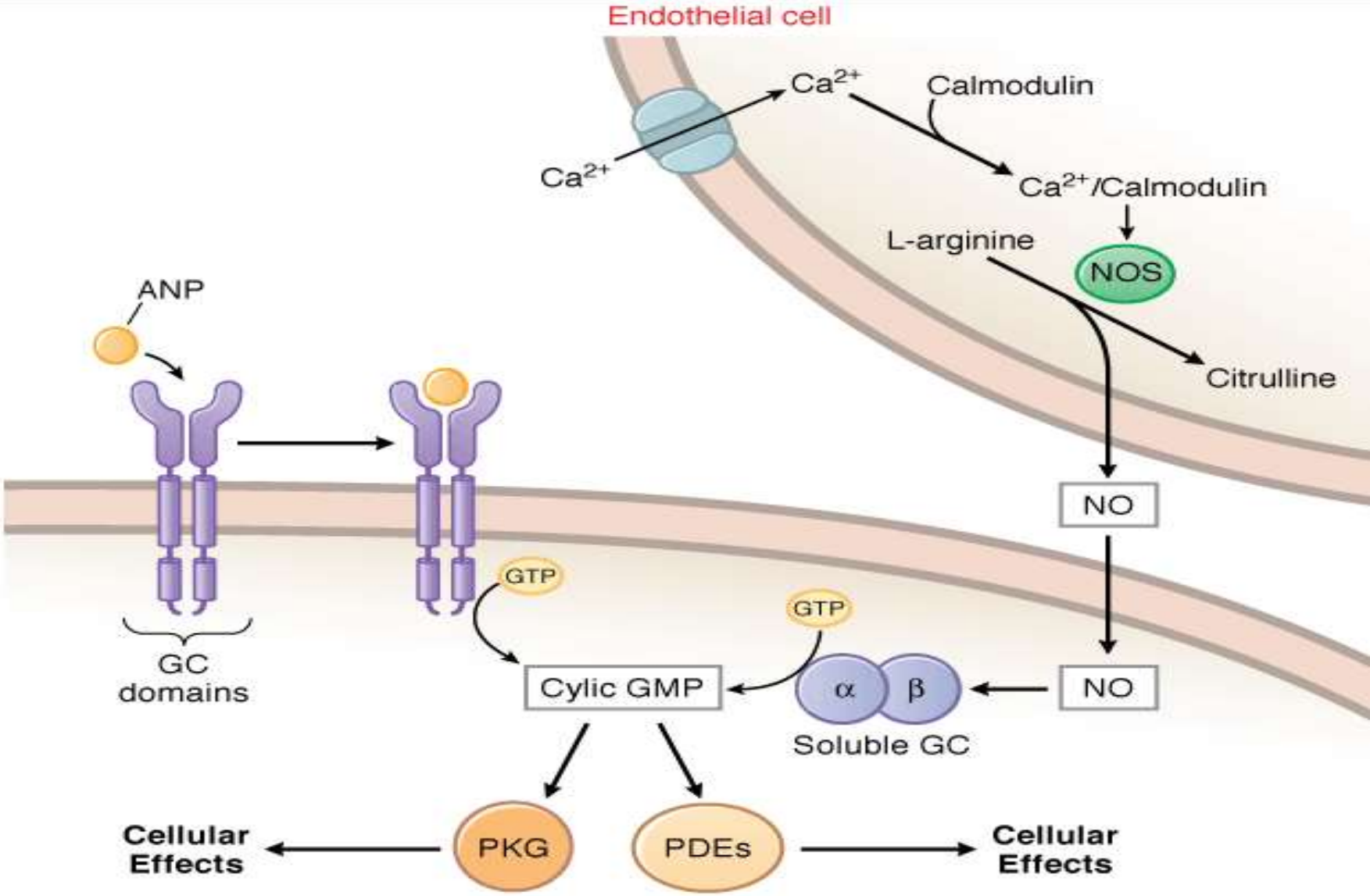
Binding of the cytokine causes dimerization of the receptor and recruits the Janus Kinases (JAKs) to the cytoplasmic tails of the receptor. JAKs trans-phosphorylate and lead to the phosphorylation of the signal transducers and activators of transcription (STATs). The phosphorylated STATS translocate to the nucleus and regulate transcription. There are proteins termed suppressors of cytokine signaling (SOCS) that inhibit the JAK-STAT pathway



Source: Brunton LL, Chabner BA, Knollmann BC: *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Edition:* www.accessmedicine.com

STRUCTURAL FAMILY	FUNCTIONAL FAMILY	PHYSIOLOGICAL LIGANDS	EFFECTORS AND TRANSDUCERS	EXAMPLE DRUGS
Intracellular enzymes	Soluble GC	NO, Ca^{2+}	Cyclic GMP	Nitrovasodilators

NATRIURETIC PEPTIDE RECEPTORS



Source: Brunton LL, Chabner BA, Knollmann BC: *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Edition:* www.accessmedicine.com

NO SYNTHASE AND SOLUBLE GUANYLATE CYCLASE

The cellular effects of cyclic GMP on the vascular system are mediated by a number of mechanisms, but especially by PKG. For example, in vascular smooth muscle, activation of PKG leads to vasodilation by:

- Inhibiting IP_3-mediated Ca^{2+} release from intracellular stores.
- Phosphorylating voltage-gated Ca^{2+} channels to inhibit Ca^{2+} influx.
- Phosphorylating phospholamban, a modulator of the sarcoplasmic Ca^{2+} pump, leading to a more rapid reuptake of Ca^{2+} into intracellular stores.
- Phosphorylating and opening the Ca^{2+}-activated K^+ channel leading to hyperpolarization of the cell membrane, which closes L-type Ca^{2+} channels and reduces the flux of Ca^{2+} into the cell.

STRUCTURAL FAMILY	FUNCTIONAL FAMILY	PHYSIOLOGICAL LIGANDS	EFFECTORS AND TRANSDUCERS	EXAMPLE DRUGS
Nuclear receptors	Steroid receptors	Estrogen, testosterone	Co-activators	Estrogens, androgens, cortisol
	Thyroid hormone receptors	Thyroid hormone		Thyroid hormone

Receptors regulating gene expression

- Several biologic ligands are sufficiently lipid-soluble to cross the plasma membrane and act on intracellular receptors.

  e.g.

  steroids (corticosteroids, mineralocorticoids, sex steroids, vitamin D), and thyroid hormone, whose receptors stimulate the transcription of genes by binding to specific DNA sequences near the gene whose expression is to be regulated.

- Target DNA sequences called **response elements.**

- In the absence of hormone, the receptor is bound to **hsp90**, a protein that appears to prevent normal folding of several structural domains of the receptor.

- Binding of hormone to the ligand-binding domain triggers release of hsp90.

- This allows the DNA-binding and transcription-activating domains of the receptor to fold into their functionally active conformations, so that the activated receptor can initiate transcription of target genes.

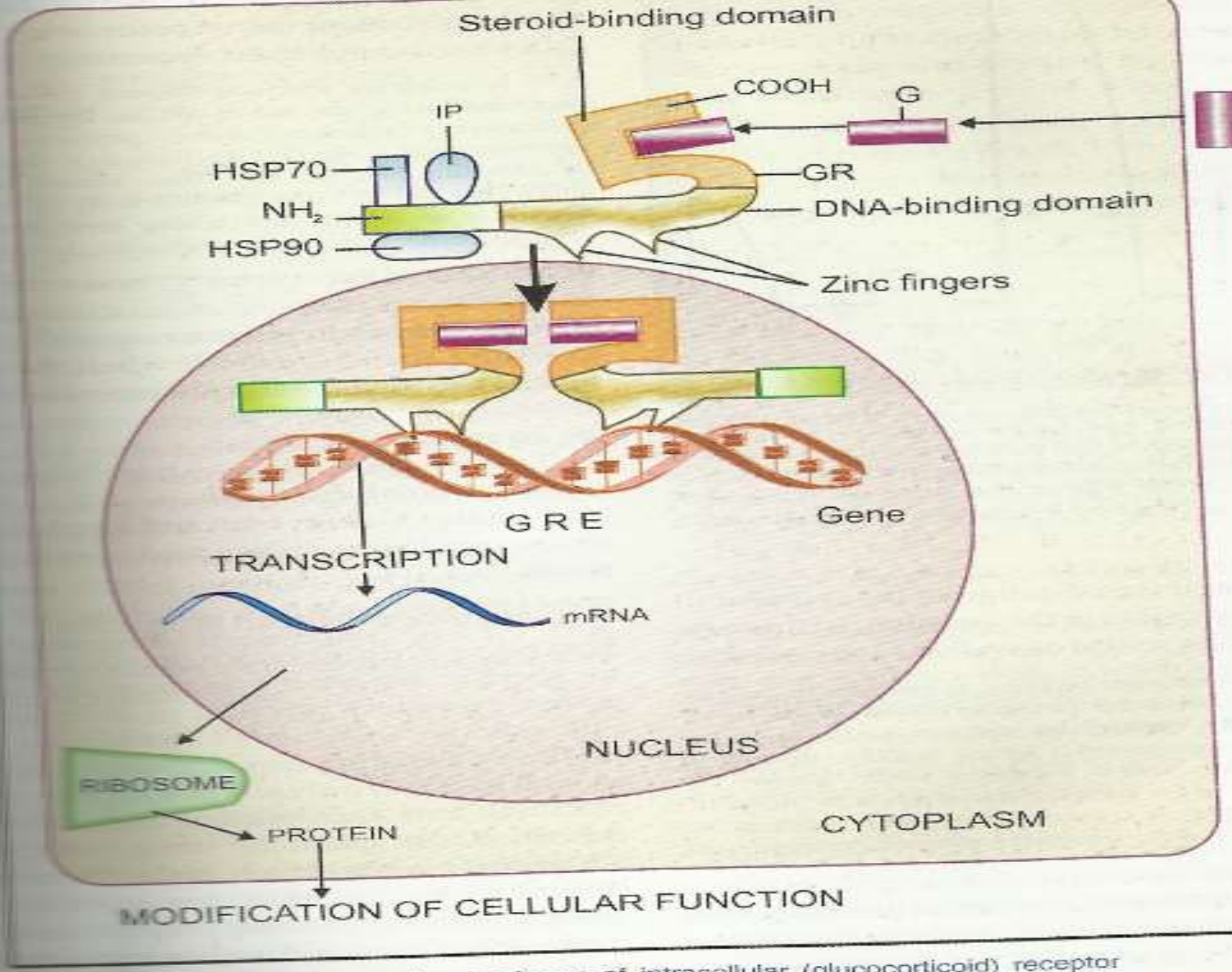
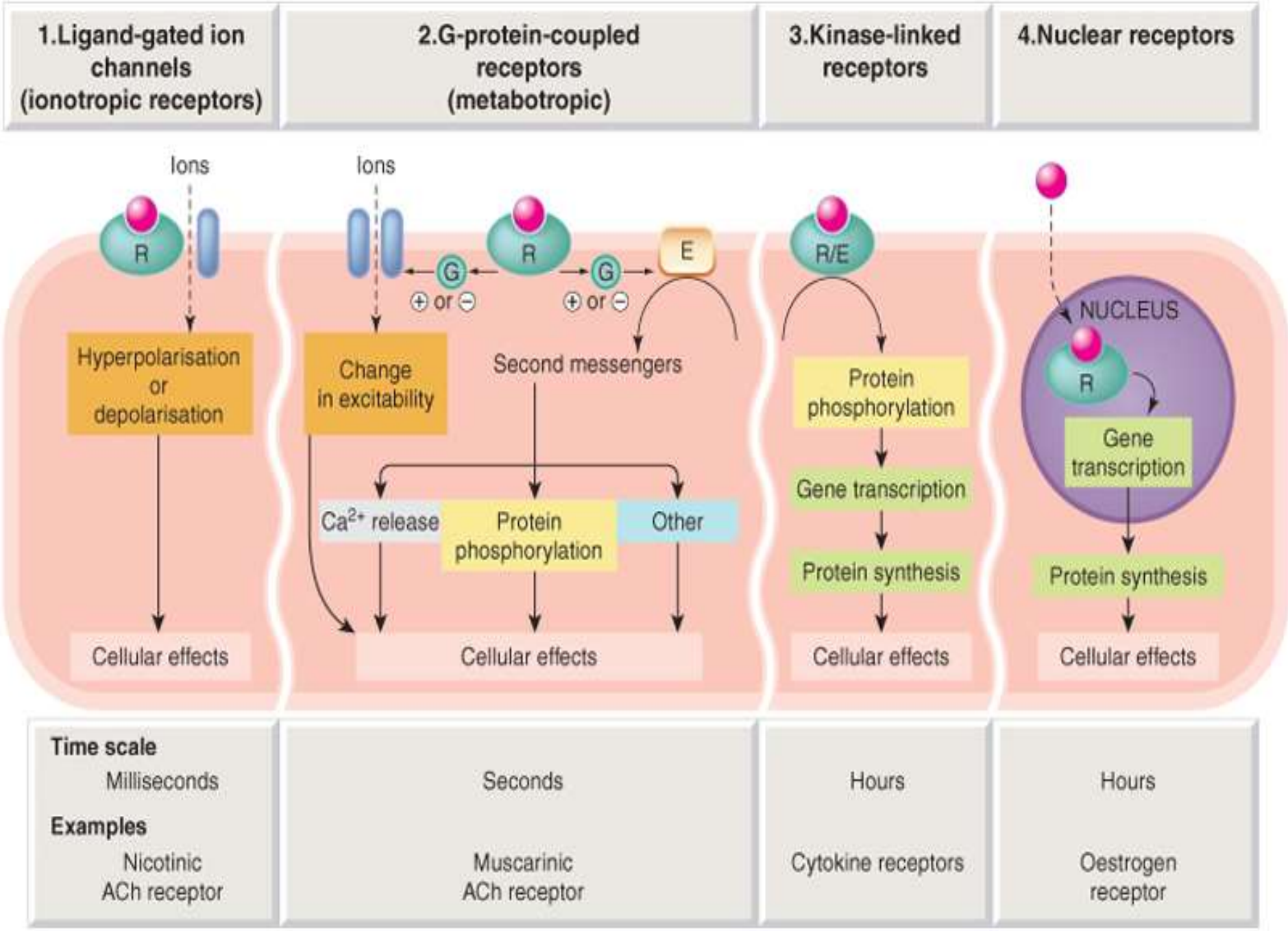
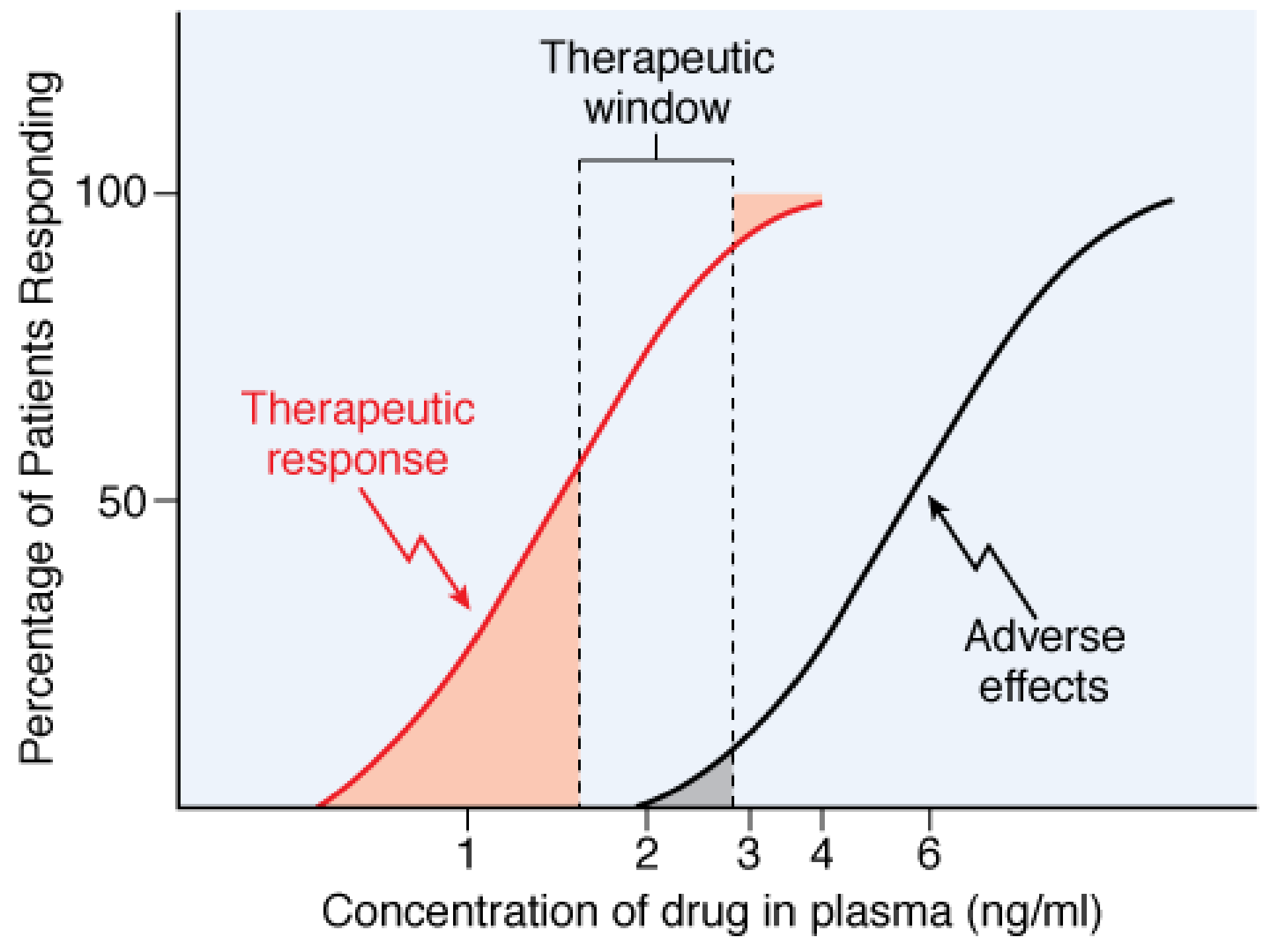


Fig. 4.9: Operational scheme of intracellular (glucocorticoid) receptor


1.Ligand-gated ion channels (ionotropic receptors)
2.G-protein-coupled receptors (metabotropic)
3.Kinase-linked receptors
4.Nuclear receptors
Ions
R
Hyperpolarisation or depolarisation
Cellular effects
Ions
R
G
G
E
⊕ or ⊖
⊕ or ⊖
Change in excitability
Second messengers
Ca^{2+} release
Protein phosphorylation
Other
Cellular effects
R/E
Protein phosphorylation
Gene transcription
Protein synthesis
Cellular effects
NUCLEUS
R
Gene transcription
Protein synthesis
Cellular effects
Time scale
Milliseconds
Seconds
Hours
Hours
Examples
Nicotinic ACh receptor
Muscarinic ACh receptor
Cytokine receptors
Oestrogen receptor

Therapeutic window

- Therapeutic effect is seen only with in a narrow range of plasma conc.
- Both above & below this range, beneficial effect is suboptimal.
- Drugs having low therapeutic window
  - Theophylline
  - Carbamazepine
  - Digoxin
  - Lithium
  - Phenytoin


Therapeutic window
Therapeutic response
Adverse effects
Percentage of Patients Responding
100
50
1
2
3
4
6
Concentration of drug in plasma (ng/ml)

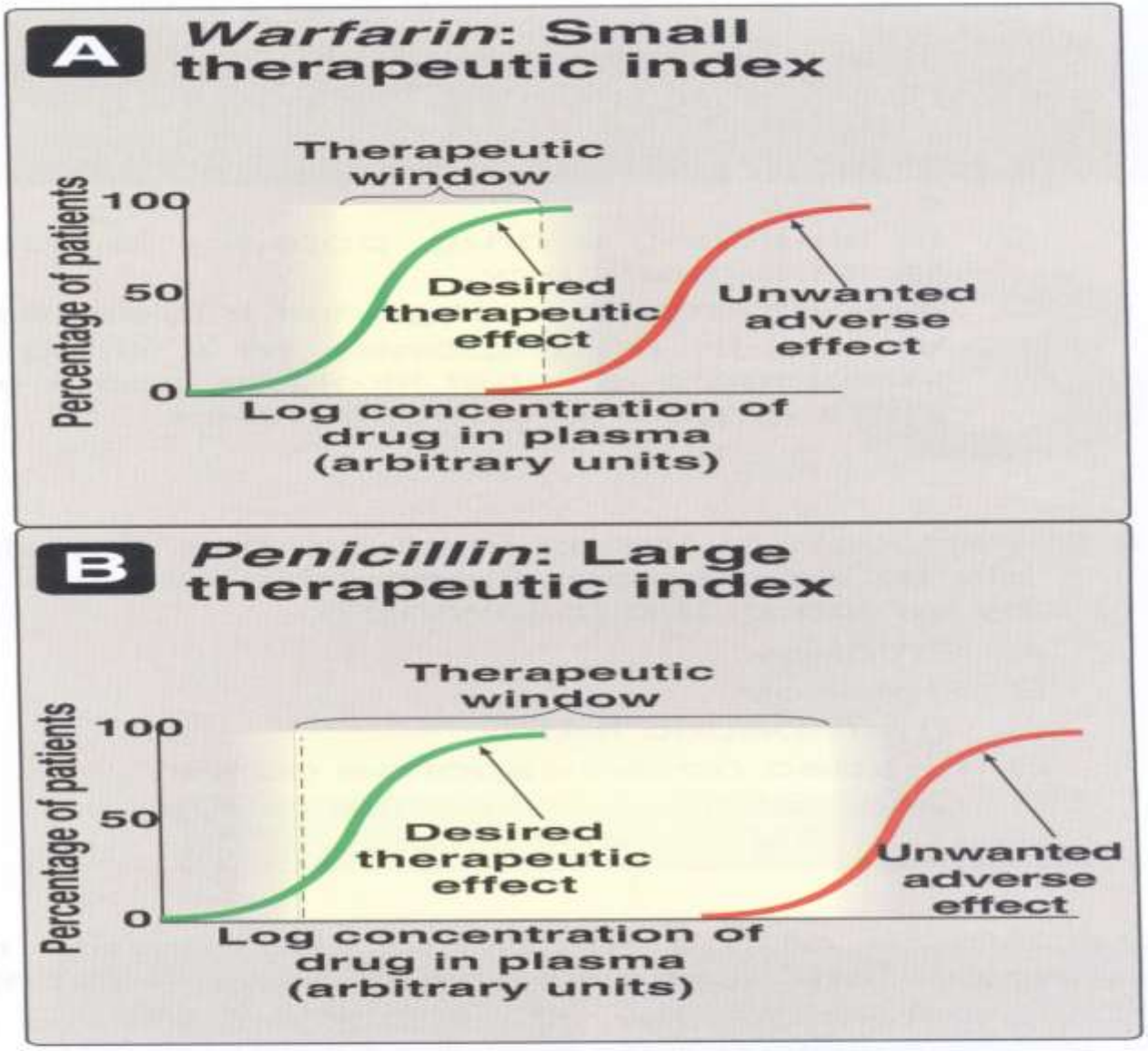


Figure 2.11
Cumulative percentage of patients responding to plasma levels of a drug.

Therapeutic Index

- **Is the ratio of the LD50 to ED50**
- **Represent an estimate of the safety of a drug.**

Importance of graded dose-response curves

1.Calculation of the ED50 (The dose that produces 50% of the maximum response in one animal

- Comparing ED50 of different drugs on the same animal gives an idea about the equieffective doses i.e the doses that produce the same effect.
- Comparing ED50 of the same drug in different patients gives an idea about the relative sensitivity of the patients to the same drug i.e the degree of biological variation inherent in a population.

2. Calculation of the maximum response

- Comparing the maximum response obtained by different drugs on the same organ gives an idea about the intrinsic activity.

3. Determination of the stepiness of the dose response

- Any small change in the drug concentration produces significant increase in the tissue response. This means that maximum response to the drug could be reached very fast and the toxicity could be reached very fast too.

SYNERGISM

(Greek: Syn-together; ergon-work)

- When the action of one drug is facilitated or increased by the other, they are said to be synergistic.

- In a synergistic pair, both the drugs can have action in the same direction or given alone one may be inactive but still enhance the action of the other when given together.

Additive

The effect of the two drugs is in the same direction and simply adds up:

effect of drugs A + B = effect of drug A + effect of drug B

Additive drug combinations	
Aspirin + paracetamol	as analgesic/ antipyretic
Nitrous oxide + halothane	as general anaesthetic
Amlodipine + atenolol	asantihypertensive
Glibenclamide + metformin	as hypoglycaemic
Ephedrine + theophylline	as bronchodilator

Supraadditive (potentiation)

The effect of combination is greater than the individual effects of the components:

effect of drug A+ B > effect of drug A+ effect of drug B

This is always the case when one component is inactive as such.

Supraadditive drug combinations	
Drug pair	*Basis of potentiation*
Acetylcholine + physostigmine	Inhibition of break down
Levodopa + carbidopa/ benserazide	Inhibition of peri-pheral metabolism
Adrenaline + cocaine/ desipramine	Inhibition of neuronal uptake
Sulfamethoxazole + trimethoprim	Sequential blockade
Antihypertensives (enalapril+ hydrochlorothiazide)	Tackling two contributory factors
Tyramine + MAO inhibitors	Increasing release-able CA store

Antagonism

Competitive antagonist

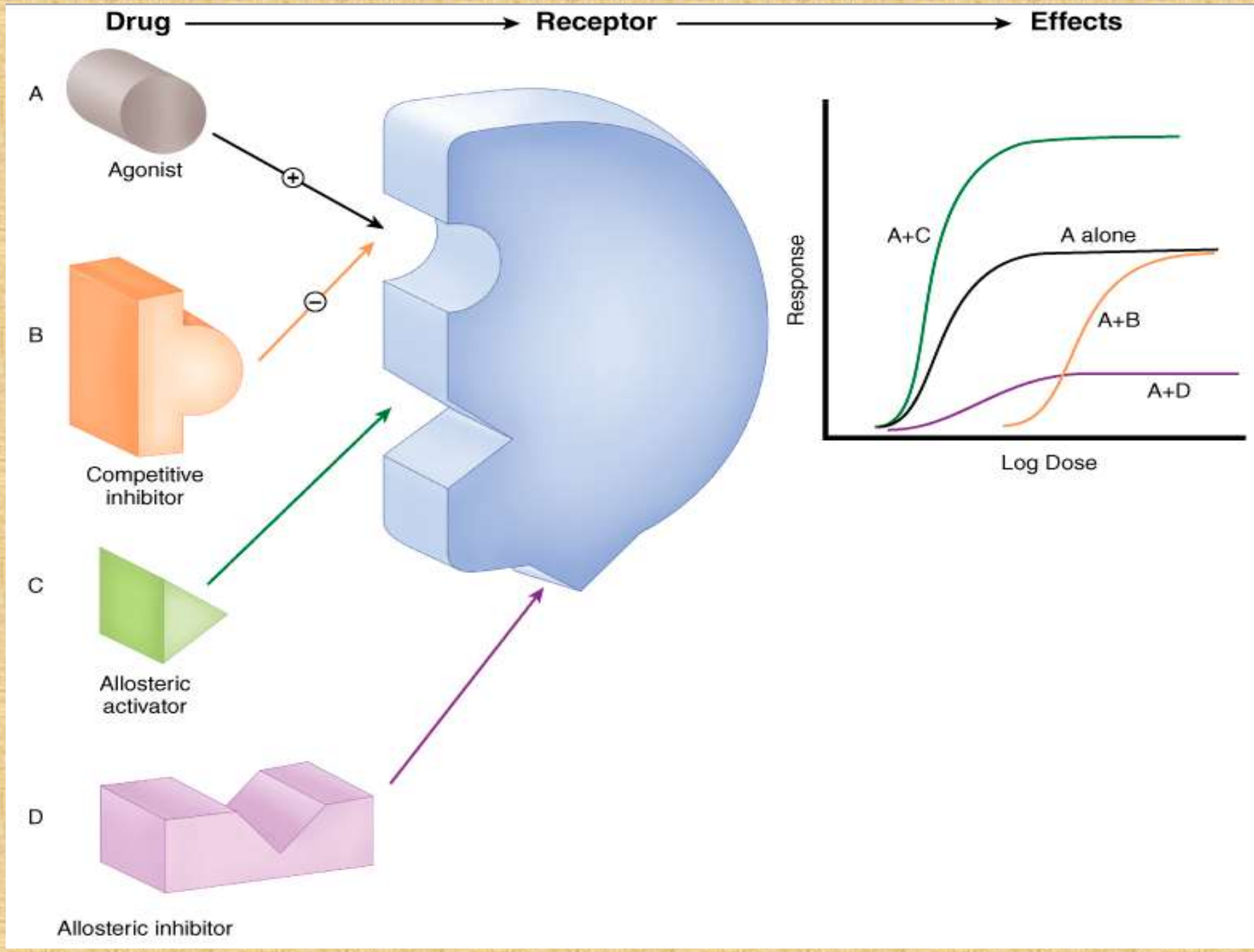
- Same binding site as of agonist
- resembles chemically with agonist
- Right shift of DRC
- Surmountable antagonism by increasing agonist dose
- Inactivation of certain agonist molecules
- Response depends on concentration of both

e.g Ach – Atropine, Morphine - Naloxone

Non-Competitive antagonist

- Different binding site as of agonist.
- Not resembles chemically with agonist.
- Flattening/downward shift of DRC.
- Unsurmountable antagonism (Maximum response is suppressed).
- Inactivation of certain receptors.
- Maximum response depends on concentration of antagonist

  e.g. Diazepam - Bicuculline


Drug
Receptor
Effects
A
Agonist
B
Competitive inhibitor
C
Allosteric activator
D
Allosteric inhibitor
Response
A+C
A alone
A+B
A+D
Log Dose

Body size

It influences the concentration of the drug attained at the site of action. The average adult dose refers to individuals of medium built. For exceptionally obese or lean individuals and for children dose may be calculated on body weight (BW) basis:

$$\text{Individual dose} = \frac{\text{BW (kg)}}{70} \times \text{average adult dose}$$

Age

The dose of a drug for children is often calculated from the adult dose

$$\text{Child dose} = \frac{\text{Age}}{\text{Age} + 12} \times \text{adult dose} \quad \text{... (Young's formula)}$$

$$\text{Child dose} = \frac{\text{Age}}{20} \times \text{adult dose} \quad \text{...(Dilling's formula)}$$

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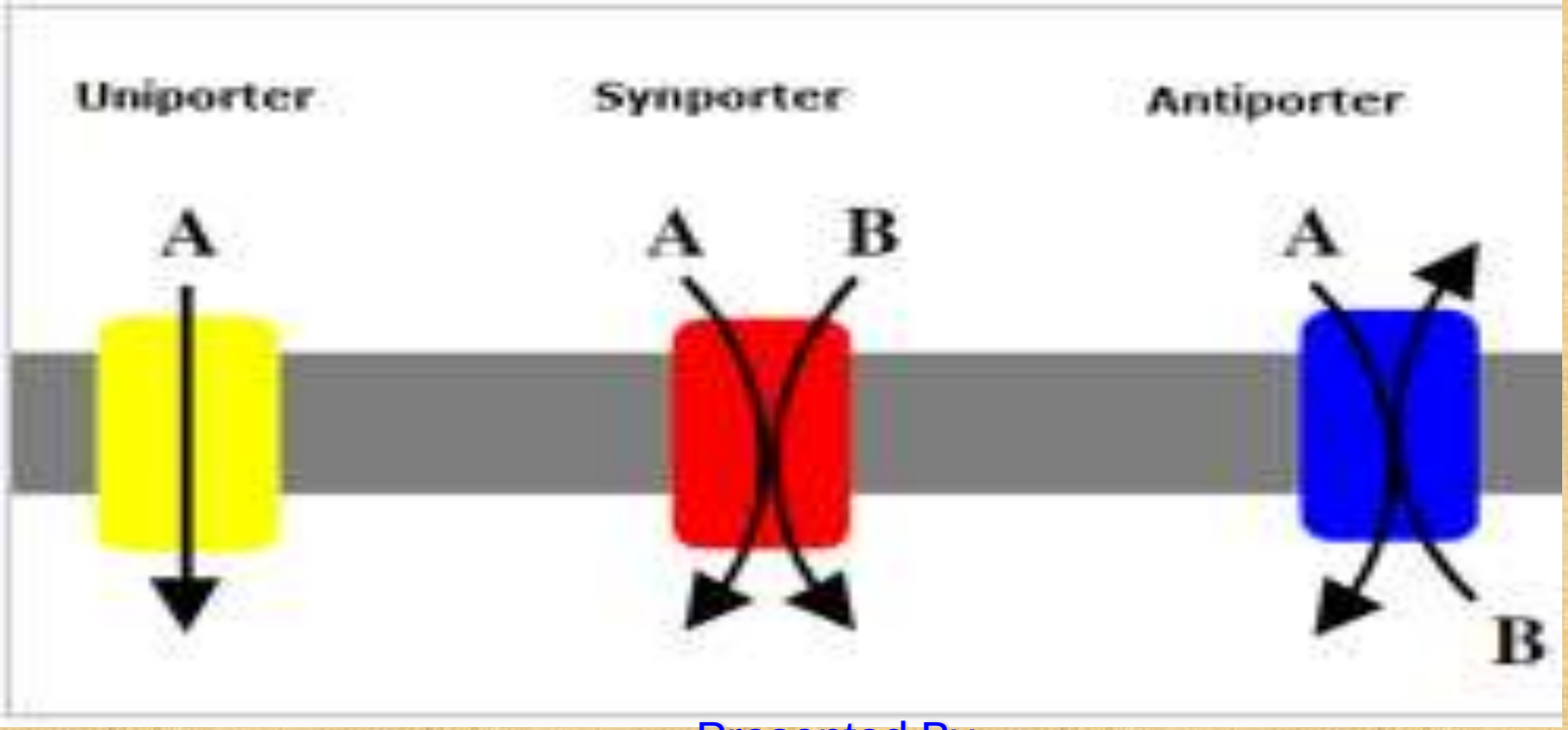
- Principles of Pharmacology 2nd edition by HL Sharma and KK Sharma
- Review of Pharmacology by Gobind Sparsh

PHARMACOKINETICS



Presented By
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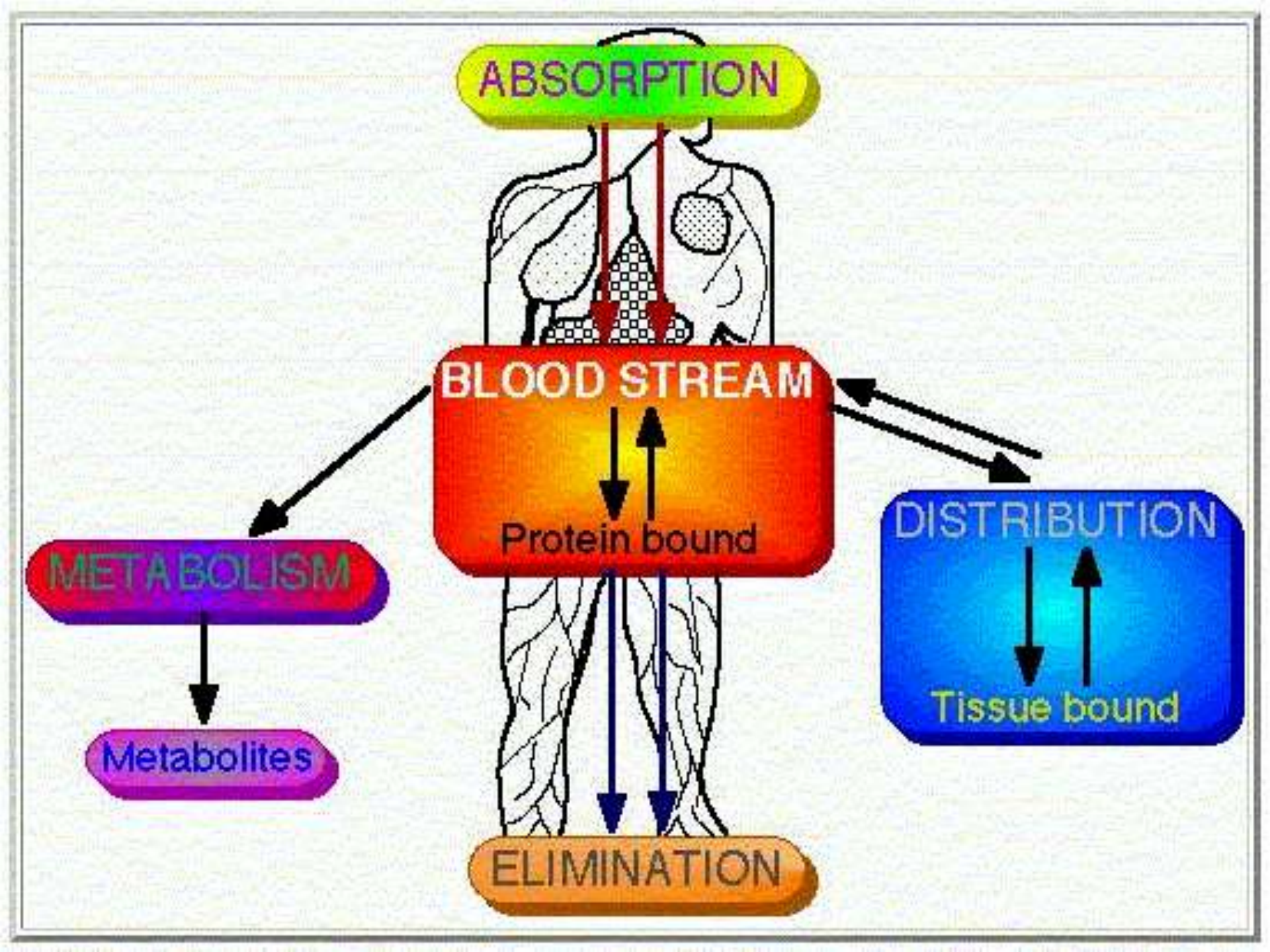
HOD , Department of Pharmacology
PDEA's Shankarrao Ursal College of Pharmaceutical Sciences & Research Center, Kharadi.

Pharmacokinetics of drugs (ADME)

Are studies of

- **A**bsorption
- **D**istribution
- **M**etabolism
- **E**xcretion of drugs


ABSORPTION
BLOOD STREAM
Protein bound
METABOLISM
Metabolites
DISTRIBUTION
Tissue bound
ELIMINATION

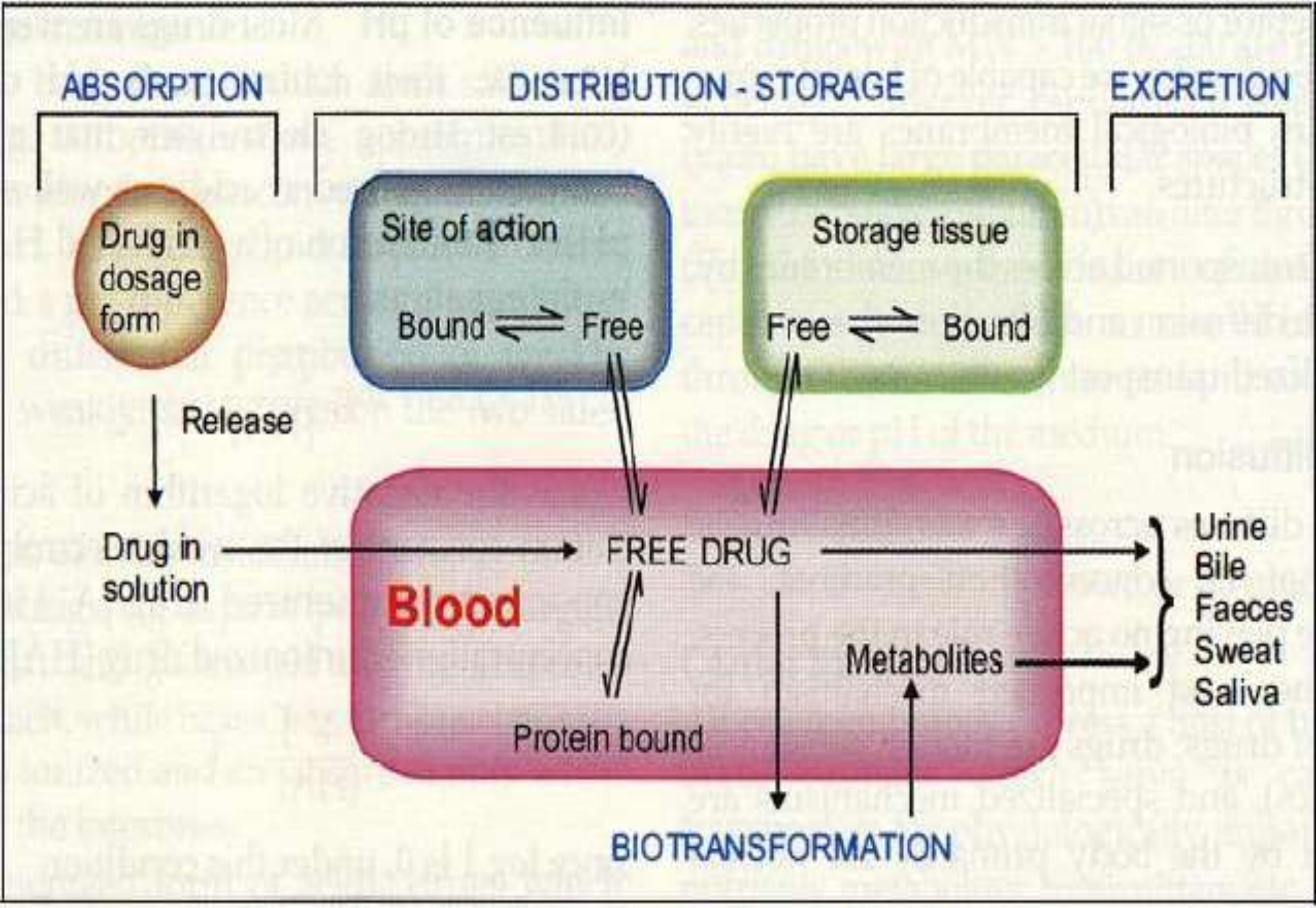


Fig. 2.1: Schematic depiction of pharmacokinetic processes

Importance of PK studies

- **Patients may suffer:**
  - **Toxic drugs may accumulate**
  - **Useful drugs may have no benefit because doses are too small to establish therapy**
  - **A drug can be rapidly metabolized.**

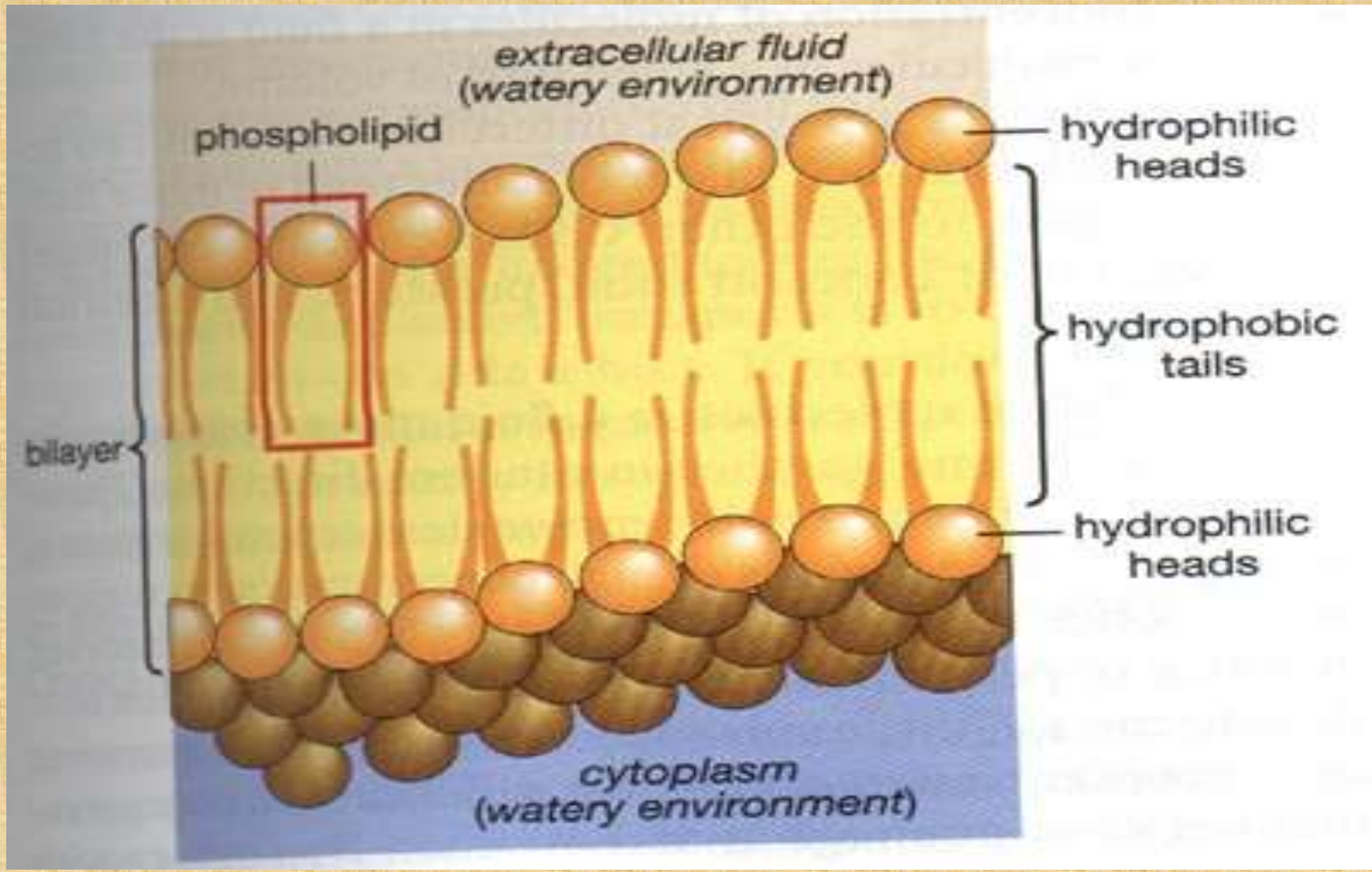
DRUG ABSORPTION

Is the passage of drug through cell membranes to reach its site of action.

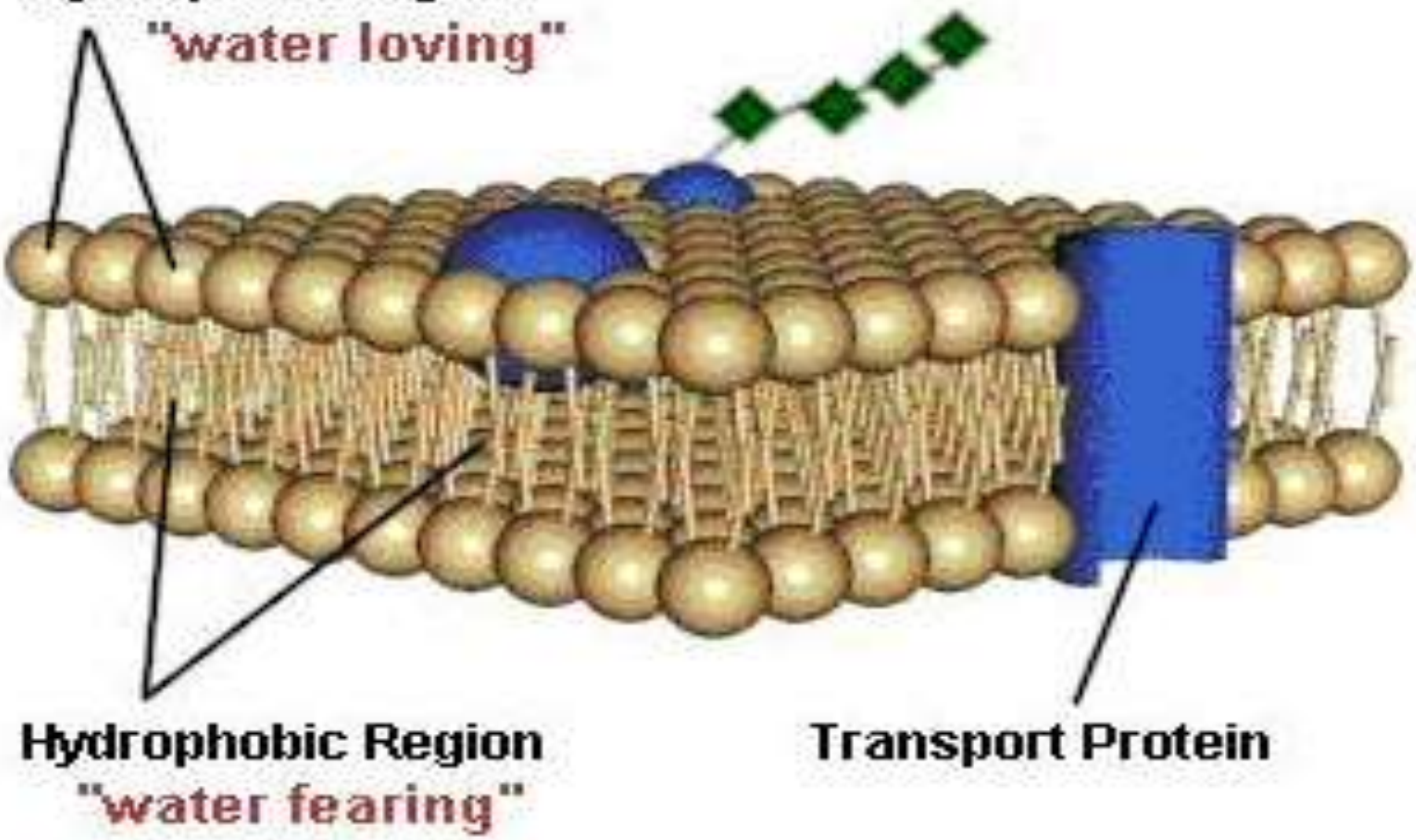
Mechanisms of drug absorption

1. **Simple diffusion = passive diffusion.**
2. **Active transport.**
3. **Facilitated diffusion.**
4. **Pinocytosis (Endocytosis).**

Cell membrane



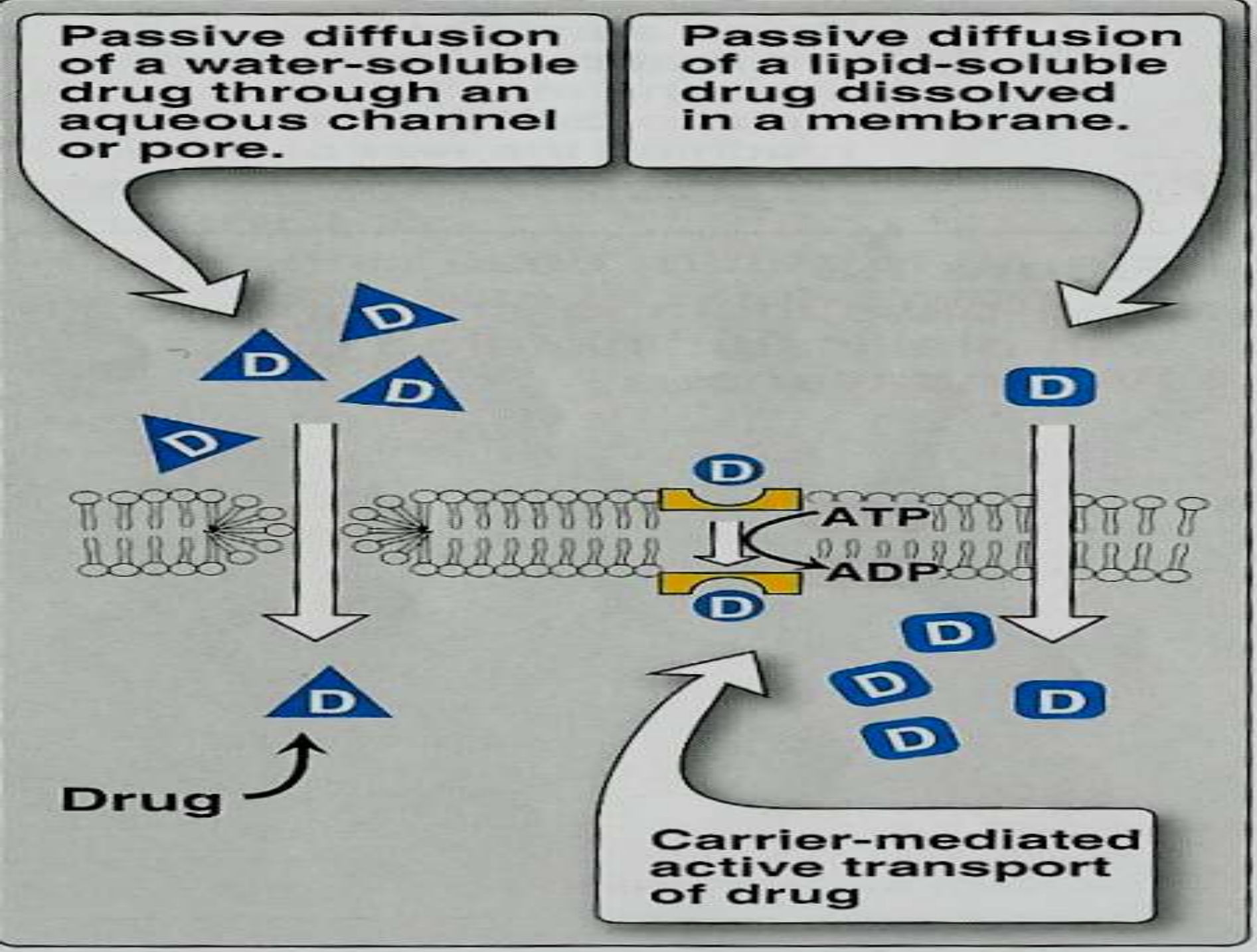
Cell Membrane

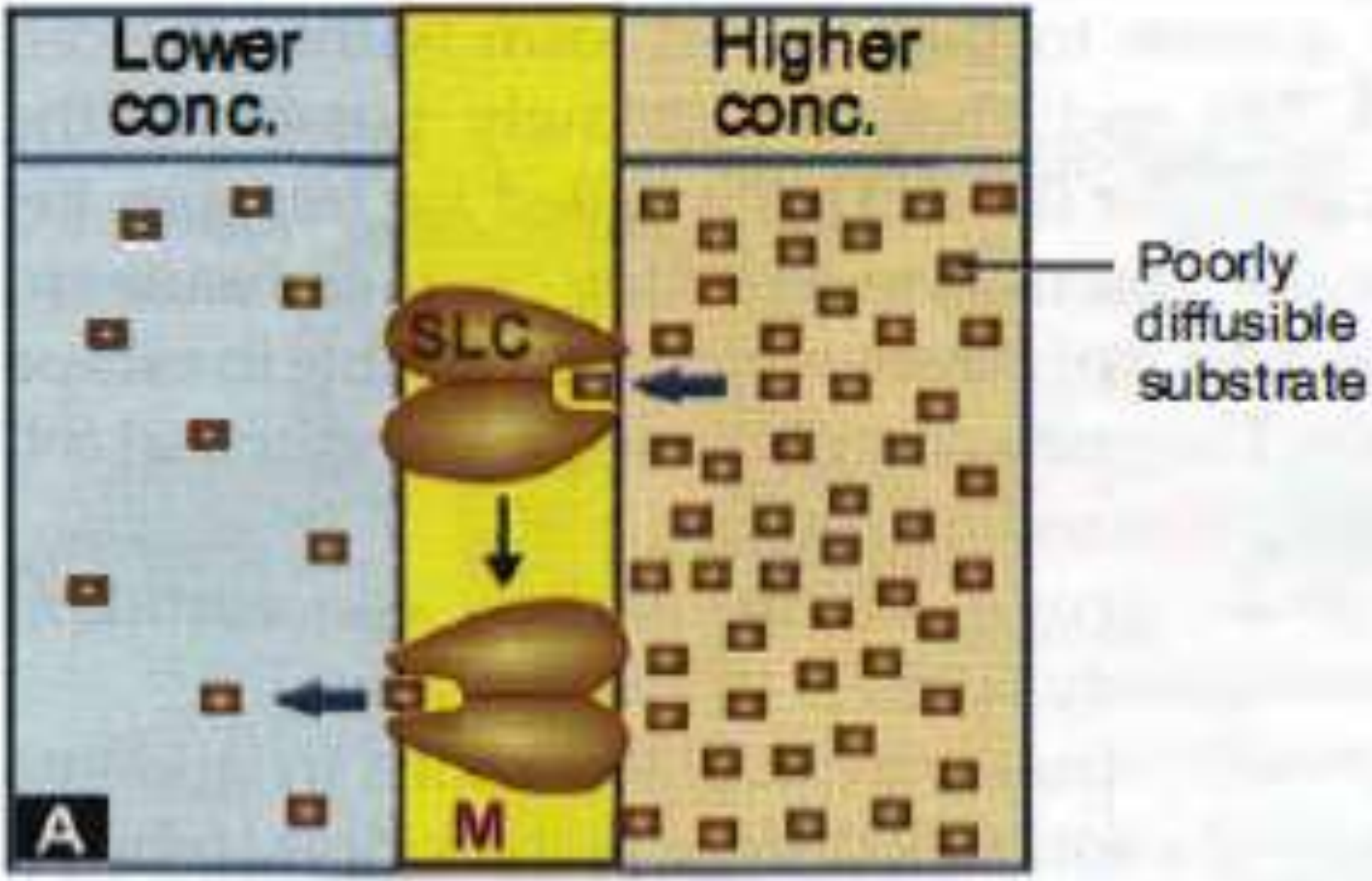


Simple or passive diffusion

- **water soluble drug (ionized or polar) is readily absorbed via aqueous channels or pores in cell membrane.**

- **Lipid soluble drug (nonionized or non polar) is readily absorbed via cell membrane itself.**


Passive diffusion of a water-soluble drug through an aqueous channel or pore.
Passive diffusion of a lipid-soluble drug dissolved in a membrane.
D
ATP
ADP
Drug
Carrier-mediated active transport of drug



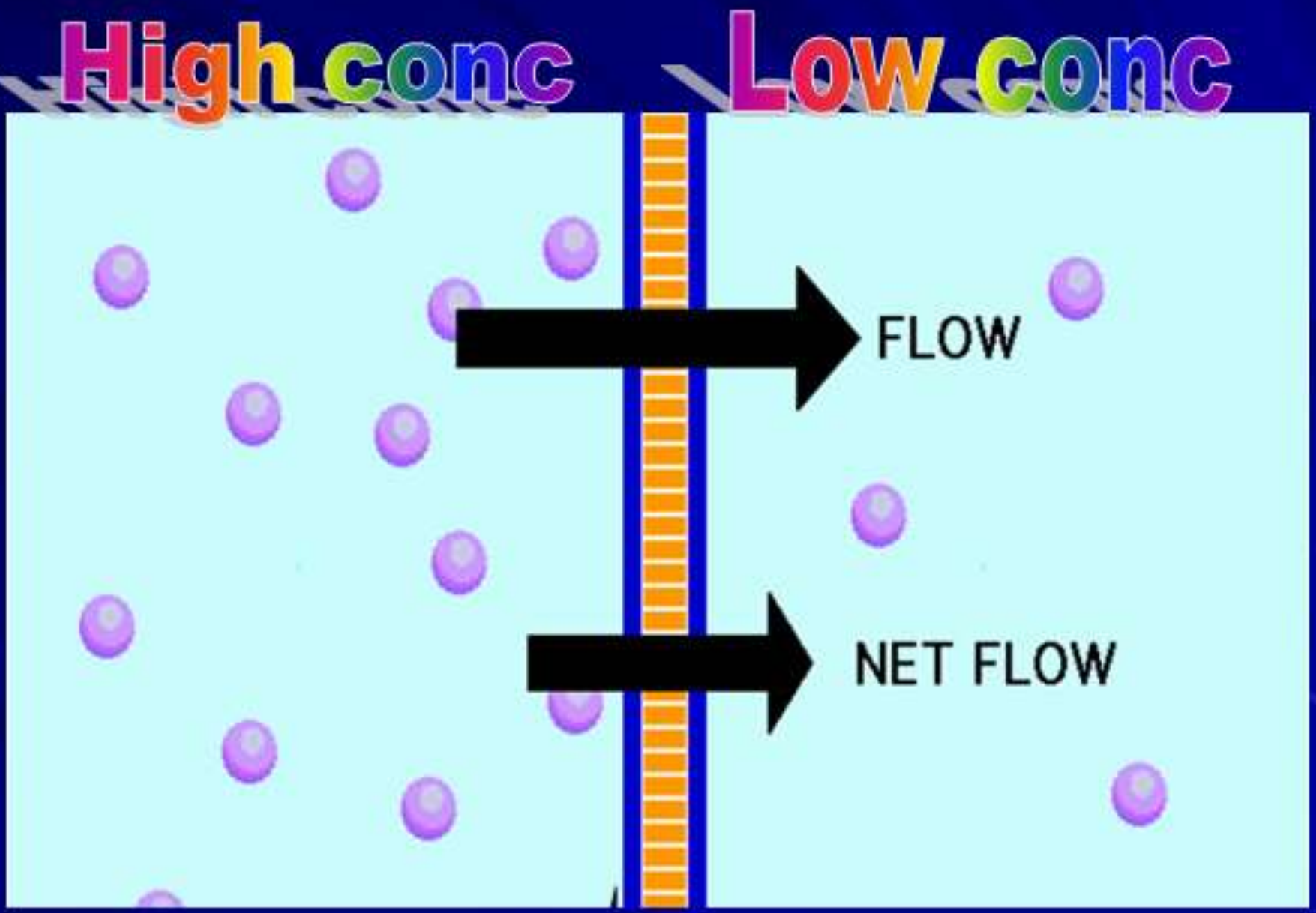
A. Facilitated diffusion: the carrier (SLC) binds and moves the poorly diffusible substrate along its concentration gradient (high to low) and does not require energy

Simple diffusion

Characters

- common.
- Occurs along concentration gradient
- Non selective
- Not saturable
- Requires no energy
- No carrier is needed
- Depends on lipid solubility
- Depends on pka of drug - pH of medium

Simple diffusion



Simple diffusion

Drugs exist in two forms ionized (water soluble & nonionized forms (lipid soluble) in equilibrium.

Drug ⇌ ionized + nonionized

- Only nonionized form is absorbable.
- Nonionized / ionized fraction is determined by pH and pKa according to Henderson-Hasselbach

pKa- pH= log protonated / non-protonated

The ionization of a weak acid HA is given by the equation

$$pH = pKa + \log \frac{[A^-]}{[HA]} \quad \ldots(1)$$

pKa is the negative logarithm of acidic dissociation constant of the weak electrolyte. If the concentration of ionized drug $[A^-]$ is equal to concentration of unionized drug [HA], then

$$\frac{[A^-]}{[HA]} = 1$$

since log 1 is 0, under this condition

$$pH = pKa \quad \ldots(2)$$

Thus, pKa is numerically equal to the pH at which the drug is 50% ionized.

PKa of the drug

(Dissociation or ionization constant):

pH at which half of the substance is ionized & half is unionized.

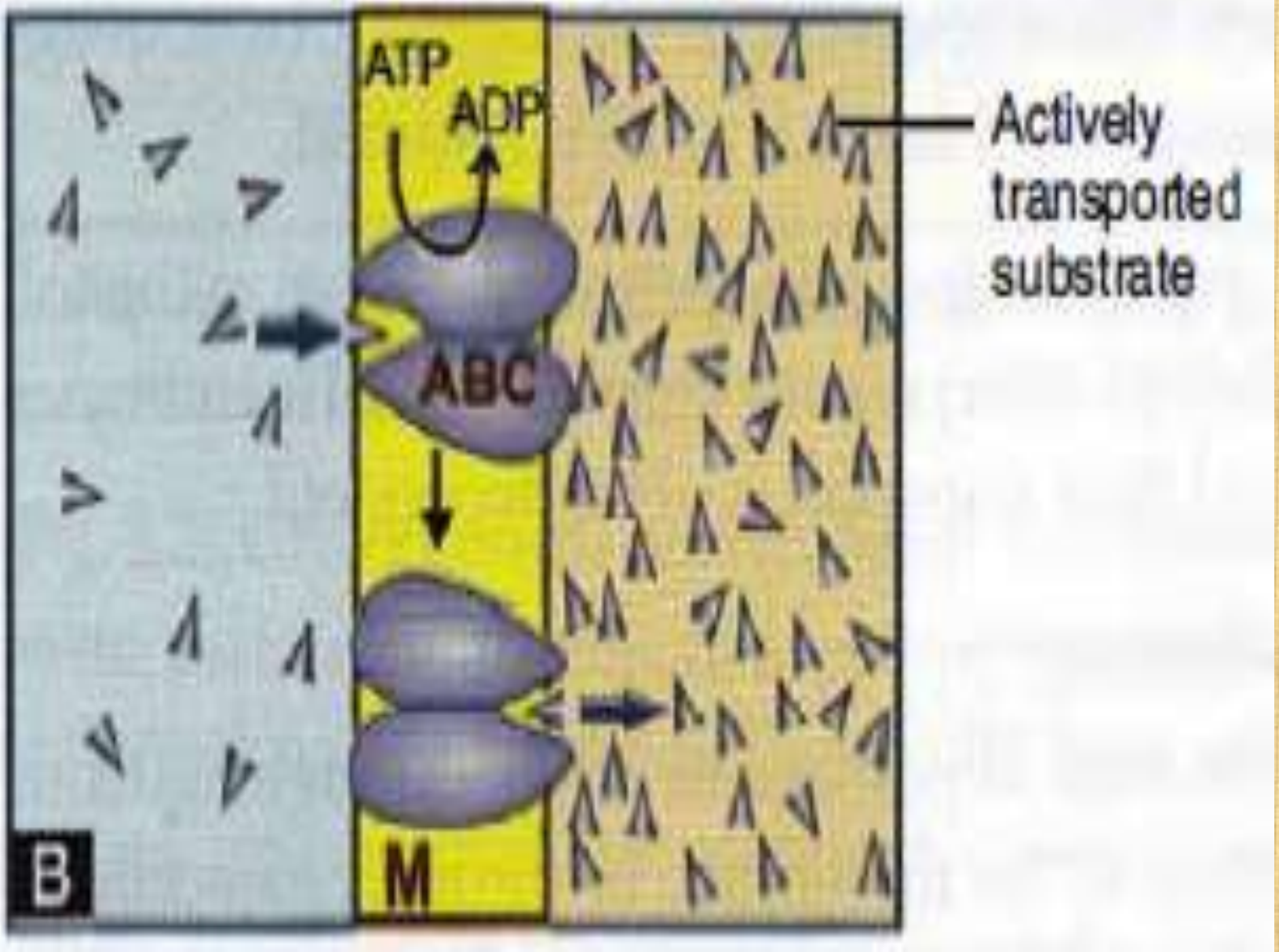
pH of the medium

Affects ionization of drugs.

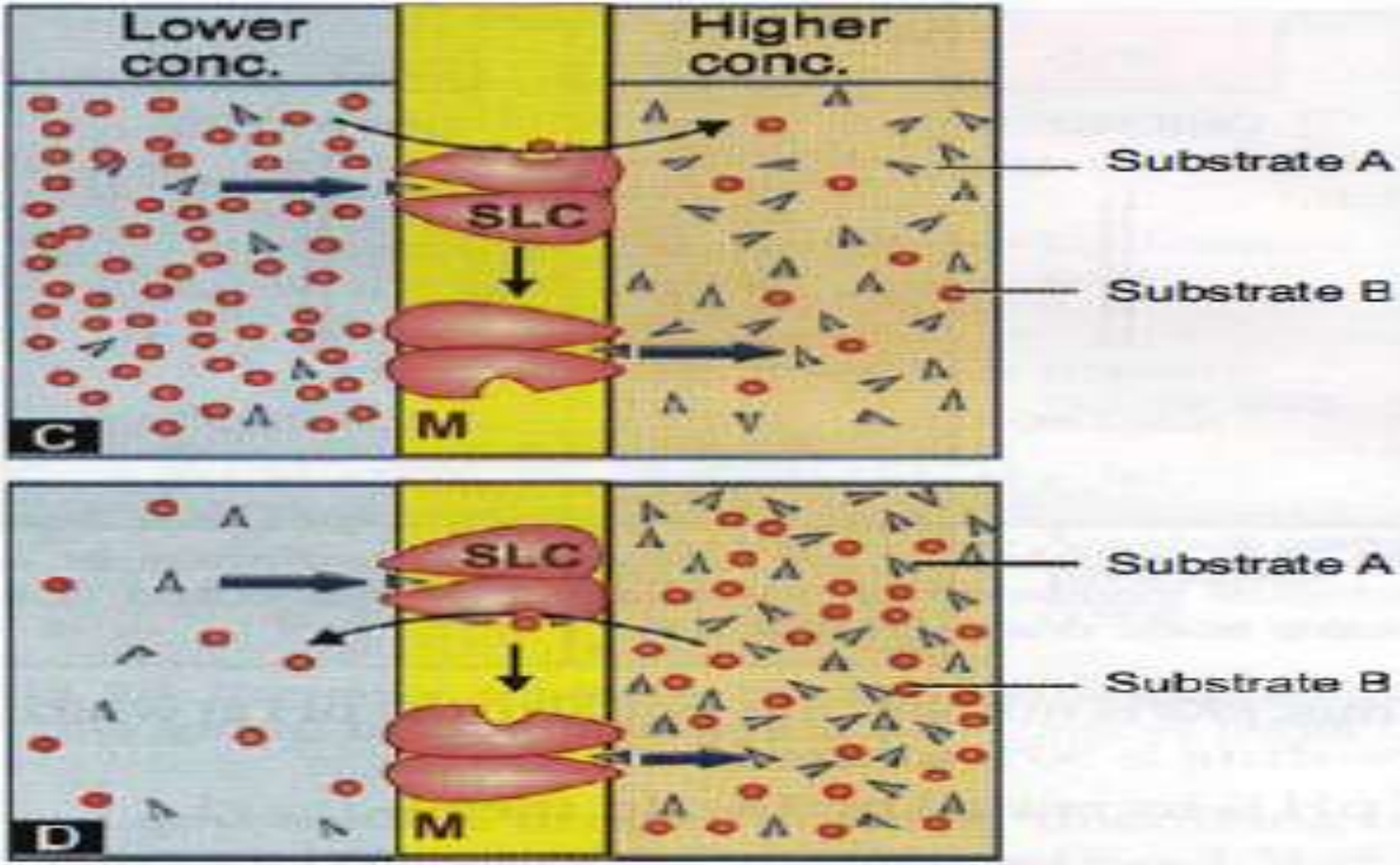
- **Weak acids → best absorbed in stomach.**
- **Weak bases → best absorbed in intestine.**

Active Transport

- Occurs against concentration gradient.
- Requires carrier and energy.
- Specific
- Saturable.
- Iron absorption.
- Uptake of levodopa by brain.



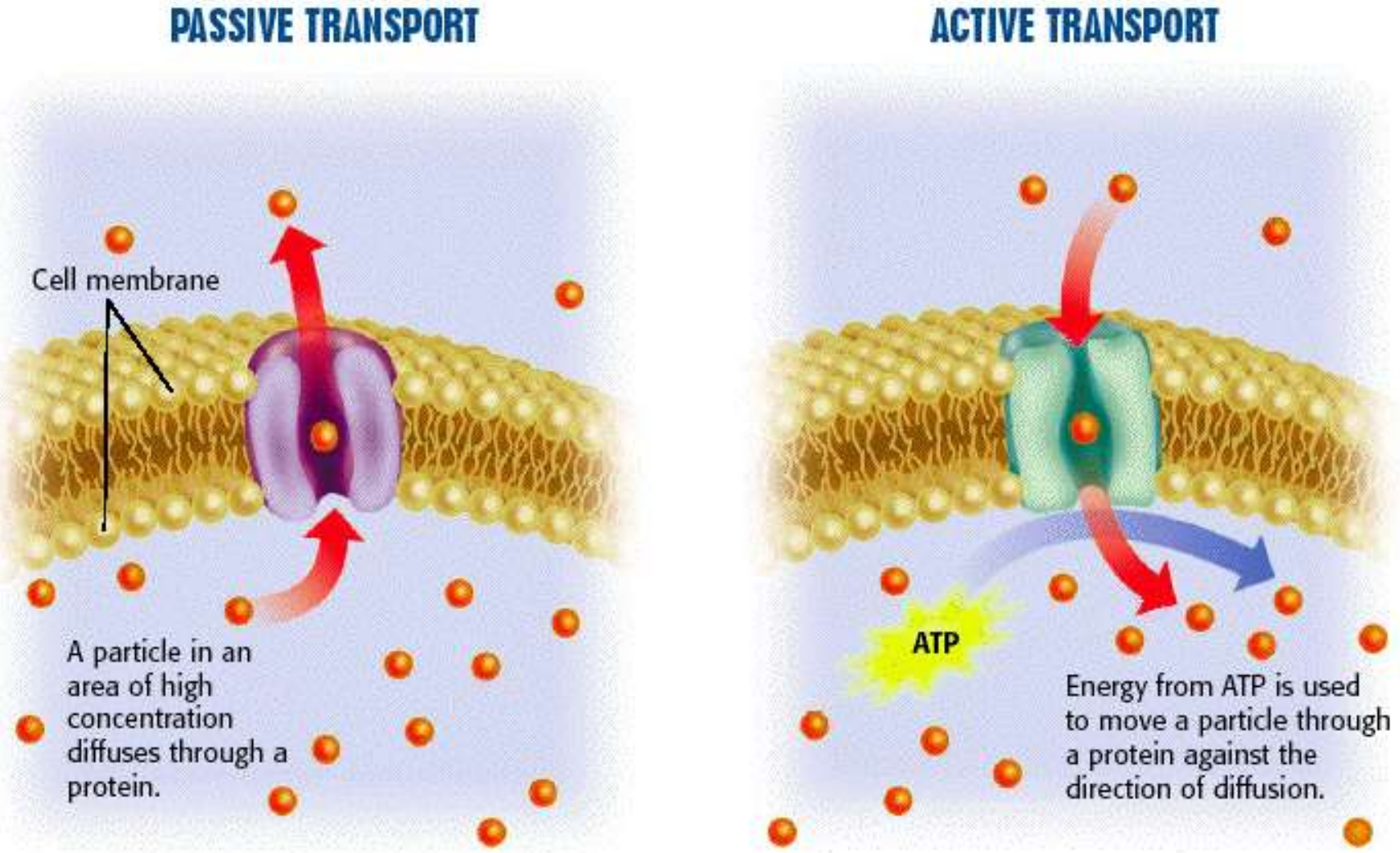
B. Primary active transport: the carrier (ABC) derives energy directly by hydrolysing ATP and moves the substrate against its concentration gradient (low to high)



C. Symport: the carrier moves the substrate 'A' against its concentration gradient by utilizing energy from downhill movement of another substrate 'B' in the same direction

D. Antiport: the carrier moves the substrate 'A' against its concentration gradient and is energized by the downhill movement of another substrate 'B' in the opposite direction

Passive and Active Transport



Carrier-mediated Facilitated Diffusion

- Occurs along concentration gradient.
- Requires carriers
- Selective.
- Saturable.
- No energy is required.

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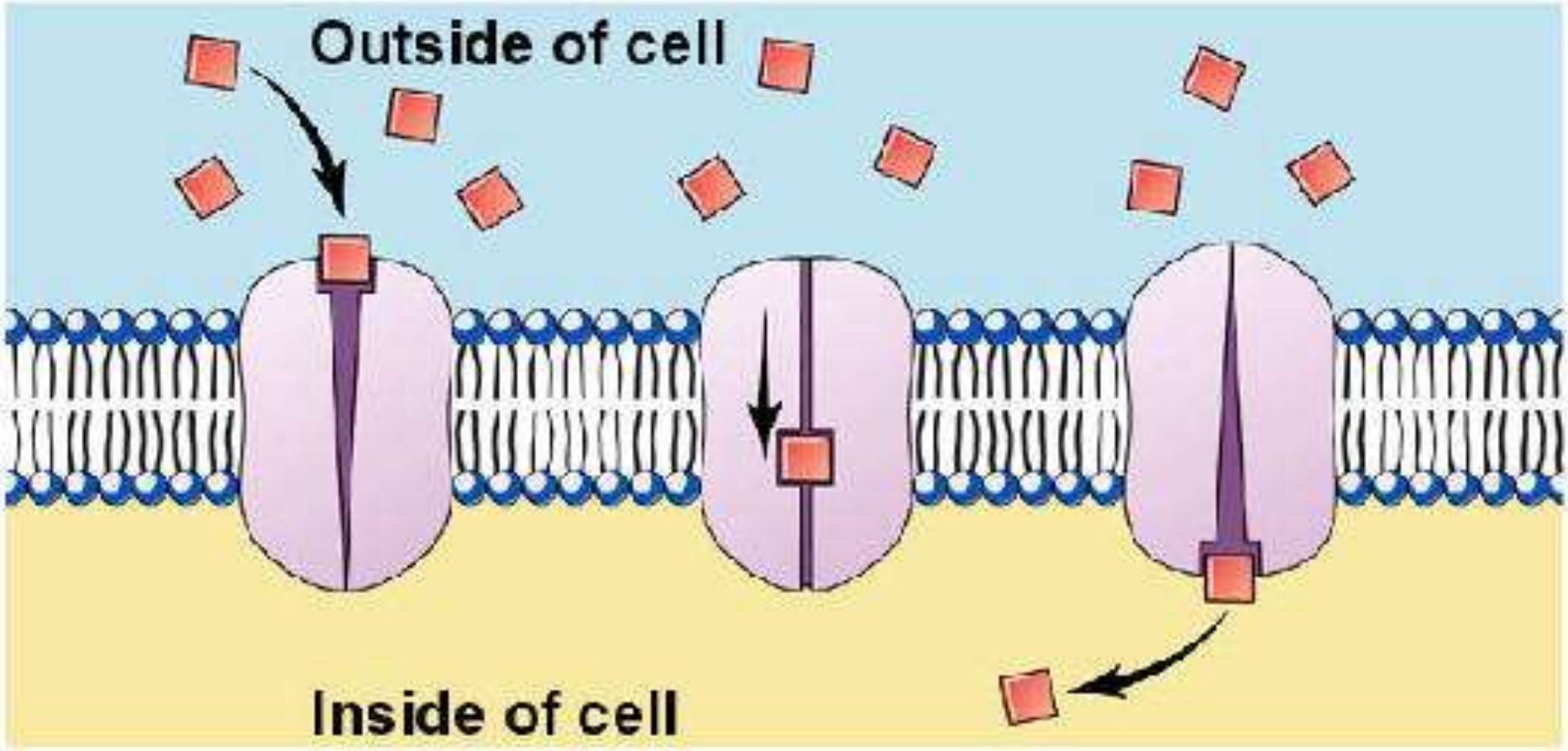
Facilitated Diffusion



Passive transport	Active transport
Along concentration gradient (From high to low)	against concentration gradient (From low to high)
No carriers	Needs carriers
Not selective Not saturable	Selective, saturable
No energy	energy is required

Active transport	Carrier-mediated facilitated diffusion
Against concentration gradient (From low to high)	along concentration gradient (From high to low)
Needs carriers	Needs carriers
Selective, saturable	Selective, saturable
Energy is required	No energy is required

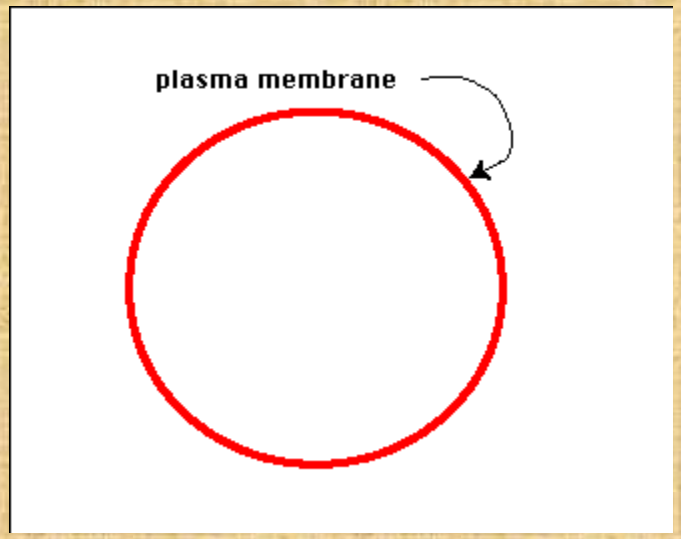
Phagocytosis (Endocytosis & Exocytosis)

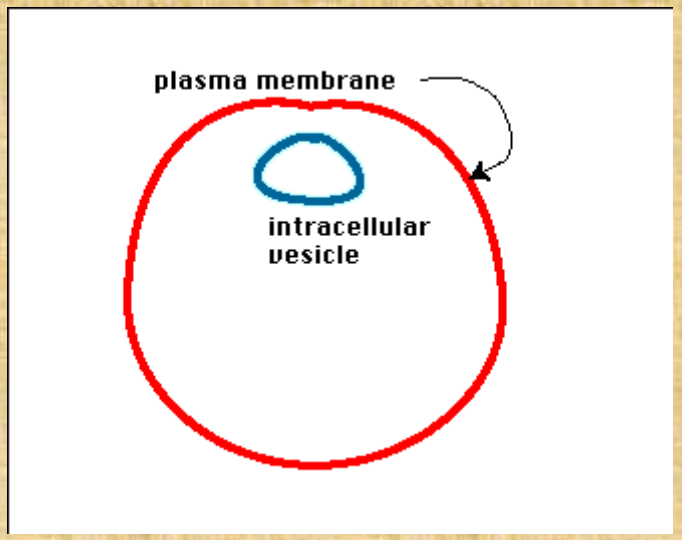
Endocytosis: uptake of membrane-bound particles.

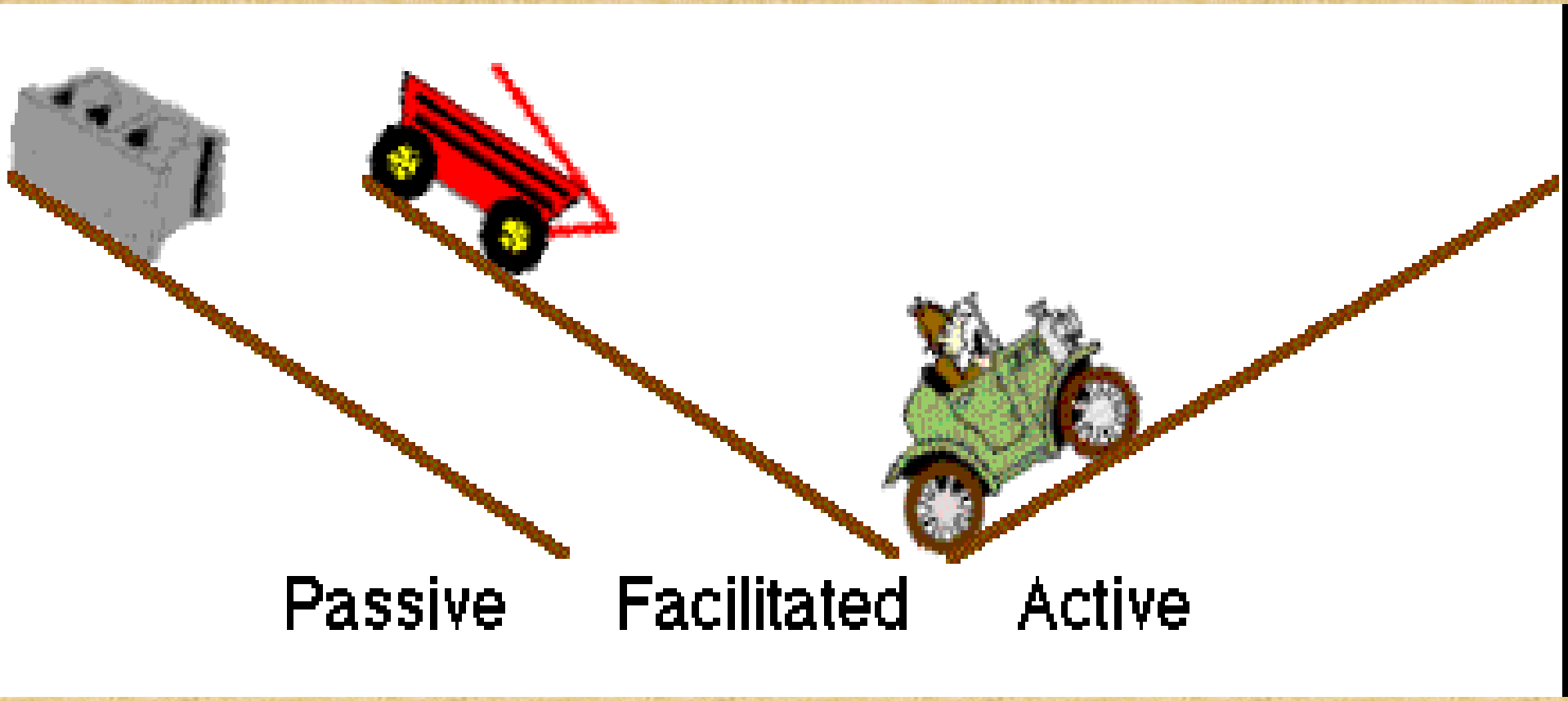
Exocytosis: expulsion of membrane-bound particles.

High molecular weight drugs or

Highly lipid insoluble drugs



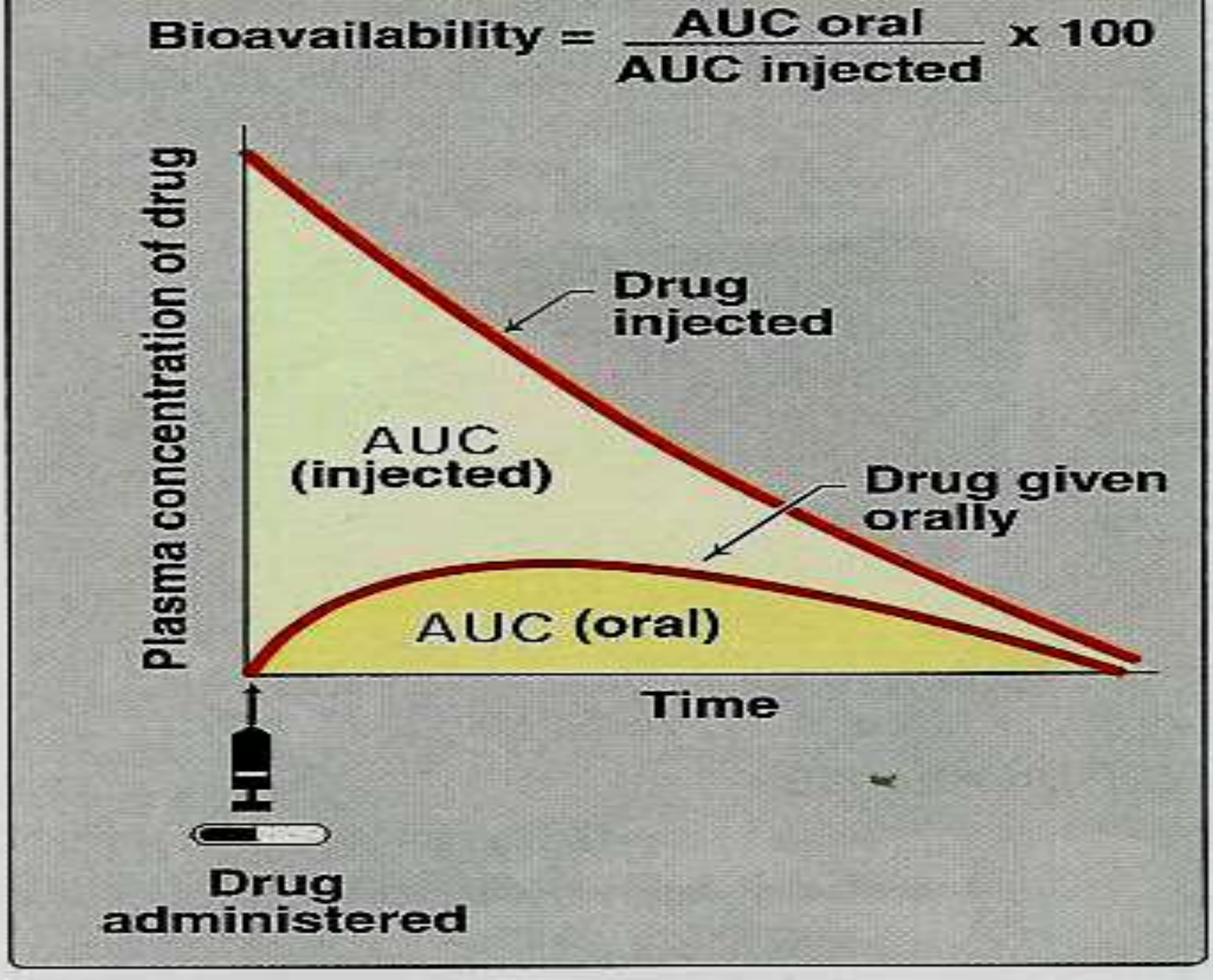



Passive
Facilitated
Active

Bioavailability

- Is the fraction of unchanged drug that enters systemic circulation after administration and becomes available to produce action
- I.V. provides 100% bioavailability.
- Oral usually has less than I.V.

- Bio = AUC oral / AUC IV X 100


Bioavailability = AUC oral / AUC injected x 100
Plasma concentration of drug
Drug injected
AUC (injected)
Drug given orally
AUC (oral)
Time
Drug administered

Factors Affecting Bioavailability

- **Molecular weight of drug.**
- **Drug Formulation (ease of dissolution).**
  (solution > suspension > capsule > tablet)
- **Solubility of the drug**
- **Chemical instability in gastric pH**
  (Penicillin & insulin)
- **First pass metabolism reduces bioavailability**

Factors Affecting Bioavailability (BAV):

- **Blood flow to absorptive site**
  - Greater blood flow increases bioavailability
  - Intestine has greater blood flow than stomach
- **Surface area available for absorption.**
  - Intestinal microvilli increases it
- **Rate of gastric emptying**
  - rapid gastric emptying ⟶ fast transit to intestine
- **pH of gut**

Factors Affecting Bioavailability (BAV)

- **Intestinal motility (Transit Time)**
  - **Diarrhea reduce absorption**
- **Drug interactions**
- **Food**
  - **slow gastric emptying**
  - **generally slow absorption**
  - **Tetracycline, aspirin, penicillin V**

Bioequivalence

- Two related drugs are bioequivalent if they show comparable bioavailability and similar times to achieve peak blood concentrations. Two related drugs with a significant difference in bioavailability are said to be bioinequivalent.

Therapeutic equivalence

- Two similar drugs are therapeutically equivalent if they have comparable efficacy and safety. [Note: Clinical effectiveness often depends on both the maximum serum drug concentrations and on the time required (after administration) to reach peak concentration. Therefore, two drugs that are bioequivalent may not be therapeutically equivalent.]

Drug Distribution

- Once in the bloodstream, a drug is distributed throughout the body
- Very little of the drug is in contact with receptors at any given time
- Most of the drug is in areas remote from the site of action (of interest), such as
  - Plasma binding sites
  - Muscle tissue
  - Adipose tissue (fat)
  - Liver
  - Kidneys

VOLUME OF DISTRIBUTION

- **Apparent volume of distribution (V_d) Presuming that the body behaves as a single homogeneous compartment with volume V into which drug gets immediately and uniformly distributed**

$$V = \frac{\text{dose administered i.v.}}{\text{plasma concentration}}$$

Drug distribution

- Factors that affect absorption also affect storage or accumulation in tissue

- Drugs will accumulate greatly in adipose or fat tissue (18-28% of body is fat)
  - Usually are not active in fat unless person begins using the fat tissue (dieting, starving); will slowly reenter the bloodstream
  - E.g., thiopental, an anesthetic is very lipid soluble
    - Enters brain quickly, has rapid onset
    - Enters muscle tissue quickly too, & follows its concentration gradient out of the brain back into circulation
    - Enters fat tissue and is stored there
    - Therefore, effect is short, but may linger for many hours

- Women have less water (54 vs. 60%) and more fat (28 vs. 18%) than men: drug action differs

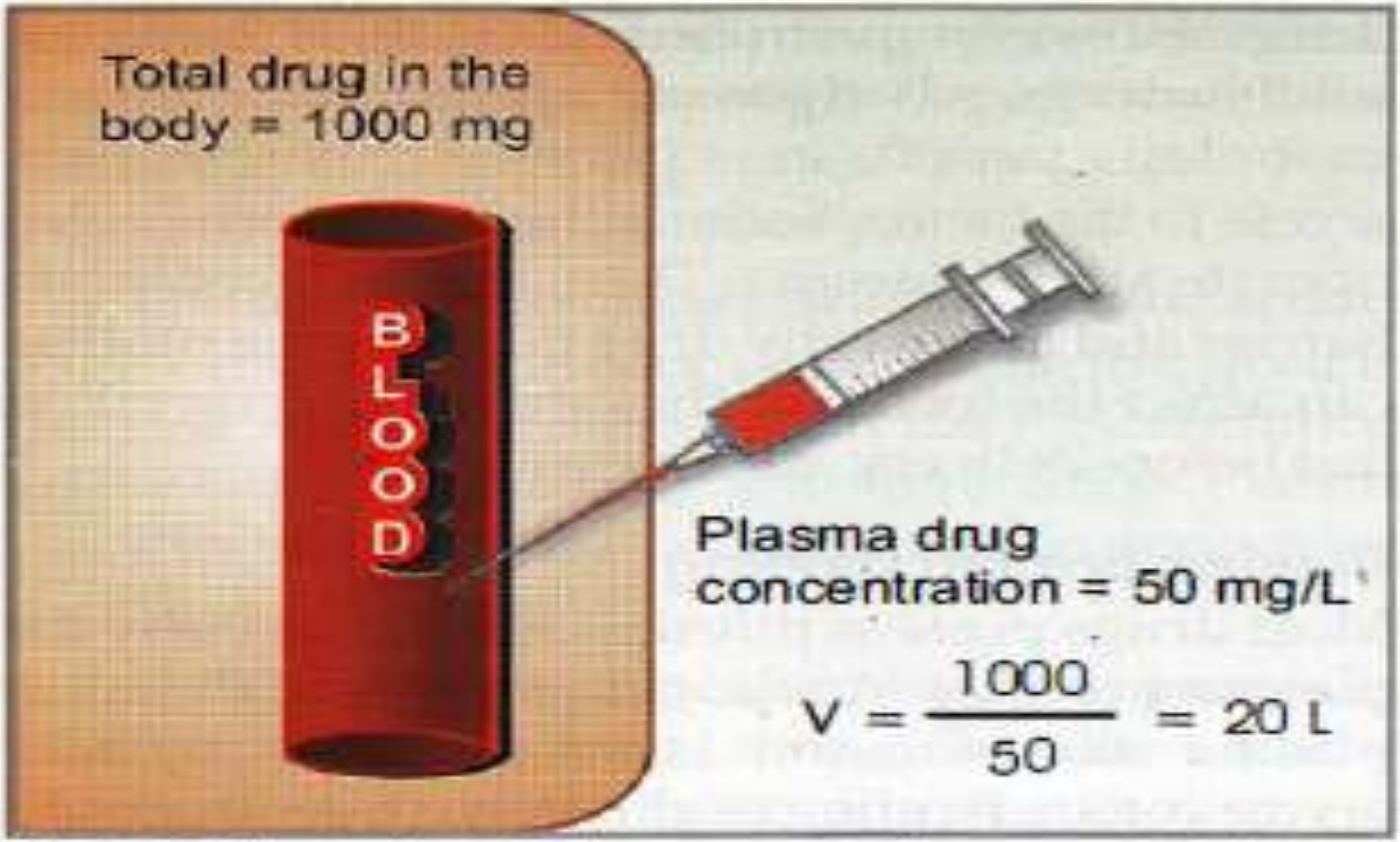


Fig. 2.7: Illustration of the concept of apparent volume of distribution (V).
In this example, 1000 mg of drug injected i.v. produces steady-state plasma concentration of 50 mg/L, apparent volume of distribution is 20 L

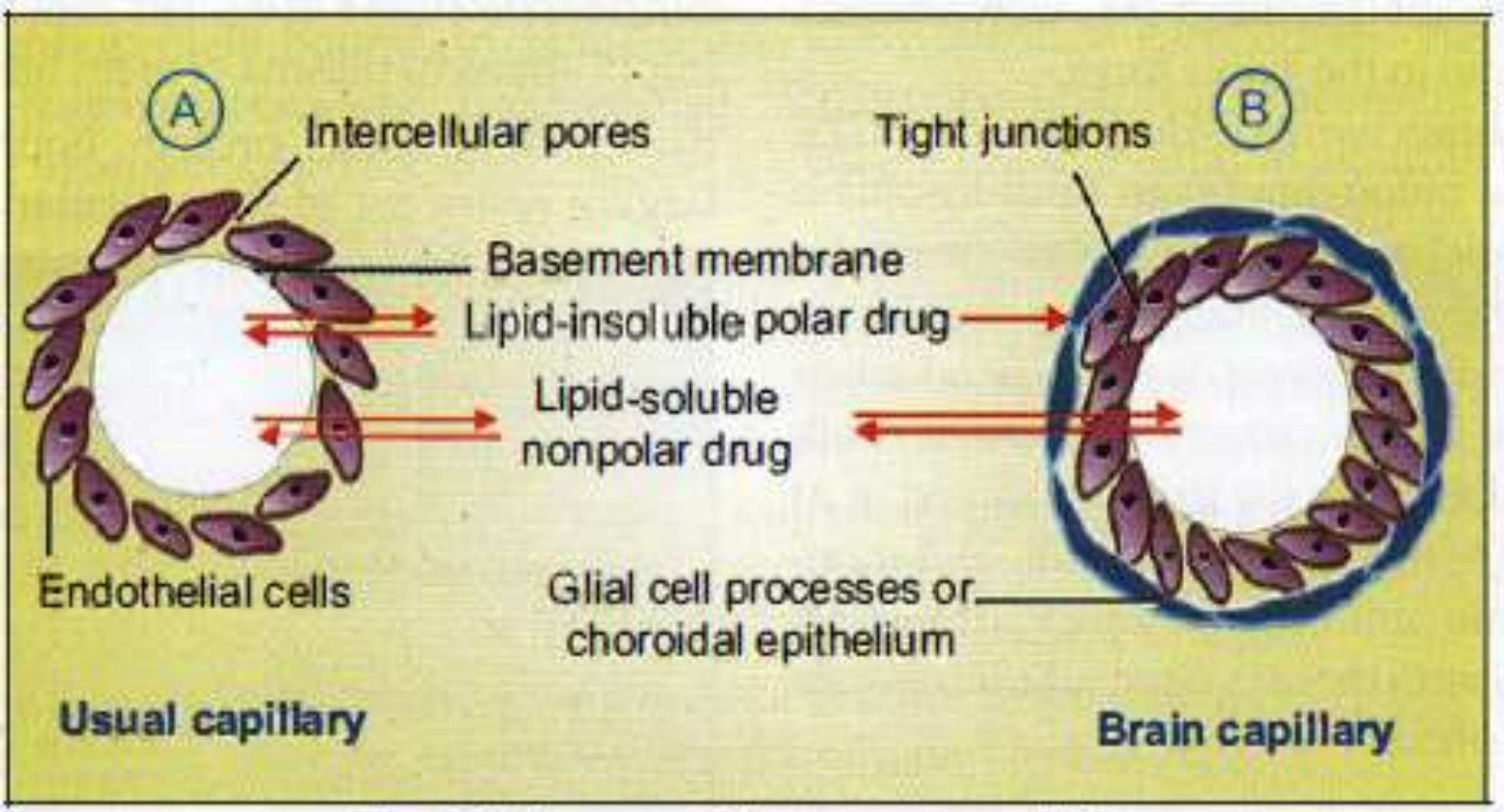


Fig. 2.8: Passage of drugs across capillaries

A. Usual capillary with large intercellular pores through which even large lipid-insoluble molecules diffuse

B. Capillary constituting blood brain or blood-CSF barrier. Tight junctions between capillary endothelial cells and investment of glial processes or choroidal epithelium do not allow passage of non lipid-soluble molecules/ions

Metabolism

- Liver is the primary site for metaboloism.
- Most of the drugs are inactivated by metabolism.
- Some drugs may be activated from the inactive compunds (**Prodrugs**) and others may give rise to active metabolites from the active compound.
- Metabolism may occur with help of **microsomal or non-microsomal enzymes.**
- Microsomal enzymes (**monooxygenase, cytochrome P450 and glucoronyl transferase**) may be induced or inhibited by other drugs whereas **non-microsomal** enzymes are not subjected to these interactions.

Enzyme inducers

- Increases the metabolism of other drugs and reduce their effect.

G	**G**riseofulvin
P	**P**henytoin
R	**R**ifampicin
S	**S**moking
Cell	**C**arbamazepine
Phone	**P**henobarbitone

Enzyme inhibitors

Vitamin	Valproate
K	**K**etoconazole
Cannot	**C**imetidine
Cause	**C**iprofloxacin
Enzyme	**E**rythromycin
Inhibition	**I**NH

Prodrug

Few drugs are inactive as such and need conversion in the body to one or more active metabolites. Such a drug are called a **prodrug .**

The prodrug may offer advantages over the active form in being more stable, having better bioavailability or other desirable pharmacokinetic properties or less side effects and toxicity.

Some prodrugs are activated selectively at the site of action.

Prodrug		Active form
Levodopa	—	Dopamine
Enalapril	—	Enalaprilat
α-Methyldopa	—	α-methylnorepinephrine
Dipivefrine	—	Epinephrine
Sulindac	—	Sulfide metabolite
Proguanil	—	Cycloguanil
Prednisone	—	Prednisolone
Bacampicillin	—	Ampicillin
Sulfasalazine	—	5-Aminosalicylic acid
Cyclophos-phamide	—	Aldophosphamide, phosphoramide mustard, acrolein
Fluorouracil	—	Fluorouridine monophosphate
Mercaptopurine	—	Methylmercaptopurine ribonucleotide
Acyclovir	—	Acyclovir triphosphate

Phases of Drug Metabolism

- **Phase I (non-synthetic**) – in this reaction functional group get attached with drug molecule.

  After phase I reaction, drug may become water soluble or lipid soluble.

- **Phase II (synthetic)** – in this reaction a conjugate is attached to drug and make it water soluble.

Metabolic Reactions

Phase I (non-synthetic)	Phase II (synthetic)
Functional group get attached with drug molecule	Conjugate is attached to drug .
Drug may become water soluble or lipid soluble.	Make it water soluble
Include **oxidation, reduction, hydrolysis, cyclization, and decyclization etc**.	Include **glucuronidation, acetylation, methylation, sulfation,and glycine conjugation** etc.

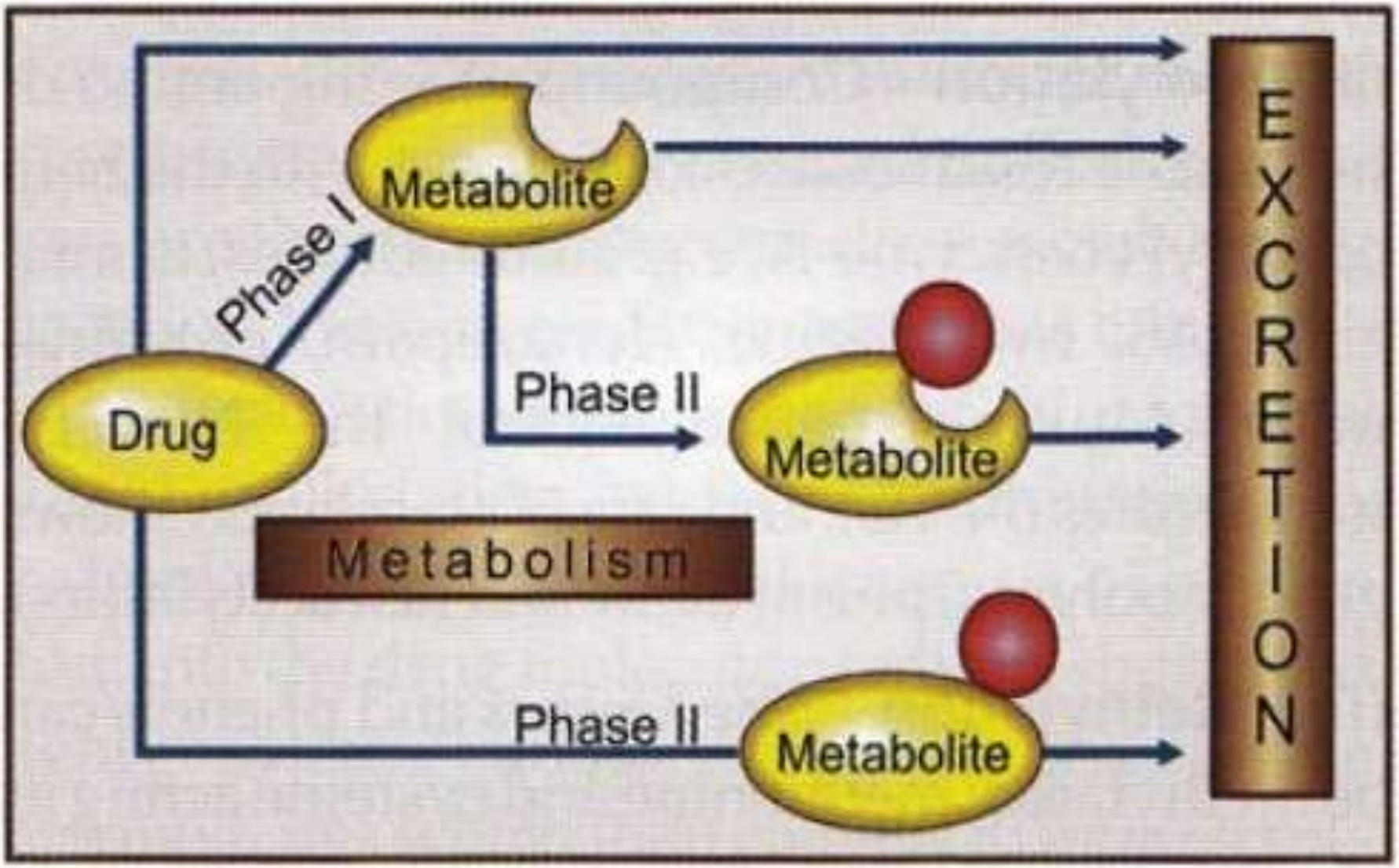


Fig. 3.1: Simultaneous and/or sequential metabolism of a drug by phase I and phase II reactions

Excretion

- Kidney is the major route of excretion.
- Excretion through kidneys occurs by glomerular filtration, tubular reabsorption and tubular secretion.
- **Glomerular filtration** depend on the plasma protein binding and renal blood flow.
- It does not depend on the lipid solubility because all substances (wether water soluble or lipid soluble) can cross fenestrated glomerular membrane.
- **Tubular reabsorption** depends on lipid solubility.
- If a drug is lipid soluble, more of it will be reabsorbed and less will be excreted. Opposite is true for lipid insoluble drugs.

Excretion

- As lipid solubility depends on ionization, the ionized drug will be excreted by the kidney.

- Thus, in acidic drug poisoning (salicylate, barbiturates etc.) urine should be alkalinized with sodium bicarbonate because weak acids are in ionized from in alkaline urine and thus are easily excreted.

- Similarly for basic drug poisoning (e.g. morphine, amphetamine etc.), urine should be acidified using ammonium-chloride.

Excretion

Tubular secretion

- Does not depend on lipid solubility or plasma protein binding.

- In the nephron, separate pumps are present for acidic and basic drugs.
- Drugs utilizing the same transporter may show drug interactions e.g. Probenecid decreases the excretion of penicillin and increases the excretion of uric acid.

- Remember, exogenous substances e.g. Penicillins are removed whereas endogenous substances like uric acid are retained by these pumps.

Kinetics Of Elimination

- Rate of elimination is the amount of drug eliminated (in units of weight like grams) per unit time.
- If it is seen as a function of plasma concentration, we derive an important parameter known as clearance **(CL).**
- Thus **clearance** is the rate of elimination of a drug divided by its plasma concentration.

First Order kinetics (Linear kinetics)	Zero Order kinetics (Non linear kinetics)
1. Constant fraction of drug is eliminated per unit time.	1. Constant amount of the drug is eliminated per unit time.
2. Rate of elimination is proportional to plasma concentration.	2. Rate of elimination is independant of plasma concentration.
3. Clearance remains constant.	3. Clearance is more at low concentrations and less at high conc.
4. Half life remain constant.	4. Half life is less at low conc. and more at high conc.
5. Most of the drugs follow first order kinetics.	5. Very few drugs follow pure zero order kinetics e.g. alcohol.
	6. Any drug at high conc. (when metabolic or elimination pathway is saturated) May show zero order kinetics.

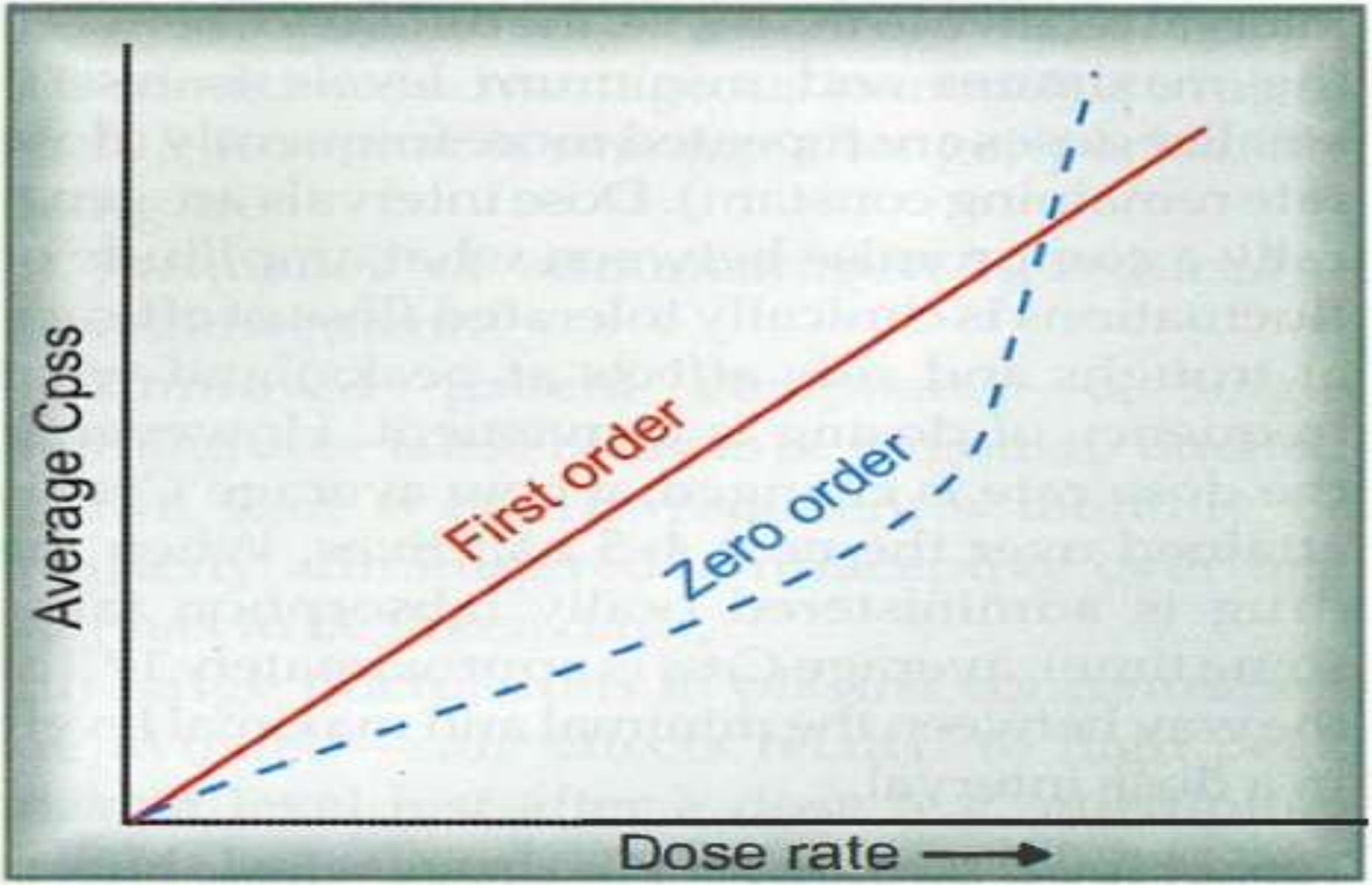


Fig. 3.5: Relationship between dose rate and average steady-state plasma concentration of drugs eliminated by first order and Michaelis Menten (zero order) kinetics

The drugs whose kinetics changes from first order to zero order at therapeutic concentration are said to follow pseudo- zero order kinetics.

Zero	Zero order kinetics shown by
W	**W**arfarin
A	**A**lcohol and **A**spirin
T	**T**heophylline
T	**T**olbutamide
Power	**P**henytoin

Plasma half-life

1. The Plasma half-life ($t_{1/2}$) of a drug is the time taken for its plasma concentration to be reduced to half of its original value.

   • initial rapidly declining (α) phase-due to distribution.

   • later less declined (β) phase-due to elimination.

2. At least two half-lives (distribution $t_{1/2}$ and elimination $t_{1/2}$) can be calculated from the two slopes.

3. The elimination half life derived from the β slope is simply called the 'half life' of the drug.

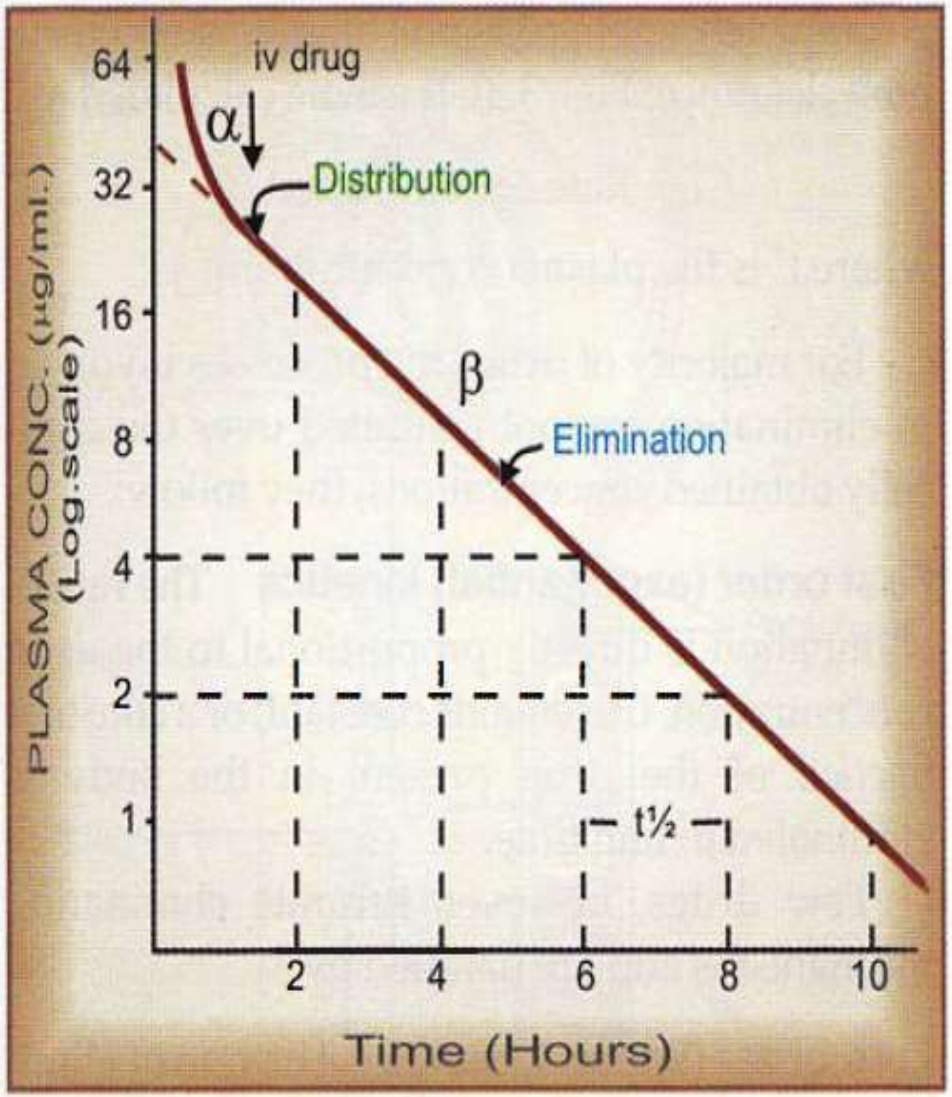


Fig. 3.4: Semilog plasma concentration-time plot of a drug eliminated by first order kinetics after intravenous injection

Plasma half-life

- A drug's half-life (measured in time) determines how often it must be taken and how long it takes for the drug to wear off and to leave one's system.

# of Half-lives	Percent eliminated
0	0
1	50
2	75
3	87.5
4	93.8
5	96.9
6	98.4

- This assumes normal liver and kidney function. Older, younger, or sick people may clear drugs more slowly.

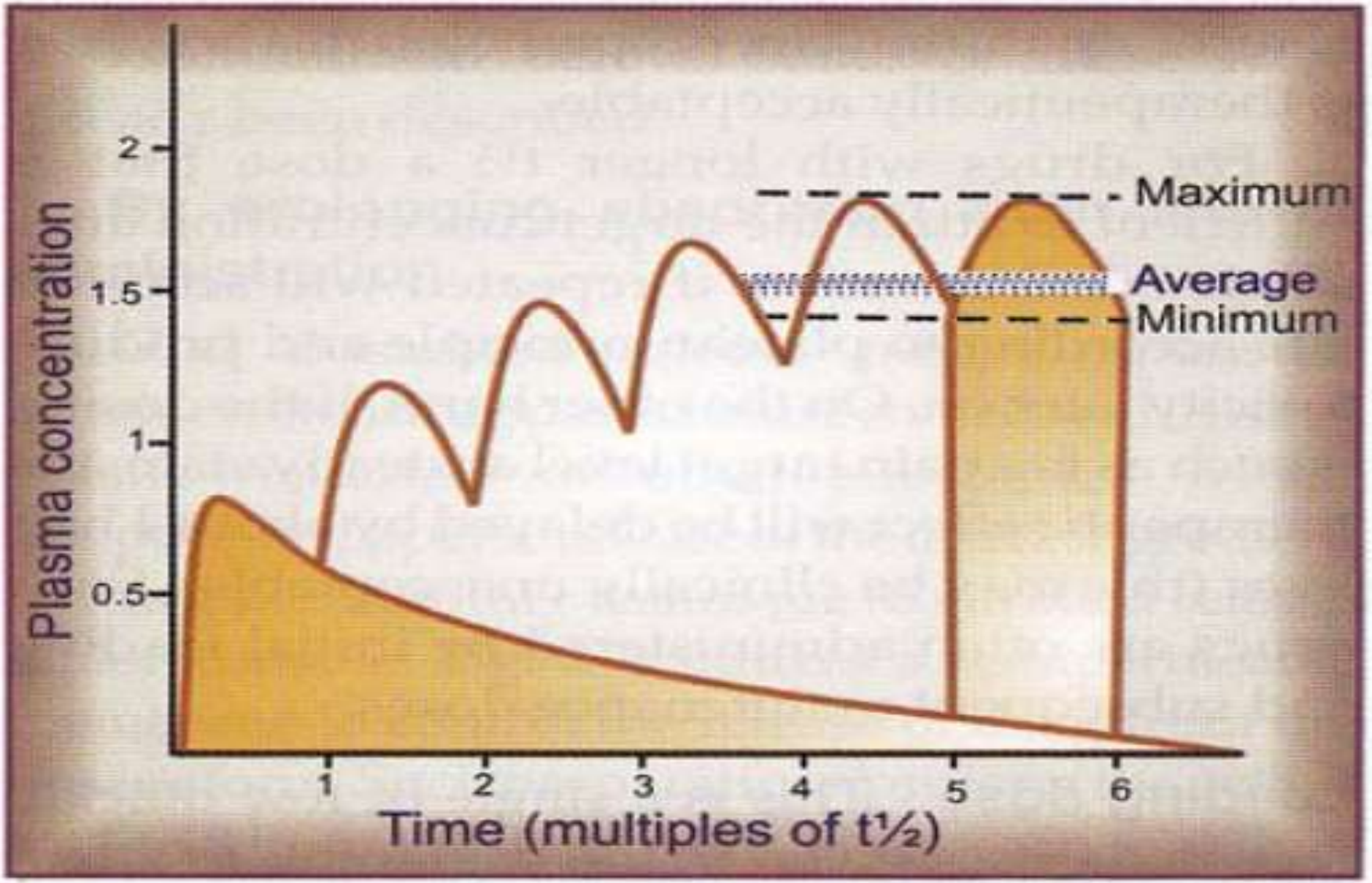


Fig. 3.6: Plateau principle of drug accumulation on repeated oral dosing.

Note. The area of the two shaded portions is equal

Dose strategy

- The drugs having high volume of distribution are given by this strategy.
- First a large dose (**loading dose**) is administered to attain the steady state quickly and later on, to maintain the plasma concentration smaller dose is given (**maintenance dose**).

- **Loading dose** : it is given to load (saturate) the tissue stores. So it is mainly dependent on $\mathbf{V_d}$.

Loading dose = V_d x Target plasma concentration

Maintenance Dose

- It is mainly dependent on **CL**.

Maintenance dose = **CL** x Target plasma conc.

Therapeutic Drug Monitoring (TDM)

- **TDM** is a process by which the dose of a drug is adjusted according to its plasma concentration.
- It is done for drugs having wide variation in pharmacokinetics ,both intra- as well as inter- individual.
- It is done for the drugs having low therapeutic index like theophylline, lithium, antiepileptics, immuno-modulators and anti-arrhythmics etc.
- **TDM** is done for those whose effect cannot be easily measured (like effect of antihypertensive drugs can be easily measured by monitoring BP, so **TDM** is not used).
- **TDM** is not done for the drugs which are activated in the body or produce active metabolites (Prodrugs).

Fixed dose ratio combination preparations

Advantages

1. Convenience and better patient compliance.

2. Certain drug combinations are synergistic, e.g. sulfamethoxazole + trimethoprim; levodopa + carbidopa/benserazide; combination oral contraceptives.

3. The therapeutic effect of two components being same may add up while the side effects being different may not, e.g. amlodipine + atenolol as antihypertensive.

4. The side effect of one component may be counteracted by the other, e.g. a thiazide + a potassium sparing diuretic.

5. Combined formulation ensures that a single drug will not be administered. This is important in the treatment of **tuberculosis** and **HIV-AIDS.**

Disadvantages of fixed dose ratio combinations

1. The patient may not actually need all the drugs present in a combination
2. The dose of most drugs needs to be adjusted and individualised. When a combined formulation is used, this cannot be done without altering the dose of the other component(s).
3. The time course of action of the components may be different: administering them at the same intervals may be inappropriate.
4. Altered renal or hepatic function of the patient may differently affect the pharmacokinetics of the components.
5. Adverse effect, when it occurs, cannot be easily ascribed to the particular drug causing it.
6. Contraindication to one component (allergy, other conditions) contraindicates the whole preparation.
7. Confusion of therapeutic aims and false sense of superiority of two drugs over one is fostered, specially in case of antimicrobials whose combinations should be avoided.

Corticosteroids should never be combined with any other drug meant for internal use.

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POISON AND ANTIDOTE

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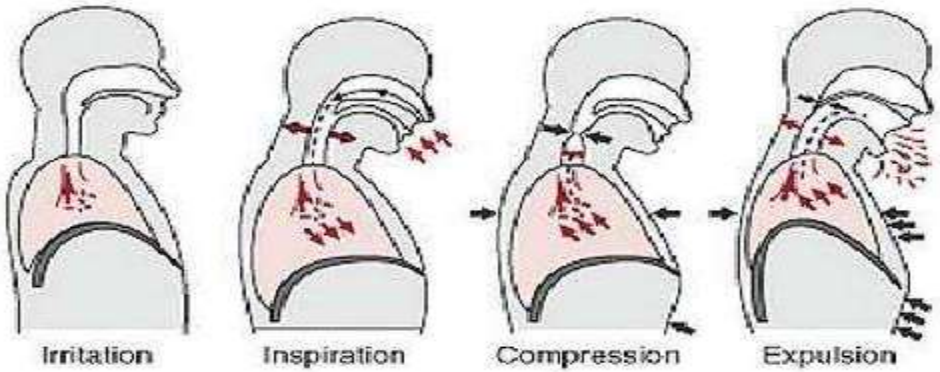
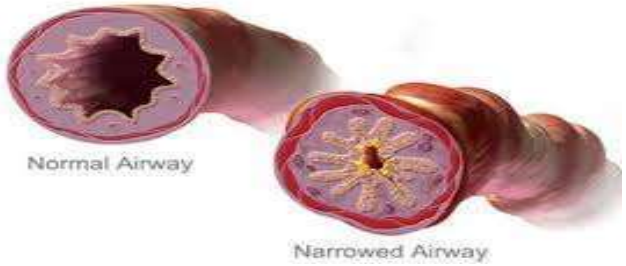
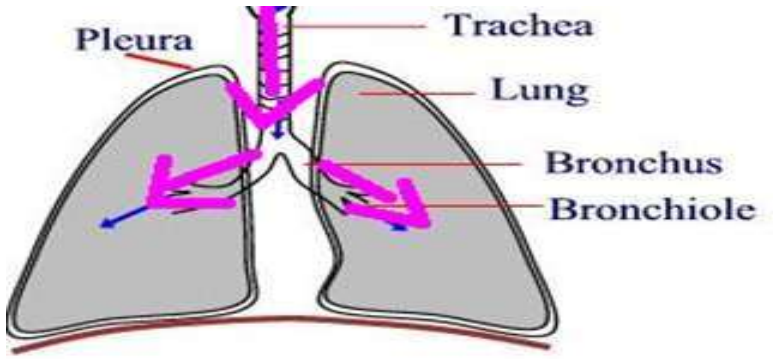
Symptoms of Respiratory Disease

- **Sputum:** it gives up when normal mucociliary clearance mechanisms are overloaded by excessive mucus production.
- **Cough:** it is part of natural defence mechanism for clearing air passages of foreign materials and excess mucus.



- **Breathlessness:** it is an unpleasant awareness about the sensation of breathing.
- **Wheeze/wheezing:** When intra-thoracic airways are narrowed, further reduction in air way calibre during expiration results in airway closure.
- **Chest pain:** due to infection, inflammation or malignant invlovement of the parietal pleura.





Expectorants

- Drugs used orally to stimulate flow of respiratory tract secretions.
- By using this ciliary motion could throw mucus towards pharynx easily or in other wards – increase the ciliary motion!
- Used in respiratory disorders in which respiratory secretions are purulent (infected), viscid (thick) or excess.

Categories: Drugs for Cough

1. **Directly acting expectorants:** Act directly on bronchial secretory cells. Mucokinetics

e.g. Terpin hydrate, Sodium/potassium citrate & acetate, Potassium iodide, Vasaka Guaiphensein etc

2. **Reflex acting expectorants:** Act by irritating gastric mucosa & then stimulate respiratory tract secretion. Mucokinetics

e.g. Ammonium Chloride/carbonate & Potassium iodide others like iodinated glycerine

3.Mucolytics: Bromhexine, Ambroxol, Acetyl cysteine, Carbocisteine

4.Antitiussive: Act by suppressing cough centers centrally.

a. **Opioids:** Codeine, Morphine
b. **Non-Opioids:** Dextromethorphane, Noscapine etc
c. **Antihistamines:** Act on histamine receptors. E.g. Chlorpheniramine, Diphenhydramine, Promethazine

5. Pharyngeal Demulcents: Lozenges, Glycerin, Linctuses containing syrups.

Ammonium Chloride

- NH4Cl, Mol. Wt. 53.49, colourless crystals/crystralline powder, cool saline taste, hygroscopic, freely soluble in water & in glycerin. Sparingly soluble in alcohol. Also known as Ammonium muriate.
- Preparation: By neutralizatin reaction, evaporation to dryness. Purification by recrystallization.
- Assay: weighed uantity dissolved in water & HCHO solution is added. Titrated with 0.1 N NaOH using phenolphthalein as indicator.
- Each ml of 0.1 N NaOH = 0.005349 gm of NH4Cl

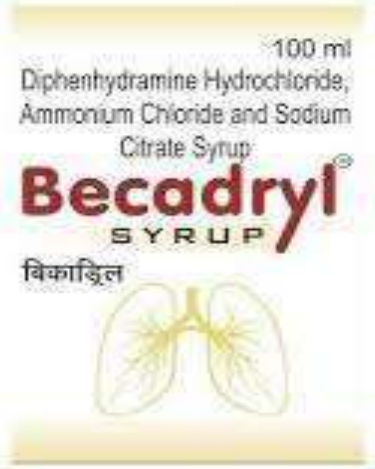
•Dose: 4 – 12 gm daily orally

Uses:

- Used as a mild diuretic, an expectorant, a weight reducing agent and a urine acidifying agent.

In Combination:

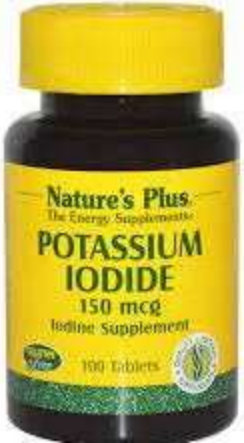
- Used to control a cough associated with a cold or other respiratory illnesses
- Dietary supplements



Potassium Iodide

- KI Molecular weight: 166.0
- Description: It occurs as a colourless crystals or white granular powder. It has a saline but slightly bitter taste. It is soluble in water, alcohol & glycerine.
- KI solution contains 10% of KI with 0.05% of Sodium thiosulfate to reduce free Iodine (yellow color) which can form on storage.
- The aueous solution of KI dissolves iodine by forming
- a dark brown solution of potassium triiodide.

$$KI + I_2 \rightarrow KI_3$$



- **Assay:** Iodometry, weighed compound dissolved in water, and add conc. HCl, $CHCl_3$ (Chloroform) & titrate with 0.05 M potassium iodate until color of $CHCl_3$ layer iodine disappears. At this stage add starch solution as indicator and titrate slowly to get a colourless solution.

Test for Iodate:

- it depends on the liberation of iodine on acidification.
- Being a strong oxidising agent, potassium iodate (KIO_3), liberates Cl from conc. HCl.
- The solution turns brown - addition of further iodate it fades as the iodine is converted into the pale coloured iodine monochloride.

$$I_2 + Cl_2 \rightarrow 2\ ICl$$

Uses: Expectorant and source of iodine

Emetics

- Vomiting – serves to relieve the upper GIT by forcible expulsion of gastric contents through the mouth.
- Nausea – an unpleasant sensation is generally associated with vomiting.
- **Emetics:** An emetic is a drug which induces vomiting. Place an important role in the treatment of poisoning cases.

•These drugs act either by irritation of gastric mucosa or

•Directly on the chemoreceptor trigger Zone (CTZ) in medulla oblongata

(i.e. centrally acting emetics)

Emetics vs Cough preparation:

- In low dose added in anti-cough preparation.
- Mild - They stimulate respiratory tract secretion.

e.g.

Emetics: Copper sulphate & Sodium potassium tartarate

Sodium Potassium Tartarate

I.P limit: It contains not less than 99.0% and not more than 102% of C_4H_4KNa calculated on the anhydrous basis.

Preparation: It is prepared by boiling a solution of sodium carbonate and potassium bitartarate for sometime and allowing the reaction mixture to stand at 60°C. The solution is filtered, concentrated and crystallized.

- Description: A colorless to blue-white crystalline salt with a saline, cooling taste. It is soluble in water and slightly soluble in alcohol

•melts at about 75°C.

Directions for use

- It is used in medicine as a mild expectorant emetic & purgative
- It is an ingredient of Fehling's Solution.
- It is used in silvering mirrors.

•It is used in piezoelectric devices,

e.g., crystal microphones and

phonograph pickup cartridges.

Copper sulphate

- Description: Blue crystalline powder which is soluble in water, glycerol and insoluble in alcohol.
- On heating at 230 ^{o}C it gives white anhydrous copper sulphate which is very hygroscopic and turns blue by absorption of water – this test may be used as a test for the presence of small amounts of water.

Uses:

- As an emetic in a dose of 300 mg.
- As an astringent in the dose range of 5 to 10 mg three times a day.

Method of Analysis:

- By Iodometric Titration
- $CuSO_4$ reacts with KI to give unstable cupric iodide (CuI_2) which decomposes to CU_2I_2 with the liberation of free iodine. The liberated iodine is titrated against sodium thiosulphate using starch as an indicator.

$$2CuSO4 + 4KI \rightarrow 2CuI2 + 2K2SO4$$

$$2CuI2 \rightarrow Cu2I2 + I2$$

$$I2 + 2Na2S2O3 \rightarrow Na2S4O6 + 2NaI$$

Procedure: KI + H20 +Acetic Acid → titrate with 0.1 N sodium thiosulphate using starch indicator → Faint blue colour → 2g Potassium thiocyanate → Titrate till blue colour disappears.

Factor: 0.1 N $Na2S2O3$ = 0.02497 g of $CuSO4.5H2O$

Preparation

- Metal oxides will dissolve in acid to form a salt and water e.g.

$$CuO + H_2SO_4 \rightarrow CuSO_4 + H_2O$$

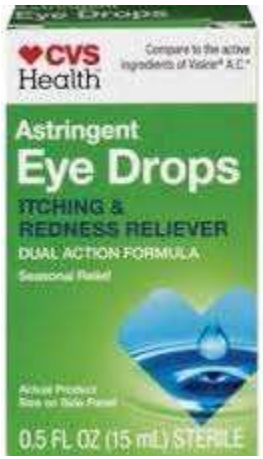
Copper oxide + Sulfuric acid → Copper sulfate + Water

Method:

- Heat the sulfuric acid until it is almost boiling.
- Add small amount of copper oxide to the hot acid solution.
- use the glass stirring rod to stir lightly after each new addition of copper oxide.
- Continue to heat the solution after the last piece of copper oxide has been added. This may take 1 – 2 min. the solution will look dark blackish blue colored.
- Set the beaker aside and allow to cool at room temperature.
- Filter the solution and set aside in a warm place for evaporation after few days or weeks collect CuSO4 crystals .

ASTRINGENTS

- Agents which are applied locally and gives protein precipitant action.
- Cause contraction and wrinkling of the tissue
- Reduces the cell permeability
- Constrict the local blood vessels
- Inhibit the transcapillary movement of plasma protein



The net effects:

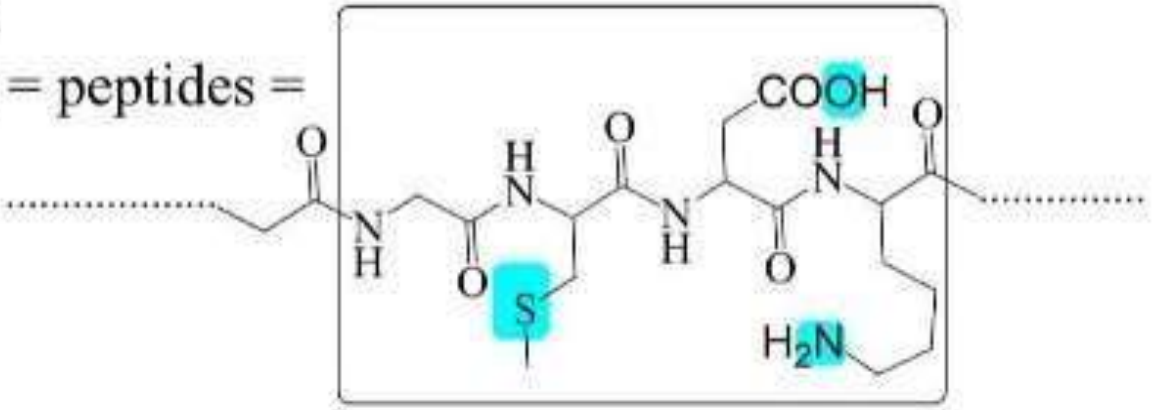
- Anti-inflammatory effect – by decreasing blood supply to site of injury
- Decrease blood lose by coagulating plasma protein
- Antiperspirant effect – by decreasing secretion of sweating by decreasing the size of pores of skin
- Antimicrobial – by protein precipitation reaction in microbes

ASTRINGENTS

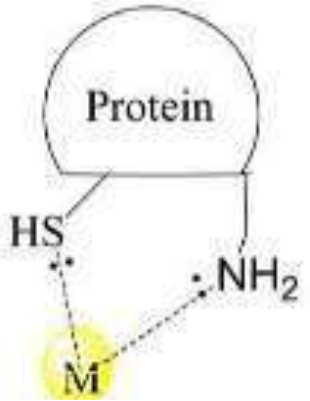
Mechanism of Protein Precipitation

Skin = Lipid + Protein

Protein = peptides =



Atoms which are capable to form metalic bonds i.e. Chelation of metal with pr.



ASTRINGENTS

Uses:

- To reduce pain (as Anti-inflammatory agents)
- To arrest hemorrhages
- Promote healing of wound
- To reduce sweating (as Antiperspirants)

Agents:

Zinc Oxide
Zinc sulphate
Calamine
Zinc chloride

Aluminium sulphate
Aluminium Chloride
Alum

ASTRINGENTS

ZINC SULPHATE

$ZnSO_4 . 7H_2O$

Properties:

Physical properties:

- Colorless crystals, efflorescent in dry air, odourless
- Water soluble, aq. soln is acidic

Chemical properties :

- Loss of *water of crystallization* at boiling water temperature

ASTRINGENTS

ZINC SULPHATE

$$ZnSO_4 \cdot 7H_2O \xrightarrow{70^oC} ZnSO_4 \cdot 6H_2O + H_2O$$

$$ZnSO_4 \cdot 7H_2O \xrightarrow{100^oC} ZnSO_4 \cdot H_2O + 6H_2O$$

$$ZnSO_4 \cdot 7H_2O \xrightarrow{200^oC} ZnSO_4 + 7H_2O$$

- Decomposition at higher temperature

$$ZnSO_4 \cdot 7H_2O \xrightarrow{800^oC} ZnO + SO_2 + O_2$$

ASTRINGENTS

ZINC SULPHATE

Preparation:

a. From zinc blend (zinc sulphide) – Oxidation in air at high temperature

$$ZnS + 2O_2 \xrightarrow{\Delta} ZnSO_4$$

b. From zinc metal

$$Zn + H_2SO_4 \xrightarrow{\Delta} ZnSO_4 + H_2\uparrow$$

- After completion of reaction, reaction mixture is treated with chloride water to remove <u>impurity of iron.</u>

ASTRINGENTS

ZINC SULPHATE

Assay:

- Principle: Complexometric titration
- Titrant: Disodium EDTA
- Indicator: eriochrome black T solution
- Titer factor: Each ml of 0.05 M disodium EDTA ≡ 0.01438 g of $ZnSO_4 . 7H_2O$

Uses:

- Astringent (Ophthalmic astringent)
- Zinc supplements
- Emetic
- Antiseptic

ASTRINGENTS

ALUM

$KAl(SO_4)_2 . 12H_2O$

Synonym: Potassium Aluminium sulphate

Properties:

Physical properties:

- Colorless transparent crystals,
- sweet astringent taste
- water soluble- gives acidic solution,
- insoluble in alcohol

Chemical properties:

- Dehydrated at high temperature

$$KAl(SO_4)_2 . 12H_2O \xrightarrow{\Delta} KAl(SO_4)_2 + 12H_2O$$

Anhydrous alum

ASTRINGENTS

ALUM

Preparation:

- Addition of hot conc. soln of potassium sulphate to a hot soln of aluminium sulphate

$$Al_2(SO_4)_3 + K_2SO_4 + 24H_2O \longrightarrow 2\,KAl(SO_4)_2.\,12H_2O$$

Assay:

- Principle: gravimetric method
- Aq. soln of sample is added in soln of ammonia and ammonium chloride solution.
- Ppt of aluminium oxide (Al_2O_3) is formed
- Ppt are filtered, washed with ammonium nitrate soln, and than dried and weighed.
- factor: 1.0g residue ≡ 9.307 g of alum

ASTRINGENTS

ALUM

Use:

- Astringent
- Pharmaceutical aid
- As a styptic for minor cuts and abrasions
- Preparation of diphtheria and tetanus toxoids

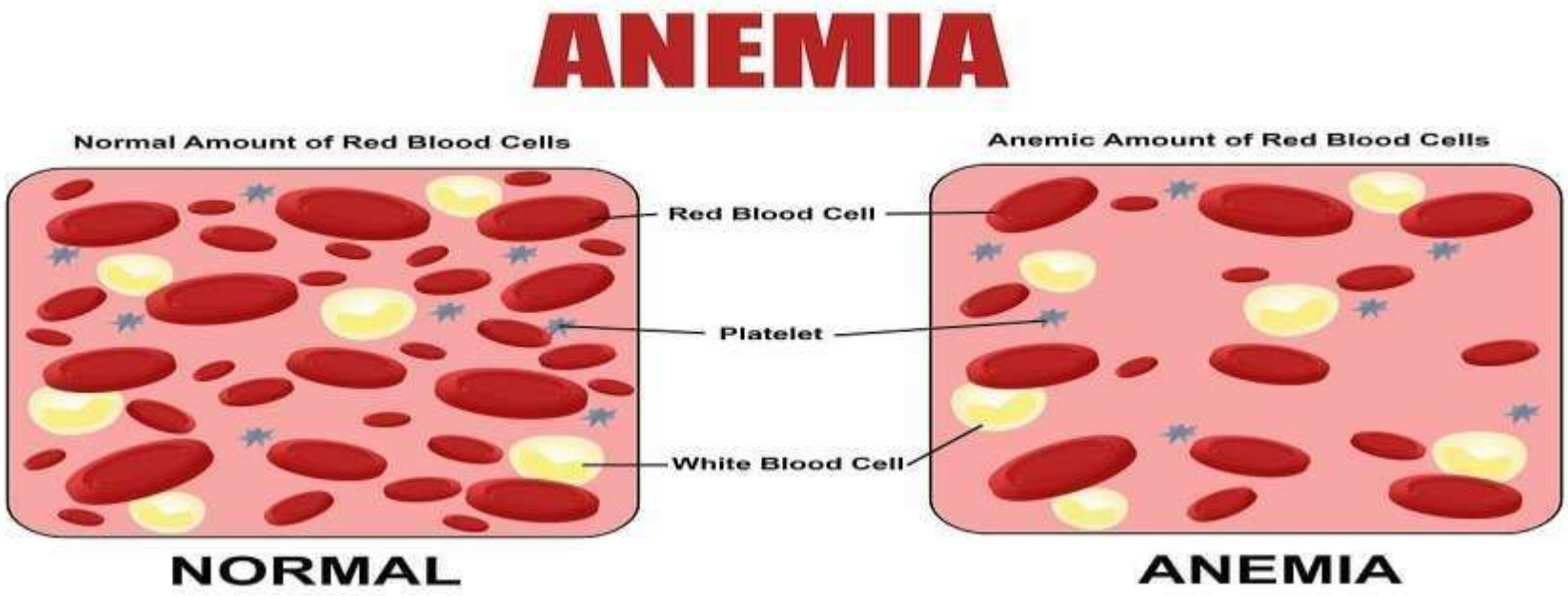
Storage:

- Well closed container

styptic - A drug that causes contraction of body tissues

Haematinics

- These are the substances required in the formation of blood, and are used in the treatment of anaemias
- Anaemia: a condition in which there is a deficiency of red cells or of haemoglobin in the blood, resulting in pallor and weariness
- Balance between production and destruction of RBCs are disturbed:



Haematinics

- The total body content of iron is estimated to be about 3 – 4g
- Major portion (70%) of body iron is present in haemoglobin and myoglobin.
- Haemoglobin helps to carry oxygen from lungs to tissues & carbon dioxide from tissue to lungs.
- Balance amount of iron consists of storage iron, transport iron & enzyme iron.
- In normal adults about 1 g of iron is lost from the body daily.
- Average daily intake of iron in a normal adult is about 14 mg.

Haematinics

Preferred Compounds:

- Ferrous Sulphate (20% hydrated salt and dried salt 32% or 65 mg)
- Ferrous Gluconate (12% iron or 28 – 36mg).

Other: Ferrous fumerate, Ferrous succinate, Ferric ammonium citrate, Iron hydroxide etc

- Preferred route: Oral
- Dosage: 200 mg daily in 3 divided doses (3 -5mg/Kg for children)

ADRs: Epigastric pain, heart burn, nausea, vomiting, staining of teeth, metallic taste, bloating, colic, Constipation

Ferrous Sulphate

- Description: Bluish green crystalline powder with metallic & astringent taste.
- It undergoes slow oxidation upon exposure to the moist air to brown coloured ferric sulphate. Hence tablets of ferrous sulphate are sugar coated in order to prevent air oxidation.
- It is soluble in water and insoluble in ethanol
- When heated at higher temp. it decomposes to ferric oxide, SO2 & H2SO4.

$$2(FeSO_4.7H_2O) \rightarrow Fe_2O_3 + SO_2 + H_2SO_4 + 13H_2O$$

- Preparation: $FeSO_4$ is prepared by adding excess of dil H_2SO_4 to iron fillings

$$Fe + H_2SO_4 \rightarrow FeSO_4 + H_2(gas)$$

The crystals of $FeSO_4$ is efflorescent in dry air and exposure to moist air undergoes slow oxidation become coated with brown basic ferric sulphate, to that the salt should be kept in well closed conatiner.

Assay: Refer Analysis Practical manual – Exp. Assay of Ferrous Sulphate.

Ferrous Gluconate

Description: Yellowish grey or pale greenish yellow fine powder having slight odour of burnt sugar.

- It is soluble in water and insoluble in ethanol
- Usual dose is 300 mg thrice a day

Preparation: Gluconic acid is preaped by oxidation of glucose and then dissolving ferrous carbonate in a calculated amount of gluconic acid. $BaCl_2$ is used to precipitagte $BaSO_4$ which is removed by filtration.

Assay: As same as $FeSO_4$. It is assayed by use of ceric ammonium sulphate.

Uses: To treat iron deficiency anaemia

Poison & Antidote

The most common poisons:

- Pain medicine, Iron pills, anticonvulsants & antidepressants
- Cleaning substances (household substances) e.g. Antimicrobials, Toilet cleaners, rust removers,
- Pesticides e.g. chemicals to kill bugs & other pests
- Cosmetics & personal care products e.g. nail glue remover & nail primer, coloring substances like lipsticks, nail polish etc
- Topical preparations e.g. topical anesthetics which keeps the blood from carrying oxygen to the tissues.
- Bites and stings e.g. snakes, ticks, spider, bees, insects bites etc or also called Venom (Animal poison delivered subcutaneously)
- Carbon monoxide & hydrocarbons like gasoline, kerosene, lighter fluid, furniture polish & paint thinner

Poison:

- Any substance that is harmful to your body or any substance that cause disturbances in organisms.
- You might swallow it, inhale it, inject it or absorb through skin.

Background

In regard to poison sources, poisons (chemicals) can be divided into three broad groups:

1. Agricultural and industrial chemicals
2. Drugs and health care products
3. Biological poisons

1. Agricultural and industrial chemicals

- The majority of agricultural chemicals are pesticides, which include insecticides, herbicides, fungicides, fumigants, and rodenticides.
- The term industrial chemicals is used to refer to chemicals used in industry, like hydrocarbons, chlorinated hydrocarbons, aldehydes, ketones, alcohols, esters etc. as well as chemicals found in or near households.
- Poisoning with industrial chemicals occurs most often by either through skin (percutaneous) or inhalation routes.

2. Drugs and health care products

- Pain medicine, Iron pills, anticonvulsants & antidepressants & cosmetics etc

3. Biotoxins or Biological poisons: can be conveniently grouped into 3 major categories:

1.Microbial toxins- poisons produced by bacteria like blue green algae, dinoflagellates, golden brown algae etc.

2. Phytotoxins-poisons produced by plants .

3. Zootoxins-Poisons produced by animal.

Substances used to counteract the action of these chemicals or form of poisoing are called

"Antidote"

Antidote

Types of Antidotes based on mechanism of action:

- Physiological antidote (Counteracts the physiological effects of poison): e.g. Sodium Nitrite converts haemoglobin into methemoglobin in order to bind cyanide and converts more toxic cyanide to less toxic cyanomethemoglobin.
- Nitrite + oxyhemoglobin → methemoglobin + cyanide

(toxic)

↓

Cyanomethemoglobin

(less toxic)

Antidote

Types of Antidotes based on mechanism of action:

- Mechanical Antidote (prevents the Absorption of the poison into the poison into the body): E.g. Activated charcoal adsorbs the poison prior absorption by means inactivates and precipitates the toxic materials by insoluble salts, thereby preventing their absorption.
- Chemical antidote (Acts by changing the Chemical nature of the poison): e.g., sodium thiosulphate causes conversion of systematic Toxic cyanide to non-toxic thiocyanide.

Antidotes in most common use:

- Cyanide poisoning: Sodium Nitrite ($NaNO_2$), Sodium Thiosulphate ($Na_2S_2O_3$)
- Universal Antidote: Activated Charcoal

Activated Charcoal

- It is similar to common charcoal but is made especially for medical use.
- During its preparation the wood charcoal is treated with various substances like steam, air, CO_2, Oxygen, $ZnCl_2$, Sulphuiric acid or phosphoric acid - helps charcoal to develop many internal spaces or "pores".
- These pores help activated charcoal trap chemicals – strong adsorptive agent.

Activated Charcoal

- It is an odourless, tasteless, fine black powder.
- It is **NOT** soluble in water or any other solvents.
- Helpful to absorbed effectively all heavy metals, viruses, bacterial & fungal toxins by binding poisons within the GIT. This allows their transfer out of the body in a harmless form.
- The most common drug poisoning is from aspirin, charcoal should be given within the first 30 min of an overdose or aspirin poisoning.
- Powdered form of it reaches its maximum rate of absorption rapidly within one min compare to other forms.
- It is mainly known both for its use in drug overdoses and chemical poisonings.
- It act to purify & cleanse the body due to its amazing ability to attract poisons to itself.

- **Uses:**
- Activated charcoal is used in treating poisoning and overdoses through oral injection.
- It is also used in air purification, gold purification, metal extraction, water purification, medicine, sewage treatment, air filters in gas masks and to treat a condition of pregnancy in which the normal flow of bile is affected (cholestasis), Prevent gas, Reduce high cholesterol & to Prevent a hangover.
- **Side effects:** constipation and black stools. Rare, side effects are a slowing or blockage of the intestinal tract & dehydration. Stomach cramps & swelling of the abdomen.
- **Dose:** adults :25-100g & children:25-50g

Sodium Nitrite

- Sodium nitrite is the inorganic compound with the chemical formula $NaNO_2$. It is a white to slightly yellowish crystalline powder that is very soluble in water and is hygroscopic.itcontains not less than 97.0% & not more than 101.0% of sodium nitrite. It can act as both oxidising & reducing agent.
- When its reacts with oxidising agent, nitric oxide (NO) is the product in acidic solution & nitrogen gas is in neutral to alkaline solutoin.

Uses: it is used as a antidote for cyanide poisoning

Side effects: head pain, blurred vision.

Dose: 30 –120mg.

Sodium Nitrite

- It give pale brown fumes of nitrous acid with dilute acids

$2NaNO_2 + H_2SO_4 \rightarrow Na_2SO_4 + 2\ HNO_2$

$3HNO_2 \leftrightarrow HNO_3 + 2\ NO\uparrow + H_2O$

$2NO + O_2 \rightarrow 2NO_2$

Method of Analysis: About 10 ml of 1% w/v aqueous solution is added by a pipette to 0.1 N $KMnO_4$ soluton acidified with sulfuric acid. The formed unstable nitrous acid (HNO_2) readily breaks down into nitric oxide & nitrogen dioxide.

$$2HNO_2 \rightarrow NO + NO_2 + H_2O$$

Sodium Nitrite

- Nitrous acid also behaves as a reducing agent for it may be oxidised to nitric acid by KMnO4.
- A known excess 25 ml of 0.1N oxalic acid is added & the mixture, after it is heated is back titrated with 0.1N KMnO4. Oxalic acid is uantitatively oxidised on warming.

$$NaNO_2 + H_2SO_4 \rightarrow HNO_2 + NaHSO_4$$

$$HNO_2 + O \rightarrow HNO_3$$

$$H_2C_2O_4 + O \rightarrow H_2O + 2CO_2\uparrow$$

Factor: Each ml of 0.1 N KMnO4 is equivalent to 0.00345 g of NaNO2.

Sodium Nitrite ($NaNO_2$)

- The main **USE** of sodium nitrite is for the industrial production of organonitrogen compounds.
- It is a reagent for conversion of amines into diazo compounds, which are key precursors to many dyes, such as diazo dyes.
- Nitroso compounds are produced from nitrites. These are used in the rubber industry.
- sodium nitrite is used as spoilage or disease causing microorganisms.
- its effectiveness depends on several factors including residual nitrite level, pH, salt concentration, reductants present and iron content.

Sodium Thiosulphate

- Sodium thiosulfate is a colorless, water-soluble salt. It is a calcium-chelating agent and has many medical uses in removing toxic substances from the body.
- Depending on the medical condition being treated, the salt can be injected, taken orally, or applied to the skin. It is also used in some vital tests for kidney patients to glean information without doing harm.
- This compound is combined with sodium nitrite and used as an antidote for cyanide poisoning in both children and adults.
- Sodium thiosulfate reacts with the cyanide and forms sodiumthioyanate, a nontoxic substance that can then be excreted harmlessly from the body.
- The salt is also used to treat arsenic poisoning. Studies show that it can be useful in removing excess copper from patients too.

Sodium Thiosulphate ($Na_2S_2O_3$)

- **Uses:** It is used as an antidote to cyanide poisoning. Thiosulfate acts as a sulfur donor for the conversion of cyanide to thiocyanate (which can then be safely excreted in the urine), catalyzed by the enzyme rhodanase.
- **Side effects:** muscle cramps, blurred vision, pain in joints & vomiting.
- **Dose**: 12.5gms in adults…..and 7gms for children

Sodium Thiosulphate

Preparation

- Few grams of the sodium carbonate are dissolved in 300ml of hot water & about five-sixths of the solution is placed in one flask and the remainder in another flask.
- Those flaks are connected in series so the sulfur dioxide gas may be passed first into the larger volume of solution, & what is there unabsorbed may pass on through the second flask.

$$Na_2CO_3 + 2SO_2 + H_2O \rightarrow 2NaHSO_3 + CO_2$$

- After a short time a marked foaming occurs in the first flask due to the escape of carbon dioxide and after this foaming ceases a similar frothing soon commences in the second flask.

Sodium Thiosulphate

Preparation

- The obtained solution of sodium bisulfite is place in a 600 ml beaker and cautiously treated with 53 g of sodium carbonate.

$$2NaHSO_3 + Na_2CO_3 \rightarrow 2Na_2SO_3 + CO_2 + H_2O$$

- The solution is boiled for 15 min then is treated with 48 g powdered sulfur wetted with small amount of ethanol and gently boiled under reflux for an hr & a half or longer.

$$Na_2SO_3 + S \rightarrow 2Na_2S_2O_3$$

- The obtained solution is filtered & the solution is left at room temperature in a crystallizing dish to set to crystallize.

Sodium Thiosulphate

Method of Analysis

Assay: Sodium thiosulpahte can analyse by using ceric ammonium sulphate and KMnO4 solution.

Reference:

Please refer: Pharmaceutical Analysis Practical Observation cum Manual book for more details.

End of Unit IV

Chapter 13

The Respiratory System

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Function of the Respiratory System

- Oversees gas exchanges (oxygen and carbon dioxide) between the blood and external environment
- Exchange of gasses takes place within the lungs in the alveoli(only site of gas exchange, other structures passageways
- Passageways to the lungs purify, warm, and humidify the incoming air
- Shares responsibility with cardiovascular system

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Organs of the Respiratory system

- Nose
- Pharynx
- Larynx
- Trachea
- Bronchi
- Lungs – alveoli

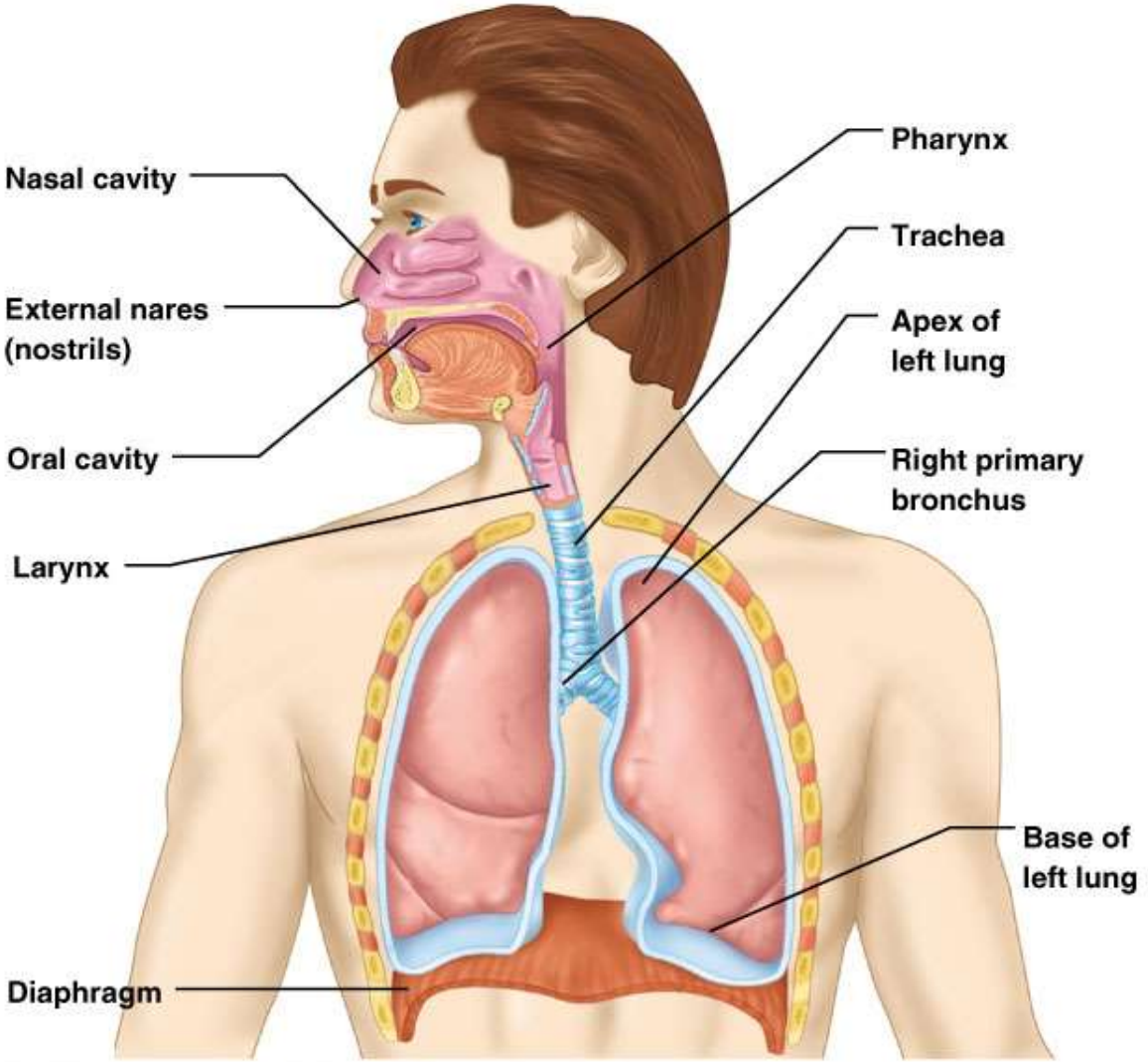

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Figure 13.1 *Slide 13.1*

Upper Respiratory Tract

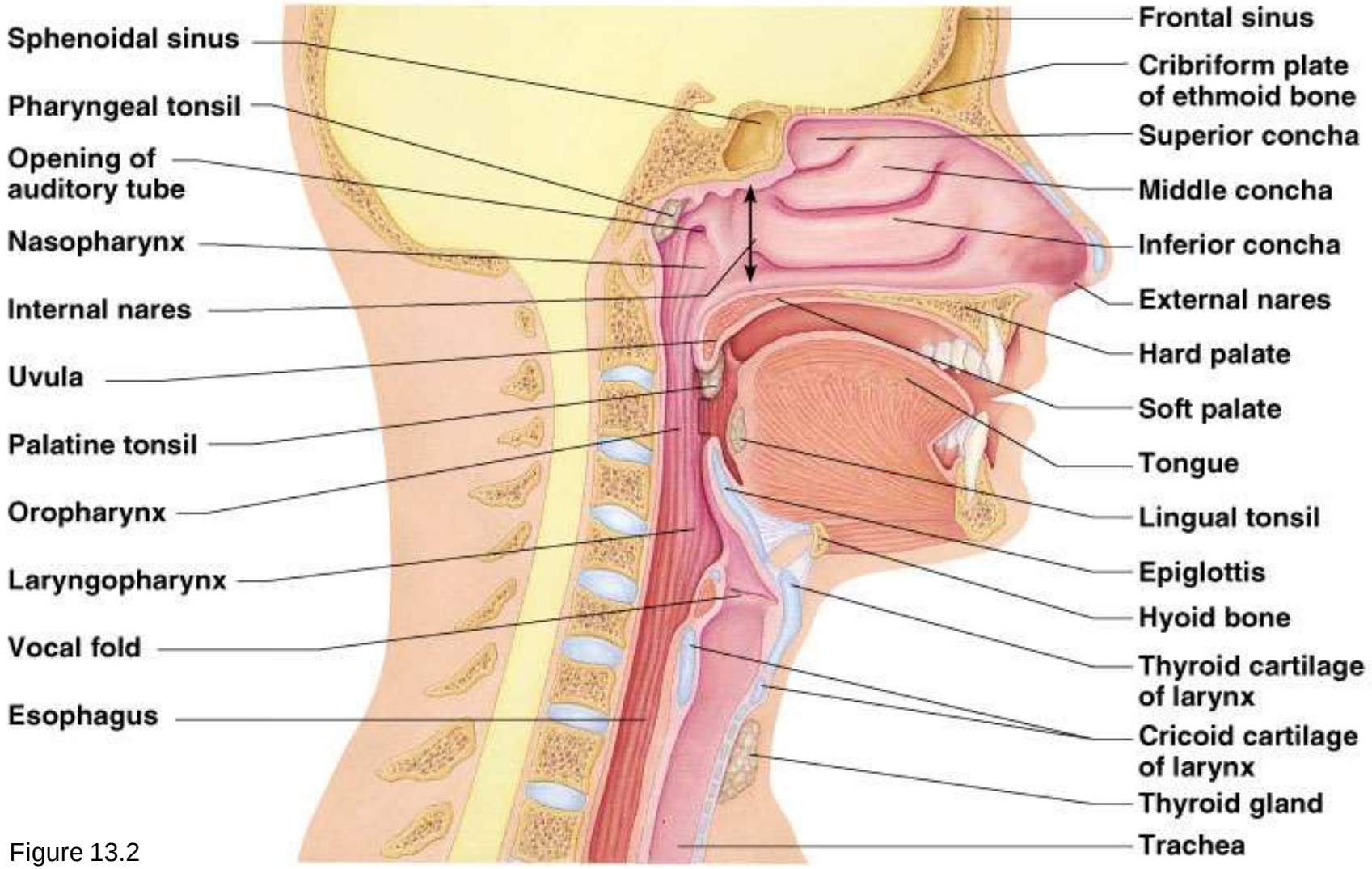

Figure 13.2

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Anatomy of the Nasal Cavity

- Olfactory receptors are located in the mucosa on the superior surface
- The rest of the cavity is lined with respiratory mucosa
  - Moistens air
  - Traps incoming foreign particles

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Anatomy of the Nasal Cavity

- Lateral walls have projections called conchae
  - Increases surface area
  - Increases air turbulence within the nasal cavity
- The nasal cavity is separated from the oral cavity by the palate
  - Anterior hard palate (bone)
  - Posterior soft palate (muscle)

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Paranasal Sinuses

- Cavities within bones surrounding the nasal cavity
  - Frontal bone
  - Sphenoid bone
  - Ethmoid bone
  - Maxillary bone

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Paranasal Sinuses

- Function of the sinuses
  - Lighten the skull
  - Act as resonance chambers for speech
  - Produce mucus that drains into the nasal cavity

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Pharynx (Throat)

- Muscular passage from nasal cavity to larynx
- Three regions of the pharynx
  - Nasopharynx – superior region behind nasal cavity
  - Oropharynx – middle region behind mouth
  - Laryngopharynx – inferior region attached to larynx
- The oropharynx and laryngopharynx are common passageways for air and food

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Upper Respiratory Tract

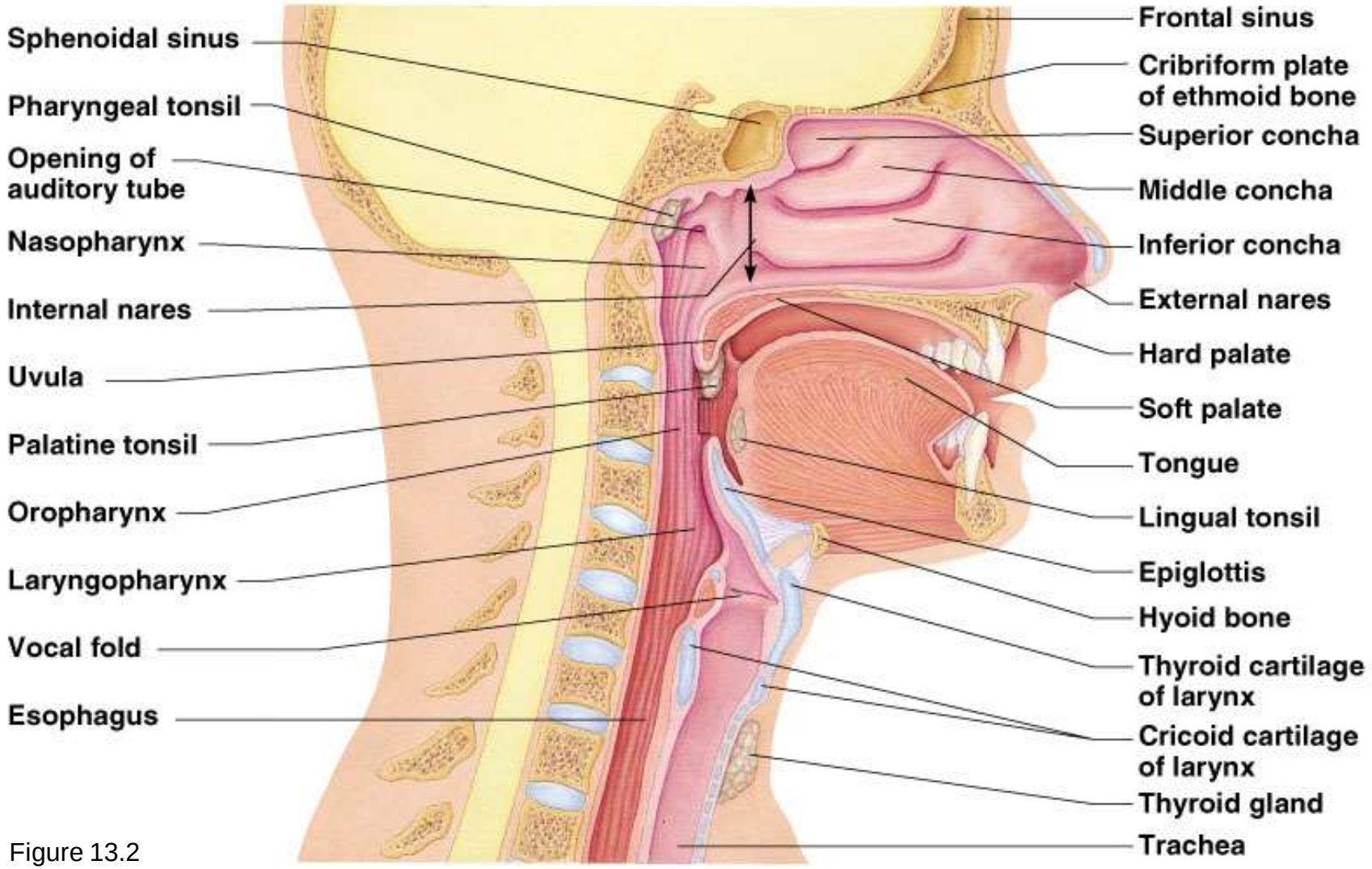

Figure 13.2

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Structures of the Pharynx

- Auditory tubes enter the nasopharynx
- Tonsils of the pharynx
  - Pharyngeal tonsil (adenoids) in the nasopharynx
  - Palatine tonsils in the oropharynx
  - Lingual tonsils at the base of the tongue

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Larynx (Voice Box)

- Routes air and food into proper channels
- Plays a role in speech
- Made of eight rigid hyaline cartilages and a spoon-shaped flap of elastic cartilage (epiglottis)

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Structures of the Larynx

- Thyroid cartilage
  - Largest hyaline cartilage
  - Protrudes anteriorly (Adam's apple)
- Epiglottis
  - Superior opening of the larynx
  - Routes food to the larynx and air toward the trachea

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Structures of the Larynx

- Vocal cords (vocal folds)
  - Vibrate with expelled air to create sound (speech)
- Glottis – opening between vocal cords

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Trachea (Windpipe)

- Connects larynx with bronchi
- Lined with ciliated mucosa
  - Beat continuously in the opposite direction of incoming air
  - Expel mucus loaded with dust and other debris away from lungs
- Walls are reinforced with C-shaped hyaline cartilage

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Primary Bronchi

- Formed by division of the trachea
- Enters the lung at the hilus (medial depression)
- Right bronchus is wider, shorter, and straighter than left
- Bronchi subdivide into smaller and smaller branches

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Lungs

- Occupy most of the thoracic cavity
  - Apex is near the clavicle (superior portion)
    - Base rests on the diaphragm (inferior portion)
  - Each lung is divided into lobes by fissures
    - Left lung – two lobes
    - Right lung – three lobes

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Lungs

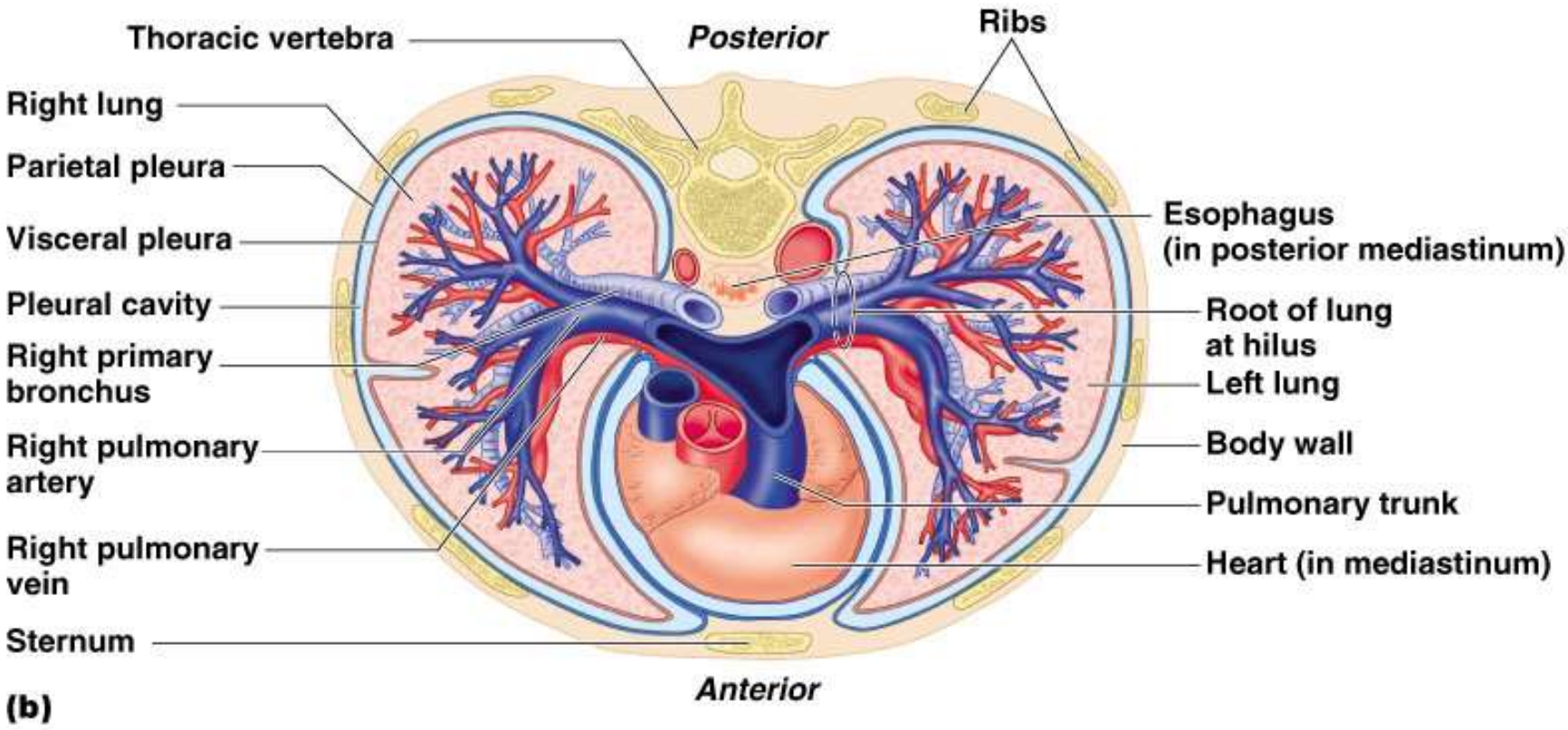

Figure 13.4b

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Coverings of the Lungs

- Pulmonary (visceral) pleura covers the lung surface
- Parietal pleura lines the walls of the thoracic cavity
- Pleural fluid fills the area between layers of pleura to allow gliding

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Respiratory Tree Divisions

- Primary bronchi
- Secondary bronchi
- Tertiary bronchi
- Bronchioli
- Terminal bronchioli

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Bronchioles

- Smallest branches of the bronchi

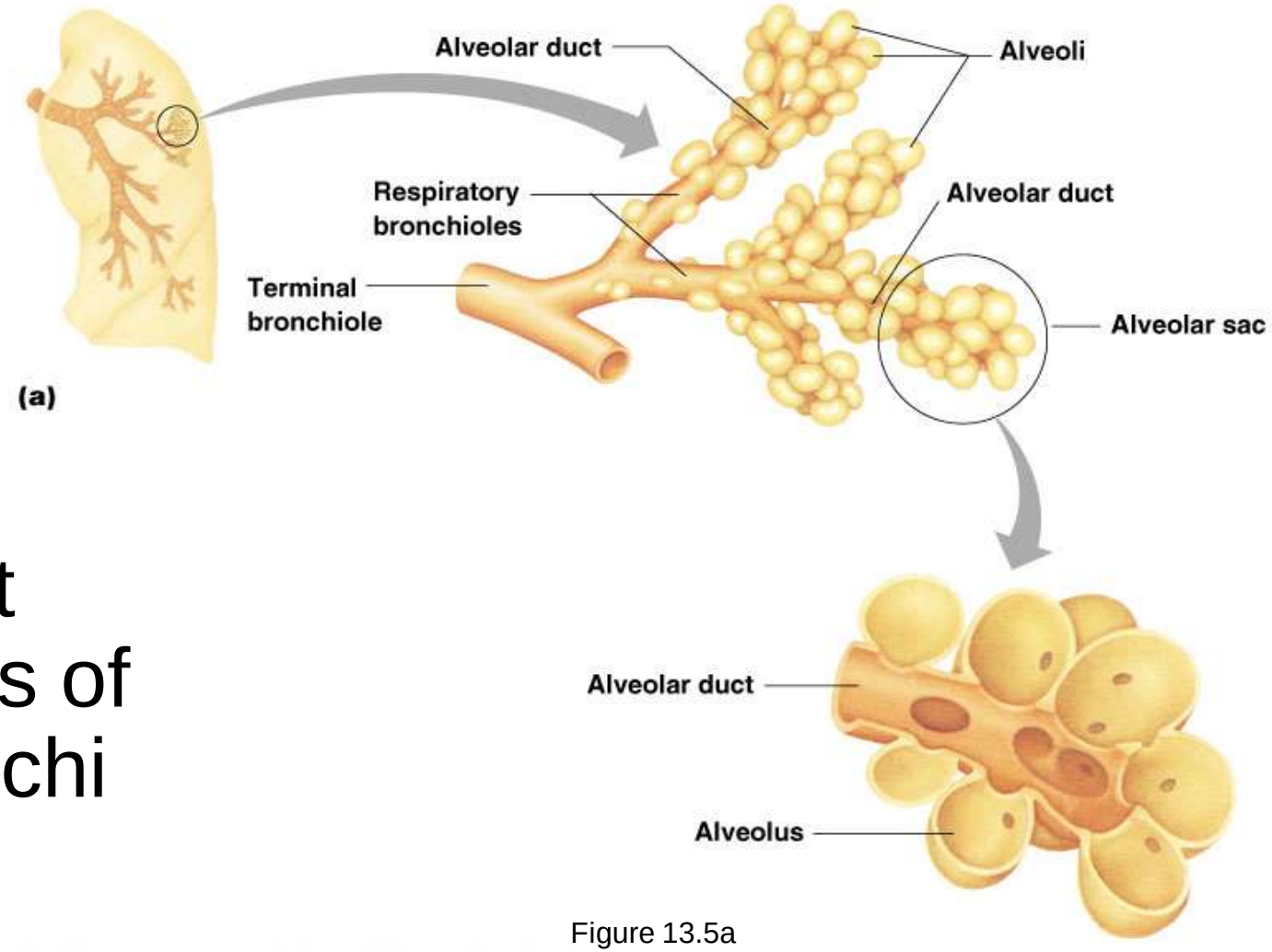

Figure 13.5a

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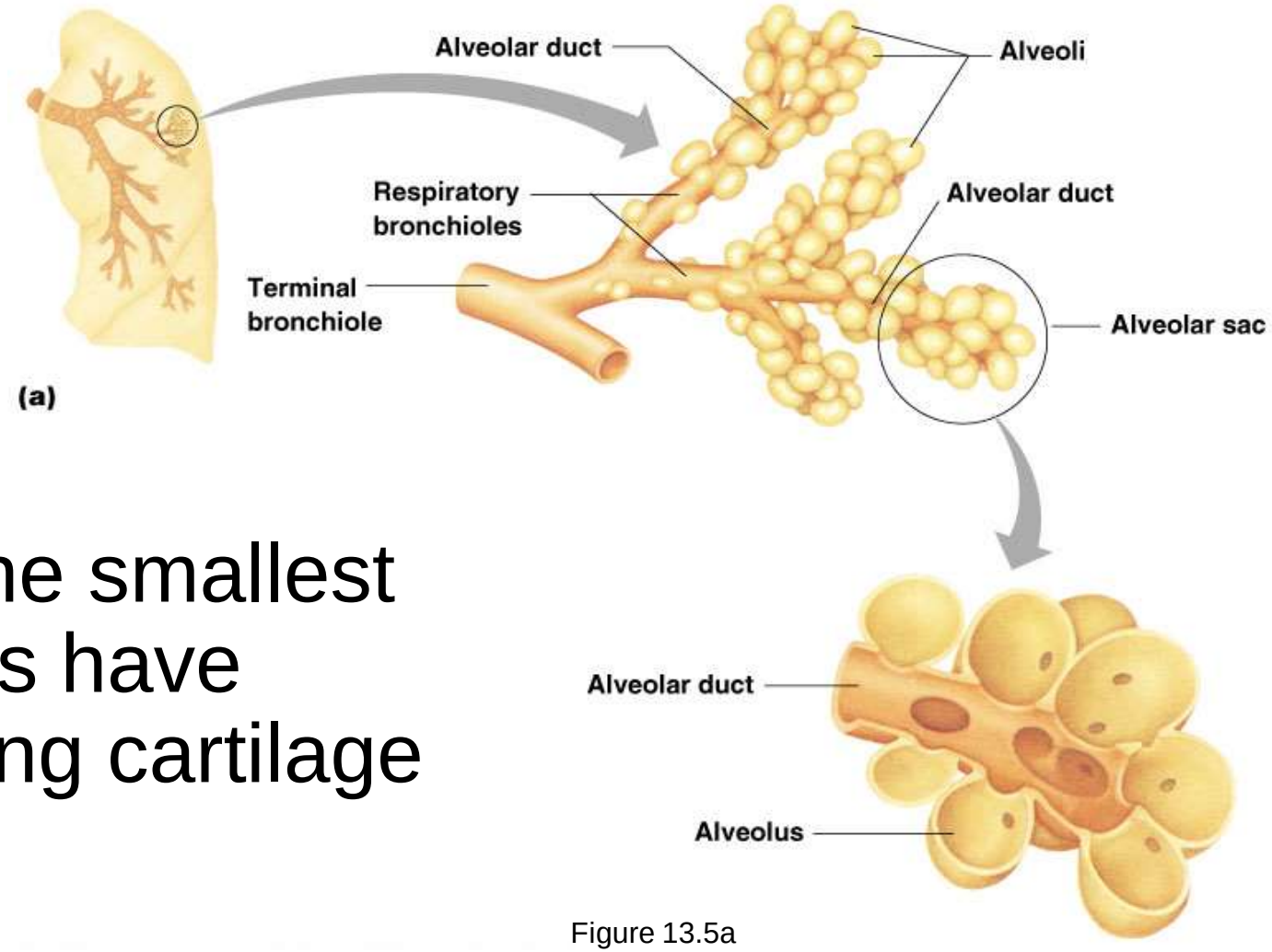
Bronchioles



- All but the smallest branches have reinforcing cartilage

Figure 13.5a

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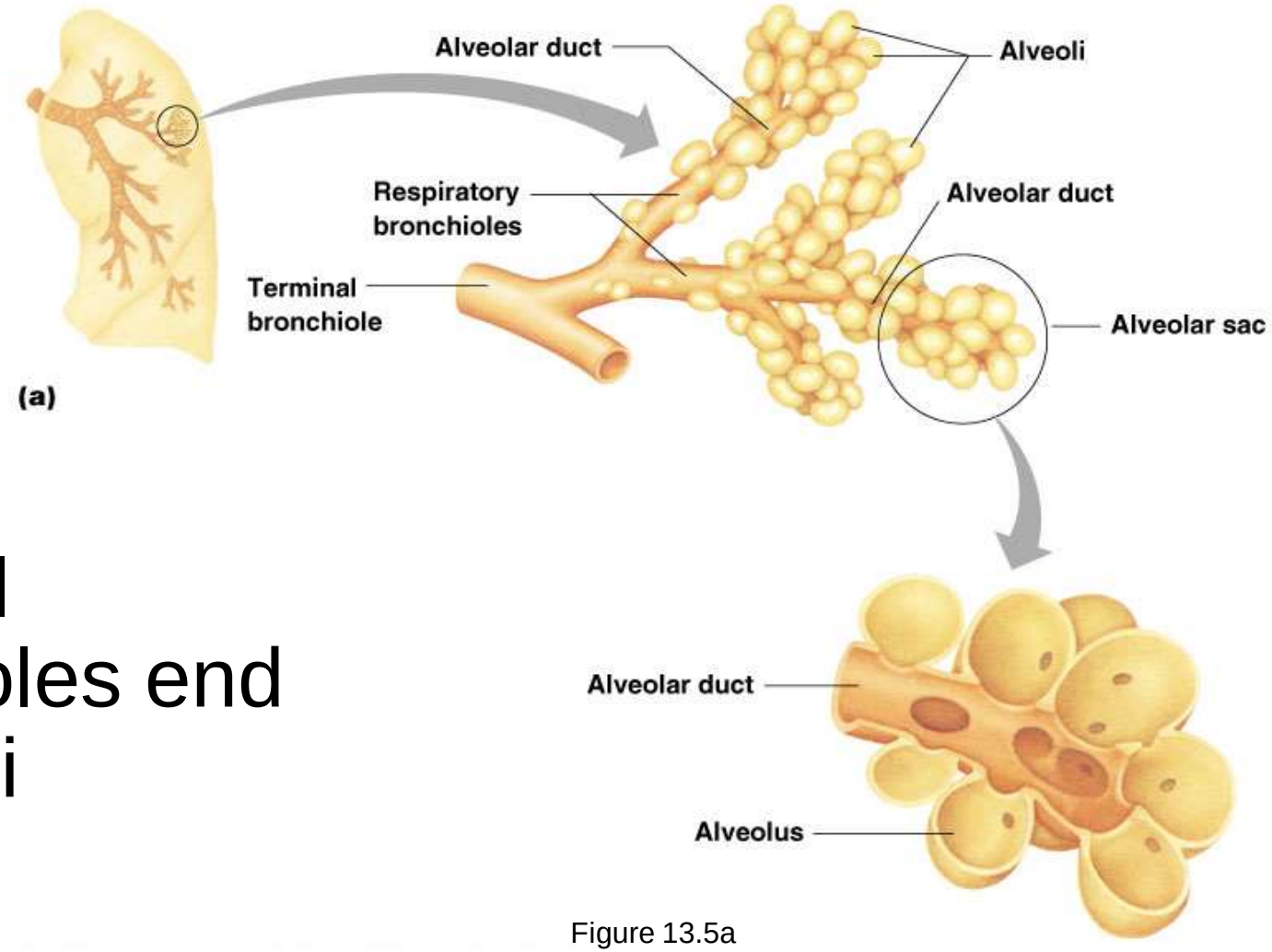
Bronchioles



- Terminal bronchioles end in alveoli

Figure 13.5a

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Respiratory Zone

- Structures
  - Respiratory bronchioli
  - Alveolar duct
  - Alveoli
- Site of gas exchange

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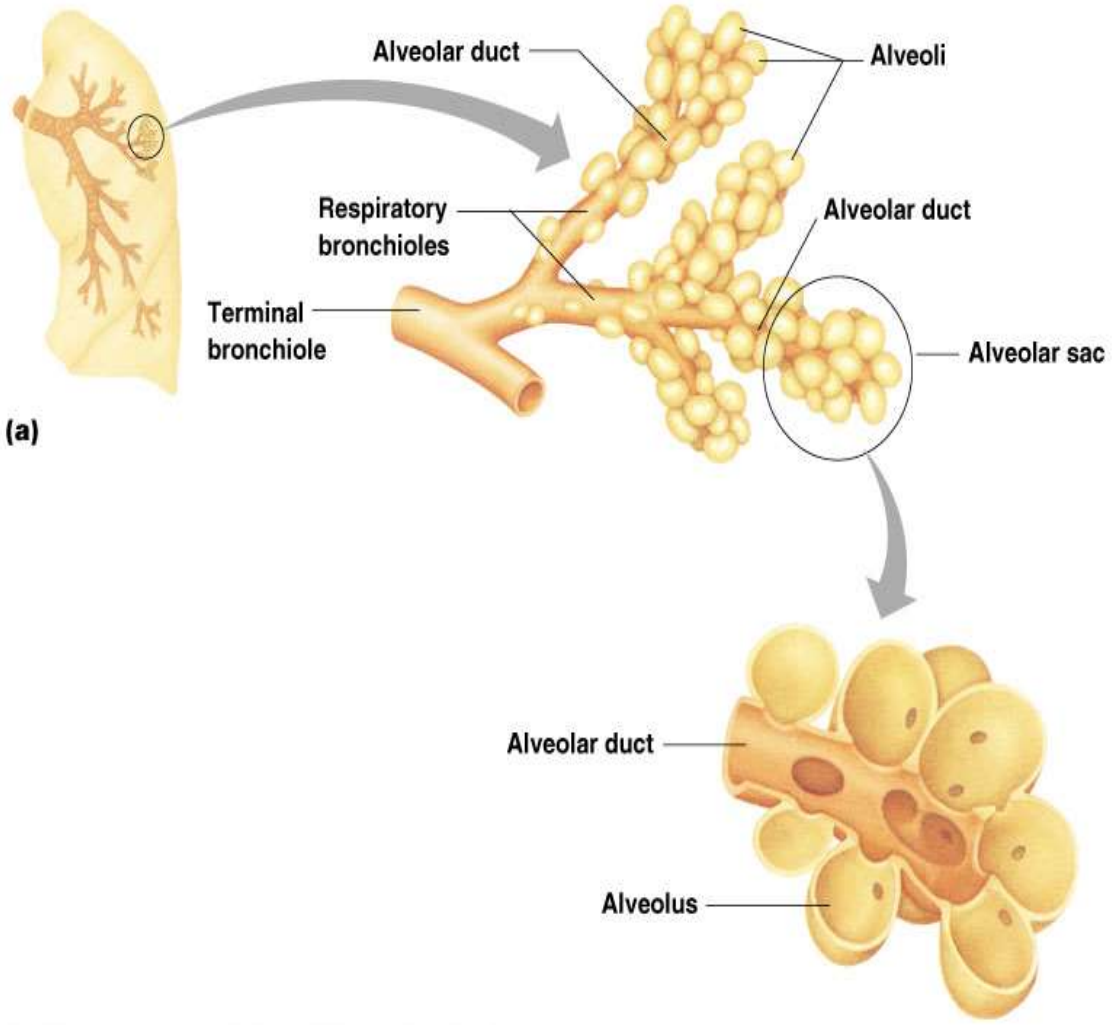
Alveoli

- Structure of alveoli
  - Alveolar duct
  - Alveolar sac
  - Alveolus
  - Gas exchange



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Respiratory Membrane (Air-Blood Barrier)

- Thin squamous epithelial layer lining alveolar walls
- Pulmonary capillaries cover external surfaces of alveoli

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Respiratory Membrane (Air-Blood Barrier)

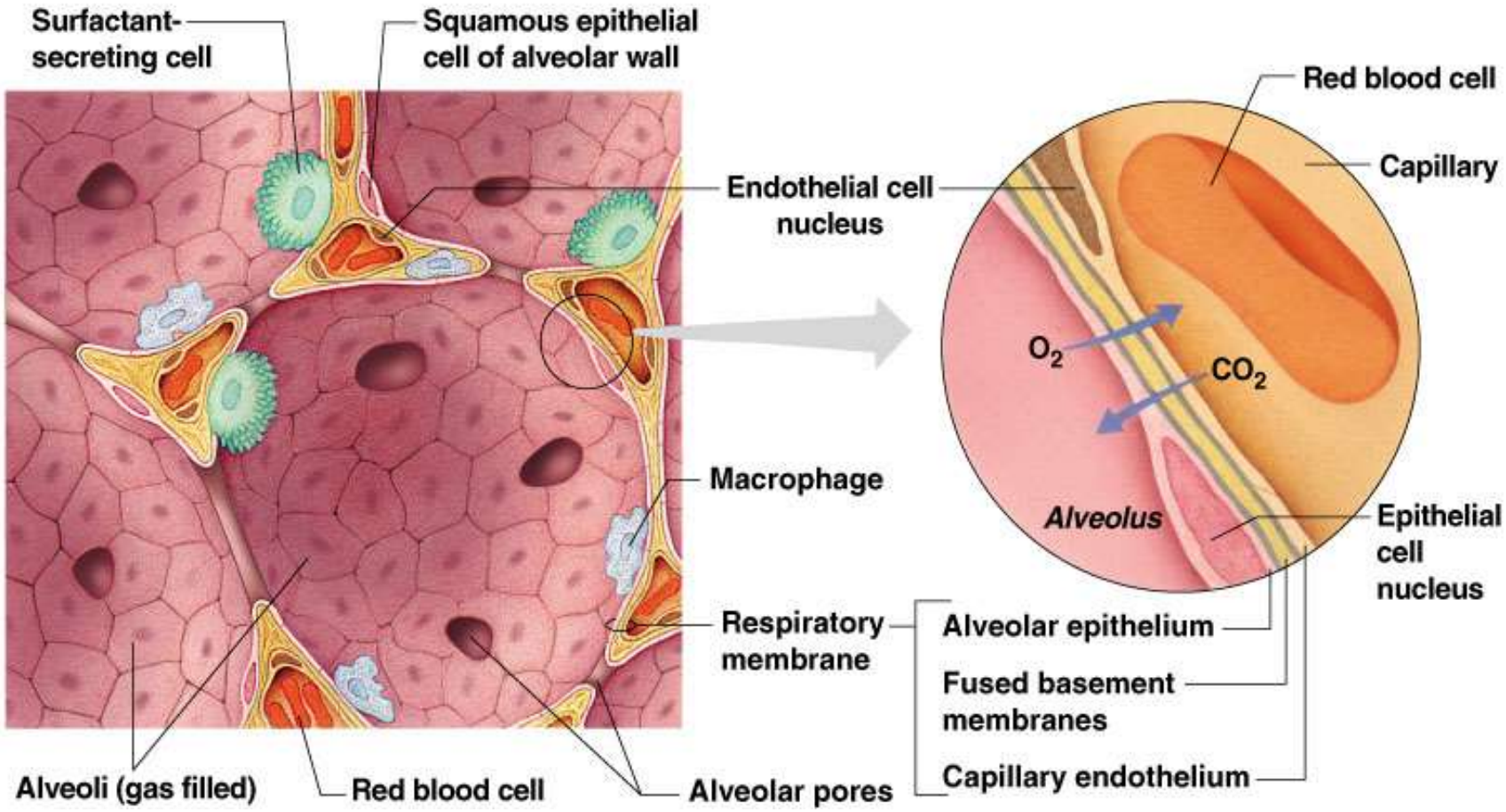

Figure 13.6

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Gas Exchange

- Gas crosses the respiratory membrane by diffusion
  - Oxygen enters the blood
  - Carbon dioxide enters the alveoli
- Macrophages add protection
- Surfactant coats gas-exposed alveolar surfaces

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Events of Respiration

- Pulmonary ventilation – moving air in and out of the lungs
- External respiration – gas exchange between pulmonary blood and alveoli

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Events of Respiration

- Respiratory gas transport – transport of oxygen and carbon dioxide via the bloodstream
- Internal respiration – gas exchange between blood and tissue cells in systemic capillaries

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Mechanics of Breathing (Pulmonary Ventilation)

- Completely mechanical process
- Depends on volume changes in the thoracic cavity
- Volume changes lead to pressure changes, which lead to the flow of gases to equalize pressure

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Mechanics of Breathing (Pulmonary Ventilation)

- Two phases
  - Inspiration – flow of air into lung
  - Expiration – air leaving lung

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Inspiration

- Diaphragm and intercostal muscles contract
- The size of the thoracic cavity increases
- External air is pulled into the lungs due to an increase in intrapulmonary volume

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Inspiration

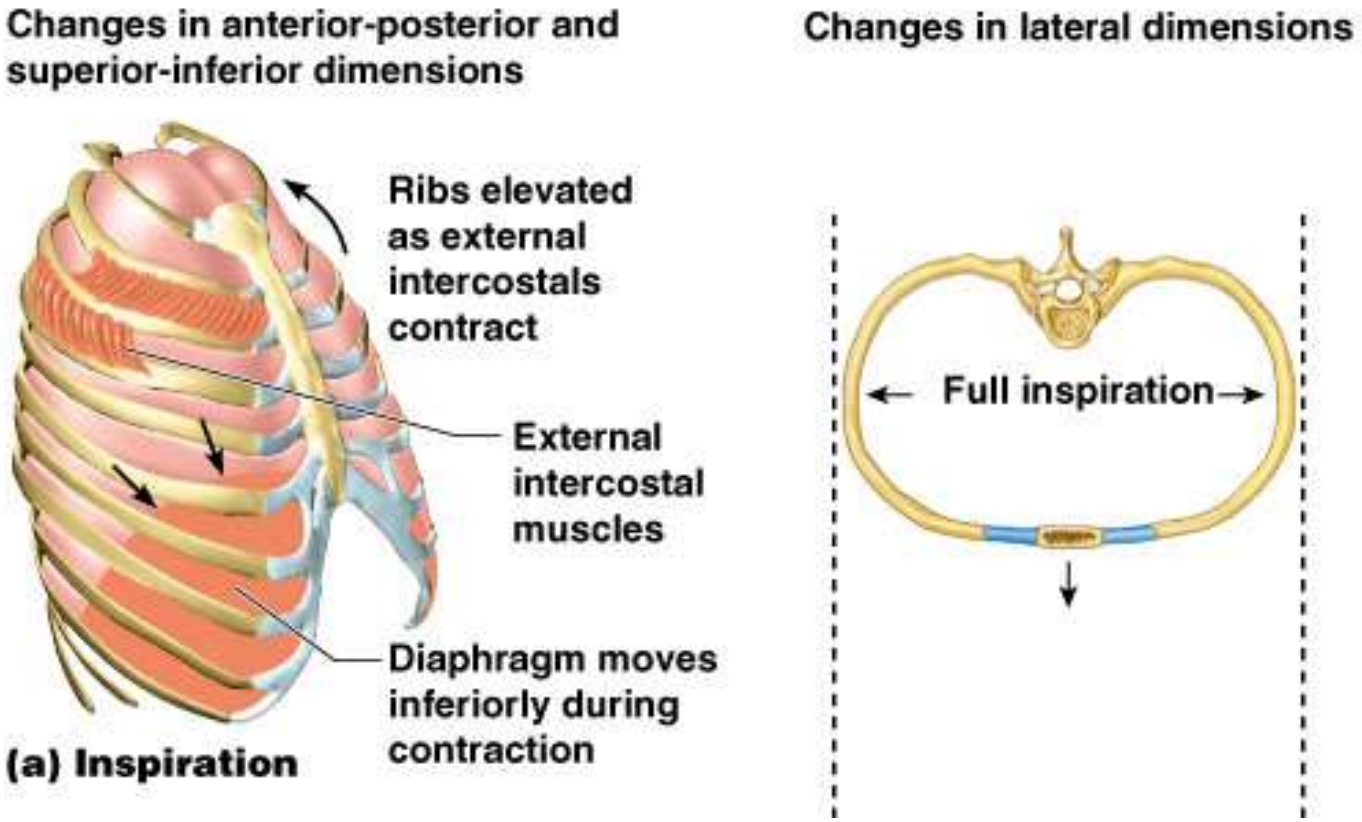

Figure 13.7a

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Exhalation

- Largely a passive process which depends on natural lung elasticity
- As muscles relax, air is pushed out of the lungs
- Forced expiration can occur mostly by contracting internal intercostal muscles to depress the rib cage

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Exhalation

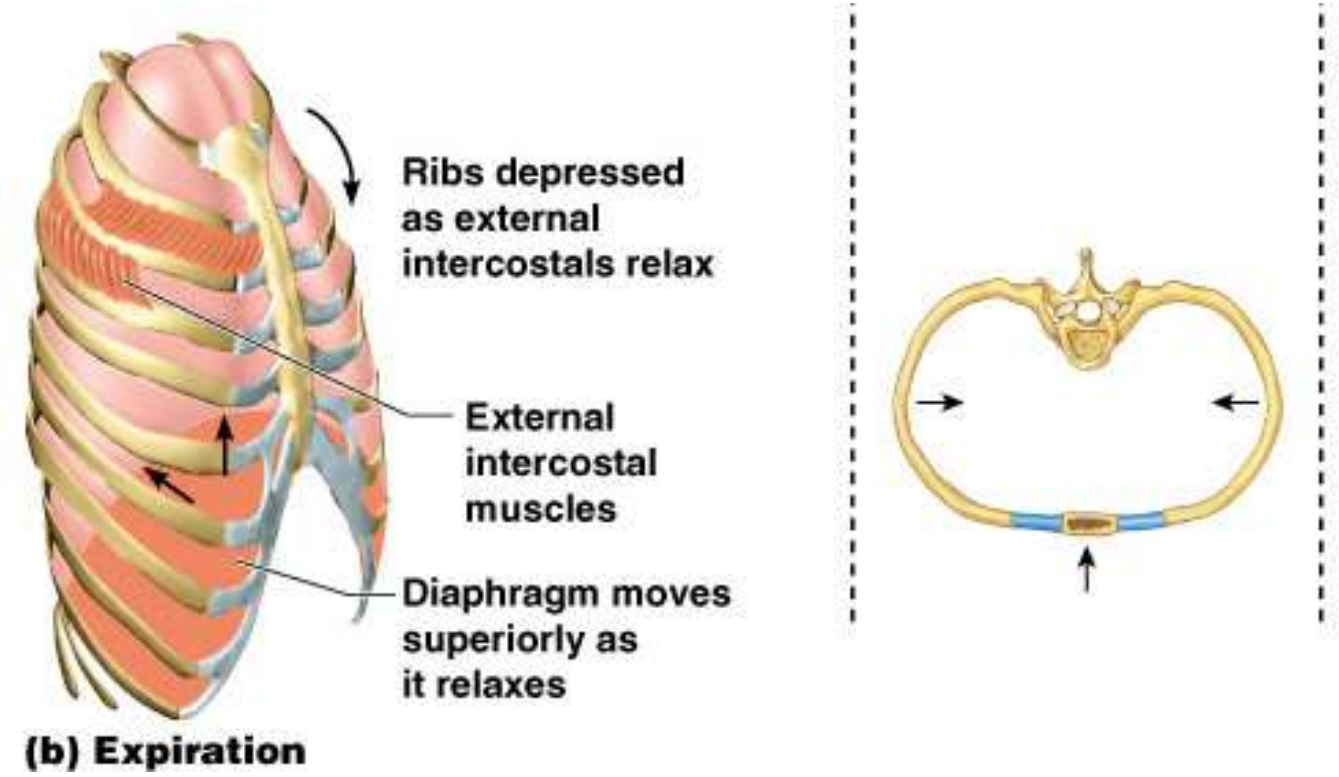

Figure 13.7b

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Nonrespiratory Air Movements

- Can be caused by reflexes or voluntary actions
- Examples
  - Cough and sneeze – clears lungs of debris
  - Laughing
  - Crying
  - Yawn
  - Hiccup

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Respiratory Volumes and Capacities

- Normal breathing moves about 500 ml of air with each breath (tidal volume [TV])
- Many factors that affect respiratory capacity
  - A person's size
  - Sex
  - Age
  - Physical condition
- Residual volume of air – after exhalation, about 1200 ml of air remains in the lungs

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Respiratory Volumes and Capacities

- Inspiratory reserve volume (IRV)
  - Amount of air that can be taken in forcibly over the tidal volume
  - Usually between 2100 and 3200 ml
- Expiratory reserve volume (ERV)
  - Amount of air that can be forcibly exhaled
  - Approximately 1200 ml

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Respiratory Volumes and Capacities

- Residual volume
  - Air remaining in lung after expiration
  - About 1200 ml

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Respiratory Volumes and Capacities

- Vital capacity
  - The total amount of exchangeable air
  - Vital capacity = TV + IRV + ERV
  - Dead space volume
    - Air that remains in conducting zone and never reaches alveoli
    - About 150 ml

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Respiratory Volumes and Capacities

- Functional volume
  - Air that actually reaches the respiratory zone
  - Usually about 350 ml
- Respiratory capacities are measured with a spirometer

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Respiratory Capacities

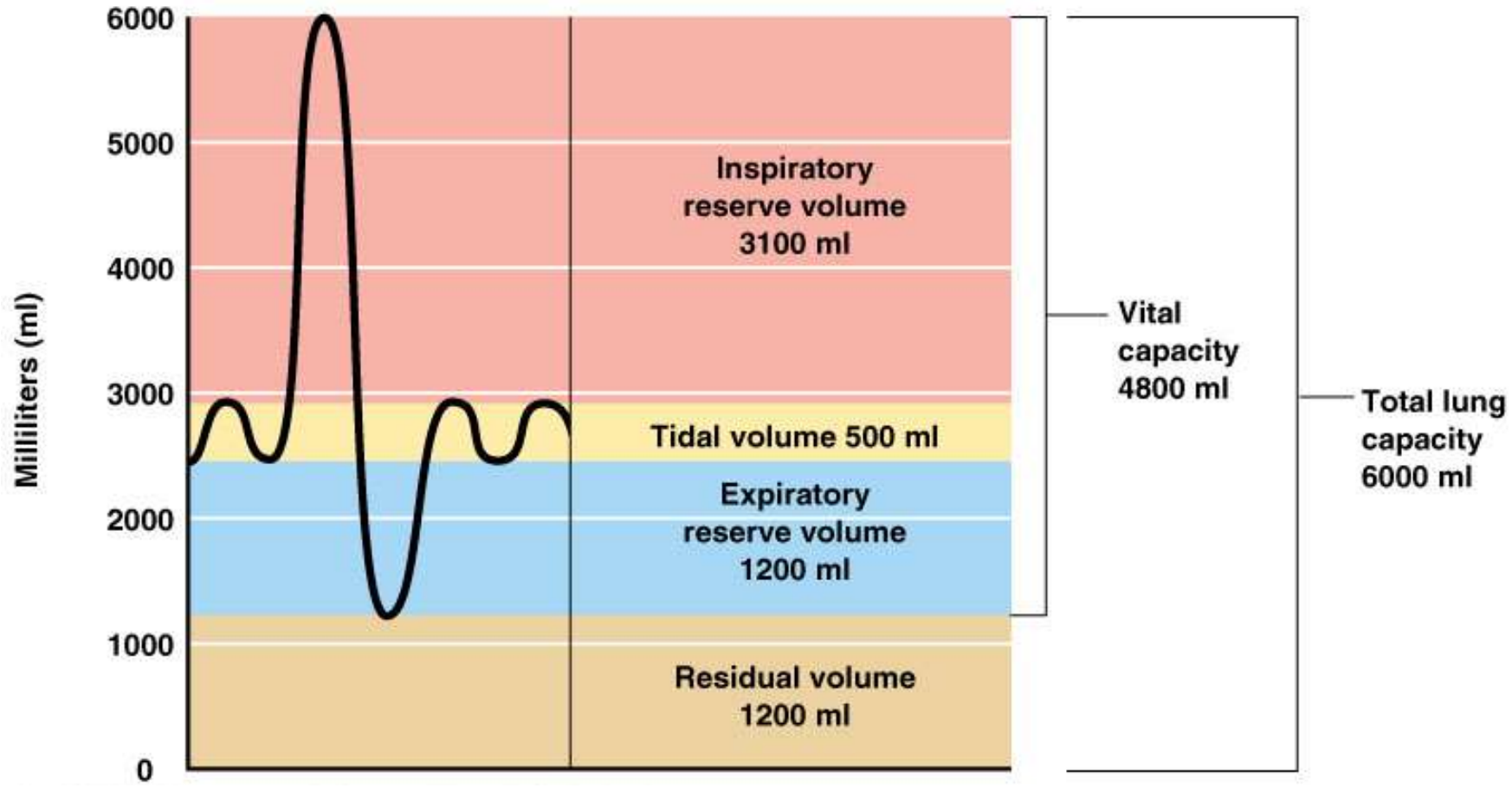

Figure 13.9

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Respiratory Sounds

- Sounds are monitored with a stethoscope
- Bronchial sounds – produced by air rushing through trachea and bronchi
- Vesicular breathing sounds – soft sounds of air filling alveoli

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External Respiration

- Oxygen movement into the blood
  - The alveoli always has more oxygen than the blood
  - Oxygen moves by diffusion towards the area of lower concentration
  - Pulmonary capillary blood gains oxygen

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External Respiration

- Carbon dioxide movement out of the blood
  - Blood returning from tissues has higher concentrations of carbon dioxide than air in the alveoli
  - Pulmonary capillary blood gives up carbon dioxide
- Blood leaving the lungs is oxygen-rich and carbon dioxide-poor

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Gas Transport in the Blood

- Oxygen transport in the blood
  - Inside red blood cells attached to hemoglobin (oxyhemoglobin [HbO_2])
  - A small amount is carried dissolved in the plasma

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Gas Transport in the Blood

- Carbon dioxide transport in the blood
  - Most is transported in the plasma as bicarbonate ion (HCO_3^-)
  - A small amount is carried inside red blood cells on hemoglobin, but at different binding sites than those of oxygen

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Internal Respiration

- Exchange of gases between blood and body cells
- An opposite reaction to what occurs in the lungs
  - Carbon dioxide diffuses out of tissue to blood
  - Oxygen diffuses from blood into tissue

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Internal Respiration

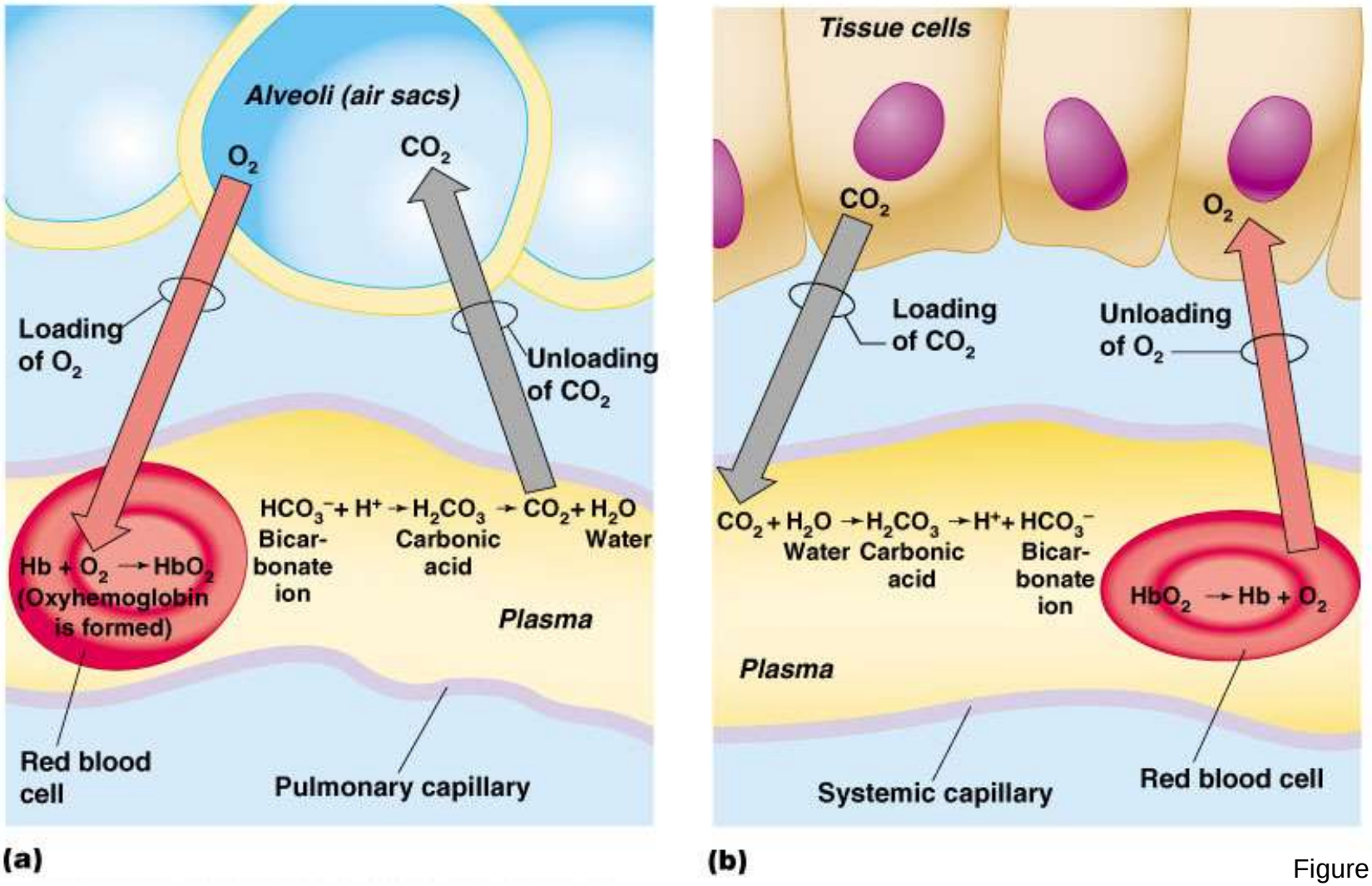

Figure 13.11

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External Respiration, Gas Transport, and Internal Respiration Summary

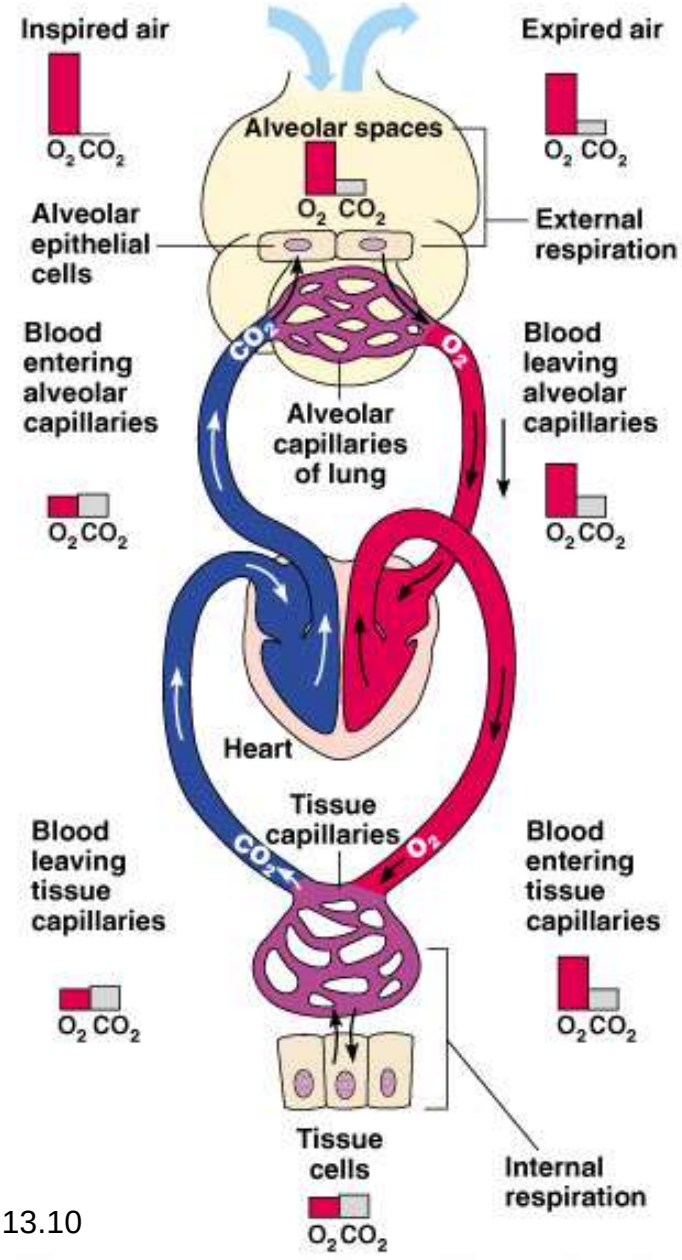

Figure 13.10

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Neural Regulation of Respiration

- Activity of respiratory muscles is transmitted to the brain by the phrenic and intercostal nerves
- Neural centers that control rate and depth are located in the medulla
- The pons appears to smooth out respiratory rate
- Normal respiratory rate (eupnea) is 12–15 respirations per minute
- Hypernia is increased respiratory rate often due to extra oxygen needs

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Neural Regulation of Respiration

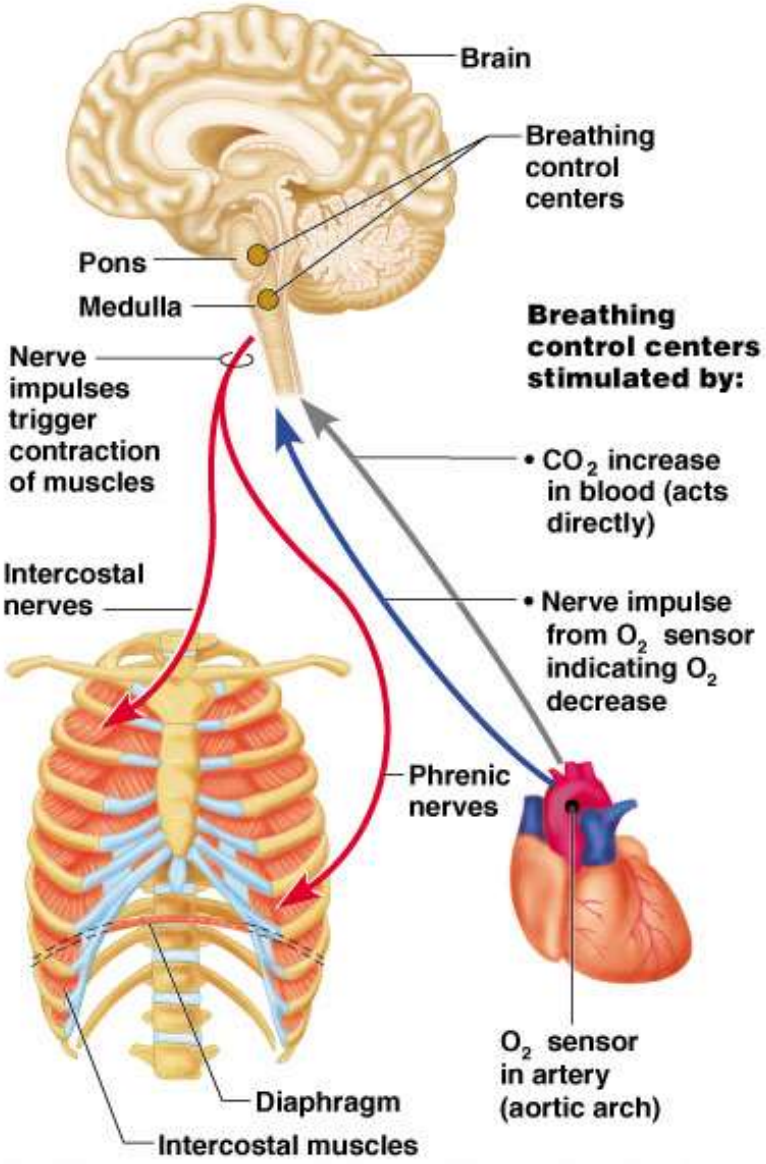

Figure 13.12

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Factors Influencing Respiratory Rate and Depth

- Physical factors
  - Increased body temperature
  - Exercise
  - Talking
  - Coughing
- Volition (conscious control)
- Emotional factors

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Factors Influencing Respiratory Rate and Depth

- Chemical factors
  - Carbon dioxide levels
    - Level of carbon dioxide in the blood is the main regulatory chemical for respiration
    - Increased carbon dioxide increases respiration
    - Changes in carbon dioxide act directly on the medulla oblongata

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Factors Influencing Respiratory Rate and Depth

- Chemical factors (continued)
  - Oxygen levels
    - Changes in oxygen concentration in the blood are detected by chemoreceptors in the aorta and carotid artery
    - Information is sent to the medulla oblongata

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Respiratory Disorders: Chronic Obstructive Pulmonary Disease (COPD)

- Exemplified by chronic bronchitis and emphysema
- Major causes of death and disability in the United States

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Respiratory Disorders: Chronic Obstructive Pulmonary Disease (COPD)

- Features of these diseases
  - Patients almost always have a history of smoking
  - Labored breathing (dyspnea) becomes progressively more severe
  - Coughing and frequent pulmonary infections are common

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Respiratory Disorders: Chronic Obstructive Pulmonary Disease (COPD)

- Features of these diseases (continued)
  - Most victimes retain carbon dioxide, are hypoxic and have respiratory acidosis
  - Those infected will ultimately develop respiratory failure

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Emphysema

- Alveoli enlarge as adjacent chambers break through
- Chronic inflammation promotes lung fibrosis
- Airways collapse during expiration
- Patients use a large amount of energy to exhale
- Overinflation of the lungs leads to a permanently expanded barrel chest
- Cyanosis appears late in the disease

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Chronic Bronchitis

- Mucosa of the lower respiratory passages becomes severely inflamed
- Mucus production increases
- Pooled mucus impairs ventilation and gas exchange
- Risk of lung infection increases
- Pneumonia is common
- Hypoxia and cyanosis occur early

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Chronic Obstructive Pulmonary Disease (COPD)

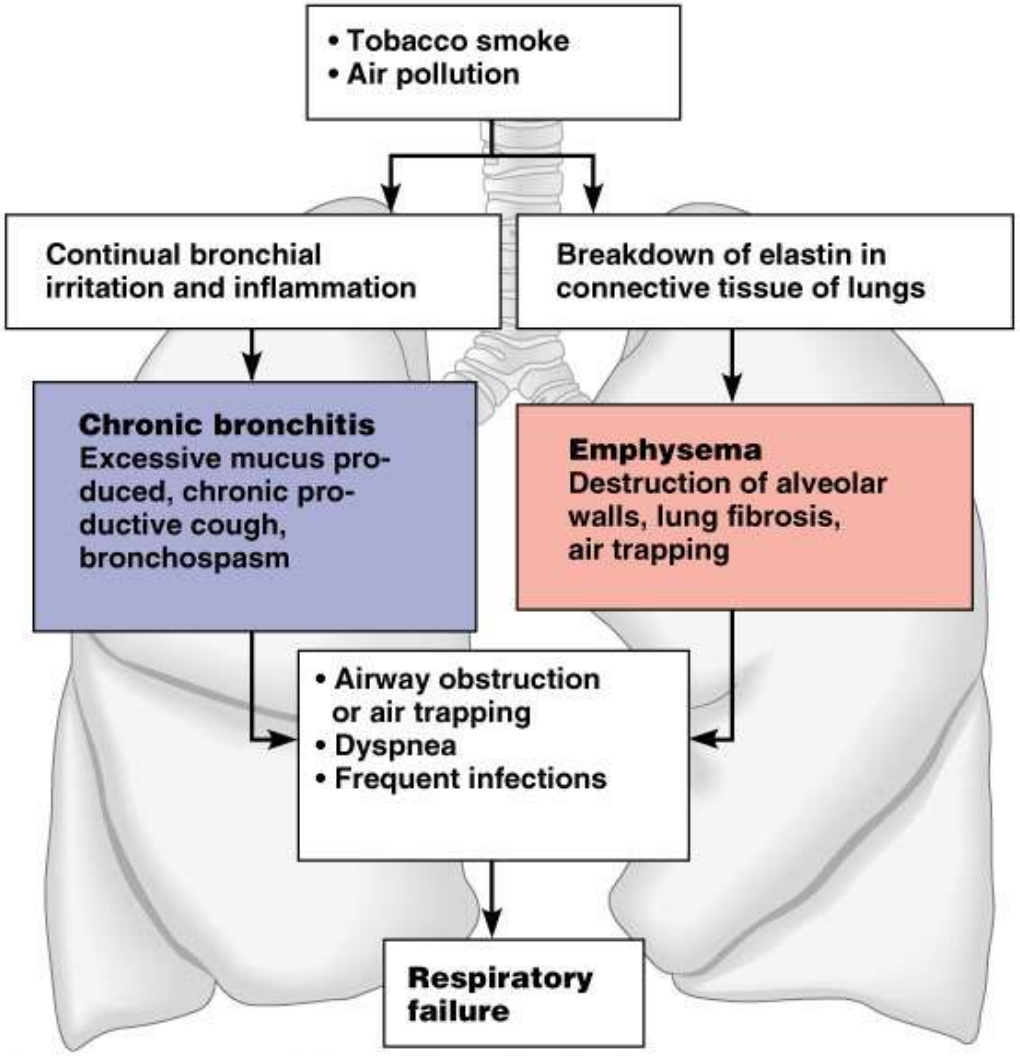

Figure 13.13

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Lung Cancer

- Accounts for 1/3 of all cancer deaths in the United States
- Increased incidence associated with smoking
- Three common types
  - Squamous cell carcinoma
  - Adenocarcinoma
  - Small cell carcinoma

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Sudden Infant Death syndrome (SIDS)

- Apparently healthy infant stops breathing and dies during sleep
- Some cases are thought to be a problem of the neural respiratory control center
- One third of cases appear to be due to heart rhythm abnormalities

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Asthma

- Chronic inflamed hypersensitive bronchiole passages
- Response to irritants with dyspnea, coughing, and wheezing

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Developmental Aspects of the Respiratory System

- Lungs are filled with fluid in the fetus
- Lungs are not fully inflated with air until two weeks after birth
- Surfactant that lowers alveolar surface tension is not present until late in fetal development and may not be present in premature babies

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Developmental Aspects of the Respiratory System

- Important birth defects
  - Cystic fibrosis – oversecretion of thick mucus clogs the respiratory system
  - Cleft palate

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Aging Effects

- Elasticity of lungs decreases
- Vital capacity decreases
- Blood oxygen levels decrease
- Stimulating effects of carbon dioxide decreases
- More risks of respiratory tract infection

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Respiratory Rate Changes Throughout Life

- Newborns – 40 to 80 respirations per minute
- Infants – 30 respirations per minute
- Age 5 – 25 respirations per minute
- Adults – 12 to 18 respirations per minute
- Rate often increases somewhat with old age

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PHARMACOGNOSY & PHYTOCHEMISTRY - I

Chapter No.: III

GLYCOSIDES

[Glycosides]

INTRODUCTION

- Primary metabolites :
- Secondary metabolites : Glycosides, Flavonoids, Alkaloids, Essential oils, Tannins.
- Phytopharmaceuticals: Significance

Glycosides

- They are secondary products formed from one or more sugar molecules added to a non-sugar biologically active molecule
  - Sugar most commonly found is glucose
  - Non-sugar molecule known as the aglycone and may be
    - amino acid derivatives
    - steroids
    - triterpenes

Glycosides

- Glycosides consist of

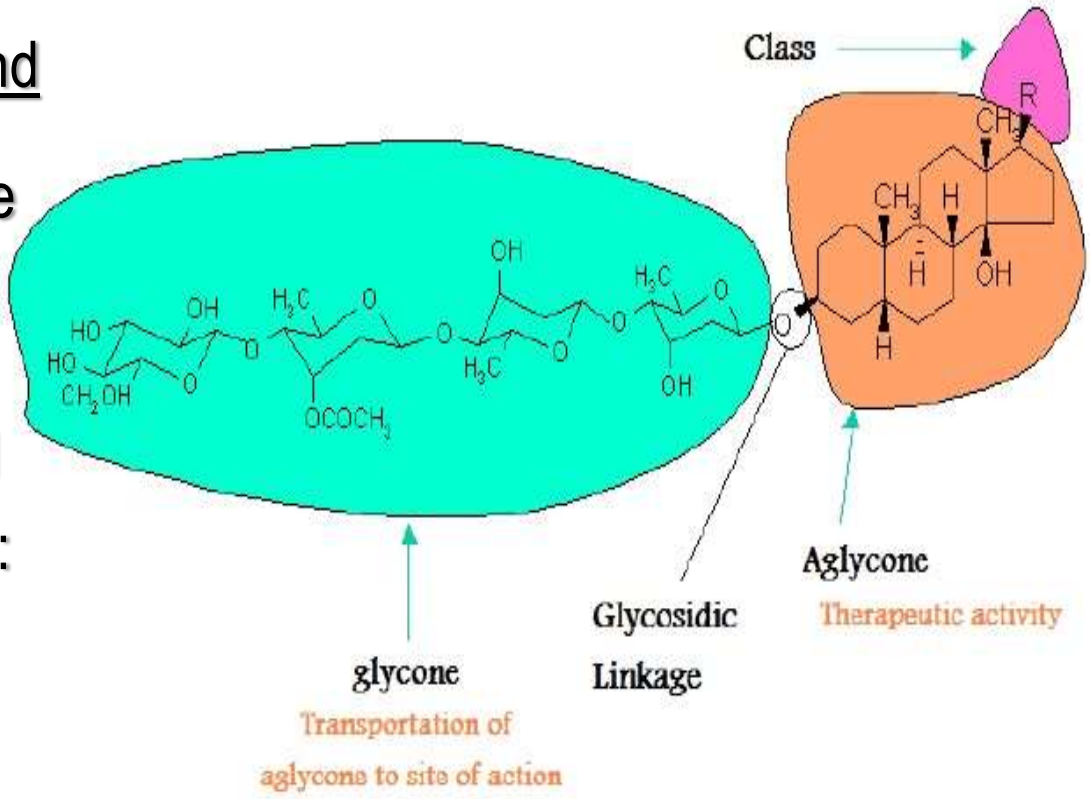
a sugar residue <u>covalently bound</u>

to a different structure called the

aglycone.

The sugar moiety can be joined to the aglycone in various ways:

1. Oxygen *(O-glycoside)*
2. Sulphur *(S-glycoside)*
3. Nitrogen *(N-glycoside)*
4. Carbon (*Cglycoside)*



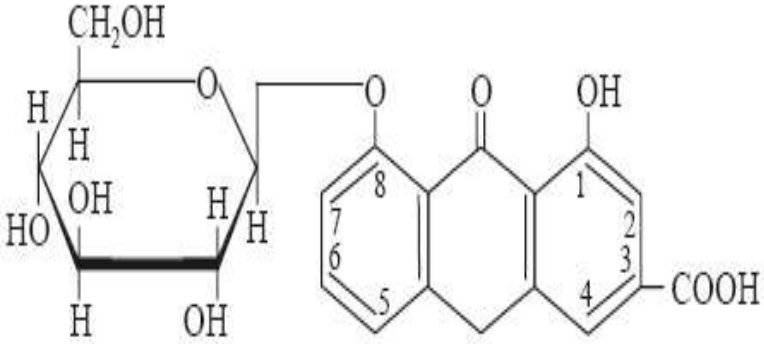
- Definition:: The organic compounds from plant or animal source which on enzymatic or acid hydrolysis give one or more sugar moieties along with non-sugar moieties.

Glycone -------- (Glycosidic linkage)-------- Aglycone (genin)

- Almost all plants that contain glycosides also contain enzymes that bring about their hydrolysis (**glycosidases**).
- α-Glycosides and β-glycosides are distinguished by the configuration of the hemiacetal hydroxyl group.
- The majority of naturally-occurring glycosides are β-glycosides.
- O-Glycosides can easily be cleaved into sugar and aglycone by hydrolysis with acids or enzymes.

1.1 O-Glycosides

- The **O-glycosides** are usually represented as follows:
- **Glycone---- (OH + H)---C—Aglycone…… *O-Glycoside***
- **Glycone---- C—Aglycone + H_2O**



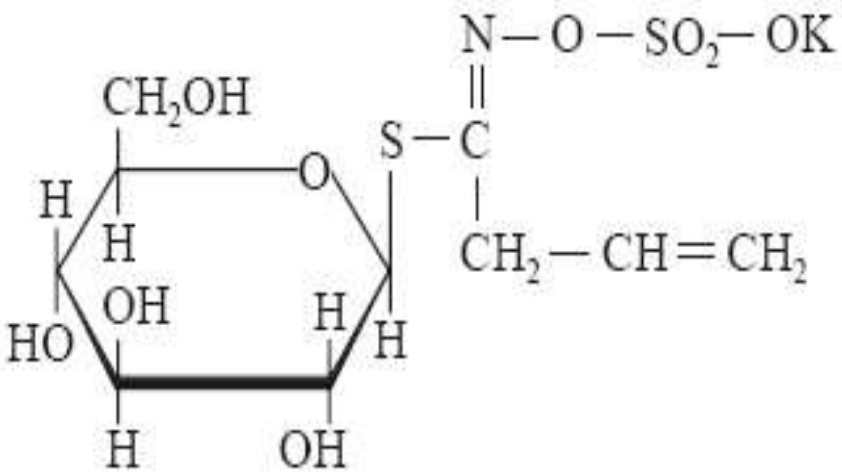
Rhein-8-Glycoside

- such as: senna, rhubarb and frangula.
- **Examples: Rhein-8-glucoside** obtained from rhubarb.
-

1.2 S-Glycosides

- The **S-glycosides** are normally designated as below:
- **Glycone---- (OH + H)---S—Aglycone…… *S-Glycoside***
- **Glycone---- S—Aglycone + H_2O**
- —SH $HO—C_6H_{11}O_5$ ——> $S—C_6H_{11}O_{5\ldots\ldots}$ S-*Glycoside*

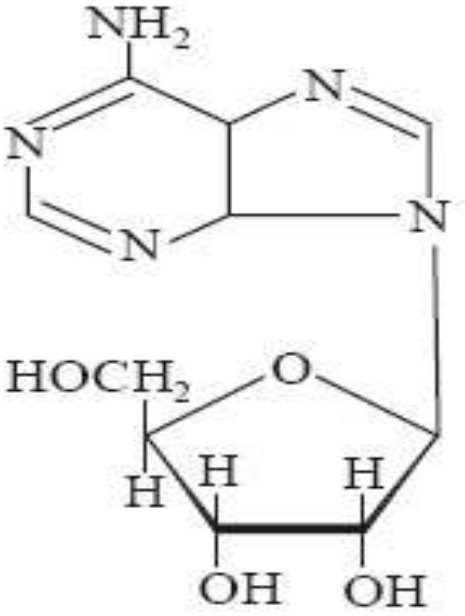
- such as: **Sinigrin,** obtained from
- black mustard seeds.
- (*i.e.*, **Brassica campestris** Family: *Cruciferae*)



Sinigrin

1.3 N-Glycosides

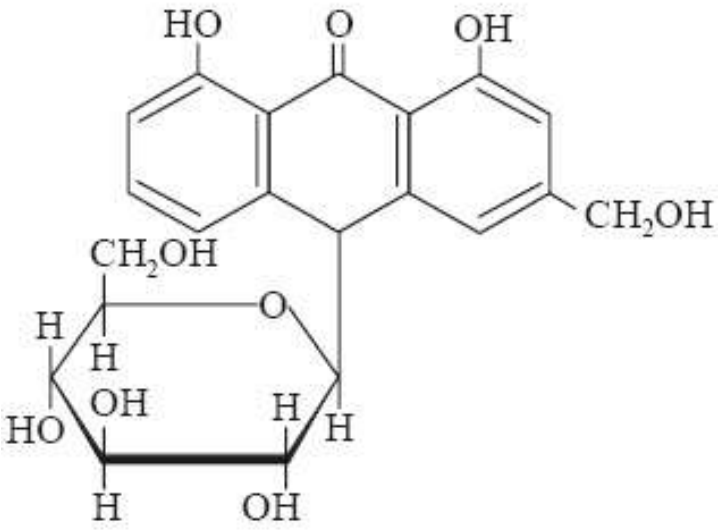
- The **N-glycosides** may be represented as shown below:
- **Glycone---- (OH + H)---N—Aglycone…… *N-Glycoside***
- **Glycone---- N—Aglycone + H_2O**
- =N—H HO—$C_6H_{11}O_5$ ——> =N—$C_6H_{11}O_5$ *N-Glycoside*
- The most typical example of N-glycosides is the nucleosides, wherein the respective amino group of the base ultimately reacts with —OH group ribose/deoxyribose.
- **Examples: Adenosine:** It is widely distributed in nature *e.g.*; from yeast nucleic acid.



Adenosine

1.4 C-Glycosides

- The **C-glycosides** may be designated as shown under:
- **Glycone---- (OH + H)---C—Aglycone…… *C-Glycoside***
- **Glycone---- C—Aglycone + H_2O**
- CH HO—$C_6H_{11}O_5$ ——> C—$C_6H_{11}O_5$ *C-Glycoside*
- C-Glycosides are present in a variety of plant substances, such as: **aloin** in Aloe; **cascaroside** in Cascara.
- E.g. **Aloin or Barbaloin:**



Aloin (or Barbaloin)

Physical Characters:

- Solids either amorphous or crystalline.
- Non volatile.
- Usually bitter in taste.
- Soluble in water and polar organic solvents.
- Reduce Fehling’s solutions only after hydrolysis.

Properties:

- Glycosides are usually soluble in water and in polar organic solvents, where as aglycone are normally insoluble or only slightly soluble in water.
- It is often very difficult to isolate intact glycosides because of their polar character.
- Many important drugs are glycosides and their pharmacological effects are largely determined by the structure of the aglycone.

Extraction and Isolation

- Because of the wide range of physical and chemical properties of glycosides and other constituents associated with them, no common general method for their isolation is recommended.
- Water, methanol, water-ethanol and ethanol are the most common solvents for extraction of glycosides.

- **Precautions before extraction**
  - **Deactivation of enzymes:**
- **Drying** for 15-30 min at **100 ^{o}C** followed by slow drying at a low temperature.
- Dipping the fresh material into **boiling water** or **boiling alcohol** for 10-20 min.
- **Boiling** the fresh plant material with **acetone**.
- Carrying out the extraction at very low temp.
- **Freeze-drying** of the plant material **before extraction** (lyophilization).
- Carrying the extraction in the presence of $(NH_4)_2SO_4$.

Classification:

- According to chemical nature of aglycone part/ therapeutic activity exhibited by the same

1. Anthraquinone / anthracene Glycosides- Senna, Aloe, Rhubarb, Cascara
2. Cardiac / sterols Glycosides- Digitalis, Squill, Strophanthus, Quabain
3. Saponin Glycosides- Dioscorea, Shatavari, Brahmi, Ginseng,
4. Cynogenetic / cynophoretic Glycosides- Bitter almond, wild cherry bark
5. Isothiocynate Glycosides- Mustard (black)
6. Flavonoids / flavonol Glycosides- Silymarin, Gingko, Citrus fruits
7. Coumarins and Furanocoumarin Glycosides- Visnaga, Psoralea
8. Aldehyde Glycosides-Vanilla, Anantmul
9. Phenol Glycosides- Bearberry
10. Steroidal Glycosides-Solanum
11. Bitter Glycosidal / miscellaneous Glycosides- Gentain, Chirata, Quassia, Kalmegh, Guduchi

Classification:

- **Atom from the aglycone involved in the glycosidic linkage:**
  - **Aglycone- O- Sugar O-glycosides**
  - **Aglycone- C- Sugar C-glycosides**
  - **Aglycone- S- Sugar S-glycosides**
  - **Aglycone- N- Sugar N-glycosides**
- **Number of sugars:**
  - **One sugar monosides e.g. Salicin.**
  - **Two sugar Biosides e.g. Diosmin.**
  - **Three sugars Triosides e.g. Digoxin.**
- **Nature of the glycoside:**
  - **Primary glycosides: Originally present in the plant e.g. Purpurea A**
  - **Secondary glycosides: Resulted from removal of one sugar from the primary glycosides e.g. Digitoxin**

- **Type of the glycosidic linkage:**
  - **α- glycosides**
  - **β- glycosides**
- **Botanical source:**
  - **Digitalis glycosides**
  - **Senna glycosides.**
- **Therapeutic use:**
  - **Analgesic glycosides.**
  - **Purgative glycosides.**
  - **Cardiac glycosides.**
- **Chemical nature of the aglycone:**
  - **Flavone glycosides.**
  - **Steroidal glycosides.**
  - **Aldehydic glycosides.**
  - **Etc…….**

Alkaloids

S.Y.B.Pharm- Sem- IV

CONTENT

- Definition
- History
- Introduction
- Chemistry
- Classification
- Occurrence
- Drugs
- Biosynthesis

Origin, History, Introduction

- the term "alkaloid" (alkali-like) is commonly used to designate basic heterocyclic nitrogenous compounds of plant origin that are physiologically active.
- The term alkaloid ot Pflanzenlkalien was coined by Meissner, a German pharmacist, in 1819.
- The mankind has been using alkaloid for various purposes like poisons, medicines, poultices, teas etc.
- The French chemist, Derosne in 1803, isolated Narcotine.

Alkaloids

Definition: the term “alkaloid” (alkali-like) is commonly used to designate basic heterocyclic nitrogenous compounds of plant origin that are physiologically active.

Deviation from Definition:

- **Basicity: Some alkaloids are not basic e.g. Colchicine, Piperine, Quaternary alkaloids.**
- **Nitrogen: The nitrogen in some alkaloids is not in a heterocyclic ring e.g. Ephedrine, Colchicine, Mescaline.**
- **Plant Origine: Some alkaloids are derived from Bacteria, Fungi, Insects, Frogs, Animals.**

Distribution and occurrence:

- Rare in lower plants.
- Dicots are more rich in alkaloids than Monocots.
- Families rich in Alkaloids: Apocynaceae, Rubiaceae, Solanaceae and Papaveracea.
- Families free from Alkaloids: Rosaceae, Labiatae

Distribution in Plant:

- All Parts e.g. Datura.
- Barks e.g. Cinchona
- Seeds e.g. Nux vomica
- Roots e.g. Aconite
- Fruits e.g. Black pepper
- Leaves e.g. Tobacco
- Latex e.g. Opium

Effects of alkaloids on humans

- High biological activity
- Produce varying degrees of physiological and psychological responses - largely by interfering with neurotransmitters
  - others interfere with membrane transport, protein synthesis or other processes
- In large doses - highly toxic - fatal
- In small doses, many have therapeutic value
  - muscle relaxants, tranquilizers, pain killers, mind altering drugs, chemotherapy

Psychoactive alkaloids

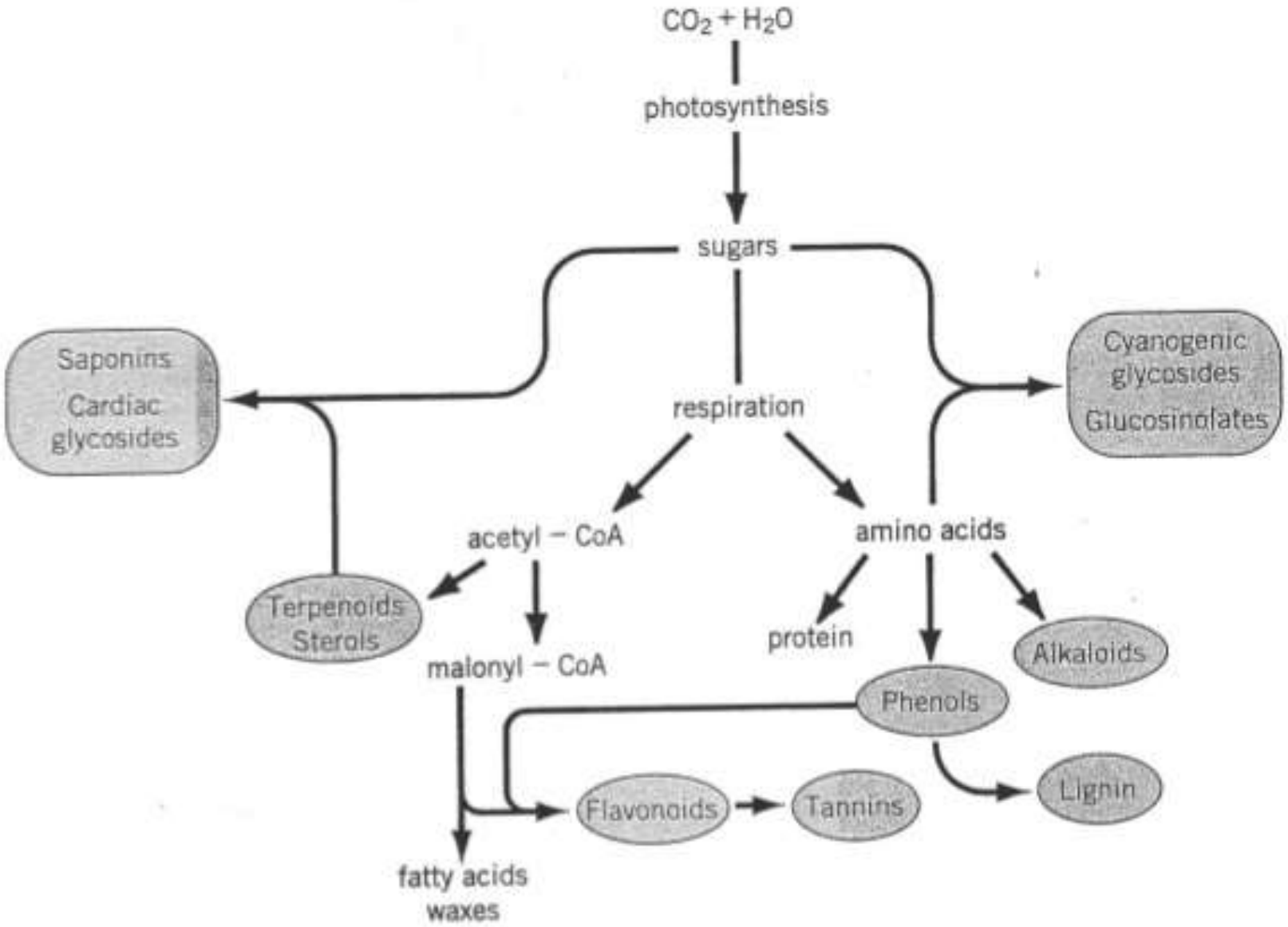
- Although only a small percent are psychoactive, these get much focus
- Affect the central nervous system - often by influencing neurotransmitters
- Categories of psychoactive cmpds
  - Stimulants
  - Hallucinogens
  - Depressants
- May also be narcotic (*addictive*)

Psychoactive alkaloids

Stimulants	Hallucinogens	Depressants
Cocaine	Tropane alkaloids	Morphine
Ephedrine	Mescaline	Codeine
Caffeine	Psilocybin	Heroin
	Ergot alkaloids (LSD)	

Alkaloid synthesis

- Most alkaloids are synthesized from a few common amino acids (tyrosine, tryptophan, ornithine or argenine, aspartic acid, & lysine)
- Nicotinic acid precursor for part of nicotine
- Purine precursor for caffeine
- Some alkaloids synthesized from terpenes - along mevalonic acid pathway
- Generally classified by the predominant ring structure and/or carbon skeleton


$CO_2 + H_2O$
photosynthesis
sugars
Saponins
Cardiac glycosides
respiration
Cyanogenic glycosides
Glucosinolates
acetyl – CoA
amino acids
Terpenoids
Sterols
malonyl – CoA
protein
Alkaloids
Phenols
Lignin
Flavonoids
Tannins
fatty acids
waxes

Function in Plants

- They may act as **protective** against insects and herbivores due to their bitterness and toxicity.
- They are, in certain cases, the final **products of detoxification** (**waste products**).
- **Source of nitrogen** in case of nitrogen deficiency.
- They, sometimes, act as **growth regulators** in certain metabolic systems.
- They may be utilized as a **source of energy** in case of deficiency in carbon dioxide assimilation.

Chemical tests

- Wagner's test: (I_2/kI):Reddish brown precipitate .
- Mayer's: ($HgCl_2$ Creamy precipitate with True alkaloid.
- Hagger's test: (Picric acid) Yellow precipitate with True alkaloid.
- Dragendorff: (Potassium Bismuth Iodide) Reddish Brown precipitate .
- Tannic acid solution: different alkaloid colored

Physical Properties:

I- State:

- **Most** alkaloids are crystalline **solids**.
- Few alkaloids are amorphous solids e.g. emetine.
- **Some** are **liquids** that are either:

  Volatile e.g. nicotine and coniine, or

  Non-volatile e.g. pilocarpine and hyoscine.

II- Color:

The **majority** of alkaloids are **colorless** but **some** are **colored** e.g.:

- Colchicine and berberine are yellow.
- betanidine is orange.
- The salts of sanguinarine are copper-red.

III- Solubility:

- Both **alkaloidal bases** and their **salts** are **soluble in alcohol**.
- Generally, the **bases** are **soluble in organic solvents** and **insoluble in water**

  Exceptions:

  - Bases **soluble in water**: caffeine, ephedrine, codeine, colchicine, pilocarpine and quaternary ammonium bases.
  - Bases **insoluble or sparingly soluble in certain organic solvents**: morphine in ether, theobromine and theophylline in benzene.
- **Salts** are usually **soluble in water** and, **insoluble or sparingly soluble in organic solvents.**

  Exceptions:

  - Salts **insoluble in water**: quinine monosulphate.
  - Salts **soluble in organic solvents**: lobeline and apoatropine hydrochlorides are soluble in chloroform

IV- Isomerization:

- Optically active isomers may show different physiological activities.
- *l*-ephedrine is 3.5 times more active than *d*-ephedrine.
- *l*-ergotamine is 3-4 times more active than *d*-ergotamine.
- *d*- Tubocurarine is more active than the corresponding *l*- form.
- Quinine (*l*-form) is antimalarial and its *d*- isomer quinidine is antiarrythmic.
- The **racemic (**optically inactive) *dl*-atropine is physiologically active.

Chemical Properties:

I- Nitrogen:

- Primary amines $R\text{-}NH_2$ e.g. Norephedrine
- Secondary amines $R_2\text{-}NH$ e.g. Ephedrine
- Tertiary amines $R_3\text{-}N$ e.g. Atropine
- Quaternary ammonium salts $R_4\text{-}N$ e.g *d*-Tubocurarine

II- Basicity:

- $R_2\text{-}NH > R\text{-}NH_2 > R_3\text{-}N$
- Saturated hexacyclic amines is more basic than aromatic amines.

Nomenclature:

Trivial names should end by **"ine"**. These names may refer to:

- The **genus** of the plant, such as Atropine from *Atropa belladona*.
- The plant **species**, such as Cocaine from *Erythroxylon coca*.
- The **common name** of the drug, such as Ergotamine from ergot.
- The name of the **discoverer**, such as Pelletierine that was discovered by Pelletier.
- The **physiological action**, such as Emetine that acts as emetic, Morphine acts as narcotic.
- A prominent **physical character**, such as Hygrine that is hygroscopic.

Prefixes and suffixes:

Prefixes:

- **"Nor-"** designates N-demethylation or N-demethoxylation, e.g. norpseudoephedrine and nornicotine.
- **"Apo-"** designates dehydration e.g. apomorphine.
- **"Iso-, pseudo-, neo-,** and **epi-"** indicate different types of isomers.

Suffixes:

- **"-dine"** designates isomerism as quinidine and cinchonidine.
- **"-ine"** indicates, in case of ergot alkaloids, a lower pharmacological activity e.g. ergotaminine is less potent than ergotamine.

IV- Stability:

- **Effect of heat**:

  Alkaloids are decomposed by heat, except **Strychnine** and **caffeine** (**sublimable**).

- **Reaction with acids:**

  1- Salt formation.

  2- Dil acids hydrolyze Ester Alkaloids e.g. Atropine

3- Conc. acids may cause:

- Dehydration:

  Atropine → Apoatropine

  Morphine → Apomorphine

- Demethoxylation:

  e.g. Codeine

- **Effect of Alkalies:**

1- Dil alkalis liberate most alkaloids from their salts e.g. NH3.

2- They may cause isomerization (racemization) of alkaloid as the conversion of hyoscyamine to atropine.

3- They also can **form salts with alkaloids containing a carboxylic group** e.g. narceine.

4- Strong alkalis: such as aqueous NaOH and KOH form salts with phenolic alkaloids.

5- Strong alkalis cause hydrolysis of Ester alkaloids (e.g. atropine, cocaine and physostigmine) and **Amide alkaloids** (colchicines).

6**- Strong alkalis cause opening of lactone ring.**

Qualitative test for Alkaloids:

Precipitation Reagents:

They are used to:

1- Indicate the absence or presence of Alkaloids

2- Test for complete of extraction

Disadvantages: Some non alkaloids interfere such as Proteins, lactones, coumarins

- **Colour Reagents:**

1- Froehd's Reagent: Phosphomolybdic acid

2- Marqui's Reagent: Formaldehyde/ Conc. H_2SO_4

3- Mandalin's Reagent: Sulphovanidic acid

4- Erdmann's Reagent: Conc. HNO_3/Conc. H_2SO_4

5- **Mecke's Reagent:** Selenious acid / conc. H_2SO_4

6- **Shaer's Reagent:** Hydrogen peroxide / conc. H_2SO_4

7- **Rosenthaler's Reagent:** Potassium arsenate / conc. H_2SO_4

8- **Conc. HNO_3**

Identification of Alkaloids:

- Melting point
- Colour test
- Optical Rotation
- Microcrystal test
- HPLC, GC, GC-MS
- UV, IR, NMR, MS.

Quantitative Determination of Alkaloids:

- **Volumetric methods:**
- These are based on reaction of alkaloidal bases with acids (Acid-Base titration).

  They include:
- **Aqueous titration:** This is carried by either:

  1- **Direct titration** of the alcoholic solution of the alkaloidal residue with standard acid, or

  2- **Back titration** by dissolving the residue in a known amount of standard acid and back titration of residual acid against standard alkali.
- **Non-aqueous titration:** This method is suitable for determination of weak bases e.g. Caffeine.

- **Gravimetric methods:**

These methods are recommended for determination of:

1- Very weak bases which can not be determined by volumetric methods e.g. caffeine and colchicine.

2- Mixtures of alkaloids that are obtained from the same plant but differ greatly in their molecular weight e.g. Cinchona and Rawolfia alkaloids.

They can be performed by either:

1- Direct Weighing of the alkaloidal mixtures

2- Precipitation of the total alkaloids and determination of the weight of the precipitate obtained.

The major **drawbacks** of the gravimetric methods are:

1- They are insensitive to microamounts of alkaloids.

2- They could not be applied in case of thermolabile and volatile alkaloids.

3- Lipophilic impurities in the residue are calculated as alkaloids.

- **Colourimetric Method:**

  e.g. Morphine + $NaNO_2$/HCl

  Ergot + p-dimethylaminobenzaldehyde

- **Spectrophotometric Methods.**

- **Polarimetric Method.**

- **Fluorimetric Method.**

- **Chromatographic Methods**

QUALITATIVE CHEMICAL TESTS FOR ALKALOIDS

General tests answered by all alkaloids are as follows:

1. ***Dragendorff's test:*** *To 2–3 mL of the alkaloid solution add few drops of Dragendorff's reagent* (potassium bismuth iodide solution). An orange brown precipitate is formed.

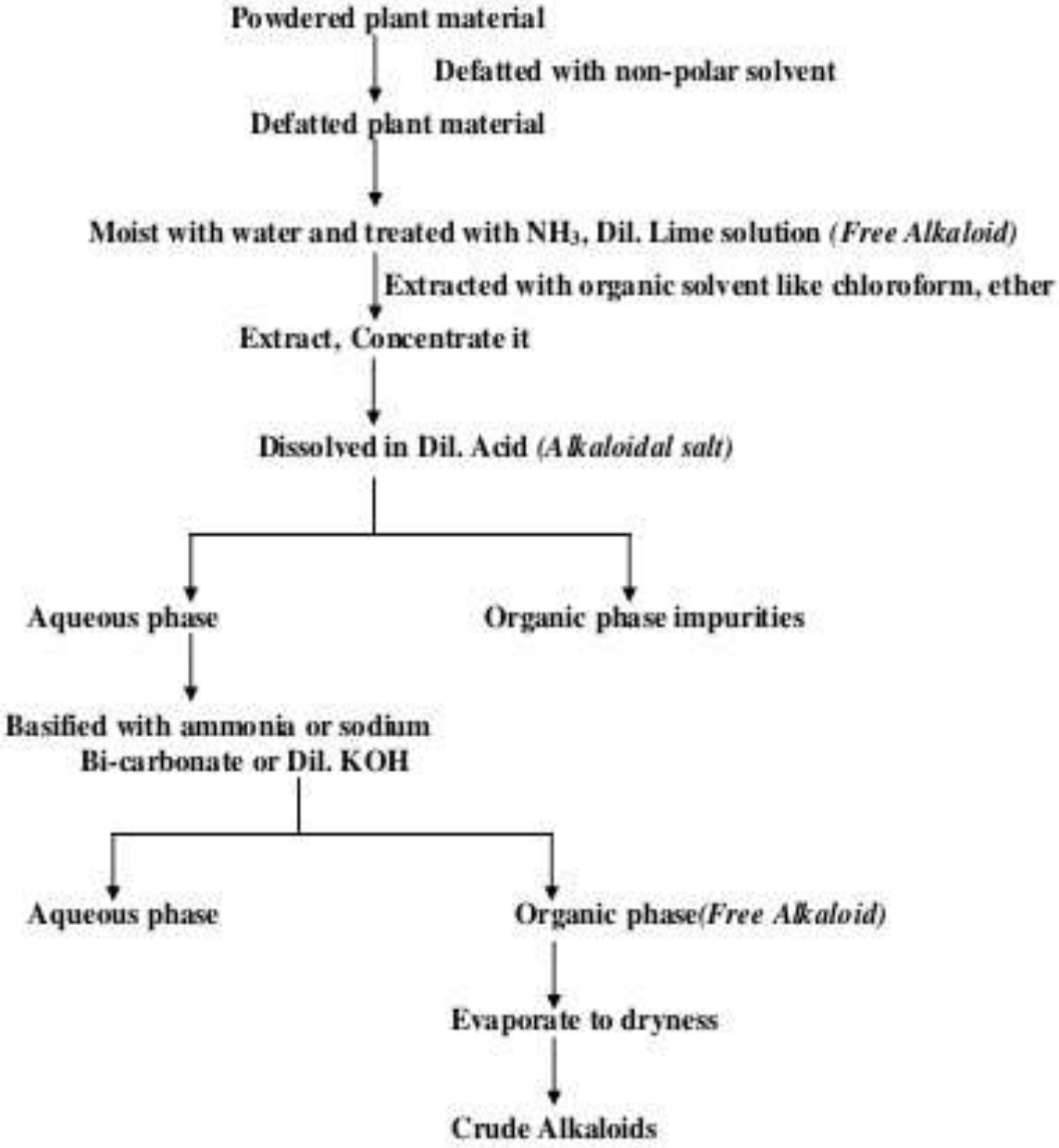
2. ***Mayer's test:*** *To 2–3 mL of the alkaloid solution add few drops of Mayer's reagent (potassium* mercuric iodide solution). White brown precipitate is formed.

3. ***Hager's test:*** *To 2–3 mL of the alkaloid solution add few drops of Hager's reagent (saturated* solution of picric acid). Yellow precipitate is formed.

4. ***Wagner's test:*** *To 2–3 mL of the alkaloid solution add few drops of Wagner's reagent (iodine–* potassium iodide solution). Reddish brown precipitate is formed.

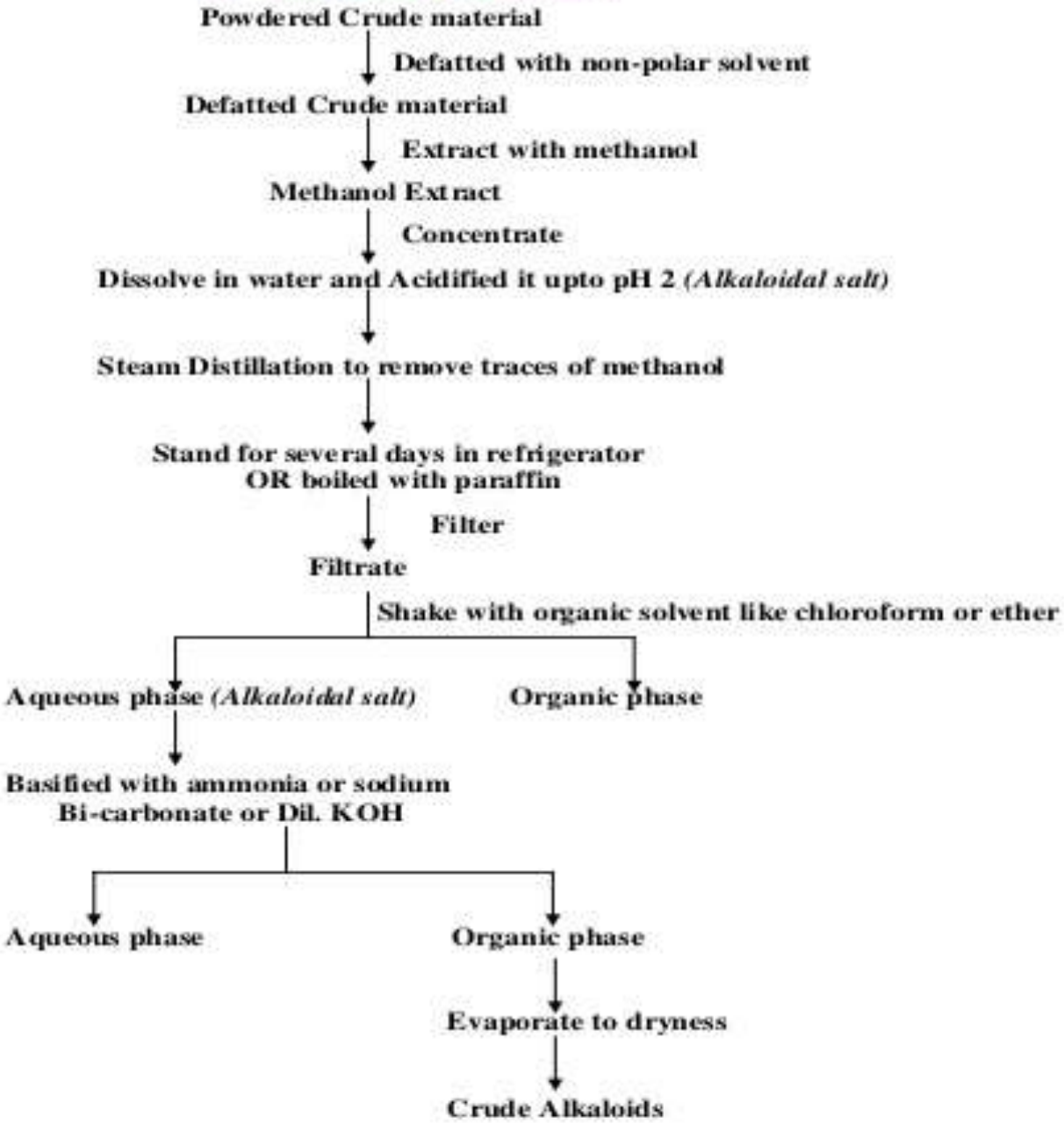
Extraction of alkaloids

1. Stass otto method



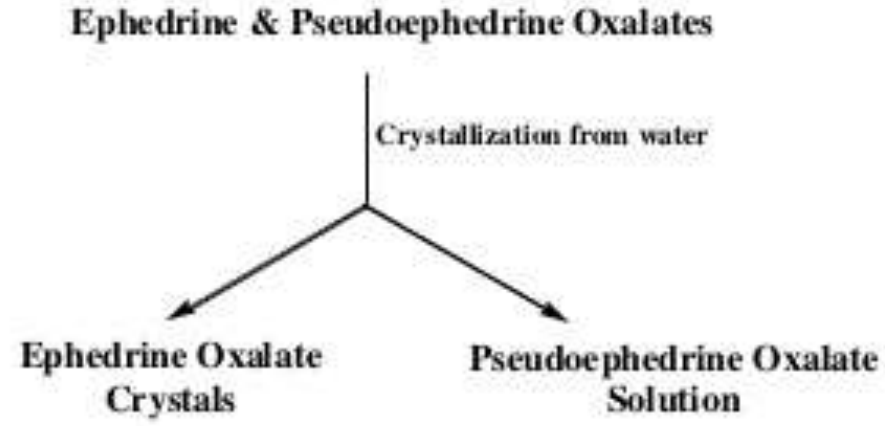
Extraction of alkaloids

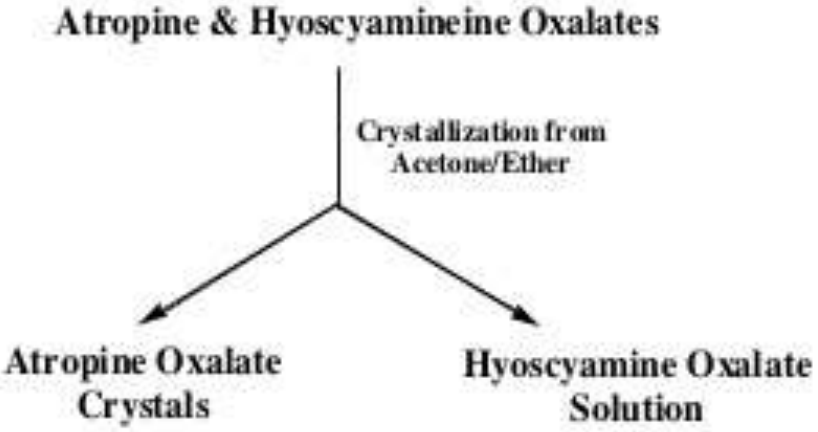
2. Manske's method



Purification of Alkaloids

1. Direct crystallization from solvent
2. Repeated acid base treatment
3. Fractional crystallization

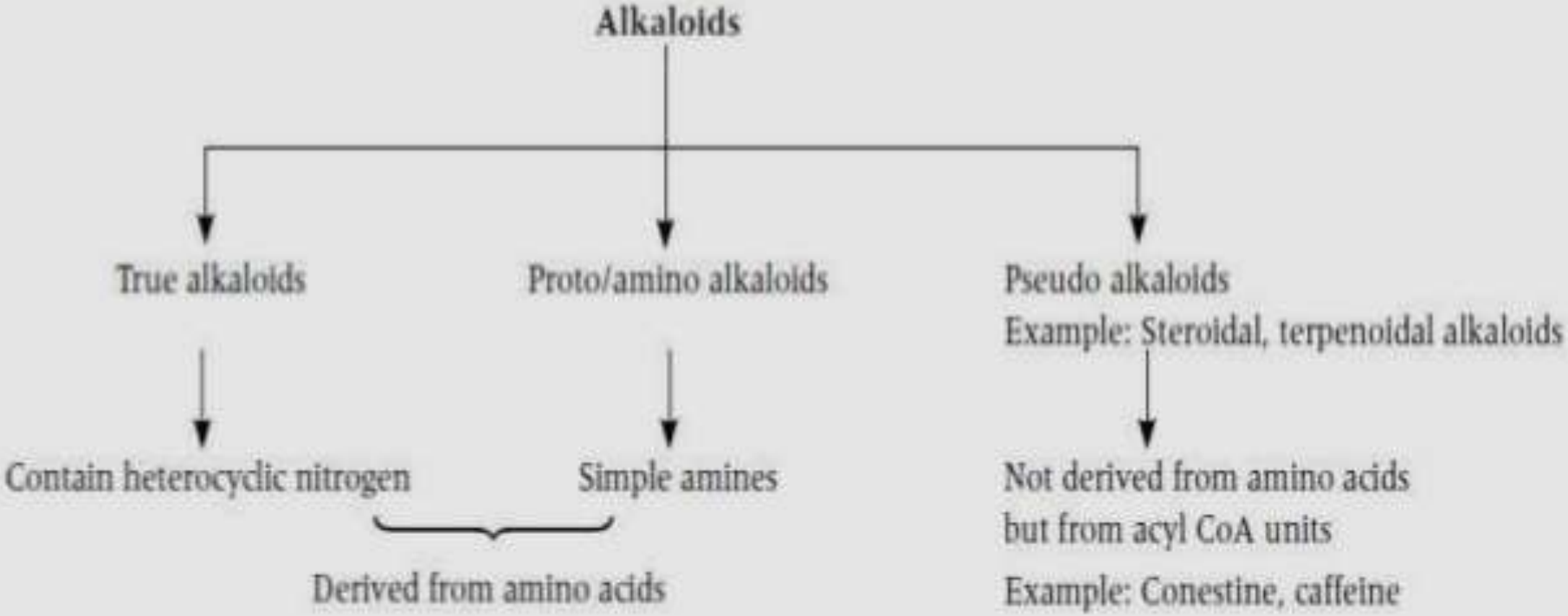




4. Chromatographic techniques
5. Gradient pH technique

Alkaloids

Definition: **the term "alkaloid" (alkali-like) is commonly used to designate basic heterocyclic nitrogenous compounds of plant origin that are physiologically active. Derived from amino acids.**



Classification of Alkaloids

❖ **True (Typical) alkaloids –**

That are derived from amino acids and have nitrogen in a heterocyclic ring. Show wide range of physiological activity and give positive test with the general alkaloid reagent. e.g. Atropine

❖ **Protoalkaloids –**

That are derived from amino acids and do not have nitrogen in a heterocyclic ring.

e.g. Ephedrine; mescaline

❖ **Pseudo alkaloids-**

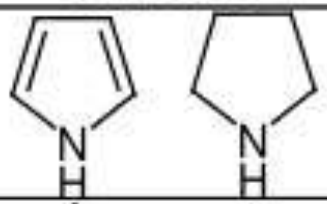
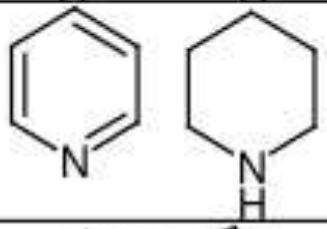
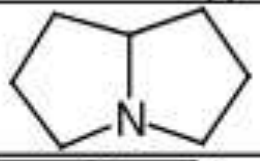
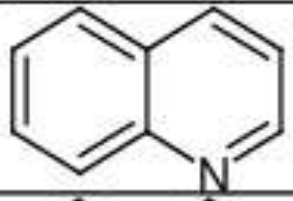
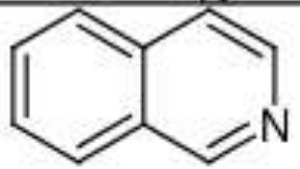
That are not derived from amino acids but have nitrogen in a heterocyclic ring. Do not show positive reaction with alkaloid reagents.

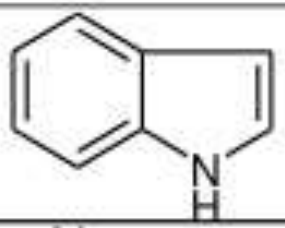
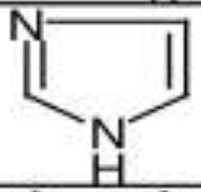
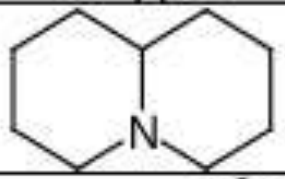
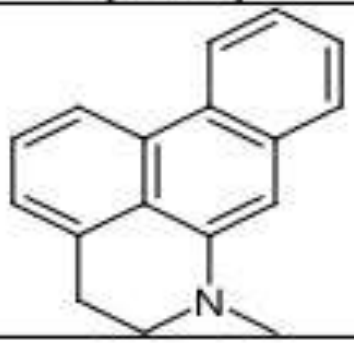
e.g. Purine alkaloids – Caffeine, steroidal alkaloids

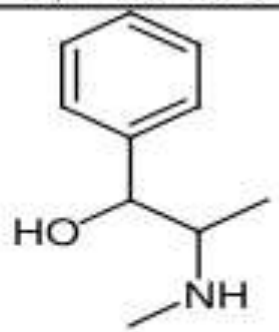
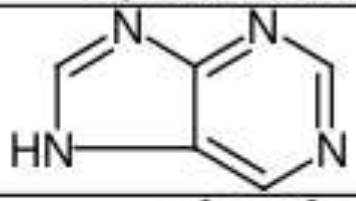
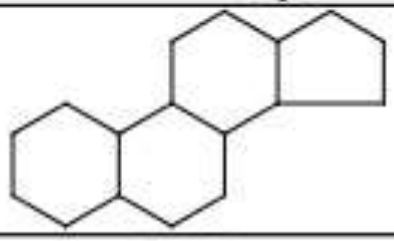
Classification of alkaloids

Chemical classification

A) True alkaloids

Sr. no.	Type	Structure	Examples
1.	Pyrrole and pyrrolidine		e.g. Hygrine, coca species
2.	Pyiridine and piperidine		e.g. Arecoline, anabasine, lobeline, conine, trigonelline
3.	Pyrrolizdine		e.g. Echimidine, senecionine, seneciphylline
4.	Tropane		e.g. Atropine, hyoscine, hyoscyamine, cocaine, pseudopelletirine
5.	Quinoline		e.g. Quinine, quinidine, cinchonine, cupreine, camptothecine
6.	Isoquinoline		e.g.Morphine, codeine, emetine, cephaline, narcotine, narceine, d-tubocurarine

7	Indole		e.g. Erotamine, ergotametriene, reserpine, vincristine, vinblastine, strychnine, brucine
8	Imidazole		e.g. Pilocrpine, isopilocarpine, pilosine
9	Norlupinane		e.g. Cystisine, laburinine
10	Piporphine (reduced isoquinoline napthalene)		e.g. Boldine

		B) PROTOALKALOID	
1.	Alkyalamine		Ephedrine, Pseudoephedrine
		C) Pseudoalkaloid	
1.	Purine		e.g. Caffeine, thophylline, theobromine
2.	Steroidal		e.g. Solanidine, conessine, protoveratrine
3.	Diterpene	$C_{20}H_{32}$	e.g. Aconitine, aconine, hypoaconine

Biosynthetic classification

1. Ornithine derived alkaloids

e.g. *Pyrrolidine alkaloid*-nicotine

Tropane alkaloid – Atropine, hyosyamine, coacaine

2. Lysine derived alkaloids

e.g. *Piperidine and pyridine alkaloid* – conine, lobaline, arecoline

Quinazolidine alkaloid- lupinine

3. Tyrosine derived alkaloids

e.g. *Isoquinoline alkaloid* – morphine, codeine, emetine, cephaline, berberine, d- tubocurine

Amino alkaloid- colchicine

4. Tryptophan derived alkaloids

e.g. *Indole alkaloid*- ergot alkaloid, vincristine, vinblastine, reserpine, strychnine, physostigmine, strychinine, brucine

Quinoline alkaloid – cinchonine, quinine, quinidine, camptothecin

5. Histidine derived alkaloids

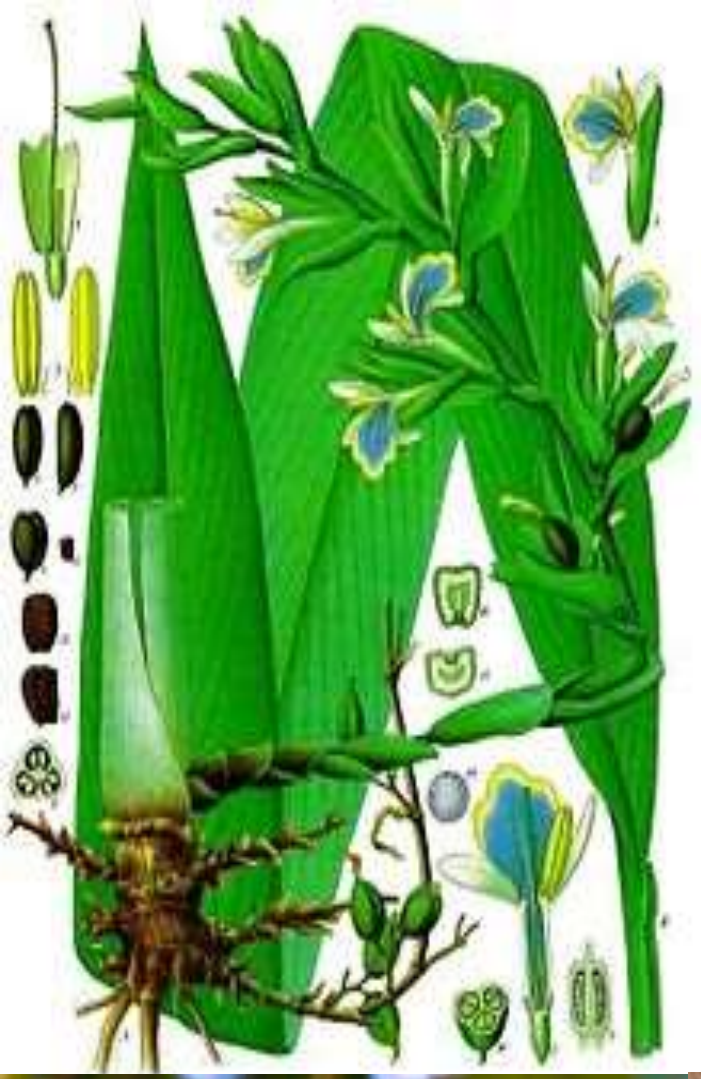
e.g. *Imidazole alkaloid* – Pilocrpine

6. Phenylalanine derived alkaloids

e.g. **Amino alkaloid-** Ephedrine

Pharmacological classification

1. Narcotic analgesic e.g.Morphine
2. Antimalerial e.g. Quinine
3. Reflux excitability e.g.Strychnine
4. Respiratory stimulant e.g.Lobeline
5. Neuralgia e.g. Aconitine
6. Oxytocic e.g. Erogotometrine
7. Bronchodilator e.g. Ephedrine, vasicine
8. Anticholinergic e.g. Atropine
9. CNS stimulant e.g. Caffeine
10. Antitussive e.g. Codeine
11. Antiarrythmic e.g.Quinidine
12. Antihypertensive e.g. Reserpine
13. Anticancer e.g. Vincristine
14. Antiglucoma e.g. Pilocarpine

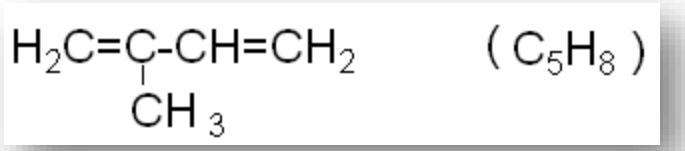


PHARMACOGNOSY & PHYTOCHEMISTRY-II

……S.Y. B. PHARM Sem-IV

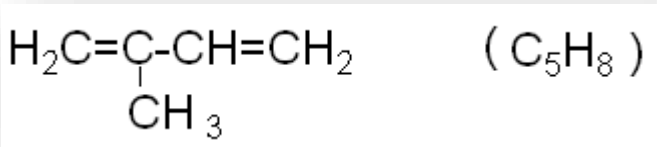
Terpenoids-Volatile oils; Resins ….

Terpenoids

1. Terpens are the **hydrocarbon and components of resins and terpentine produced from plants having general formula**  **, occur widely in nature.**

1. Terpens are modified chemical by **oxidation / rearrangement of C- skeleton** the resulting compound known as Terpenoids.
2. All the terpens are formed by inking of 2 or more isoprene unit

....derivative of **isoprene polymers,**



5. They are positional isomers in which

Functional group is located at diff. ..position on carbon skeleton.

Some compound same c- arrangement but –OH, double bond at diff. position.

6. They consist complex mixture of **terpens, alcohol, aldehyde, acid, esters**. They does not occur in free state.
7. It include constituents such as limonene, phellandrene, geraniol, citral, carvone, cineol and many others.
8. Few of the compound are used as **starting material for synthesis of other material.**

Introduction to volatile oil:

- The odorous, volatile principle of plant and animal source are known as Volatile oils.
- As they evaporate when exposed to air at ordinary temperature, they are also called as **"ethereal oils"**
- They represent essence or active constituents of plant, hence they are also known as **"essential oils"**
- Chemistry: derived from terpens and their oxygenated compounds.
- They are made up of isoprene unites (--C_5H_8) and are usually mono, sesqui- and diterpenes.

Properties:

- They are **usually lighter than water.**
- They possess **characteristic odors.**
- They have **high refractive indices.**
- Most of them are **optically active.**
- They are commonly found in species of Labiatae, **Rutaceae, Piperaceae, Zingiberaceae, Umbelliferae, Myrtaceae and Lauraceae.**
- They are present in entire plant or in any part of the plant .

Properties:

Extraction

❖Volatile oils are extracted by

…….steam distillation,

……..Solvent extraction or

……..mechanical techniques. means such as

Enfleurage, Ecuelle and

Liquid carbon dioxide method

❖**Steam distillation:**

hydro distillation- water…..? ?

(Air dried parts.. Leaves, flower exposed to steam)

❖**Solvent extraction: lipid solvent ether, benzene etc**

Effleurage:

- Method is used for extraction of delicate perfumes .
- The fresh flowers are mechanically spread on layer of fatty material,
- Allowed to imbibe and the exhausted petals are replaced by fresh material.
- The process is continued till the fatty layer saturated with volatile principles which are then extracted with lipid solvent.

Ecuelle:

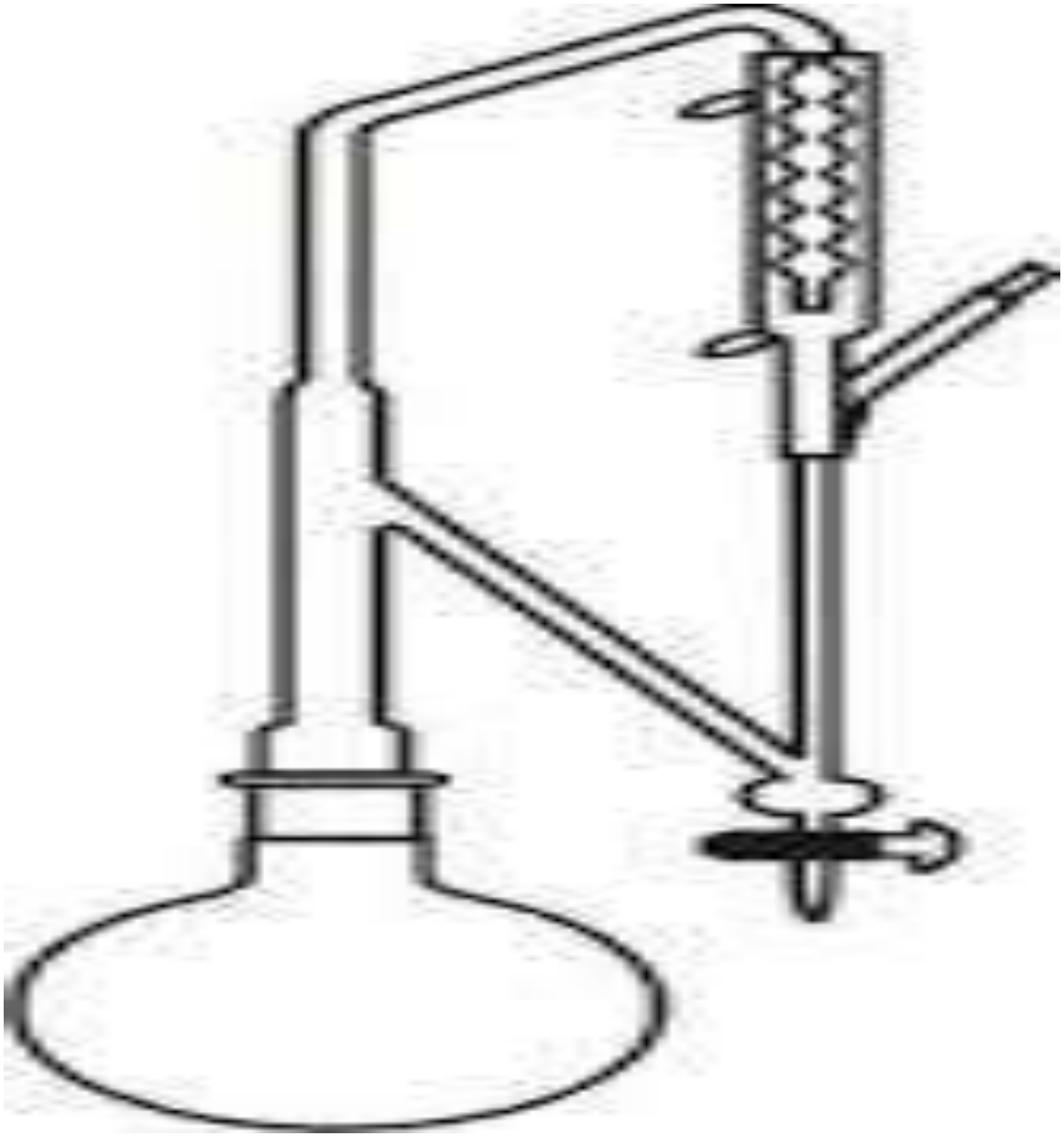
- citrus oils…oil cells are ruptured by pointed projection and get extracted

Liquid carbon dioxide:

- Its liquefied under pressure act as solvent again reduced pressure volatile oil get extract.
- CO2……pressure effect. …..? ?

Clevenger Apparatus :

Hydro distillation



Chemical test

1. To the thin section of the drug, add alcoholic solution of **Sudan III** . **Red colors obtained by** globules indicate the **presence of volatile oil.**

2. To the thin section of the drug, add a drop of **tincture alkane . Red color indicates the presence of volatile oil.**

Types of Volatile oils:

- 1. Alcohol Volatile Oils (-OH)
  e.g.. Menthol (Mentha), Santalol (Sandal wood), Coriandrol (Coriander), Borneol

- 2 . Aldehyde Volatile Oils (-CHO)
  e.g. Cinnamaldehyde (Cinnamon), Citral, Benzaldehyde

- 3. Ester Volatile oils (R-COR)
  e.g. Lavender, Mustard

- 4. Hydrocarbon Volatile Oils (–C=C--)
  e.g. Turpentine , l- phellandrene, Caryophyllene

- 5. Ketone Volatile Oils (--CO--)
  e.g. Carvone (Caraway), Camphor, Menthone, Muskone (Musk), Civetone (Civet)

- 6. Oxide Volatile Oils
  e.g. Ascaridole (Chenopodium oil), Cineol (Eucalyptus oil)

- 7.Ether Volatile Oils (--C-O-C--)
  e.g. Chavicol (Anise), Anethol (Dill)

- 8 Phenol Volatile Oils (Ar--OH)
  e.g. Eugenol (Clove)

Storage: Storage of Volatile Oils

- Certain volatile oils are liable to deteriorate on keeping.
- The deterioration is accompanied by change in color, or increase in viscosity of the oil, or change in odor of the oil.
- Therefore, volatile oils should be preserved properly in well closed, well filled containers away from light and in cool place.

Classification

All the terpens are formed by linking of 2 or more isoprene unit ....derivative of **isoprene all class as……**

no.	Class	isoprene unit	Mol. Formula	Sub class	Example
1	Isoprene / hemiterpene	1	C 5 H 8	--	Phenol, isovaleric acid
2	Monoterpenes	2	C 10 H 16	Acyclic	Ocimene, Geranial, Neral, Geraniol
				Mono cyclic	Limonene, menthol, Terpineol
3.	Sesqui terpenes	3	C 15 H 24	Mono cyclic	Zingiberene
				Bicyclic	Santonin,
4.	Di-terpenes	4	C 20 H 32	Acyclic	Phytol
				Mono cyclic	Vitamin A1
				Bicyclic	Manol
				Tri-cyclic	Abietic acid
5.	Triterpenes	6	C 30 H 48	Acyclic	Squalene
				Tetra cyclic	Lanosterol
				Penta cyclic	Amyrin

Classification…..cont..

no.	Class	isoprene unit	Mol. Formula	Sub class	Example
6	Tetraterpenes / Cardenoids	8	C 40 H 64	--	B-carotenes
7	Pentaterpenes	10	C 50 H 80	----	Bixin, Crocetin, Crocin, Picrocrocin
8	Polyterpenes / Rubber	n…	(C 5 H 8) n.	---	

Terpenoids

Depending upon type and number of rings reported in terpenoids

(except hemiterpene), they are named as

- Acyclic Terpenoids - have open-chain structure
- **Monocyclic Terpenoids –** have one-ring in structure
- **Bicyclic Terpenoids –** have two-rings in structure
- **Tricyclic Terpenoids –** have three-rings in structure

General properties of Terpenoids

- ✓ They are normally colorless liquids or solids with pleasant smell.
- ✓ They are **insoluble in water**.
- ✓ They are **soluble in alcohol, organic solvents and fixed oils**.
- ✓ They readily volatilize in steam.
- ✓ Most of them are optically active.
- ✓ Terpenoids normally contain **one or more double bonds** and form additive compounds with halogens, nitrosyl chloride, nitrosyl bromide.
- ✓ They get **oxidized by oxidizing agents.**

Isolation of Terpenoids volatile oils

- Isolation of Terpenoids volatile oils obtained in first step of distillation contain number of mono and sesqui-terpenoids.
- During fractional distillation of volatile oils, mono terpenoids hydrocarbons get distilled first followed by their oxygenated derivatives.
- **Physical methods, chemical methods (treating with sodium bisulphate, phenyl hydrazine, Tilden's reagent, Grignard's reagent), distillation under reduced pressure** and several types of **chromatographic methods are used for their isolation.**
- Proper adsorbents are used and then the chromatogram is eluted suitably.

✓Uses:

Uses

Terpenoids by virtue of their pleasant flavor are compounds of tremendous importance in

- ✓ **Perfumery and deodoridsers**
- ✓**Cosmetics,**
- ✓**Soaps,**
- ✓**Incense - sticks,**
- ✓**Foods: flvouring agents,**
- ✓**Pharmaceuticals industries: masking agent in cleaning mixtures, polishes, insecticides and medicinal values.**

EVALUATION / ANALYSIS OF OILS

If composition of volatile oil known then its possible to analyze or distinguish by special characteristic values as

- ✓Ester value for lavender oil
- ✓Phenol value for clove
- ✓Aldehyde value for cassia, cinnamon
- ✓Ketone value for caraway
- ✓Santalol constant for sandalwood oil
- ✓Geraneol constant

Following physical properties should be important for quality control of volatile oil

1. Boiling point
2. Refractive index
3. Optical rotation
4. Specific gravity
5. Freezing point
6. Solubility characteristic
7. Behavior during fractional distillation
8. GLC-----LC-GC…
9. UV, IR, NMR, GC-MS Most imp. For separation and analysis of volatile oil

PowerPoint® Lecture Slide Presentation by Vince Austin

Human Anatomy & Physiology

FIFTH EDITION

Elaine N. Marieb

The Integumentary System

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Skin (Integument)

- Consists of three major regions
  - Epidermis – outermost superficial region
  - Dermis – middle region
  - Hypodermis – deepest region

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Skin (Integument)

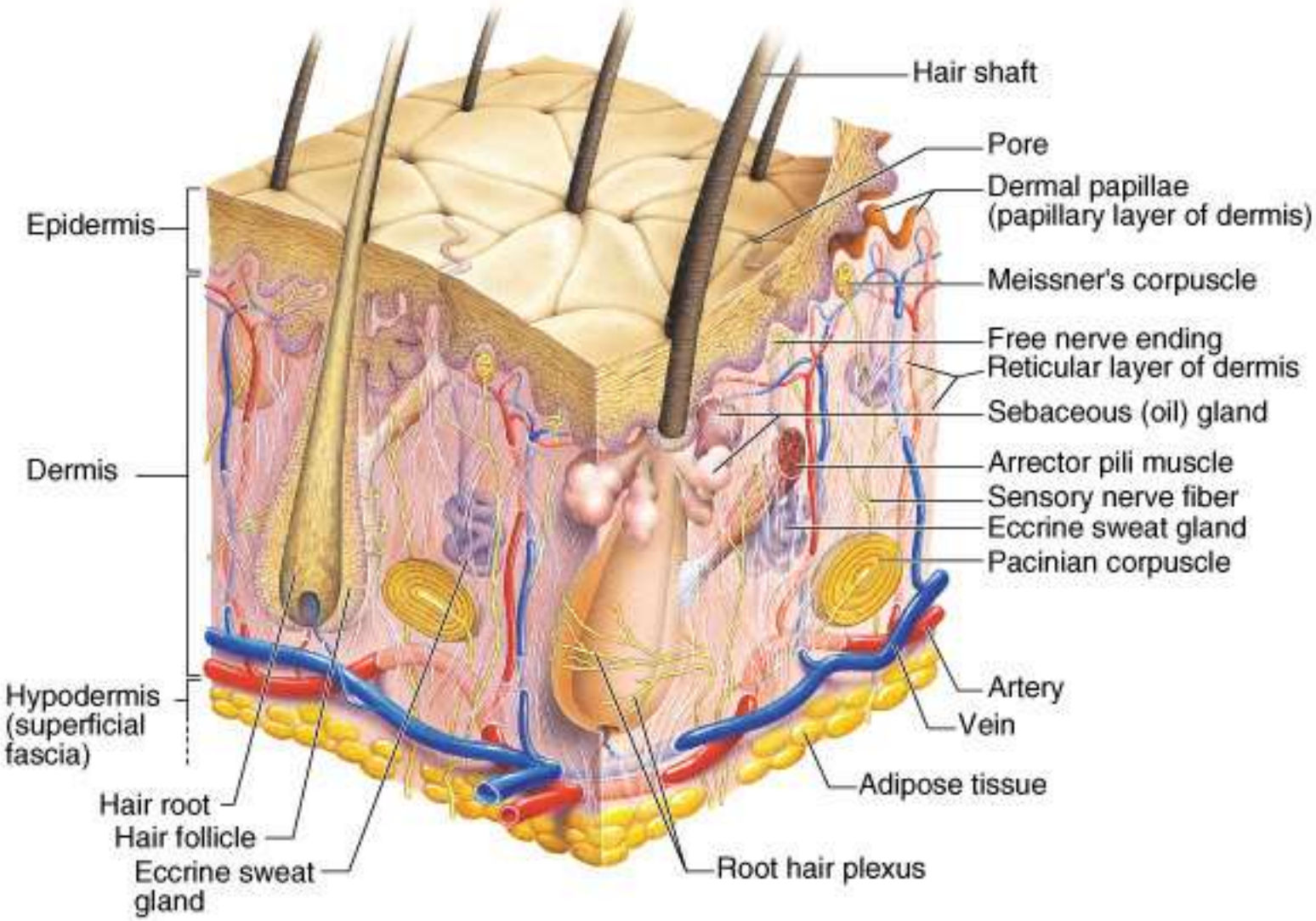

Figure 5.3

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Epidermis

- Composed of keratinized stratified squamous epithelium, consisting of four distinct cell types and four or five layers
- Cell types include keratinocytes, melanocytes, Merkel cells, and Langerhans' cells
- Outer portion of the skin is exposed to the external environment and functions in protection

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Cells of the Epidermis

- Keratinocytes – produce the fibrous protein *keratin*
- Melanocytes – produce the brown pigment *melanin*
- Langerhans' cells – epidermal macrophages that help activate the immune system
- Merkel cells – function as touch receptors in association with sensory nerve endings

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Layers of the Epidermis: Stratum Basale (Basal Layer)

- Deepest epidermal layer firmly attached to the dermis
- Consists of a single row of the youngest keratinocytes
- Cells undergo rapid division, hence its alternate name, *stratum germinativum*

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Layers of the Epidermis: Stratum Basale (Basal Layer)

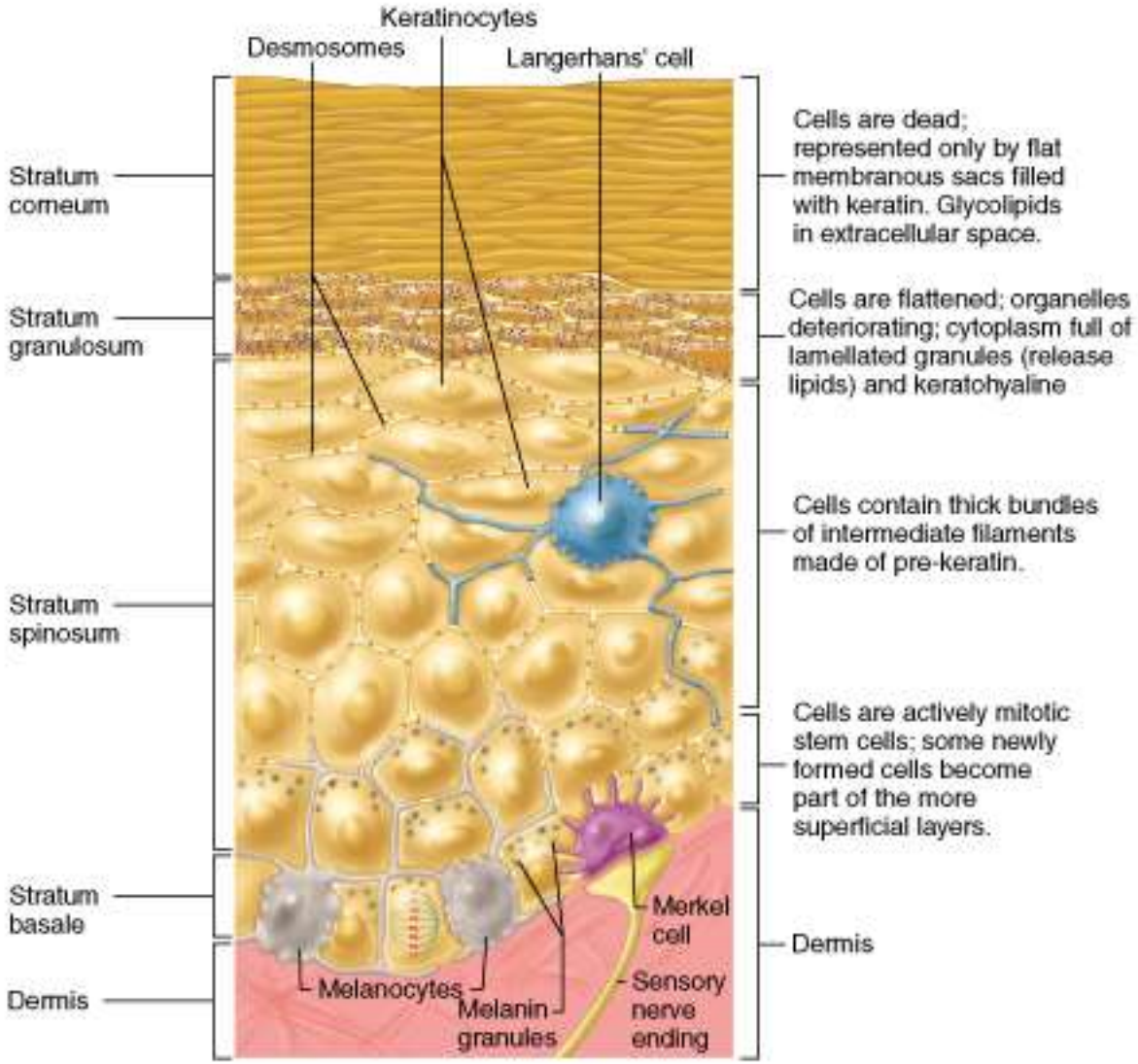

Figure 5.2

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Layers of the Epidermis: Stratum Spinosum (Prickly Layer)

- Cells contain a weblike system of intermediate filaments attached to desmosomes
- Melanin granules and Langerhans' cells are abundant in this layer

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Layers of the Epidermis: Stratum Granulosum (Granular Layer)

- Thin; three to five cell layers in which drastic changes in keratinocyte appearance occurs
- Keratohyaline and lamellated granules accumulate in the cells of this layer

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Layers of the Epidermis: Stratum Lucidium (Clear Layer)

- Thin, transparent band superficial to the stratum granulosum
- Consists of a few rows of flat, dead keratinocytes
- Present only in thick skin

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Layers of the Epidermis: Stratum Corneum (Horny Layer)

- Outermost layer of keratinized cells
- Accounts for three quarters of the epidermal thickness
- Functions
  - Waterproofing
  - Protection from abrasion and penetration
  - Renders the body relatively insensitive to biological, chemical, and physical assaults

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Dermis

- Second major skin region containing strong, flexible connective tissue
- Cell types include fibroblasts, macrophages, and occasionally mast cells and white blood cells
- Composed of two layers – papillary and reticular

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Layers of the Dermis: Papillary Layer

- Papillary layer
  - Areolar connective tissue with collagen and elastic fibers
  - Its superior surface contains peglike projections called *dermal papillae*
  - Dermal papillae contain capillary loops, Meissner's corpuscles, and free nerve endings

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Layers of the Dermis: Reticular Layer

- Reticular layer
  - Accounts for approximately 80% of the thickness of the skin
  - Collagen fibers in this layer add strength and resiliency to the skin
  - Elastin fibers provide stretch-recoil properties

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Hypodermis

- Subcutaneous layer deep to the skin
- Composed of adipose and areolar connective tissue

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Skin Structure

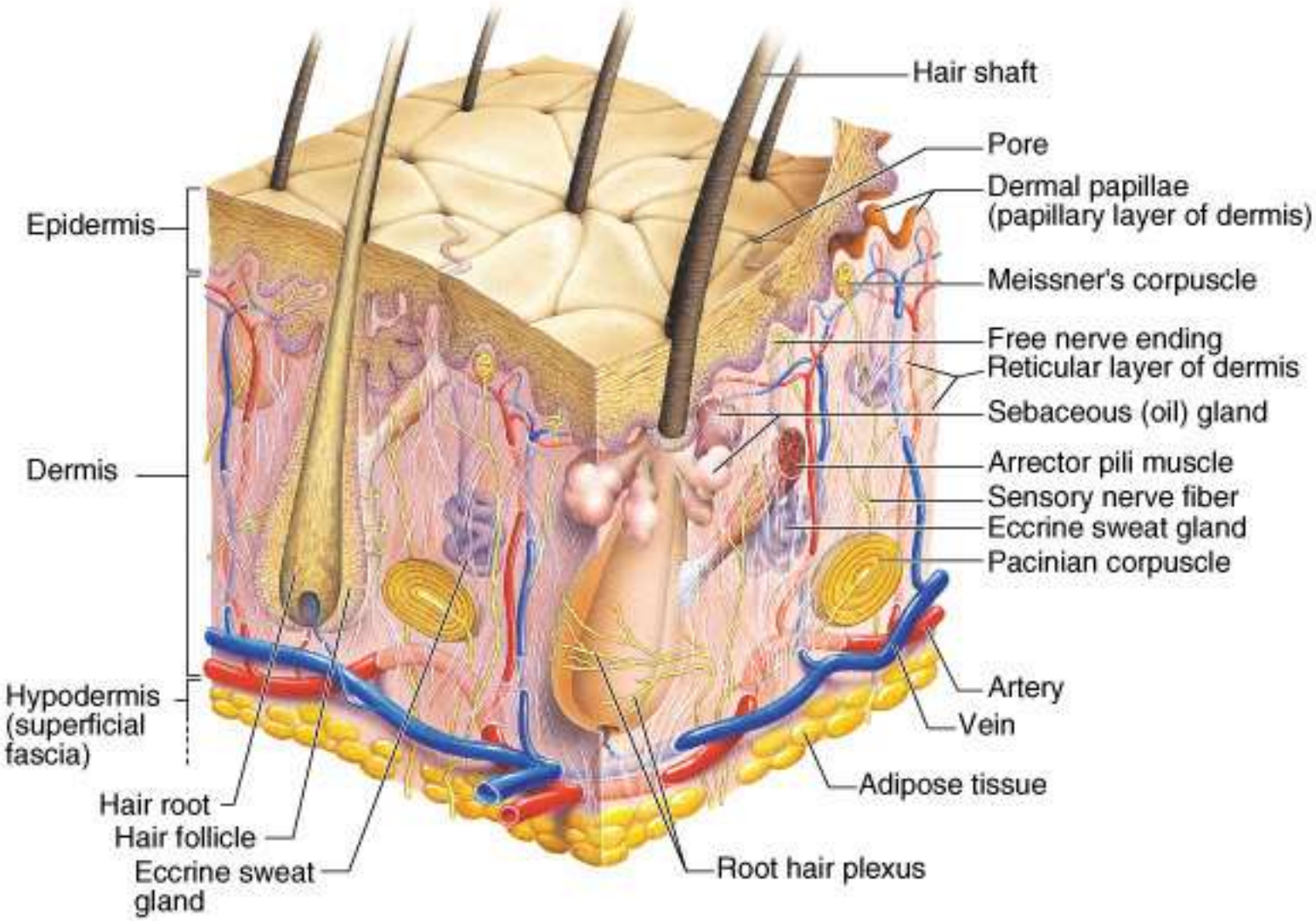

Figure 5.3

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Skin Color

- Three pigments contribute to skin color
  - Melanin – yellow to reddish-brown to black pigment, responsible for dark skin colors
    - Freckles and pigmented moles – result from local accumulations of melanin
  - Carotene – yellow to orange pigment, most obvious in the palms and soles of the feet
  - Hemoglobin – reddish pigment responsible for the pinkish hue of the skin

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Sweat Glands

- Different types prevent overheating of the body; secret cerumen and milk
  - Eccrine sweat glands – found in palms, soles of the feet, and forehead
  - Apocrine sweat glands – found in axillary and anogenital areas
  - Ceruminous glands – modified apocrine glands in external ear canal and secrete cerumen
  - Mammary glands – specialized sweat glands that secret milk

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Sebaceous Glands

- Soften skin when stimulated by hormones
- Simple alveolar glands found all over the body
- Secrete an oily secretion called sebum

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Hair

- Helps maintain warmth, alerts the body to presence of insects on the skin, and guards the scalp against physical trauma, heat loss, and sunlight
- Filamentous strands of dead keratinized cells produced by hair follicles
- Contains hard keratin, which is tougher and more durable than the soft keratin of the skin
- Made up of the shaft projecting from the skin and the root embedded in the skin
- Consists of a core called the medulla, a cortex, and an outermost cuticle

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Hair

- Pigmented by melanocytes at the base of the hair

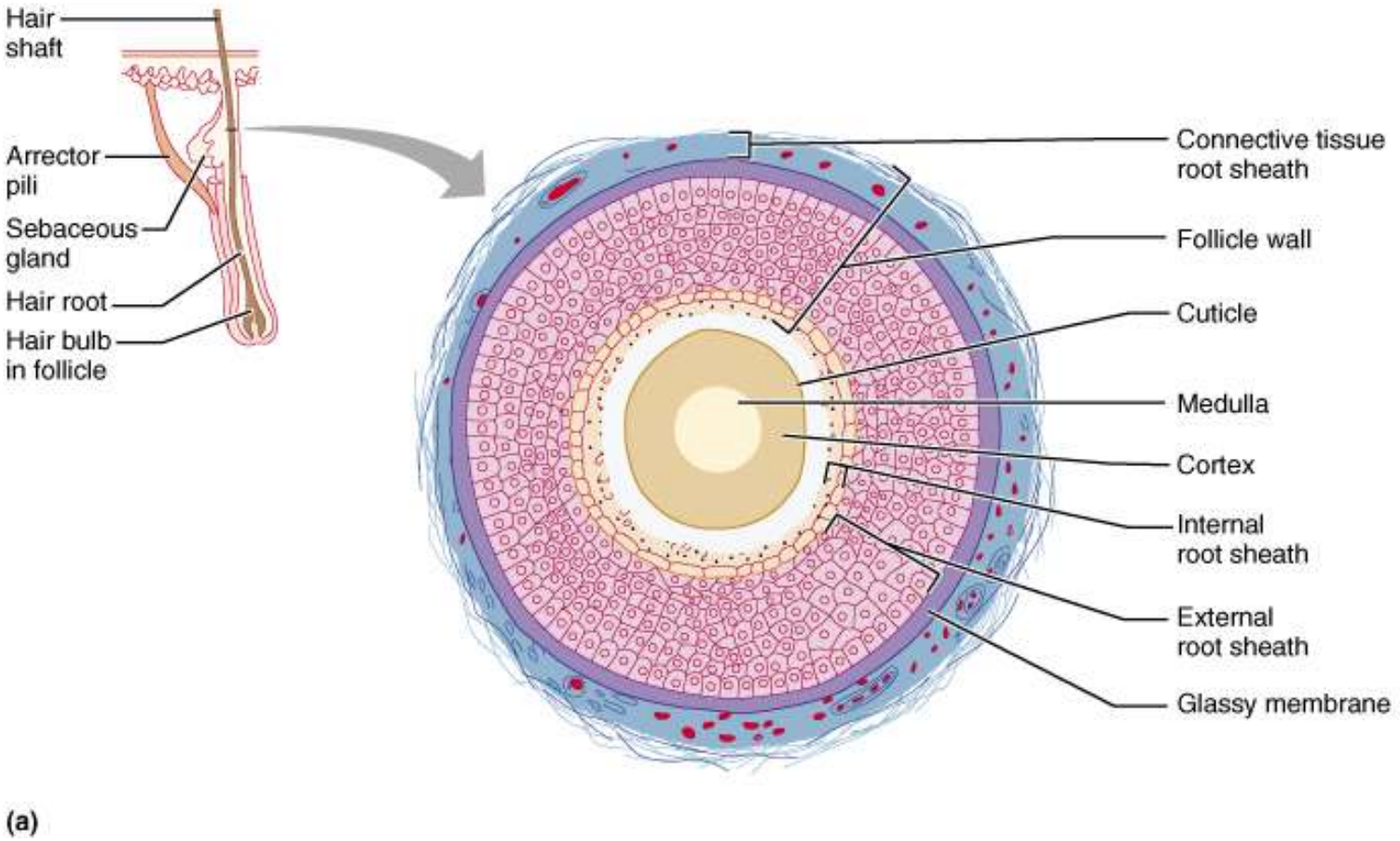

Figure 5.6a

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Hair Follicle

- Root sheath extending from the epidermal surface into the dermis
- Deep end is expanded forming a hair bulb
- A knot of sensory nerve endings (a root hair plexus) wraps around each hair bulb
- Bending a hair stimulates these endings, hence our hairs act as sensitive touch receptors

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Hair Follicle

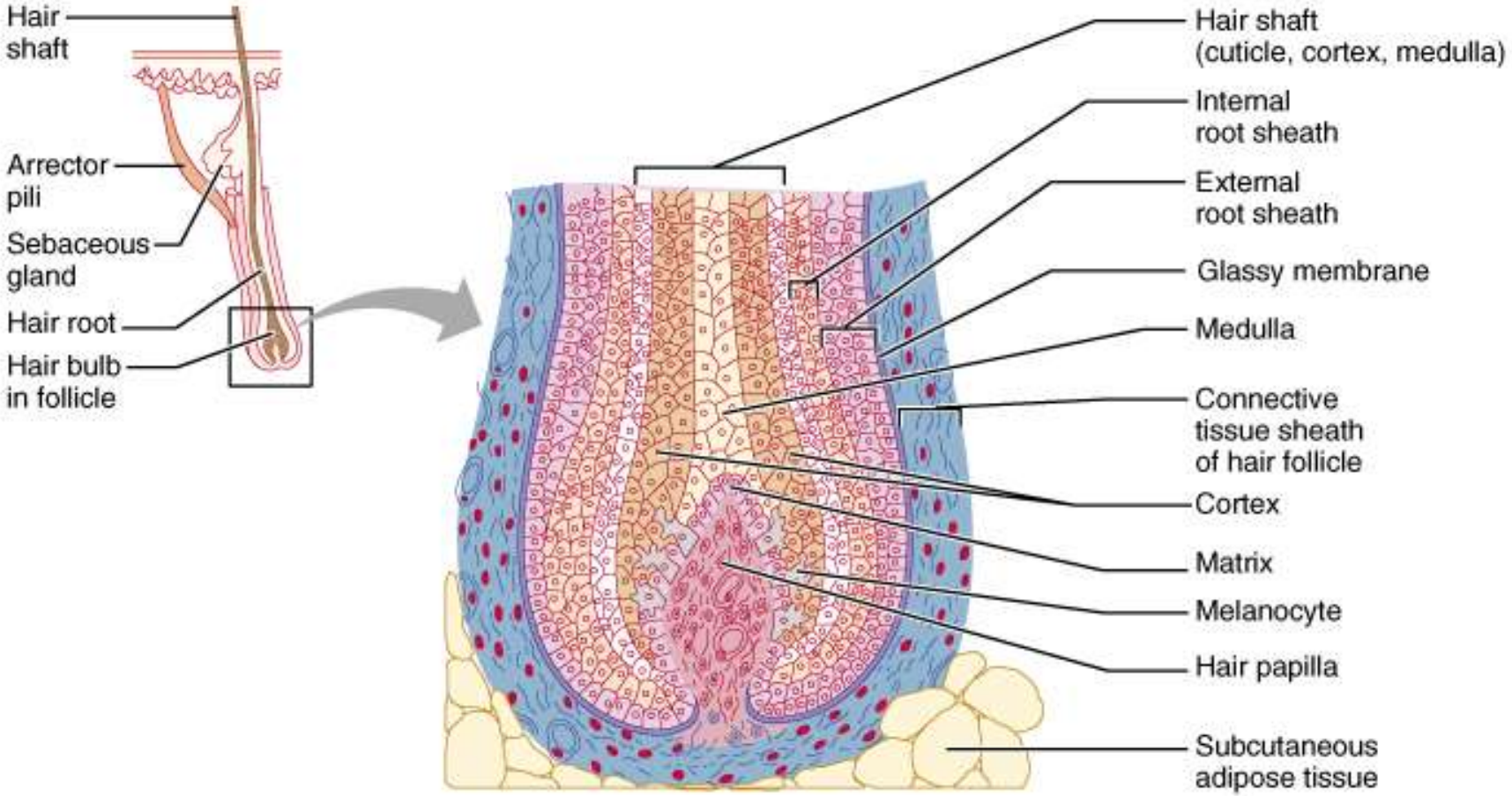

Figure 5.6c

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Types of Hair

- Vellus – pale, fine body hair found in children and the adult female
- Terminal – coarse, long hair of eyebrows, scalp, axillary, and pubic regions

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Hair Growth

Hair grows at different rates on different individuals.

Hair spends 2 to 6 years in the growth phase where keratinized cells accumulate from the hair follicle. After this they spend several months in the resting phase.

Following the resting phase, the hair will fall out and be replaced by another hair.

Some people are better able to grow long hair than others based on this phenomenon.

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Hair Thinning and Baldness

- Alopecia – hair thinning in both sexes
- True, or frank, baldness – genetically determined and sex influenced condition (i.e., male pattern baldness)
- Androgens seem to play a key role in male patterened baldeness.
- This occurs in women too. Men tend to move toward “horseshoe” pattern while women usually more uniform thinning on the top of the head

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Nails

-Plates of tightly pack, hard, keratinized epidermal cells.

-Form a clear and solid covering over dorsal surfaces of distal portions of digits

-Nails consist of:

Nail body: portion of nail that is visible

Free edge: portion that may extend past distal end

Nail Root: portion buried in a fold of skin

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…continued

-Most of nail body appears pink due to underlying capillaries

-Free edge is white because of absence of underlying capillaries

-Lunula: crescent shaped white portion at proximal end of nail

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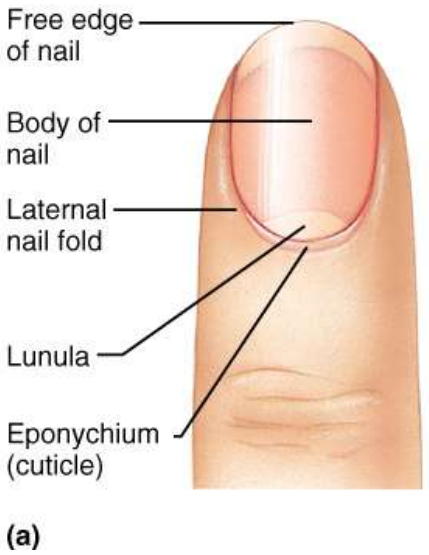
Structure of a Nail

- Scalelike modification of the epidermis on the distal, dorsal surface of fingers and toes



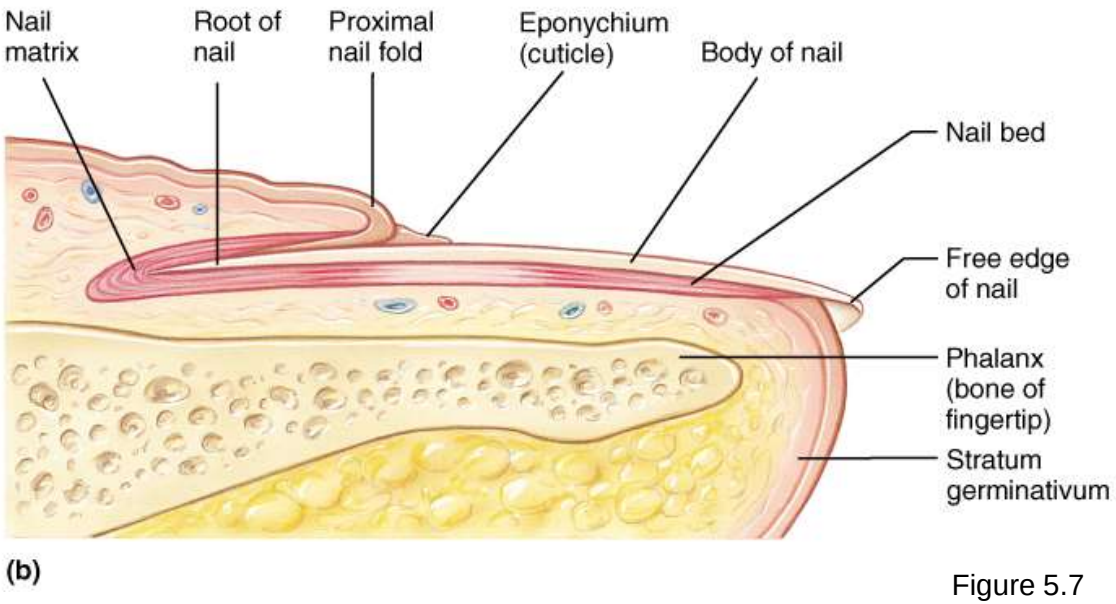


Figure 5.7

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Functions of the Integumentary System

- Protection – chemical, physical, and mechanical barrier
- Body temperature
- Regulated by dilation (cooling) and constriction (warming) of dermal vessels
- Sweat glands increase secretions to cool the body
- Cutaneous sensation – exoreceptors sense touch and pain

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Functions of the Integumentary System

- Metabolic functions – synthesis of vitamin D in dermal blood vessels
- Blood reservoir – skin blood vessels store up to 5% of the body's blood volume
- Excretion – limited amounts of nitrogenous wastes are eliminated from the body in sweat

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Skin Cancer

- Basal cell carcinoma
- Squamous cell carcinoma
- Melanoma

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Basal Cell Carcinoma

- Least malignant and most common skin cancer
- Stratum basale cells proliferate and invade the dermis and hypodermis
- Slow growing and do not often metastasize
- Can be cured by surgical excision in 99% of the cases

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Squamous Cell Carcinoma

- Arises from keratinocytes of stratum spinosum
- Arise most often on scalp, ears, and lower lip
- Grows rapidly and metastasizes if not removed
- Prognosis is good if treated by radiation therapy or removed surgically

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Melanoma

- Cancer of melanocytes is the most dangerous type of skin cancer
- Melanomas have the following characteristics (ABCD rule):
  - A: Asymmetry; the two sides of the pigmented area do not match
  - B: Border is irregular and exhibits indentations
  - C: Color (pigmented area) is black, brown, tan, and sometimes red or blue
  - D: Diameter is larger than 6 mm (size of a pencil eraser)

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Melanoma

- Treated by wide surgical excision accompanied by immunotherapy
- Chance of survival is poor if the lesion is over 4 mm thick

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Pune District Education Association , Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre, Kharadi, Pune-411014

Presentation on

SOFT GELATIN CAPSULES

Presented by :-

Mrs. Trusha P. Shangrapawar

CAPSULES

Defination

Capsule are solid dosage forms in which the active medicament are enclosed in either a hard or soft soluble container or shell of a suitable form of gelatin.

Capsules are solid dosage forms in which drug and/or inert substances are enclosed in a gelatin shell.

CLASSIFICATION OF CAPSULES

CAPSULES

HARD GELATIN CAPSULES

SOFT GELATIN CAPSULES

SOFT GELATIN CAPSULES

Definition

A **soft gelatin capsule** is a type of capsule that is usually used to contain medicine in the form of liquid or powder, and which dissolves more quickly than a hard gelatin capsule. It dissolves readily in the gastric juices of the stomach, and may work faster than other capsules.

ADVANTAGES

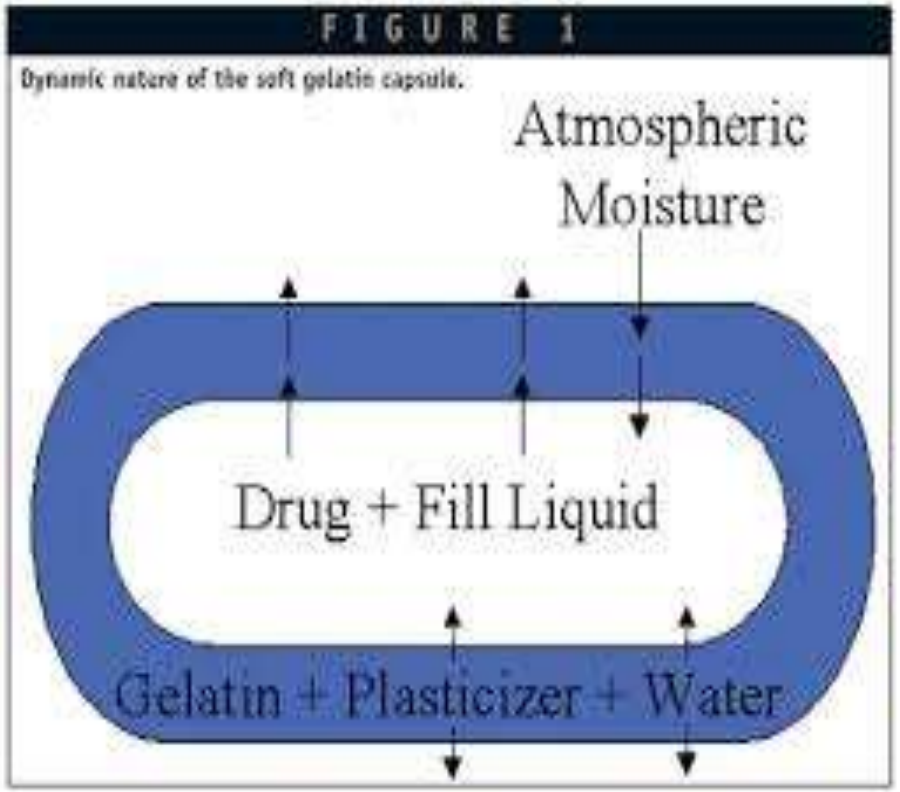
- Reproducibility
- Homogenisity
- Rapid dissolution
- Enhanced bioavailability
- Higher mean plasma levels
- Less amount of exicipients
- Elegency
- Masking of taste and odor of drugs

DISADVANTAGES

- Expensive
- Incompatability
- Loss of drug
- Softening

SHELL COMPOSITION

- Gelatin
- Plasticizer
- Colours
- Opacifier
- Flavours



GELATIN

Gelatin is heterogeneous product derived from the partial, irreversible, hydrolytic extraction of the treated animal collagens obtained from

- Animals bones
- Hide portions
- Frozen pork skin
- Connective tissues

- This long polypeptide chain yields on hydrolysis 18 amino acid, the most prevalent of which are glycine and alanine.
- Gelatin can vary in its chemical and physical properties depending on the manner of extraction.
- There are two basic types of gelatin

➢ Types A

➢ Types B

Pork Skin

↓

Acid hydrolysis

↓

Pharmagel A

↓

Isoelectic point : 7.0 - 9.0

Bones

↓

Alkaline hydrolysis

↓

Pharmagel B

↓

Isoelectic point : 4.8 - 5.0

PLACTISIZER

- A plasticizer or softener is a substance incorporated in a material (usually a plastic) to increase the flexibility, workability,dispensability.

- It may reduce the melt viscosity, lower temperature of second order transition or lower the elastic modulus of the product.

- Plasticizers significantly account for a large proportion of the capsule — usually, approximately 20 – 30 % of the soft gel.

TYPES OF PLASTICIZERS:

Phthalates
Adipates
Citrates
Phosphate esters
Polymerics
Esters of glycol and polyhydric alcohols
Sebacate nad azelate esters
Secondary plasticizers
Trimellitates

COLORS

The colors used in shell has to be darker than the color of an encapsulating materials. Colors may be natural or synthetic. They are useful in making the capsules more attractive.

OPAFICERS

The opacifier initiates the formation of a thick cover. Furthermore, it disrupts any potential photodegradation of the light susceptible ingredients. Using Titanium oxide as individual constituent results in the formation of a white but opaque shell. Elsewhere, when you incorporate other substituents, then you get a colored one.

Thank You!!

Pune District Education Association's
Shankarrao Ursal College of
Pharmaceutical Sciences & Research
Centre, Kharadi, Pune.

SPECTROFLUORIMETRY

Presented by : Dr.Vijaya .U. Barge

LEARNING OBJECTIVES

- Introduction to spectroflurometry.
- To study luminescence and its type.
- To learn electronic transitions and energy level diagram.
- To study fluorescence and phosphorescence and factors affecting it.
- To learn about instrumentation and working of fluorimetry.

INTRODUCTION

- Fluorescence is the molecular absorption of light energy at one wavelength and its nearly instantaneous re-emission at another, usually longer wavelength.
- Fluorometry or fluorimetry is the measurement of fluorescence.
- The instrument used to measure fluorescence is called a fluorometer.
- The emitted light is proportional to the concentration of the analyte being measured (up to a maximum concentration).

CONTIUNE…..

- Fluorescent behavior is observe in simple as well as in complex gaseous, liquid, and solid systems.
- Light is absorbed by molecules which causes electrons to become excited to a higher electronic state.
- The electrons remain in the excited state till excitied electron loses excess energy is not lost by collisions with other molecules,
- After loss of excess of energy ,the electron returns to the ground state.
- Energy is emitted during the electrons return to their ground state.

TYPES OF LUMINESCENCE

1) **Photoluminescence** - As the excitation of the molecule is due to the absorption of a photon (light), these types of luminescence are called photoluminescence.

(a) **Fluorescence**: Fluorescence is the emission of visible light by a substance that has absorbed light of a different wavelength. The emitted photon has a longer wavelength.

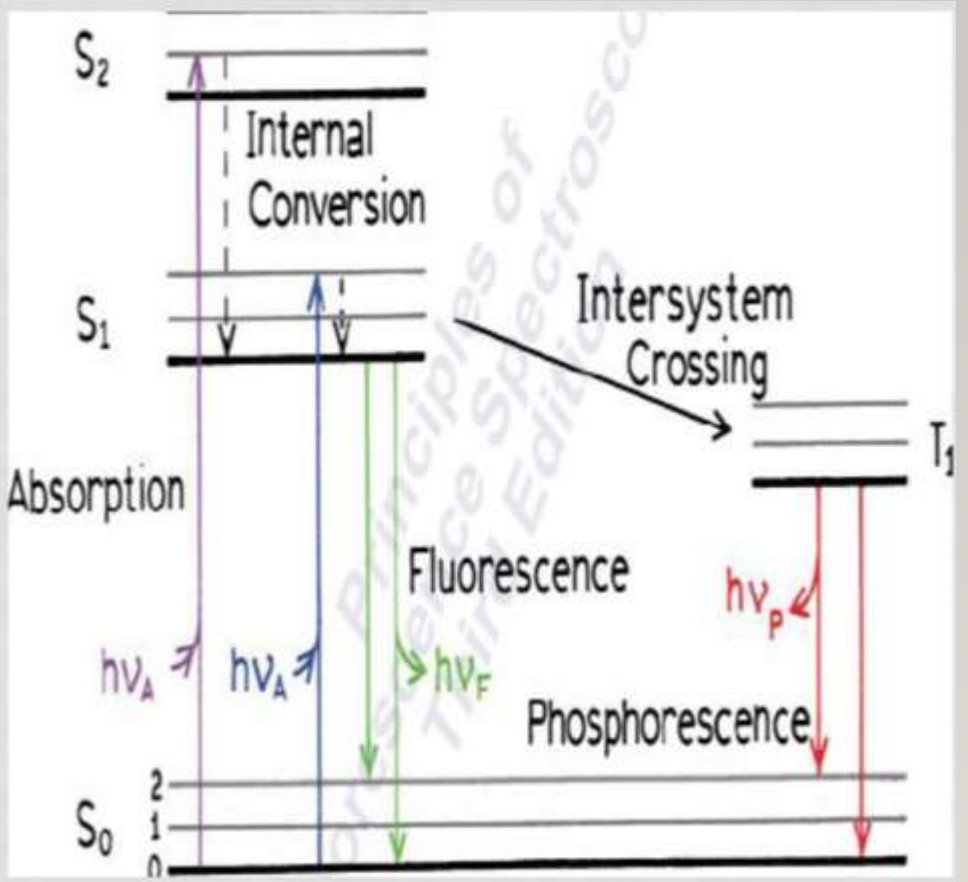
(b) **Phosphorescence**: Phosphorescence is related to fluorescence in emitting a photon however, a phosphorescent material does not immediately re-emit the radiation it absorbs.

IMPORTANT TERMS

- Singlet ground state: state in which electrons in a molecule are paired.
- Singlet excited state: state in which electrons are unpaid but of opposite spins.
- Triplet state: state in which unpaired electrons of same spin are present.
- Excitation process: absorption of energy or light followed by conversion from ground state to excite state.
- Relaxation process: process by which atom or molecule losses energy & returns to ground state.

ENERGY LEVEL DIAGRAMS FOR PHOTOLUMINESCENT MOLECULES

- A Jablonski diagram is basically an energy diagram, arranged with energy on a vertical axis.
- The diagram is arranged into columns.
- Every column usually represents a specific spin multiplicity for a particular species.
- As electronic energy states increase, the difference in energy becomes continually less.
- Additionally, as the electronic energy levels get closer together, the overlap of vibronic energy levels increases.



DEACTIVATION PROCESSES

- An excited molecule can return to its ground state by a combination of several mechanistic steps. The basic two of these steps are fluorescence and phosphorescence, involve the release of a photon of radiation.
- The other deactivation methods are-

1. Vibrational relaxation
2. Internal conversion
3. External conversion
4. Intersystem crossing

1. Vibrational relaxation

- Collisions between the molecules of the excited species and those of the solvent lead to rapid energy transfer and a very small increase in temperature of the solvent.
- Vibrational relaxation process is so efficient that the average lifetime of a vibrationally excited molecule is very less. As a consequence, fluorescence from solution always involves a transition from the lowest vibrational level of an excited state.

2. Internal conversion

- The term internal conversion is employed to describe inter molecular processes by when a molecule passes to a lower energy electronic state without emission of radiation.
- Internal conversion takes when two electronic energy levels are sufficiently close the existence of an overlap in vibrational levels.

3. External conversion

- Deactivation of an excited electronic state may involve interaction and energy transfer between the excited molecule and the solvent or other solutes. These processes are called external conversions.
- Evidence for external conversion includes the marked effect on the fluorescent intensity of most species.

4. Intersystem crossing

- It is a process in which the spin of an excited electron is reversed and a change à multiplicity of the molecule results.
- It most common in molecules that contain heavy atoms, such a iodine or bromine. Spin and orbital interactions increase in the presence of such atoms, and change in spin is thus more favored.

FACTORS AFFECTING FLUORESCENCE AND PHOSPHORESCENCE INTENSITY

1. **Adsorption** : Sensitivity requires very dilute solutions that is 10-100 times weaker than those employed in absorption spectroscopy which leads to adsorption of the fluorescent substance on the container walls.
2. **Intensity of incident light**: Increase in intensity of incident light on the sample increases fluorescence intensity.

   Intensity of incident light depends on:

   (a) Intensity of light emitted from the lamp.

   (b) Excitation monochromator transmission properties.

   (c) Excitation slit width.

3. **Turbidity** : Fluorescence measurements are significantly more immune to the effects of turbidity compared to absorption techniques like UV/VIS spectrophotometers. If the interfering substance is reflective, turbidity can create light scatter and reading. If the interfering substance absorbs light, fluorescence will be reduced.
4. **Bubbles in the Sample**: Since fluorescence is such a sensitive measurement, and since accuracy of readings depend on light exciting the molecules of the compound, bubbles in the sample can result in fluctuating readings
5. **Effect of pH**:Fluorescence can be affected by pH. The effect of pH depends on the chemical structureof the molecule.
6. **Temperature**:Fluorescence is affected by changes in temperature. As temperature increases, fluorescence decreases. And wise vesa.
7. **Viscosity**:Increase viscosity leads to decreased collisions of molecules which lead to enhancement of fluorescence intensity and vice a versa.

QUENCHING

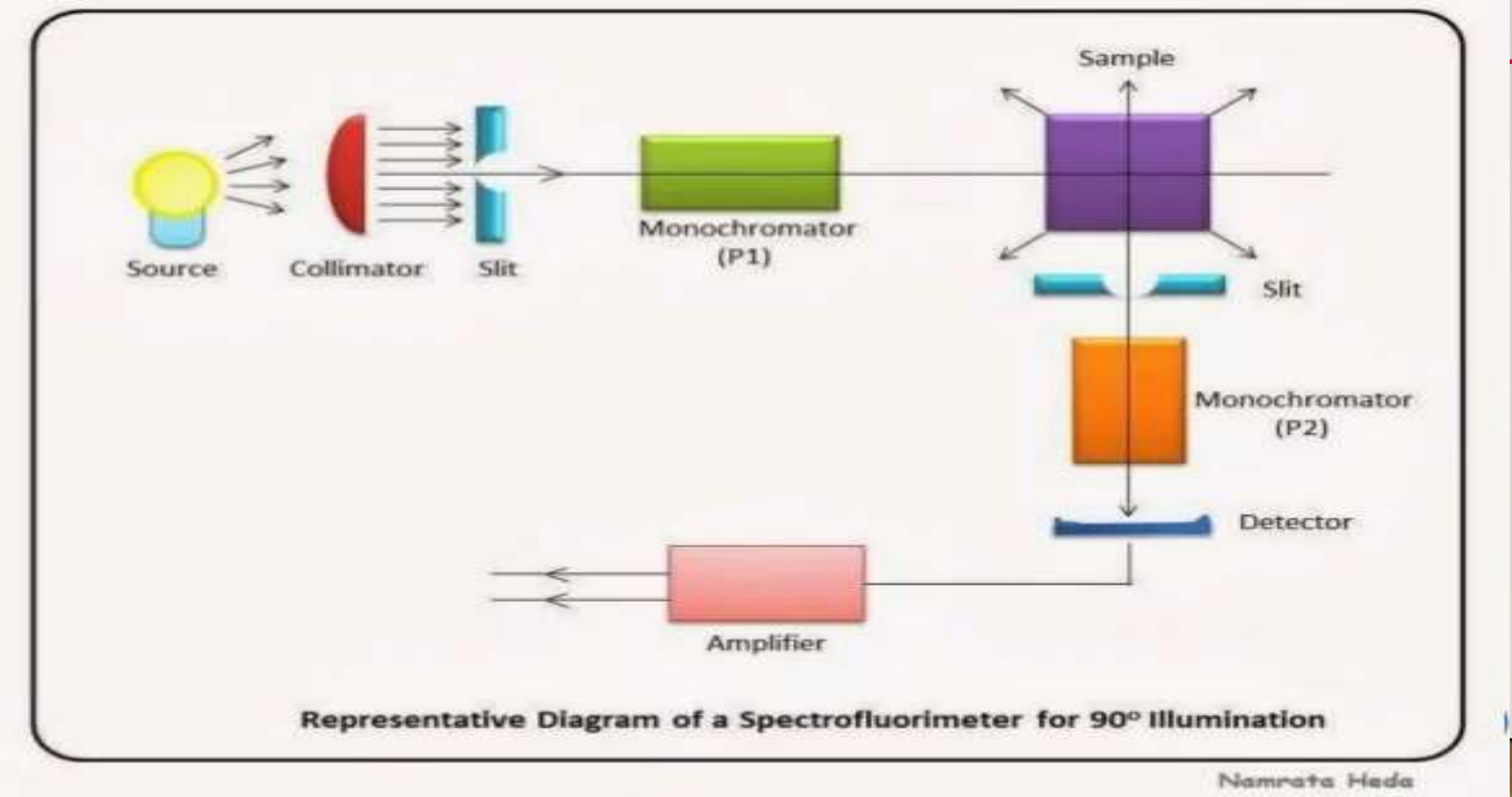
- It is a process that decrease the fluorescence intensity of given substance. It may occur due to various factors ike pH, temperature, viscosity, complex formation.

FLUROSCENCE CAN BE LOST BY

1. **Concentration quenching**: At low concentration linearity is observed. But as the concentration of the same substance increased in the sample fluroscence intensity decreases.

2. **Chemical quenching**: it may be of various factor like
   a) **pH**: aniline at pH 5-13 gives blue flouresence But at pH 5 and pH >13 it does not exhibit flouresence
   b) **Oxygen**: presence of oxygen leads to quenching because of its parmegnatic property.
   c) **Halides and electron withdrawing group**: Halides likechloride bromide, iodide and electron withdrawing group like nitro and carboxylic group decreases the flouresence intensity.
   d) **Heavy metals**: Presence of heavy metals also leads toquenching because of collision and triplet ground state.
3. **Static quenching**: In these process the quenching agent forms a non-fluorescent complex .
4. **Collisional quenching**: Quenching take place when number of collision are increased due to several factor like presence of halides, heave metals increased temperature and decrease in viscosity.

INSTRUMENTATION

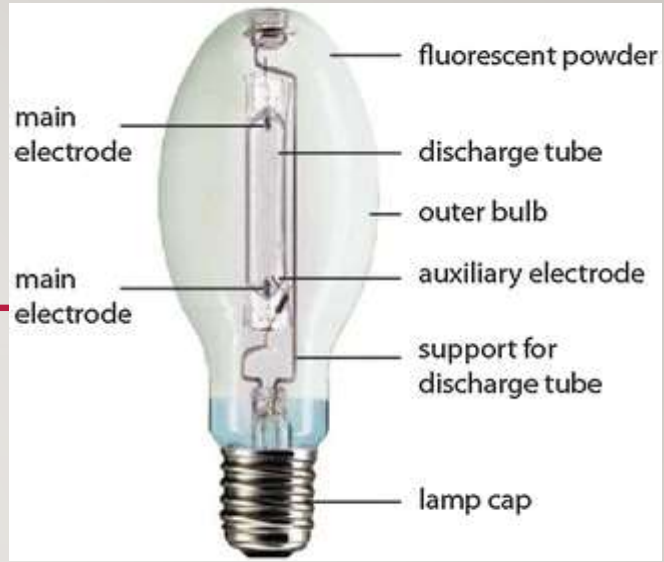


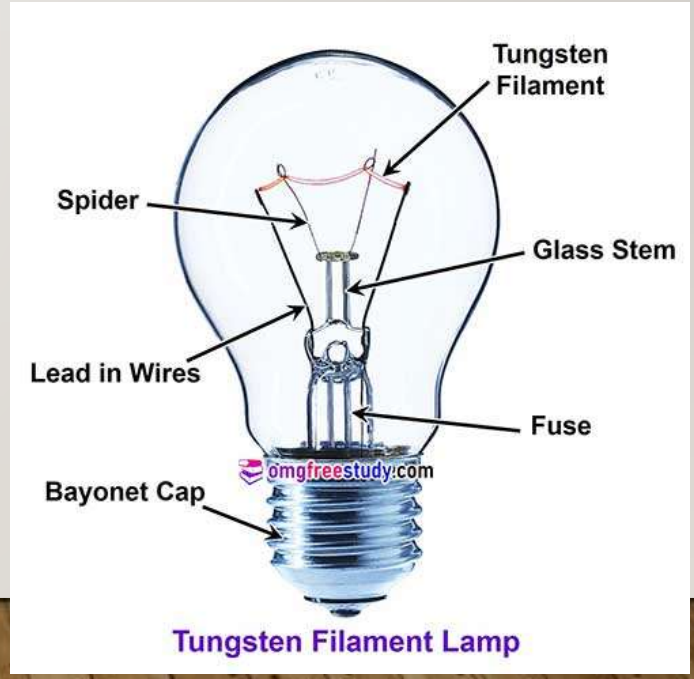
1. LIGHT SOURCE

a) Mercury vapour lamp: Mercury vapour at high pressure give intense lines on continuous background above 350nm.low pressure mercury vapour gives an additional line at 254nm.it is used in filter fluorimeter.

b) xenon arc lamp: It give more intense radiation than mercury vapour lamp. it is used in spectrofluorimeter.

c) tungsten lamp:- If excitation has to be done in visible region this can be used. It is used in low cost instruments.





2. MONOCHROMATOR /FILTER

- Filters: these are nothing but optical filters works on the principle of absorption of unwanted light and transmitting the required wavelength of light. In inexpensive instruments fluorimeter primary filter and secondary filter are present.
- Monochromators: they convert polychromatic light into monochromatic light. They can isolate a specific range of wavelength or a particular wavelength of radiation from a source.

3. SAMPLE CELLS

- These are ment for holding liquid samples. These are made up of quartz which are known as cuvettes and can have various shapes ex: cylindrical or rectangular etc.

4. LIGHT DETECTOR

- The light detector is most often a photomultiplier tube, though photodiodes are increasingly being used. The light passing through the emission filter is detected by the photomultiplier or photodiode. The light intensity, which is directly proportional (linear) to the compound's concentration, is registered as a digital readout.

APPLICATION OF SPECTROFLUOROMETRY

- Determinaton of inorganic substance.
- Determination of uranium salt.
- Used in acid base titration.
- Oraganic anaylsis.
- Pharmaceutical analysis.
- Liquid chromatography.
- Determination of vitamin B1 and B2.

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THANK YOU

Pune District Education Association's Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre.

Thermogravimetric Analysis

Presented by : Dr. Vijaya U. Barge

(Vice Principal & Professor)

Contents

- Introduction
- Types
- Principal
- TGA Curve
- Instrumentation
- Factors affecting results
- Advantages
- Limitations
- Pharmaceutical applications

Thermogravimetric analysis(TGA)

- Thermogravimetric analysis or thermal gravimetric analysis (TGA) is a method of thermal analysis in which the mass of a sample is measured over time as the temperature changes.

- This measurement provides information about physical phenomena, such as phase transitions, absorption and desorption as well as chemical phenomena including chemisorptions, thermal decomposition, and solid-gas reactions (e.g., oxidation or reduction)

Three types of thermogravimetry:

1.Isothermal or static thermogravimetry: In this technique the sample weight is recorded as function of time at constant temperature.

2.Quasistatic thermogravimetry: In this technique the sample is heated to constant weight at each of series of increasing temperatures.

3.Dynamic thermogravimetry: In this technique the sample is heated in an environment whose temperature is changing in a predetermined manner generally at linear rate. This type is generally used.

Principle of TGA:

- In thermo-gravimetric analysis, the sample is heated in a given environment (air, N_2, CO_2, He, Ar, etc.) at controlled rate. The change in the weight of the substance is recorded as a function of temperature or time.

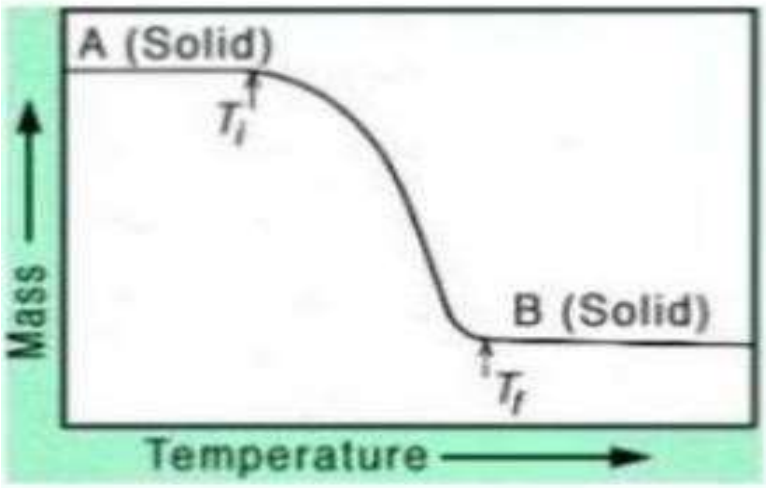
- The temperature is increased at a constant rate for a known initial weight of the substance and the changes in weights are recorded as a function of temperature at different time interval.

- This plot of weight change against temperature is called thermo-gravimetric curve or thermo-gram, this is the basic principle of TGA.

TGA curve:

- The instrument used for themo-gravimetry is a programmed precision balance for rise in temperature known as Thermo-balance.

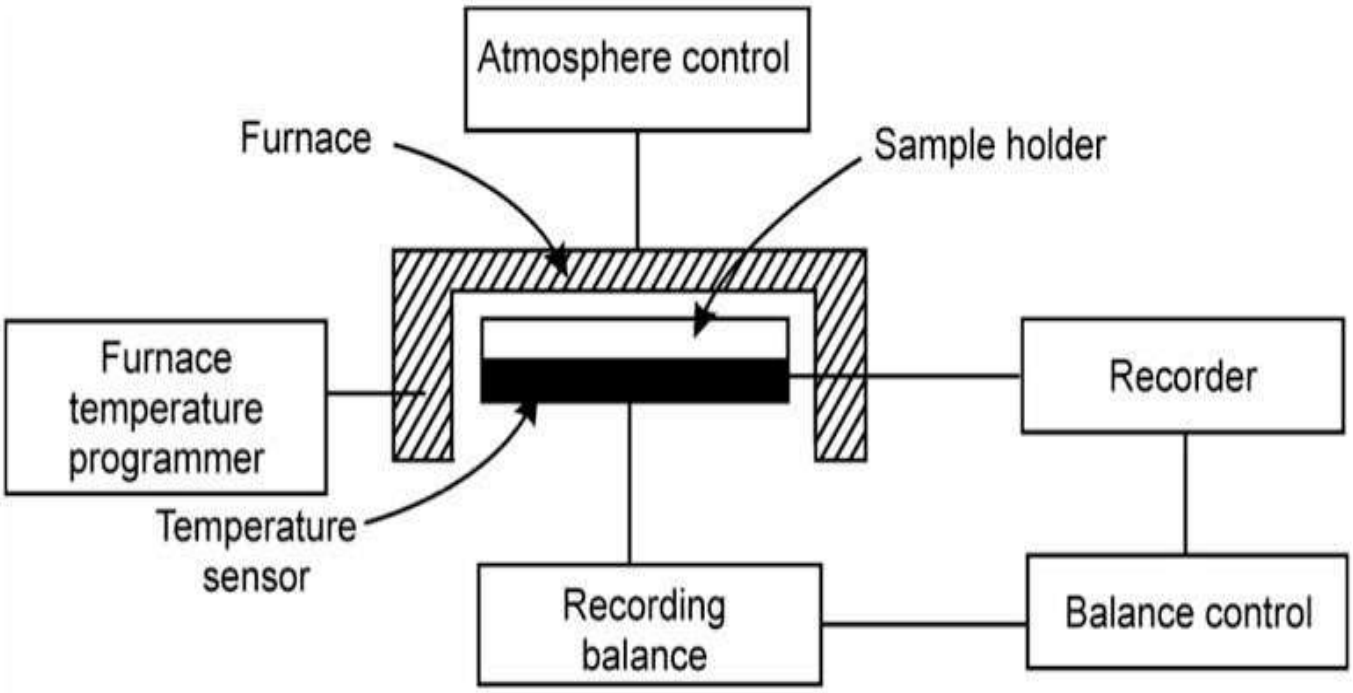
- Results are displayed by a plot of mass change versus temperature or time and are known as Thermogravimetric curves or TG curves.



TGA curve:

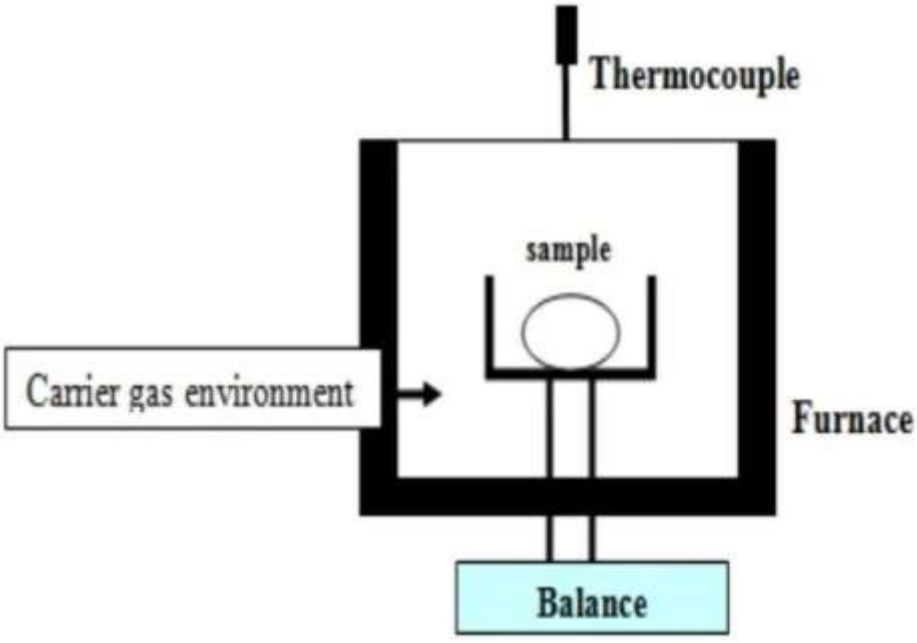
- TG curves are normally plotted with the mass change (Dm) in percentage on the y-axis and temperature (T) or time (t) on the x-axis.
- There are two temperatures in the reaction, Ti(procedural decomposition temp.) and Tf(final temp.) representing the lowest temperature at which the onset of a mass change is seen and the lowest temperature at which the process has been completed respectively.
- The reaction temperature and interval (Tf-Ti) depend on the experimental condition; therefore, they do not have any fixed value.

Instrumentation of TGA:



Instrumentation of TGA:

- Recording balance
- Sample holder
- Furnace
- Temperature programmer /controller (thermocouple)
- Recorder



Recording balance : A microbalance is used to record a change in mass of sample/ substance.

An ideal microbalance must possess following features:

•It should accurately and reproducibly record the change in mass of sample in ideal ranges of atmospheric conditions and temperatures.

• It should provide electronic signals to record the change in mass using a recorder.

• The electronic signals should provide rapid response to change in mass.

Recording balance :

- It should be stable at high ranges, mechanically and electrically.

- Its operation should be user friendly. After the sample has been placed on microbalance, it is left for 10-15min to stabilize.

Recorder balances are of to types:

I. Deflection-type instruments and
II. Null-type instrument

Recording balance :

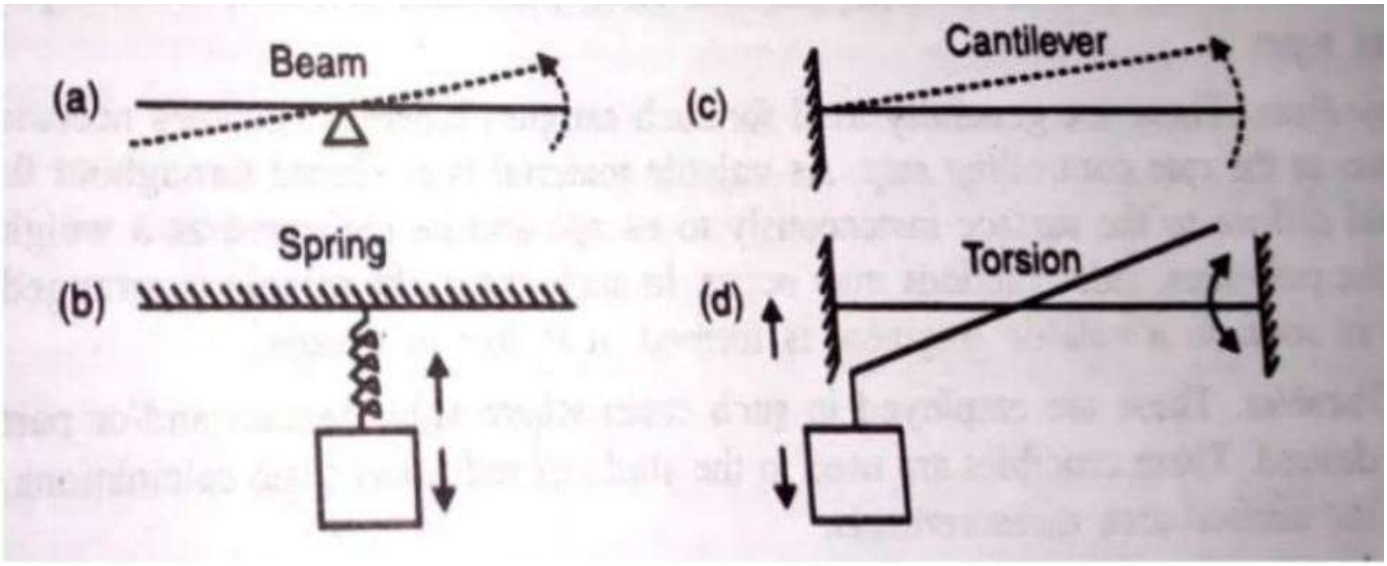
I. **Deflection balances :** They are following types-

i. Beam type

ii. Helical type

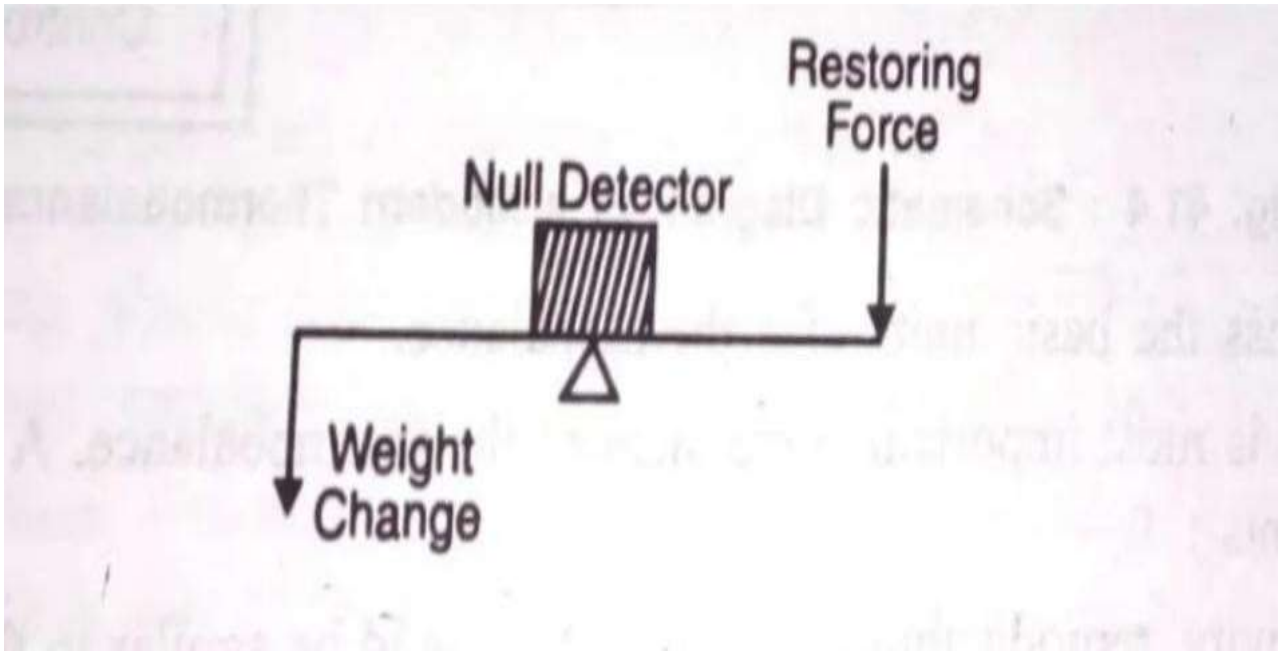
iii. Cantilevered beam

iv. Torsion wire



II. Null point balances:

It consist of a sensor which detects the deviation from the null point and restores the balance to its null points by means of restoring force.



Sample holder :

- The sample to be studied is placed in sample holder or crucible. It is attached to the weighing arm of microbalance.

- There are different varieties of crucibles used. Some differ in shape and size while some differ in materials used.

- They are made up from platinum, aluminum, quartz or alumina and some other materials like graphite, stainless steel, glass, etc

Sample holder:

Crucibles:

Crucibles should have temperature at least 100K greater than temperature range of experiment and must transfer heat uniformly to sample. Therefore the shape, thermal conductivity and thermal mass of crucibles are important which depends on the weight and nature of sample and temperature range.

There are different types of crucibles.

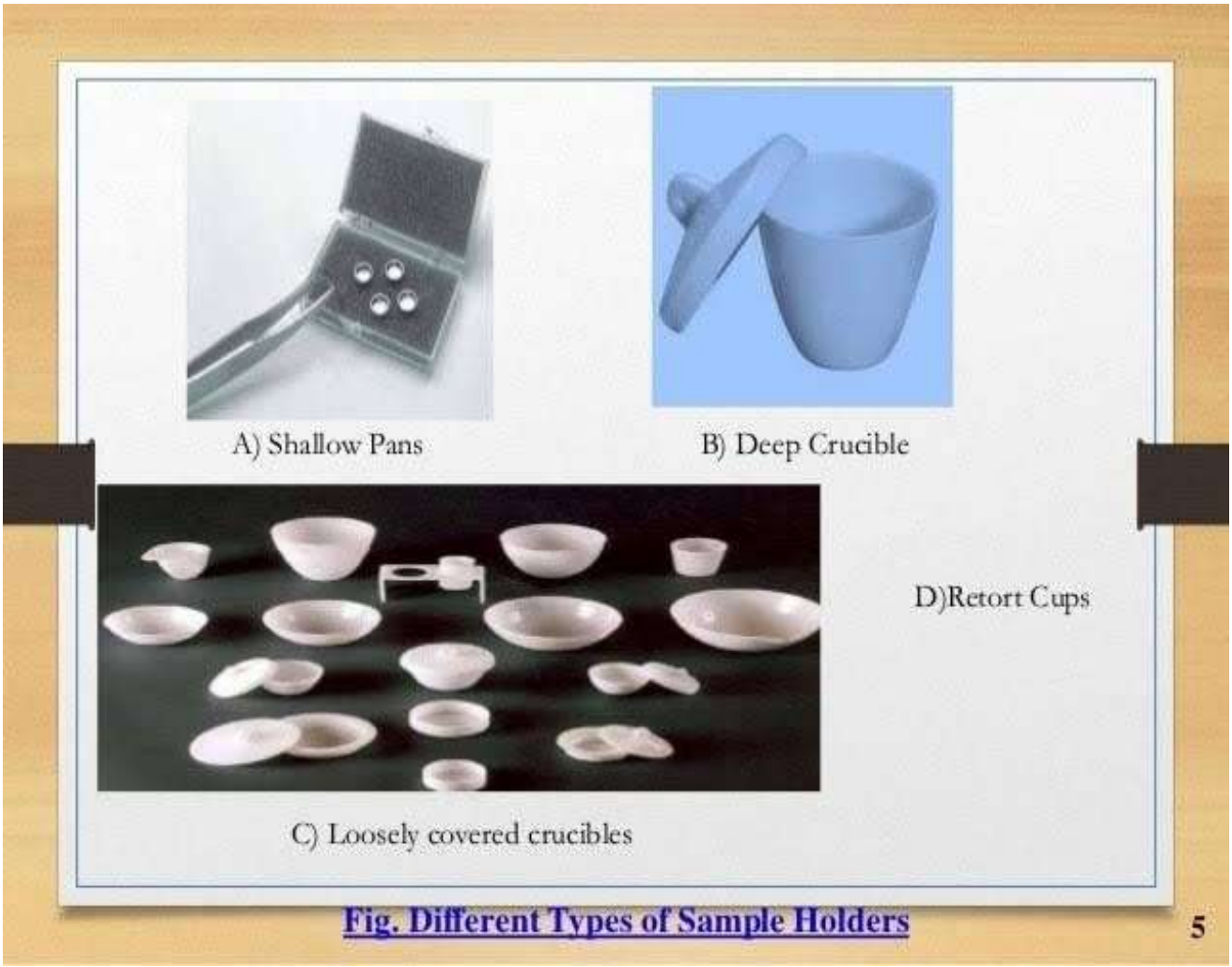
1.Shallow pans(used for volatile substances)

2. Deep crucibles (Industrial scale calcination)

3. Loosely covered crucibles (self generated atm. Studies)

4. Retort cups (Boiling point studies)

Types of crucibles:



Furnace :

- The furnace should be designed in such way that it produces a linear heating range.

- It should have a hot zone which can hold sample and crucible and its temperature corresponds to the temperature of furnace.

- There are different combinations of microbalance and furnace available. The furnace heating coil should be wound in such a way that there is no magnetic interaction between coil and sample or there can cause apparent mass change.

Temperature programmer/controller:

- Temperature measurement is done in no. of ways thermocouple is the most common technique.

- The position of the temperature measuring device relative to the sample is very important.

- The major types are:

a . The thermocouple is placed near the sample container and it has no contact with the sample container. This isn't a good arrangement where low-pressure are employed.

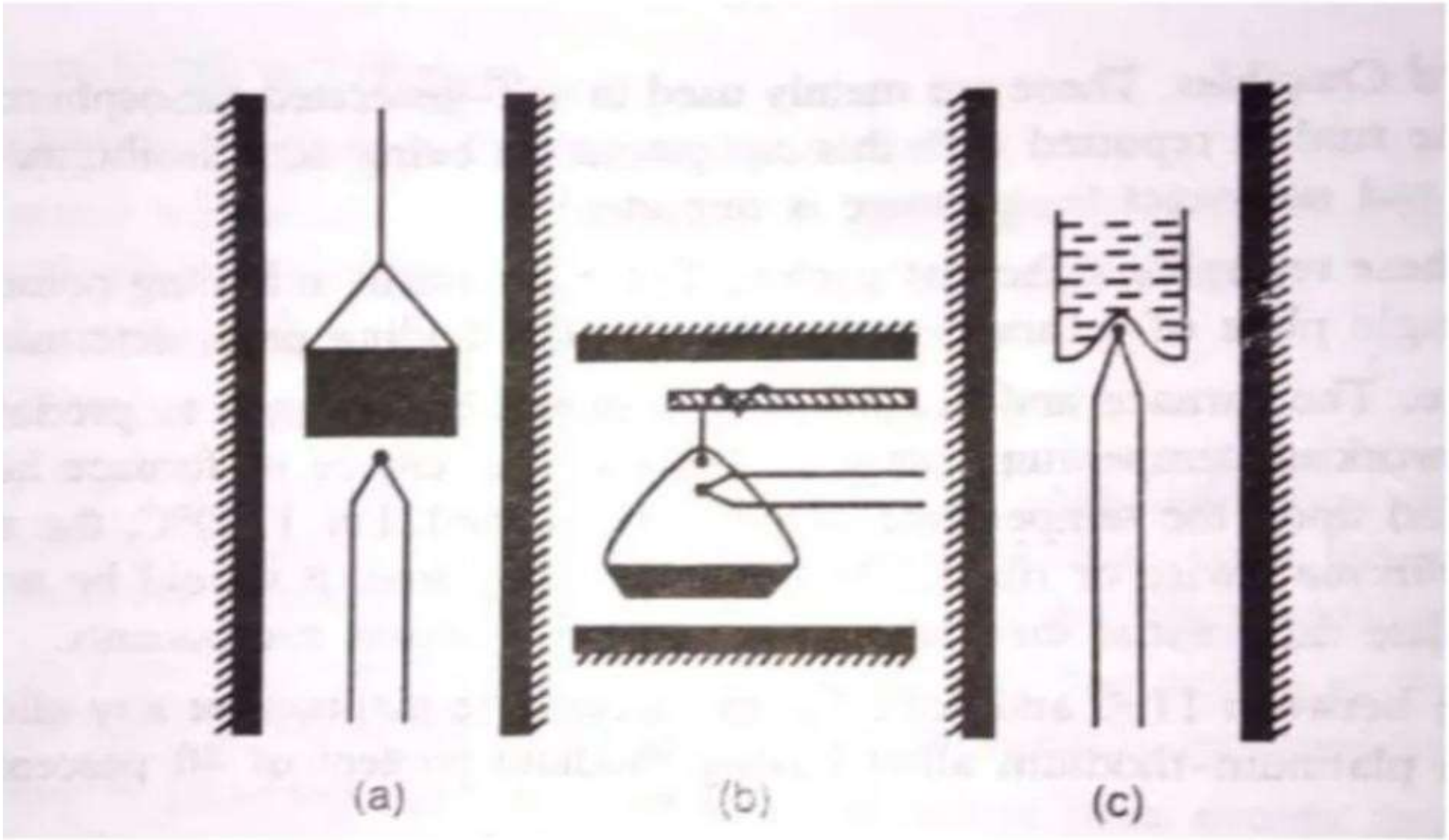
Temperature programmer/controller:

b. The sample is kept inside the sample holder but not in contact with it. This arrangement is better than that of (a) because it responds to small temperature changes.

c. The thermocouple is placed either in contact with sample or with the sample container. This is the best arrangement of sample temperature detection.

Temperature programmer/controller:

Thermocouple in a thermo-balance:



Recorder:

The recording systems are mainly of 2types

1.Time-base potentiometric strip chart recorder.

2. X-Y recorder.

- In some instruments, light beam galvanometer, photographic detail recorders or one recorder with two or more pens are also used.

- In the X-Y recorder, we get curves having plot of weights directly against temperatures. However, the percentage mass change against temperature or time would be more useful.

Factors affecting TGA:

The factors which may affect the TGA curves are classified into two main groups.:

(1) Instrumental factors:

(a) Furnace heating rate

(b) Furnace atmosphere

(2) Sample characteristics includes :

(a) Weight of the sample

(b) Sample particle size

Factors affecting TGA:

1.Instrumental factors :

a.Furnace Heating rate: The temperature at which the compound (or sample) decompose depends upon the heating rate. When the heating rate is high, the decomposition temperature is also high. A heating rate of 3.5℃ per minute is usually recommended for reliable and reproducible TGA.

b.Furnace atmosphere: The atmosphere inside the furnace surrounding the sample has a profound effect on the decomposition temperature of the sample. A pure N2 gas from a cylinder passed through the furnace which provides an inert atmosphere.

Factors affecting TGA:

2.Sample characteristics:

a.Weight of the sample: A small weight of the sample is recommended using a small weight eliminates the existence of temperature gradient throughout the sample.

b.Particle size of the sample: The particle size of the sample should be small and uniform. The use of large particle or crystal may result in apparent, very rapid weight loss during heating. 25

Other factors affecting TGA curve:

- Sample holder
- Heat of reaction
- Compactness of sample
- Previous history of the sample

Advantages of TGA:

- A relatively small set of data is to be treated.

- Continuous recording of weight loss as a function of temperature ensures Equal weightage to examination over the whole range of study.

- As a single sample is analyzed over the whole range of temperature, the variation in the value of the kinetic parameters, if any, will be indicated.

Limitations of TGA:

- The Chemical or physical changes which are not accompanied by the change in mass on heating are not indicated in thermo- gravimetric analysis.

- During TGA, Pure fusion reaction, crystalline transition, glass transition, crystallization and solid state reaction with no volatile product would not be indicated because they provide no change in mass of the specimen.

Applications of TGA:

- From TGA, we can determine the purity and thermal stability of both primary and secondary standard.

- Determination of the composition of complex mixture and decomposition of complex OR composition of complex systems.

- For studying the sublimation behaviour of various substances.

- TGA is used to study the kinetics of the reaction rate constant.

- The effect of reactive or corrosive atmosphere on materials.

- Analysis of the dosage form.

- Used in the study of catalyst: The change in the chemical states of the catalyst may be studied by TGA techniques. (Zn- $ZnCrO_4$) Zinc-Zinc chromate is used as the catalyst in the synthesis of methanol.
- Oxidative stability of materials.

- Estimated lifetime of a product.

- Moisture and volatiles contents on materials.

- TGA is often used to measure residual solvents and moisture, but can also be used to determine solubility of pharmaceutical materials in solvents.

thank
you

The Human Digestive System

By
Mr. Krunal G. Kanase
HOD & Assistant Professor
Department of Pharmacology
PDEA's SUCOPSRC, Pune

Digestion

- Phases Include
  1. Ingestion
  2. Movement
  3. Mechanical and Chemical Digestion
  4. Absorption
  5. Elimination

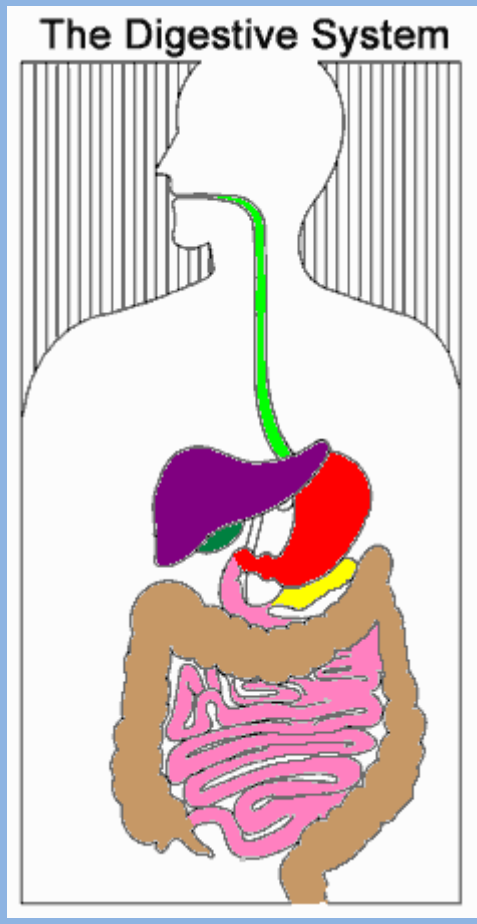
  Digestion- is the process of breaking down food into a form your body can use.

Digestion

- Types
  - Mechanical (physical)
    - Chew
    - Tear
    - Grind
    - Mash
    - Mix
  - Chemical
    - Enzymatic reactions to improve digestion of
      - Carbohydrates
      - Proteins
      - Lipids

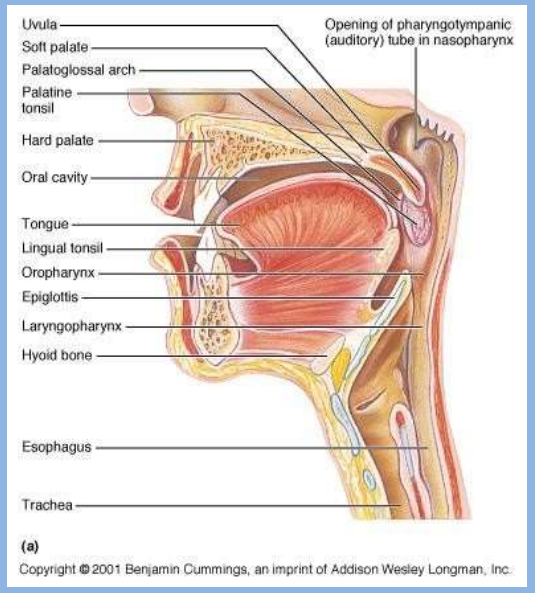
Digestive System Organization

- Gastrointestinal (Gl) tract
  - Tube within a tube
  - Direct link/path between organs
  - Structures
    - Mouth
    - Pharynx
    - Esophagus
    - Stomach
    - Small intestine
    - Large Intestine
    - Rectum

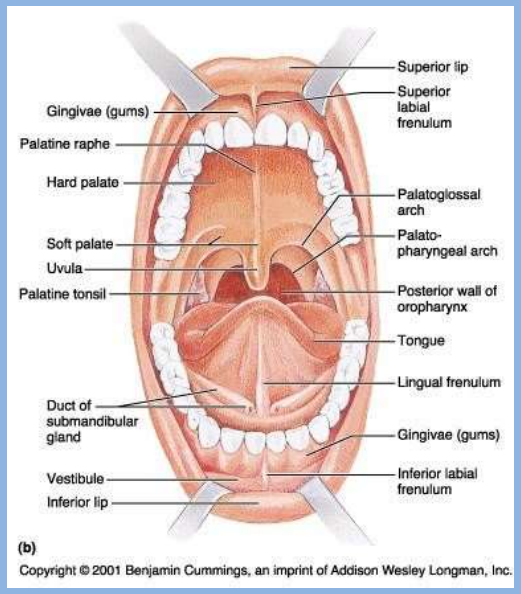


Mouth

- Teeth mechanically break down food into small pieces. Tongue mixes food with saliva (contains amylase, which helps break down starch).



- Epiglottis is a flap-like structure at the back of the throat that closes over the trachea preventing food from entering it.

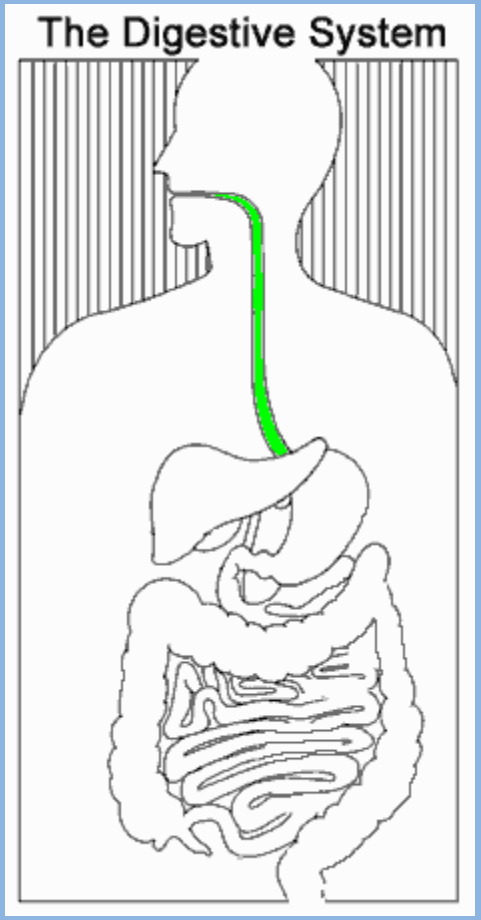


Esophagus

- Approximately 10” long
- Functions include:

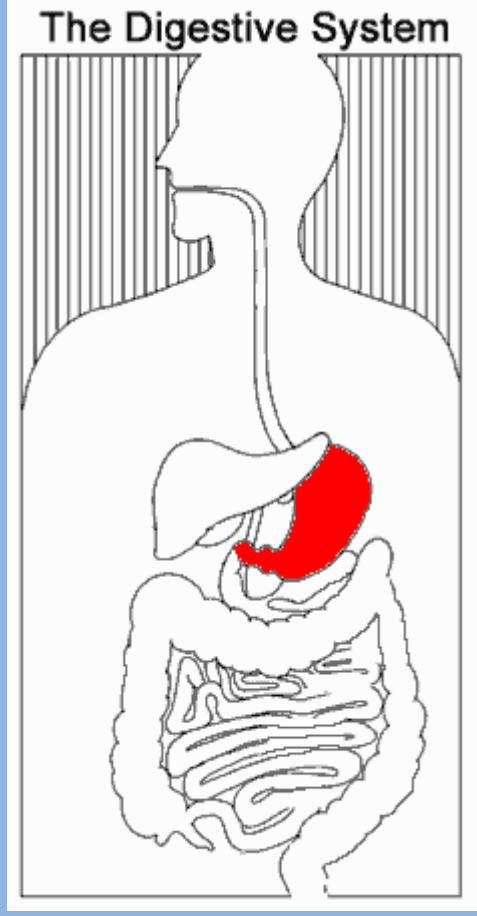
1. Secrete mucus
2. Moves food from the throat to the stomach using muscle movement called peristalsis

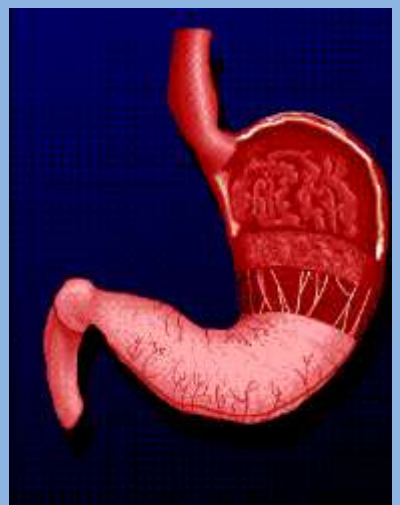
- If acid from the stomach gets in here that’s heartburn.



Stomach

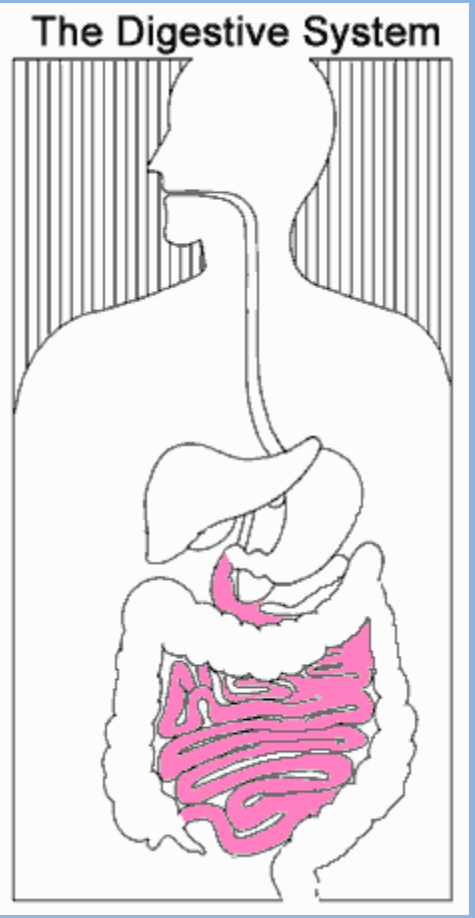
- J-shaped muscular bag that stores the food you eat, breaks it down into tiny pieces.
- Mixes food with digestive juices that contain enzymes to break down proteins and lipids.
- Acid in the stomach kills bacteria.
- Food found in the stomach is called chyme.

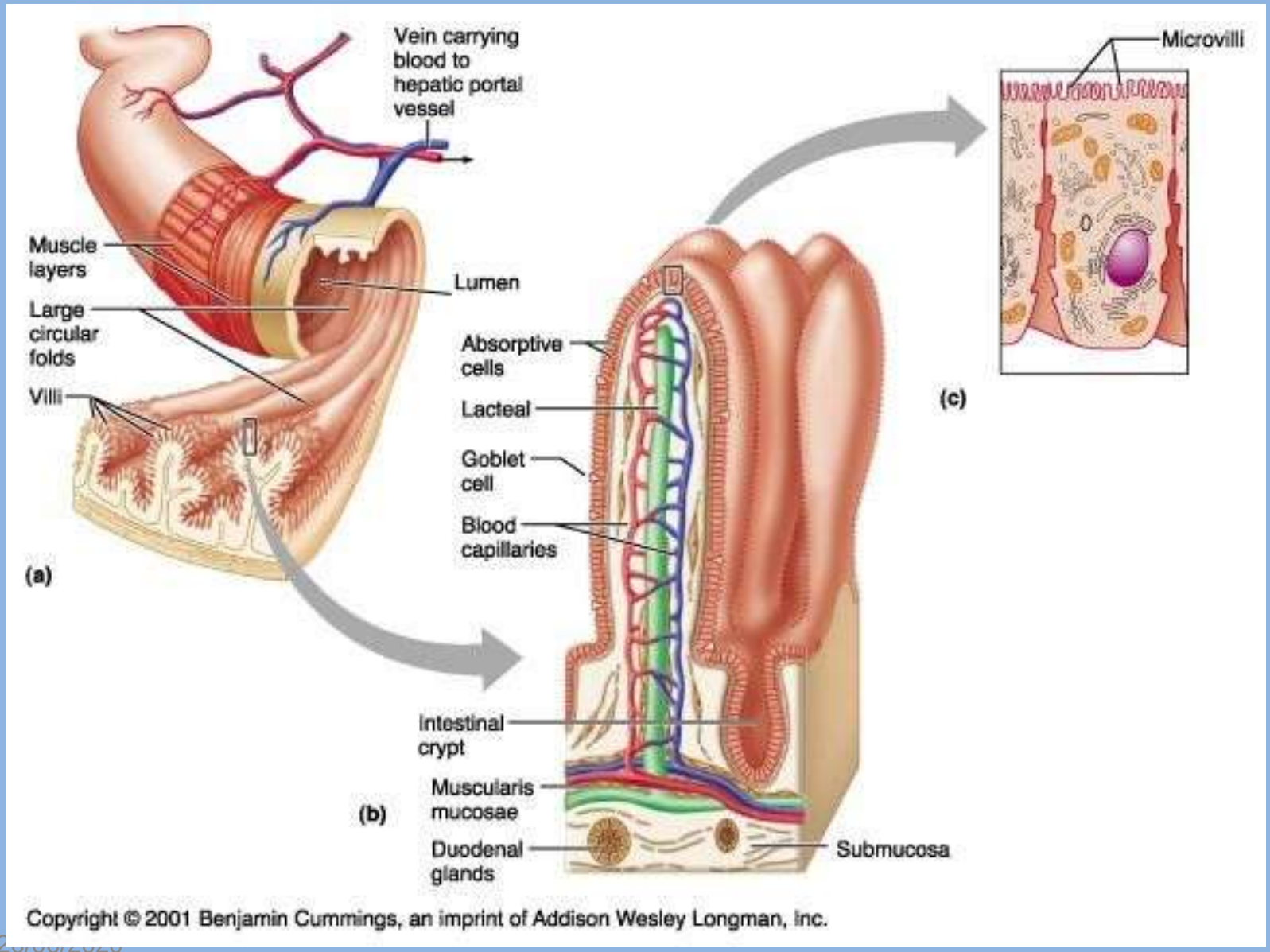




Small Intestine

- Small intestines are roughly 7 meters long
- Lining of intestine walls has finger-like projections called villi, to increase surface area.
- The villi are covered in microvilli which further increases surface area for absorption.




Vein carrying blood to hepatic portal vessel
Muscle layers
Lumen
Large circular folds
Villi
(a)
Absorptive cells
Lacteal
Goblet cell
Blood capillaries
Intestinal crypt
Muscularis mucosae
Duodenal glands
Submucosa
(b)
Microvilli
(c)
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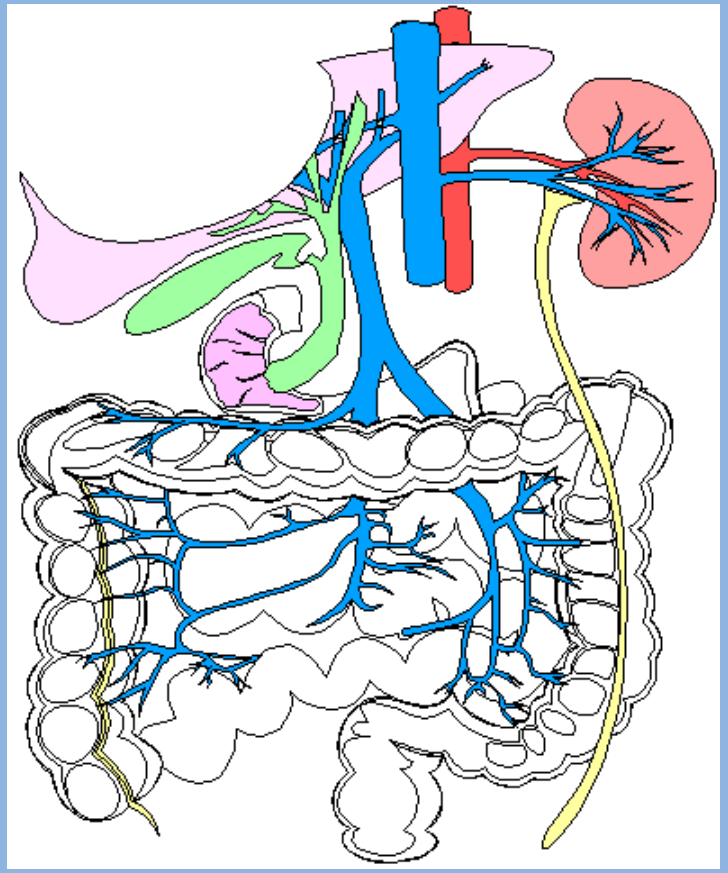
Small Intestine

- Nutrients from the food pass into the bloodstream through the small intestine walls.

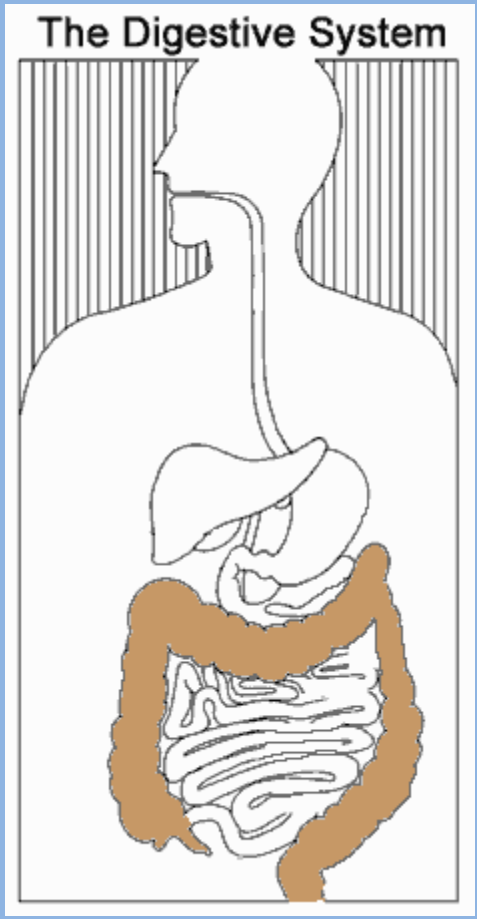
- Absorbs:
  - 80% ingested water
  - Vitamins
  - Minerals
  - Carbohydrates
  - Proteins
  - Lipids

  - Secretes digestive enzymes



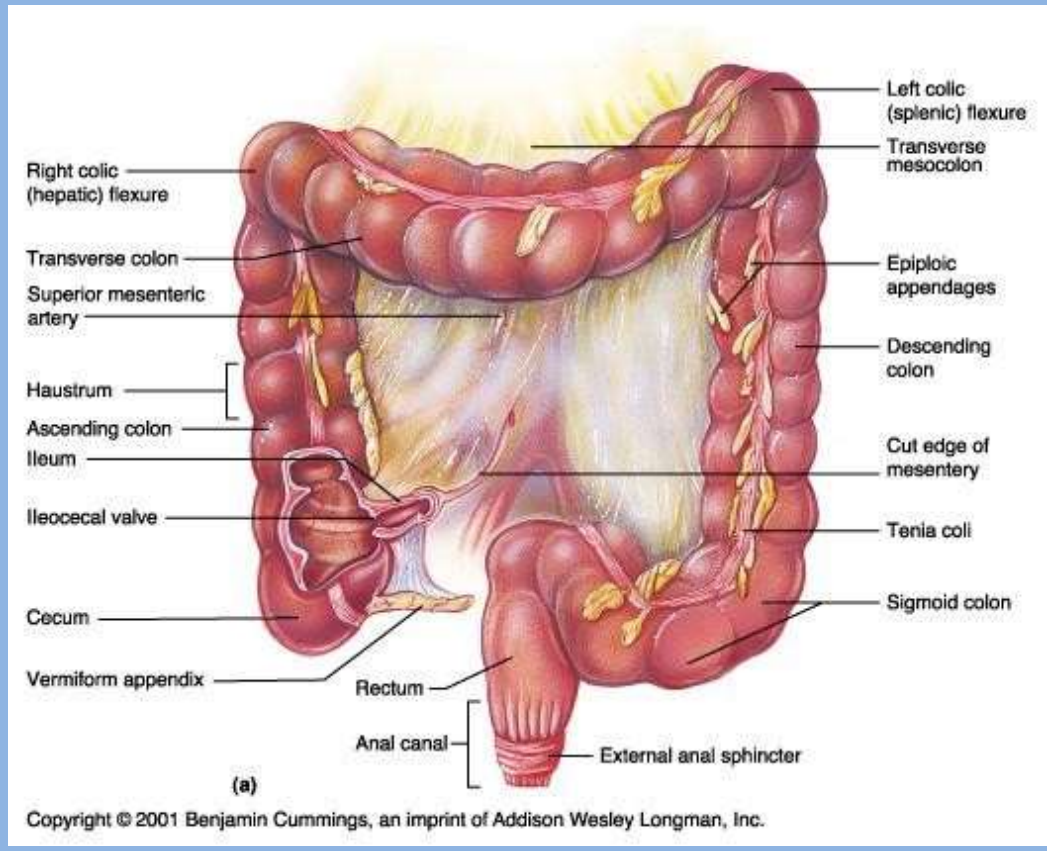
Large Intestine

- About 5 feet long
- Accepts what small intestines don't absorb
- Rectum (short term storage which holds feces before it is expelled).



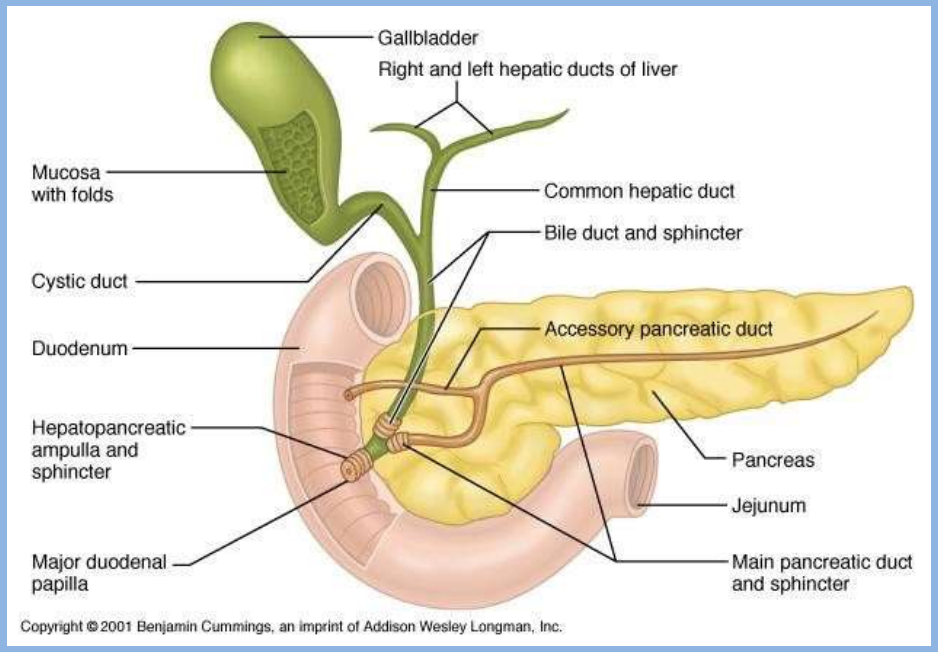
Large Intestine

- Functions
  - Bacterial digestion
    - Ferment carbohydrates
    - Protein breakdown
  - Absorbs more water
  - Concentrate wastes



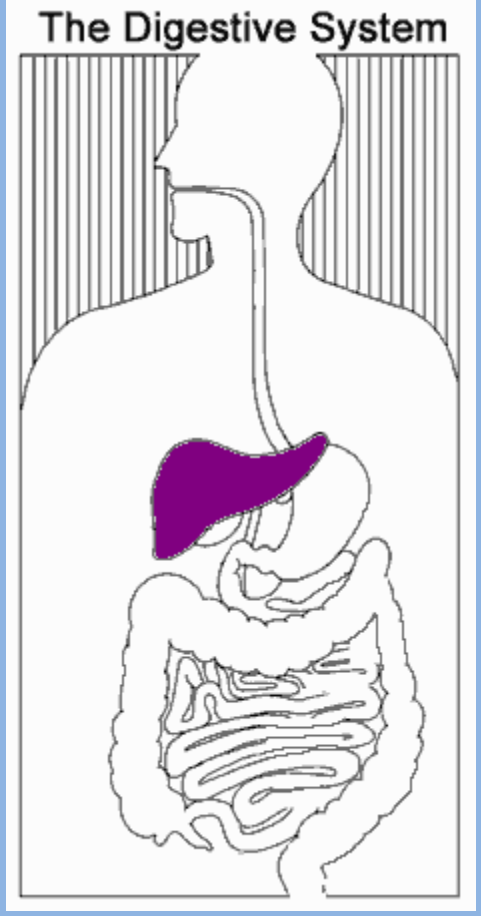
Accessory Organs

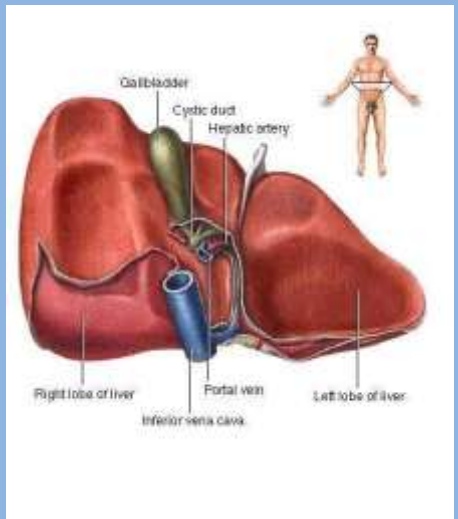
- Not part of the path of food, but play a critical role.
- Include: Liver, gall bladder, and pancreas



Liver

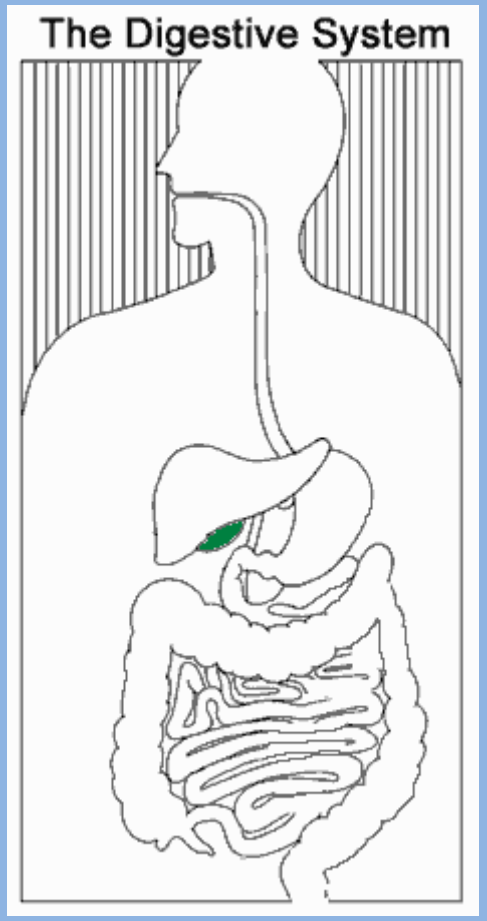
- Directly affects digestion by producing bile
  - Bile helps digest fat
  - filters out toxins and waste including drugs and alcohol





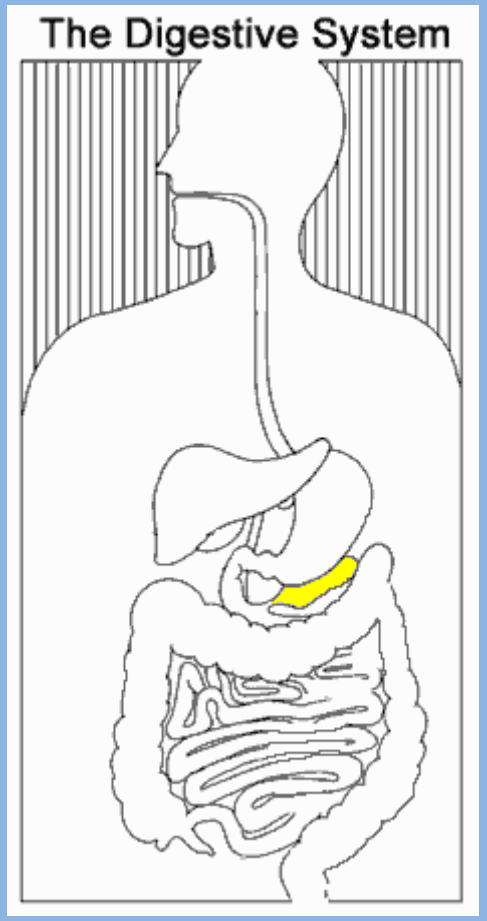
Gall Bladder

- Stores bile from the liver, releases it into the small intestine.
- Fatty diets can cause gallstones



Pancreas

- Produces digestive enzymes to digest fats, carbohydrates and proteins
- Regulates blood sugar by producing insulin



Fun Facts

- HOW LONG ARE YOUR INTESTINES? At least 25 feet in an adult. Be glad you're not a full-grown horse -- their coiled-up intestines are 89 feet long!
- Food drying up and hanging out in the large intestine can last 18 hours to 2 days!
- In your lifetime, your digestive system may handle about 50 tons!!

Tissues

Presented By
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PDEA's Shankarrao Ursal College of Pharmaceutical Sciences & Research Center, Kharadi.

Tissues

- **What is a tissue?**

  group of cells with similar structure and function plus extracellular substance (matrix)

- **Histology:**

  study of tissues

Types of Tissues

1. **Epithelial**
2. **Connective**
3. **Muscular**
4. **Nervous**

Epithelial Tissues

- **Location:**
  - cover body (internal and external)
  - Ex. Skin, kidney, trachea, glands, etc.

- **Characteristics:**
  - cells close together (very little extracellular matrix)
  - form most glands
  - have free surface
  - **Basal surface:**

    attaches epithelial cells to underlying tissues

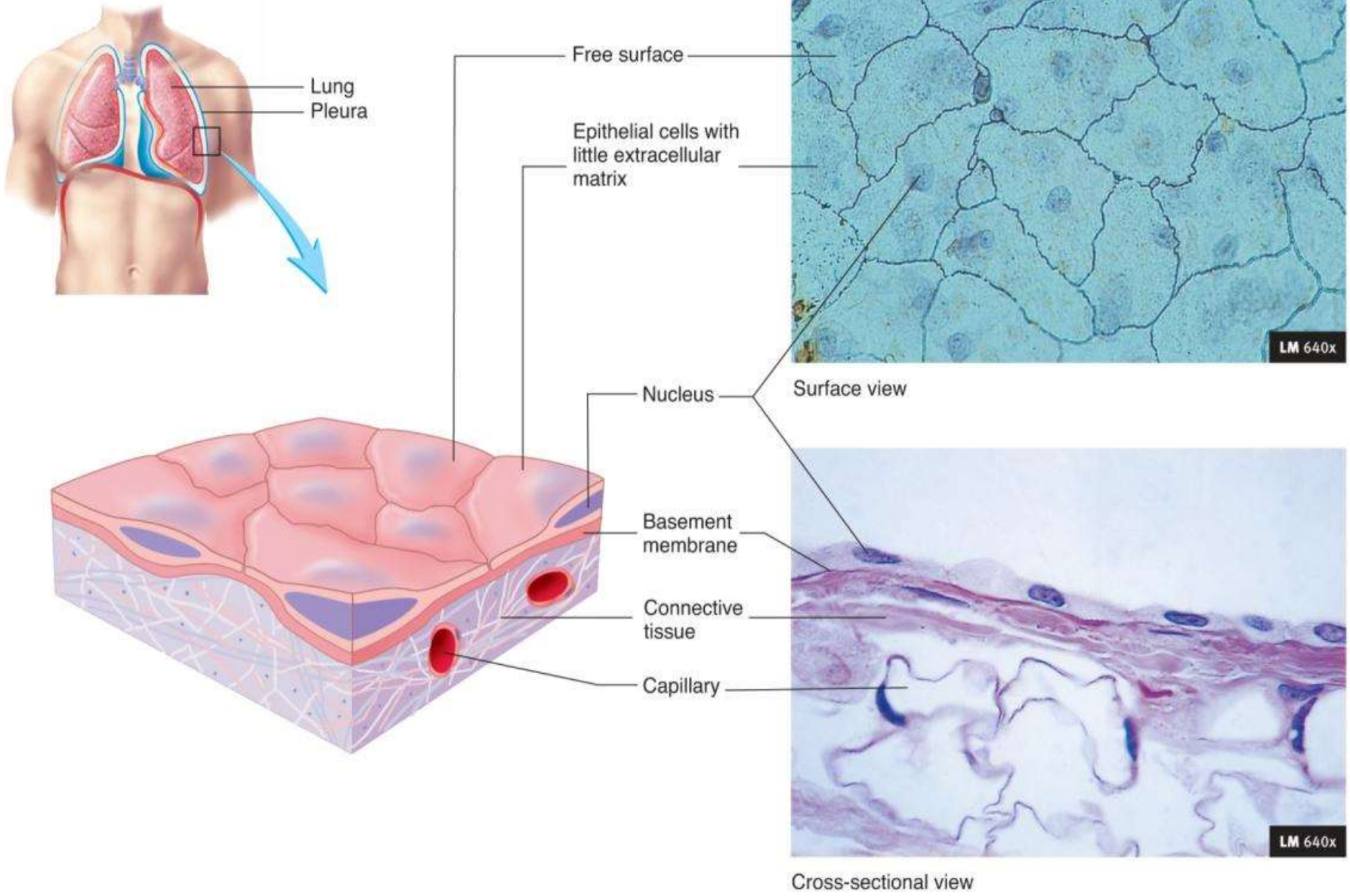

Figure 4.1 Characteristics of Epithelium

Surface and cross-sectional views of epithelium illustrate the following characteristics: little extracellular material between cells, a free surface, and a basement membrane attaching epithelial cells to underlying tissues. The capillaries in connective tissue do not penetrate the basement membrane. Nutrients, oxygen, and waste products diffuse across the basement membrane between the capillaries and the epithelial cells.

Functions of Epithelial Tissues

1. **Protect:**
   Ex. Skin
2. **Act as a barrier:**
   Ex. Skin keeps bacteria out
3. **Diffusion and Filtration:**
   Ex. Lungs and kidneys
4. **Secretion:**
   Ex. Sweat glands
5. **Absorption:**
   Ex. Small intestine

Classification of Epithelial Tissue

- **Classified according to number of cell layers and cell shape**
- Simple and stratified = number of cell layers
- Squamous, cuboidal, columnar, transitional= cell shape

TABLE 4.1 Classification of Epithelia

Number of Layers	Cell Shape
Simple (one layer)	Squamous Cuboidal Columnar
Pseudostratified (a modified form of simple epithelium)	Columnar
Stratified (more than one layer)	Squamous Cuboidal Columnar
Transitional (a type of stratified epithelium)	Roughly cuboidal to columnar when not stretched and squamouslike when stretched

Types of Epithelial Tissues

- **Simple Epithelium**

  Structure: 1 layer of cells

- **Stratified Epithelium**

  Structure: many layers of cells

- **Simple Squamous**

  Structure: 1 layer of flat, tile-like cells

  Function: diffusion and filtration

  Location: blood vessels, lungs, heart, kidneys

- **Simple Cuboidal**

  Structure: 1 layer of square-shaped cells

  Function: secretion

  Location: glands, ovaries, kidneys

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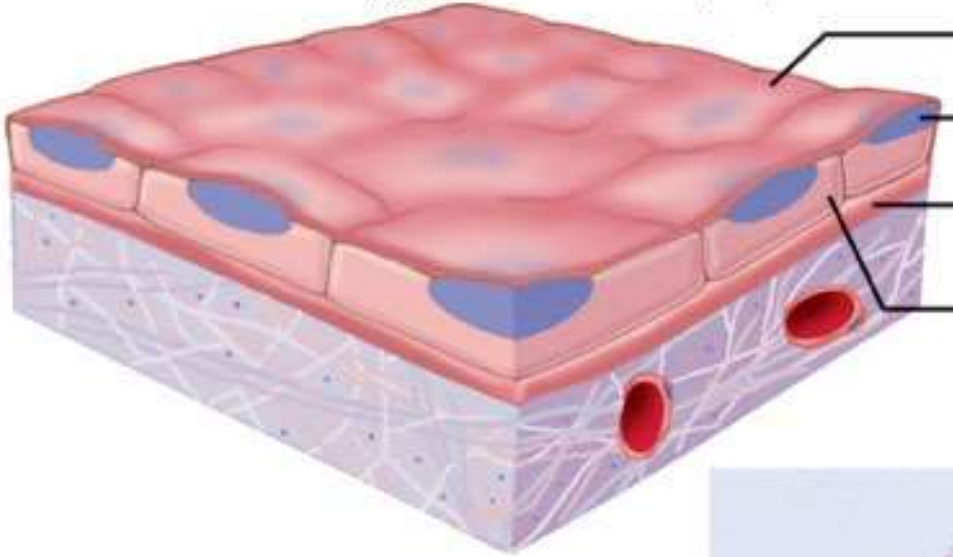

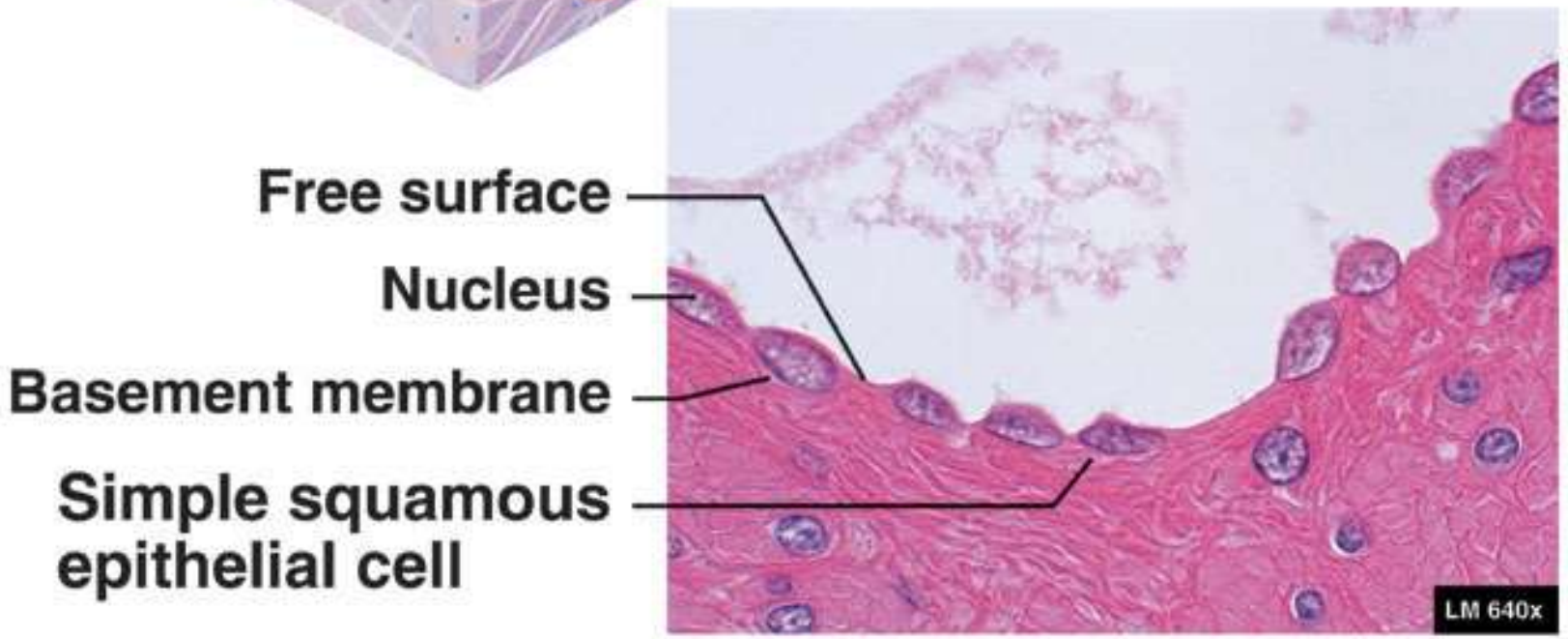

(a) Simple squamous epithelium

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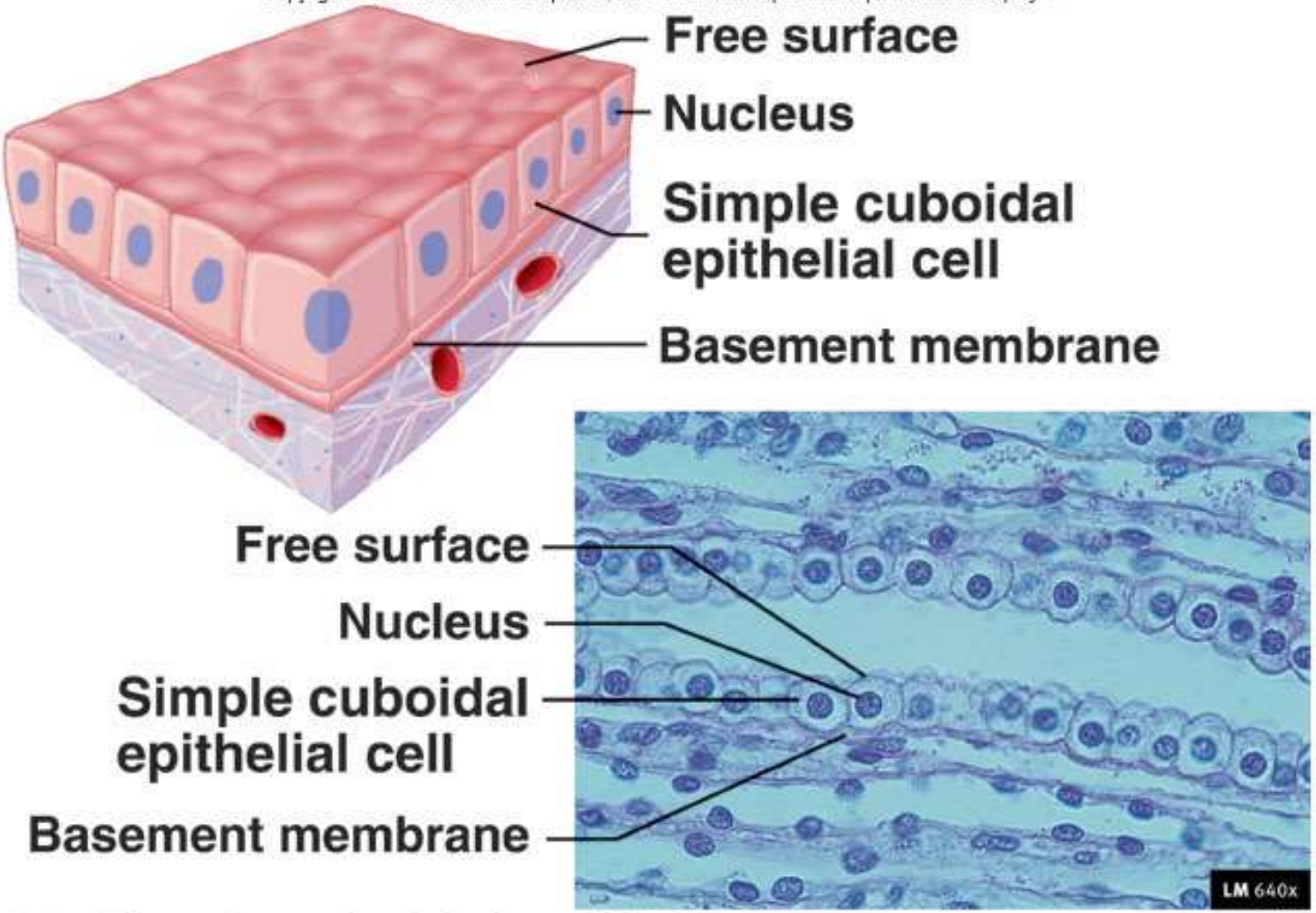

(b) Simple cuboidal epithelium

- **Simple Columnar**

  Structure: 1 layer of tall, narrow cells

  Function: secrete mucus and absorption

  Location: stomach, intestines, resp. tract

- **Pseudostratified Columnar**

  Structure: 1 layer of tall, narrow cells
  appears stratified but isn't

  Function: secrete mucus and propel debris out of resp. tract (cilia)

  Location: nasal cavity and trachea

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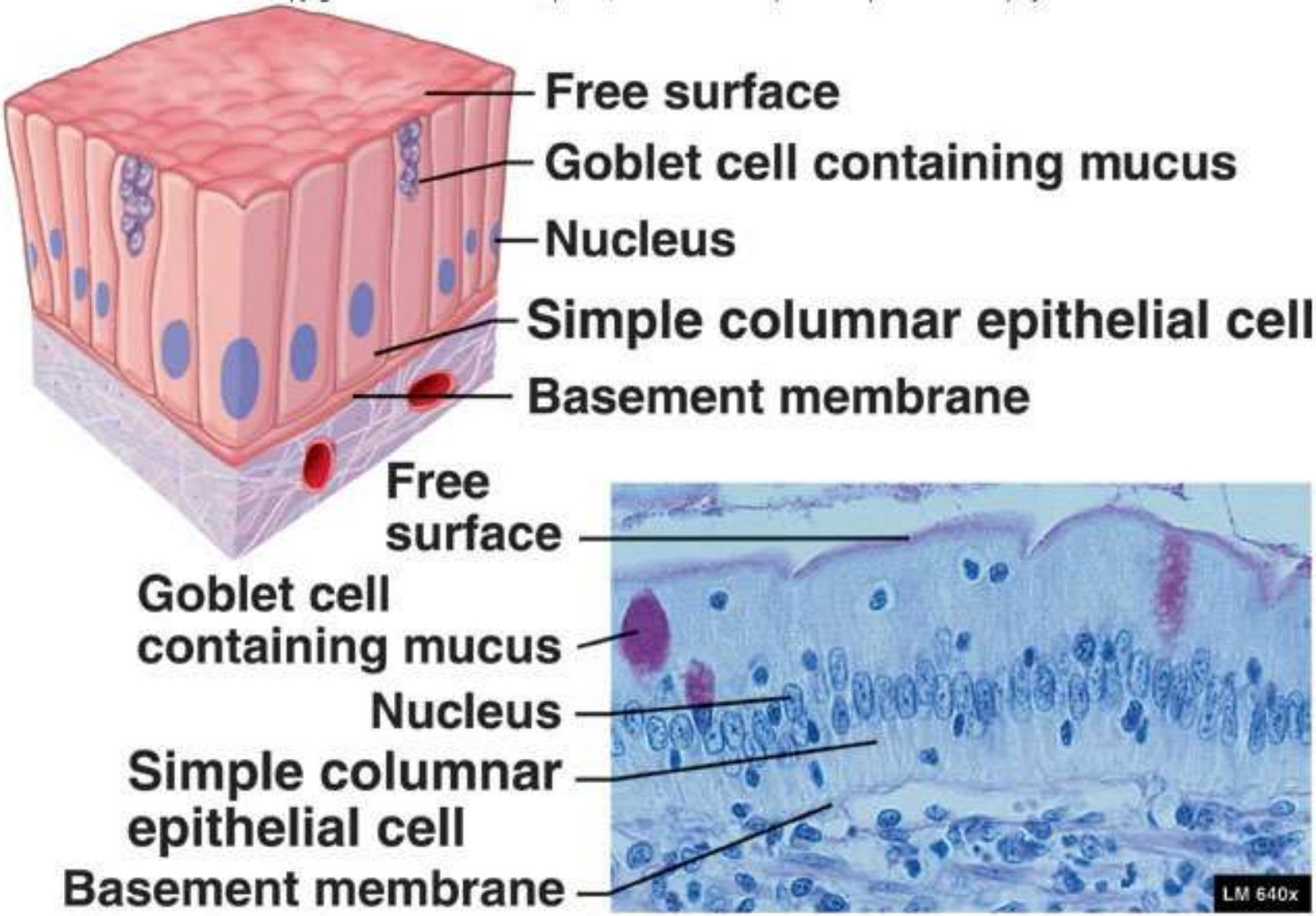

(c) Simple columnar epithelium

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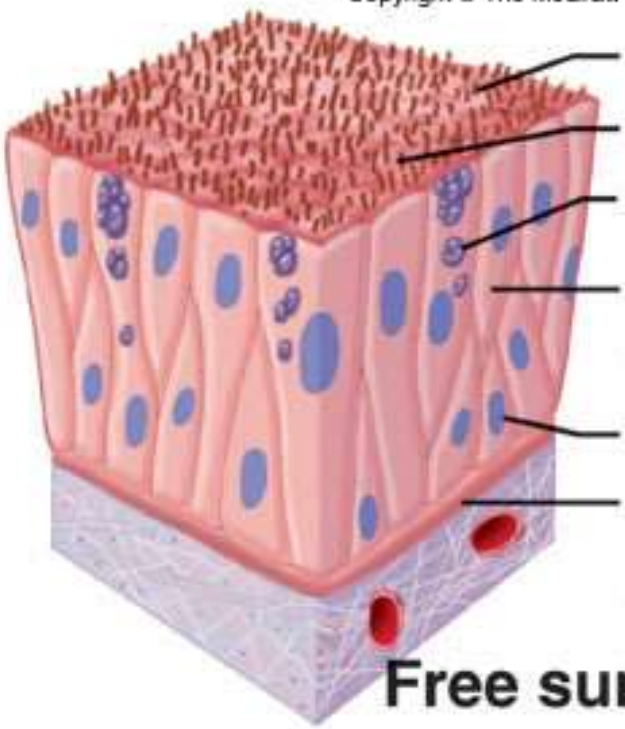

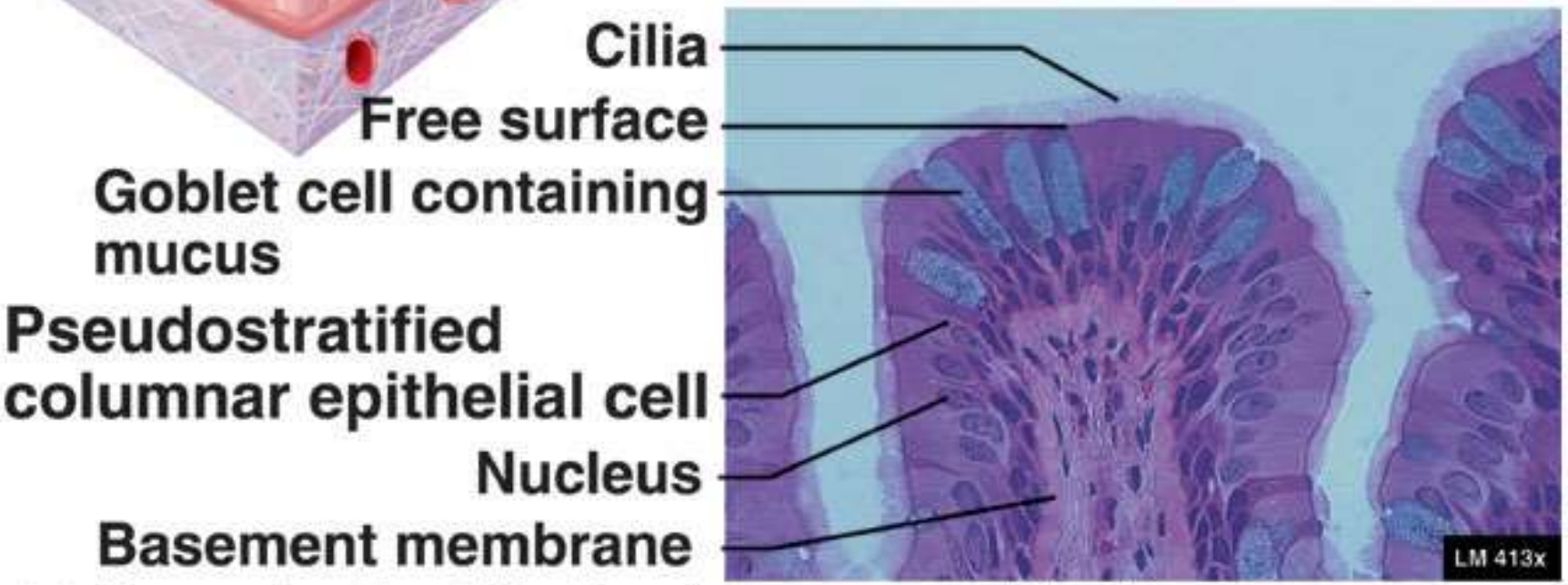

(g) Pseudostratified columnar epithelium

- **Stratified Squamous**

  Structure: many layers of flat, tile-like cells

  Function: protect and acts as a barrier

  Location: skin, mouth, throat, esophagus

- **Transitional**

  Structure: special type of stratified epi. changes shape (stretched squamous, not stretched cuboidal)

  Function: hold fluids

  Location: urinary bladder

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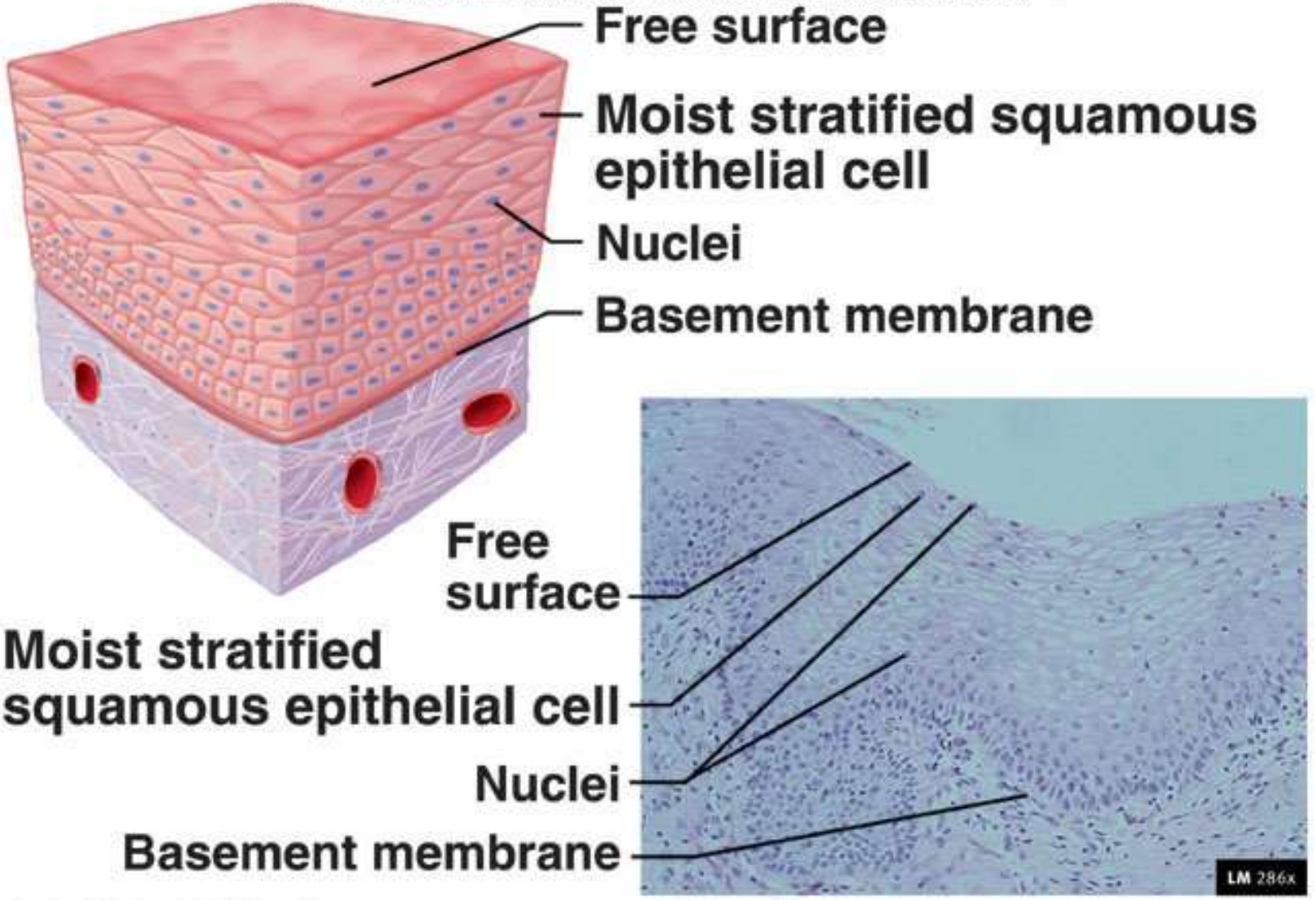

(d) Stratified squamous epithelium

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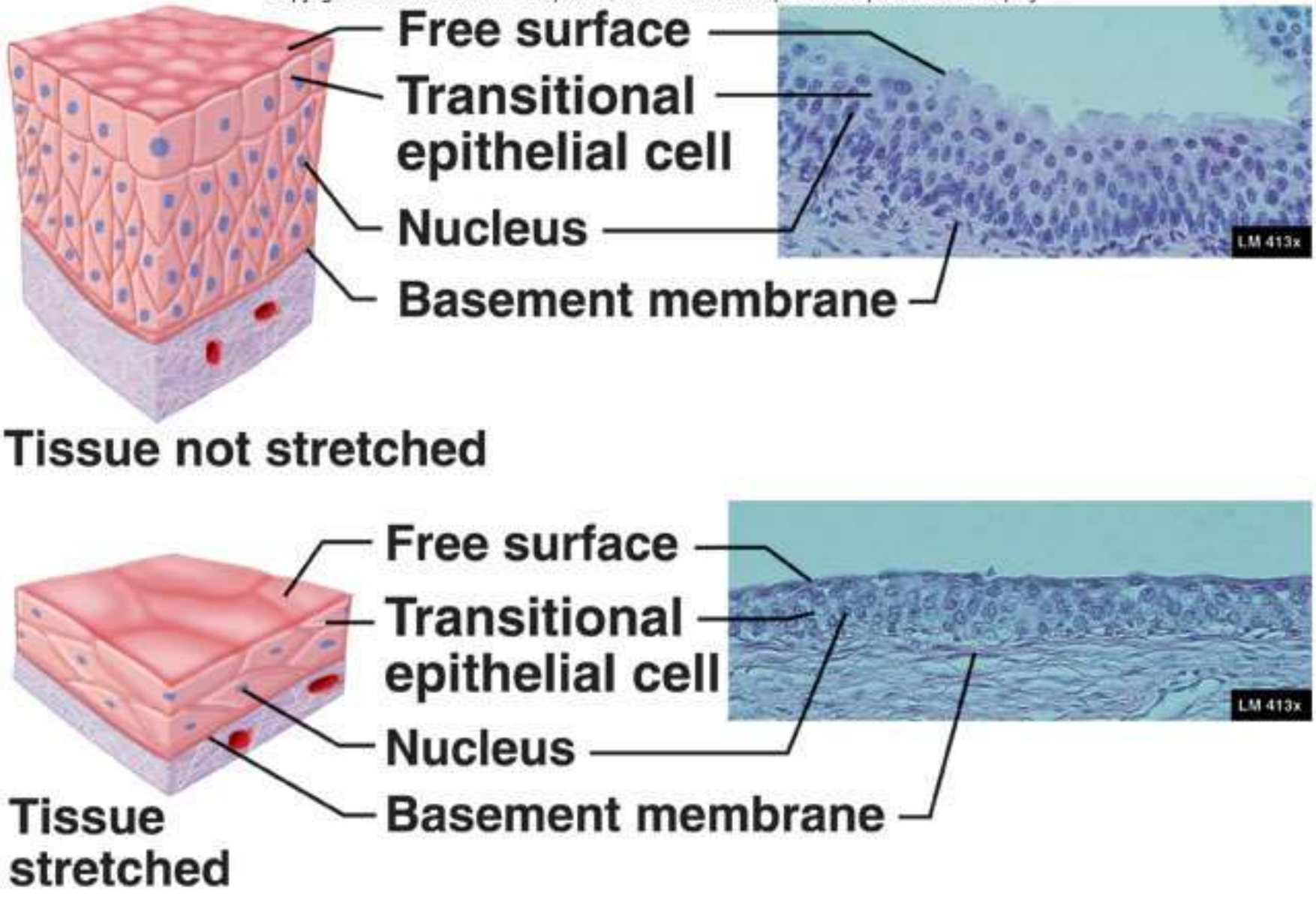

(h) Transitional epithelium

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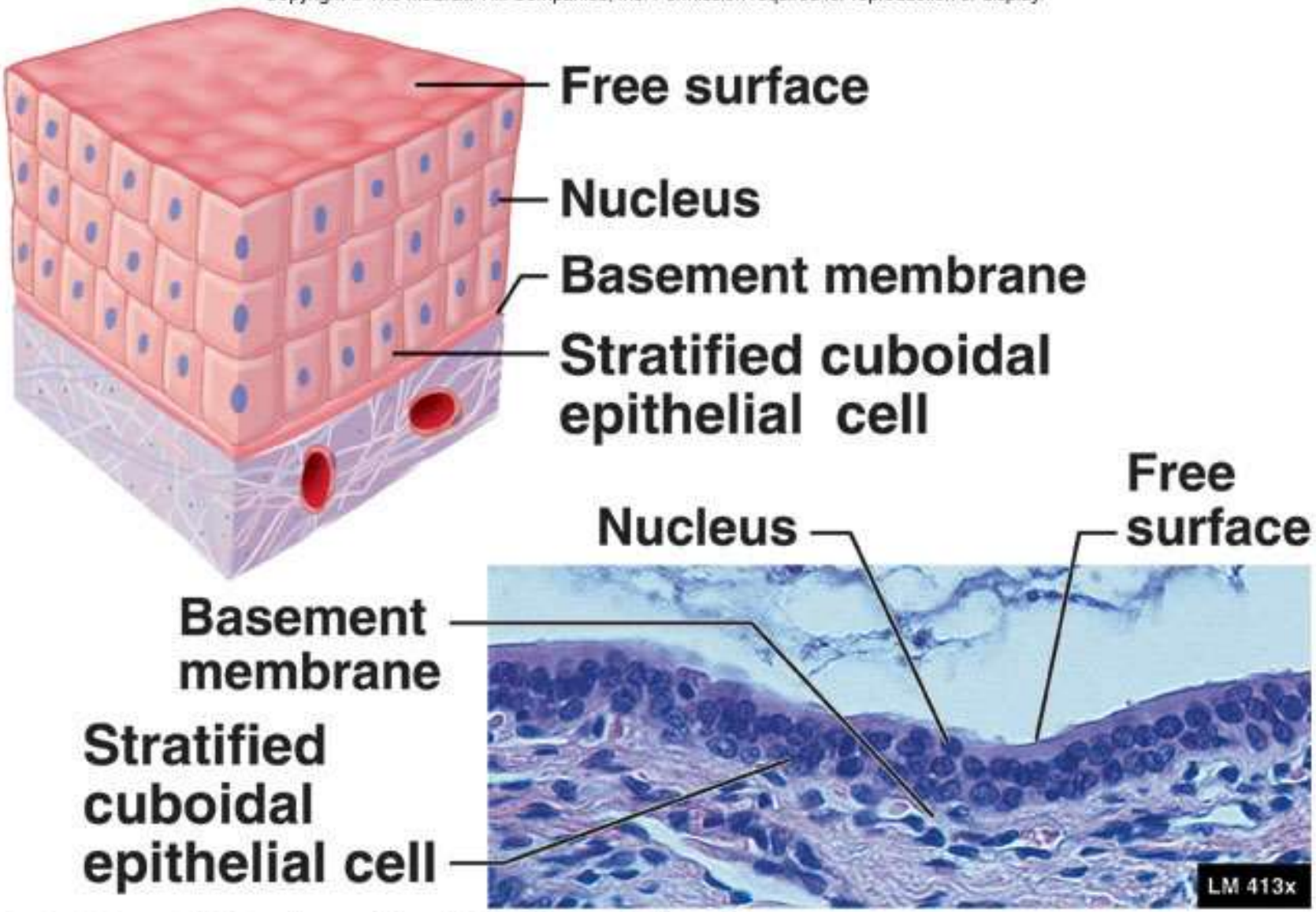

(e) Stratified cuboidal epithelium

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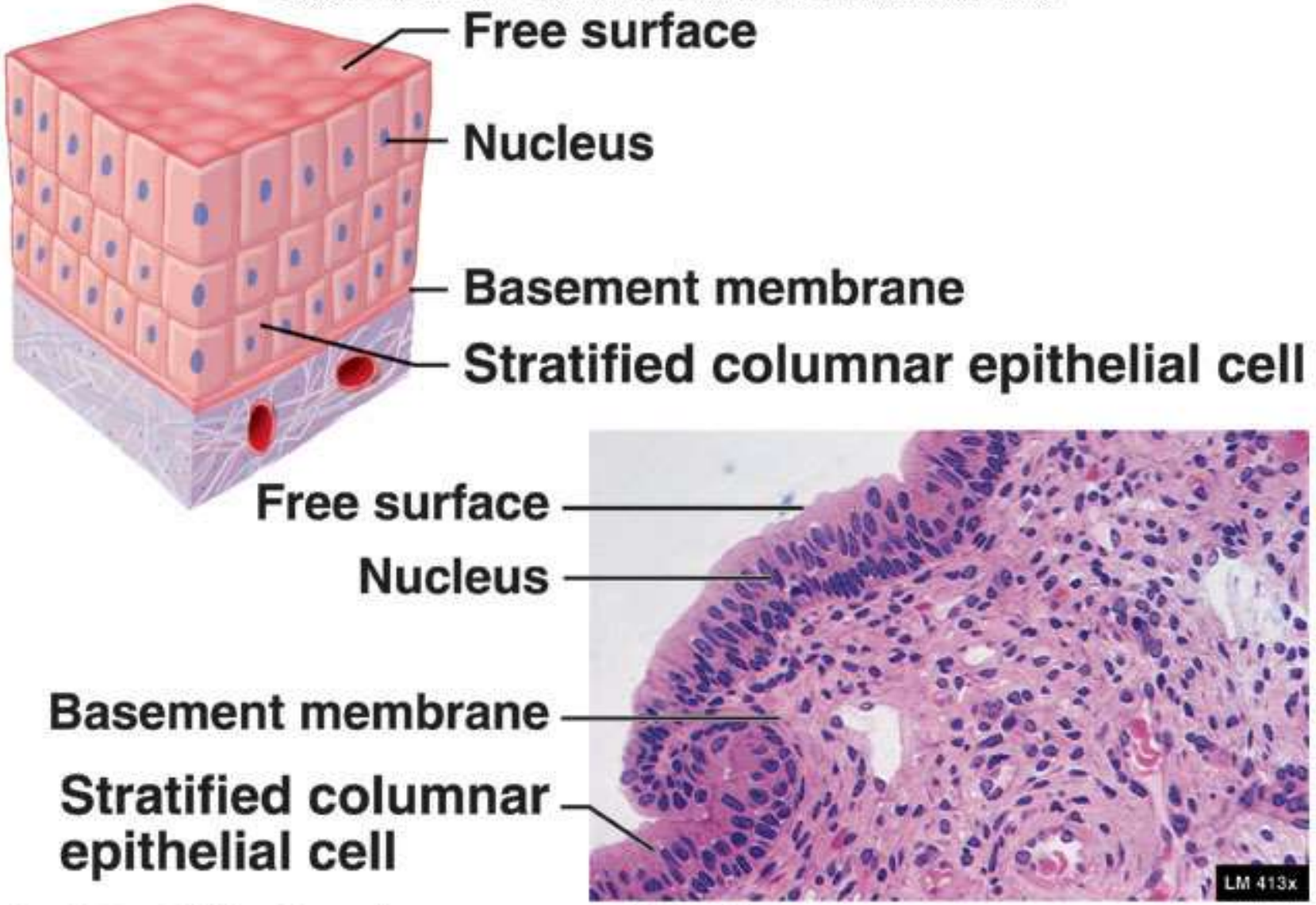

(f) Stratified columnar epithelium

Free Cell Surfaces

- **Surface not in contact with other cells**

- **Smooth to reduce friction, Ex. Blood vessels**

- **Microvilli:**
  - increase cell's surface area
  - Ex. Small intestine

- **Cilia:**
  - move materials across cell's surface
  - Ex. Trachea

- **Goblet cells:**
  - produce mucus
  - Ex. Stomach

Cell Connections

- **Tight junctions:**
  - bind adjacent cells together
  - Ex. Intestines

- **Desmosomes:**

  mechanical links that bind cells

- **Hemidesmosomes:**
  bind cells to basement membrane

- **Gap junctions:**
  - small channels that allow molecules to pass between cells
  - allow cells to communicate
  - most common

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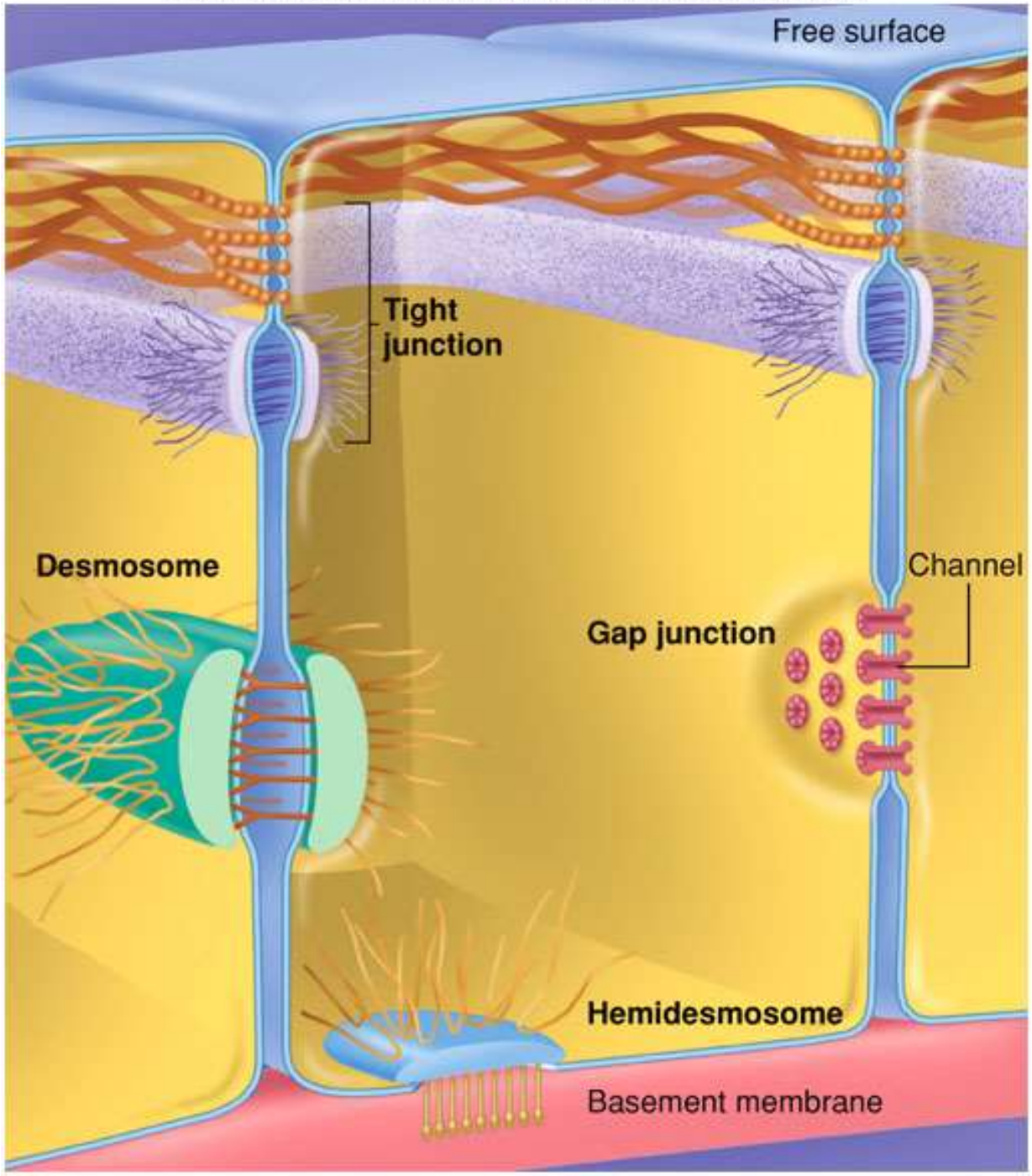

Glands

- **What are they?**

  structures that secrete substances onto a surface, into a cavity, or into blood

- **Exocrine glands:**

  - glands with ducts
  - Ex. Sweat or oil glands

- **Endocrine glands:**

  – no ducts (directly into bloodstream)

  – Ex. Thyroid, thymus, pituitary glands, etc.

Types of Exocrine Glands

- **Simple:**
  no branches
- **Compound**:
  many branches
- **Tubular:**
  end of duct
- **Alveolus:**
  sac-like structure

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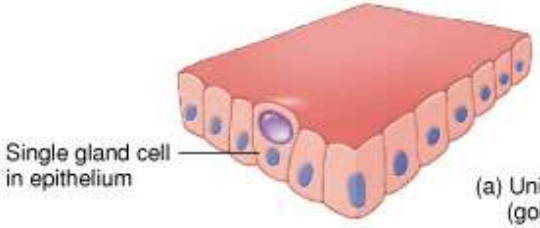
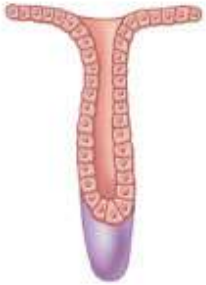
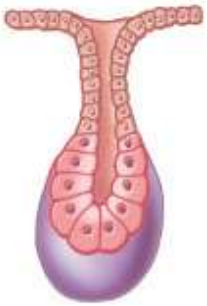


(a) Unicellular
(goblet cells in large and small intestine and respiratory passages)

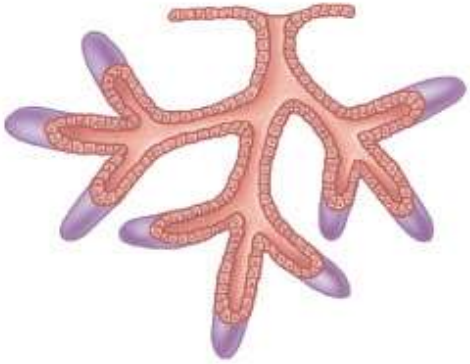


(b) Simple straight tubular
(glands in stomach and colon)

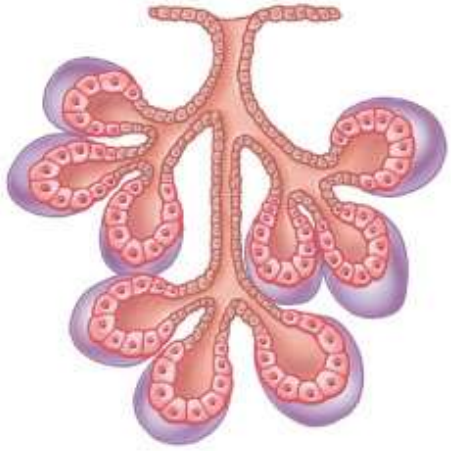
(c) Simple coiled tubular
(lower portion of stomach and small intestine)



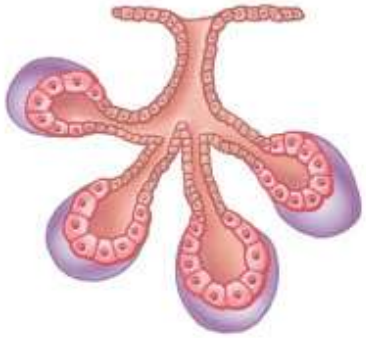
(d) Simple acinar or alveolar
(sebaceous glands of skin)



(e) Compound tubular
(mucous glands of duodenum)



(f) Compound acinar or alveolar
(mammary glands)



(g) Compound tubuloacinar or tubuloalveolar (pancreas)

Connective Tissues Characteristics

- Cells far apart
- Contain large amounts of extracellular matrix
- **Classified based on type of extracellular matrix and function**
- Ex. Blast cells build, clast cells carve
- Extracellular matrix contains 3 components (in varying amounts): **protein fibers, ground substance, fluid**
- **Ground substance:** proteins and sugars

Types of Protein Fibers

- **Collagen fibers:**

  look like ropes and are flexible but resist stretching
- **Reticular fibers:**

  supporting network that fills spaces between organs and tissues
- **Elastic fibers:**

  recoil after being stretched

Functions of Connective Tissue

1. **Enclose and separate:**
   Ex. around organs and muscles

2. **Connect tissues:**
   Ex. Tendons: connect bone to muscle
   Ex. Ligaments: connect bone to bone

3. **Support and Movement:**
   Ex. bones

4. **Storage:**

   Ex. bones store calcium and adipose tissue stores fat

5. **Cushion and insulate:**

   Ex. adipose tissue protects organs and helps conserve heat

6. **Transport:**

   Ex. Blood

7. **Protect:**

   Ex. Immune cells

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Table 4.4 Classification of Connective Tissues

- Loose (areolar) connective tissue
- Adipose tissue
- Dense connective tissue
  - Dense collagenous connective tissue
    - Collagen fibers arranged in the same direction
    - Collagen fibers arranged in many directions
  - Dense elastic connective tissue
    - Elastic fibers arranged in the same direction
    - Elastic fibers arranged in many directions
- Cartilage
  - Hyaline cartilage
  - Fibrocartilage
  - Elastic cartilage
- Bone
- Blood

Types of Ordinary Connective Tissue

- **Loose**

  Location: between organs, muscles, glands, skin

  Structure: collagen fibers far apart

  Function: support and protect

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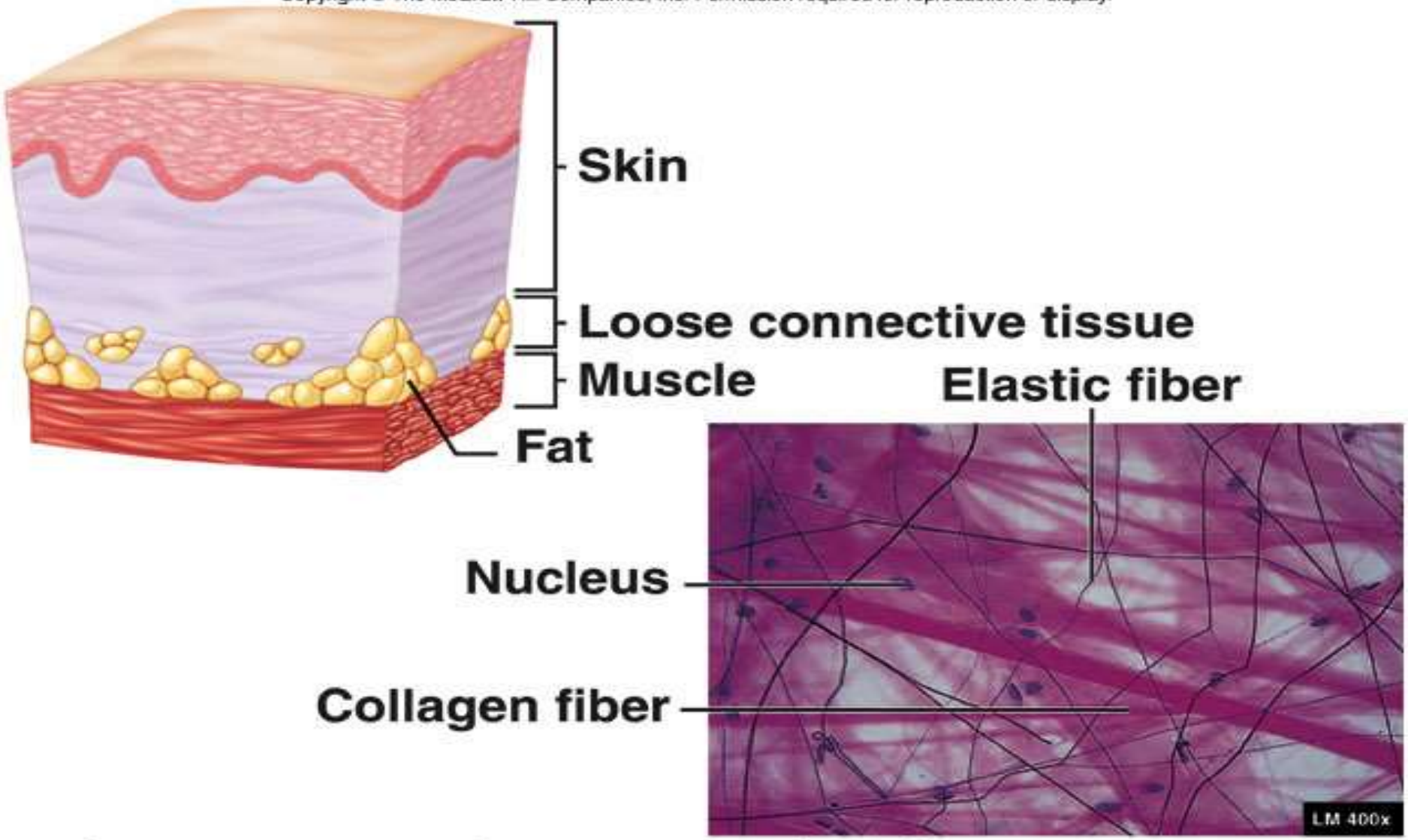

(a) Loose, or areolar, connective tissue

- **Dense**

  Location: tendons, ligaments, skin

  Structure: collagen fibers packed close together

  Function: connect and can withstand pulling forces

- **Adipose**

  Location: under skin and around organs

  Structure: collagen and elastic fibers, cells filled with lipids

  Function: storage, insulate, cushion

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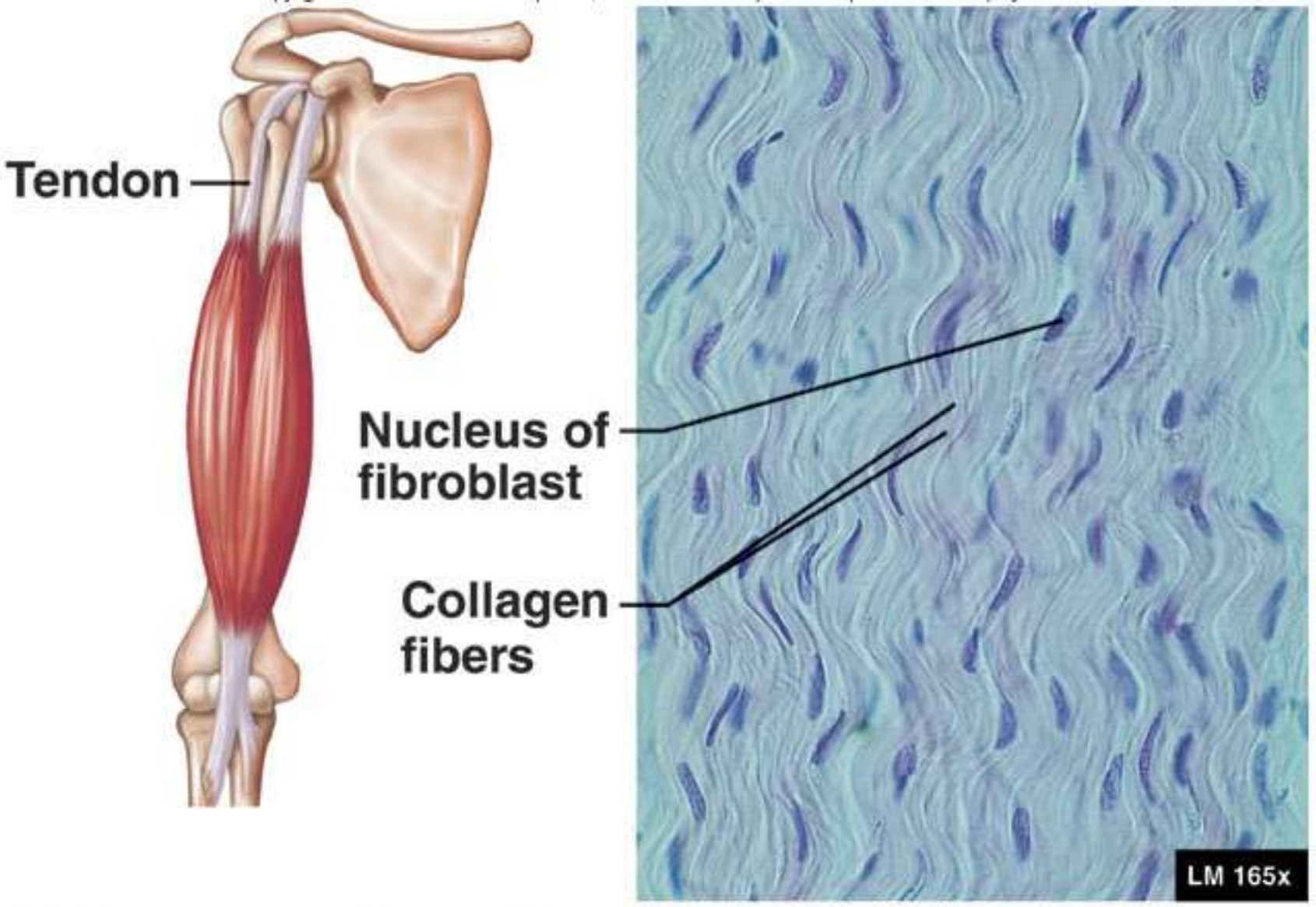

(b) Dense regular collagenous connective tissue

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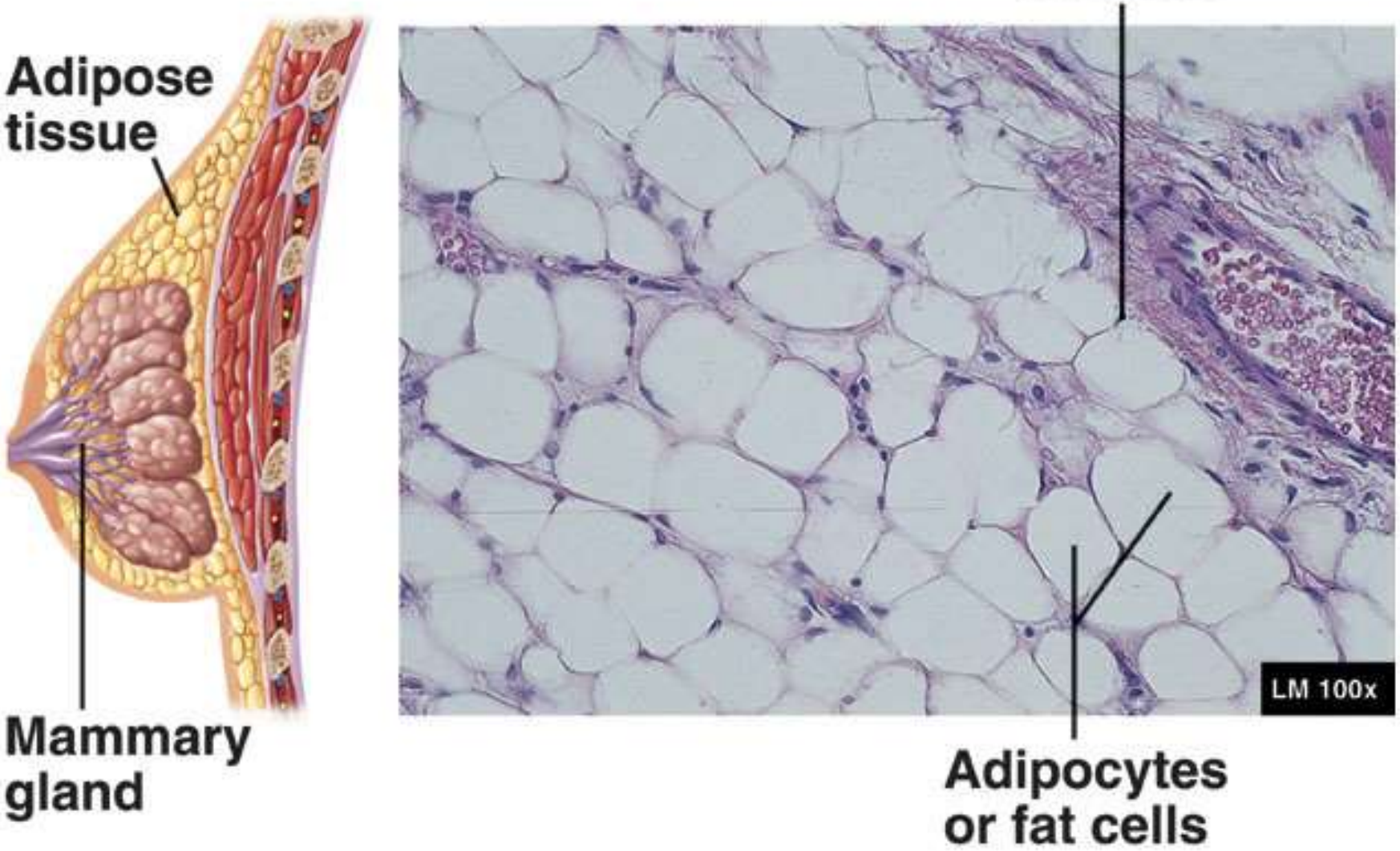


(f) Adipose tissue

Cartilage

- Type of connective tissue
- Composed of chondrocytes
- Contains collagen
- Withstands compressions
- Provides support, flexibility, strength

Types of Cartilage

- **Hyaline cartilage**

  Location: covers ends of bones

  Structure: some collagen fibers

  Function: reduces friction (cushion)

- **Fibrocartilage**

  Location: between vertebra

  Structure: lots of collagen fibers

  Function: can withstand compression

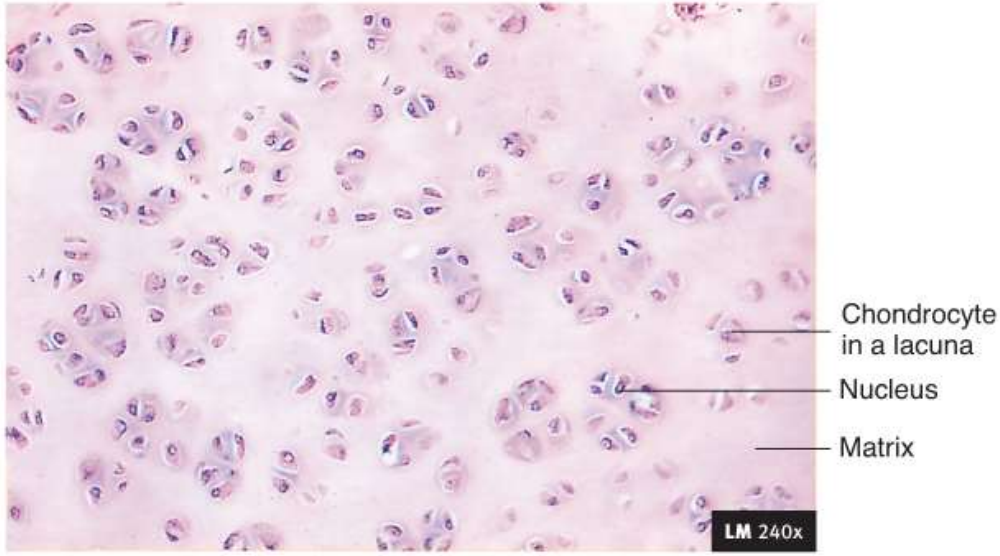
TABLE 4.8 Connective Tissue: Cartilage

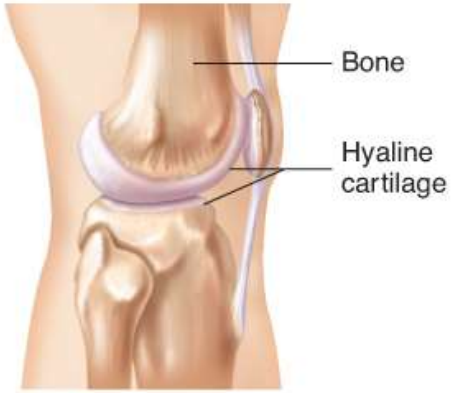
(a) Hyaline Cartilage

Structure: Collagen fibers are small and evenly dispersed in the matrix, making the matrix appear transparent; the cartilage cells, or chondrocytes, are found in spaces, or lacunae, within the firm but flexible matrix

Function: Allows growth of long bones; provides rigidity with some flexibility in the trachea, bronchi, ribs, and nose; forms rugged, smooth, yet somewhat flexible articulating surfaces; forms the embryonic skeleton

Location: Growing long bones, cartilage rings of the respiratory system, costal cartilage of ribs, nasal cartilages, articulating surface of bones, and the embryonic skeleton





continued

- **Elastic cartilage**

  Location: ear and tip of nose

  Structure: elastic fibers

  Function: can recoil

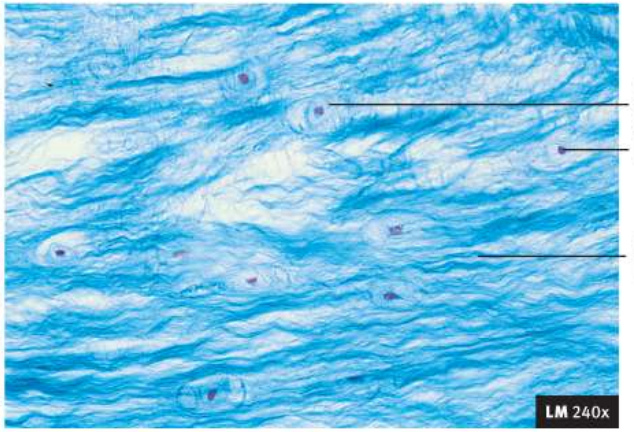
TABLE 4.8 *continued*

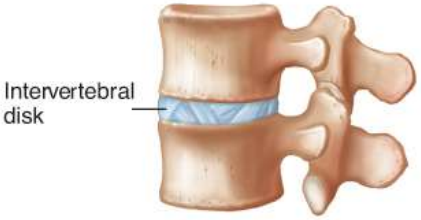
(b) Fibrocartilage

Structure: Collagen fibers similar to those in hyaline cartilage; the fibers are more numerous than in other cartilages and are arranged in thick bundles

Function: Somewhat flexible and capable of withstanding considerable pressure; connects structures subjected to great pressure

Location: Intervertebral disks, pubic symphysis, and articular disks (e.g., knees and temporomandibular [jaw] joints)



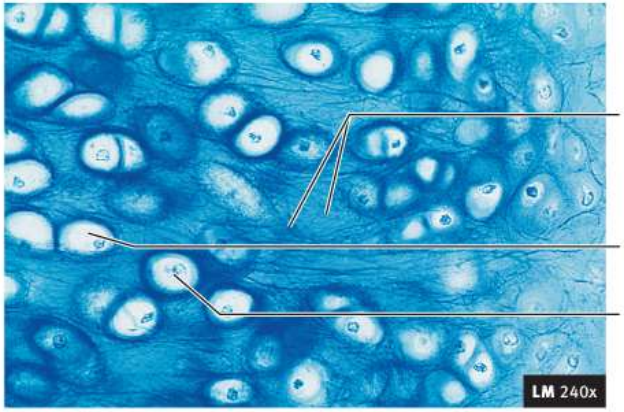


(c) Elastic Cartilage

Structure: Similar to hyaline cartilage, but matrix also contains elastin fibers

Function: Provides rigidity with even more flexibility than hyaline cartilage because elastic fibers return to their original shape after being stretched

Location: External ears, epiglottis, and auditory tubes



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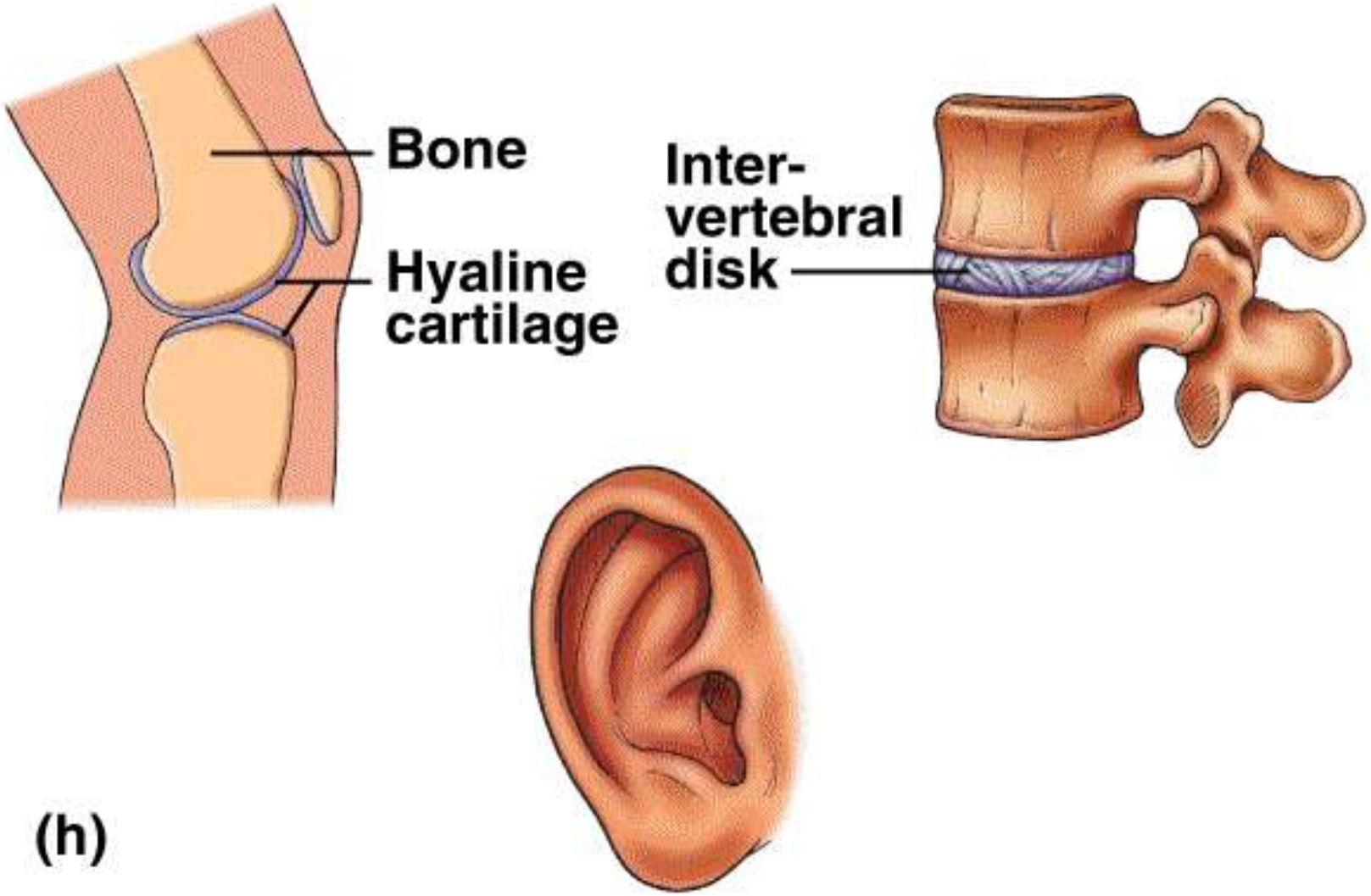
Cartilage



Bone

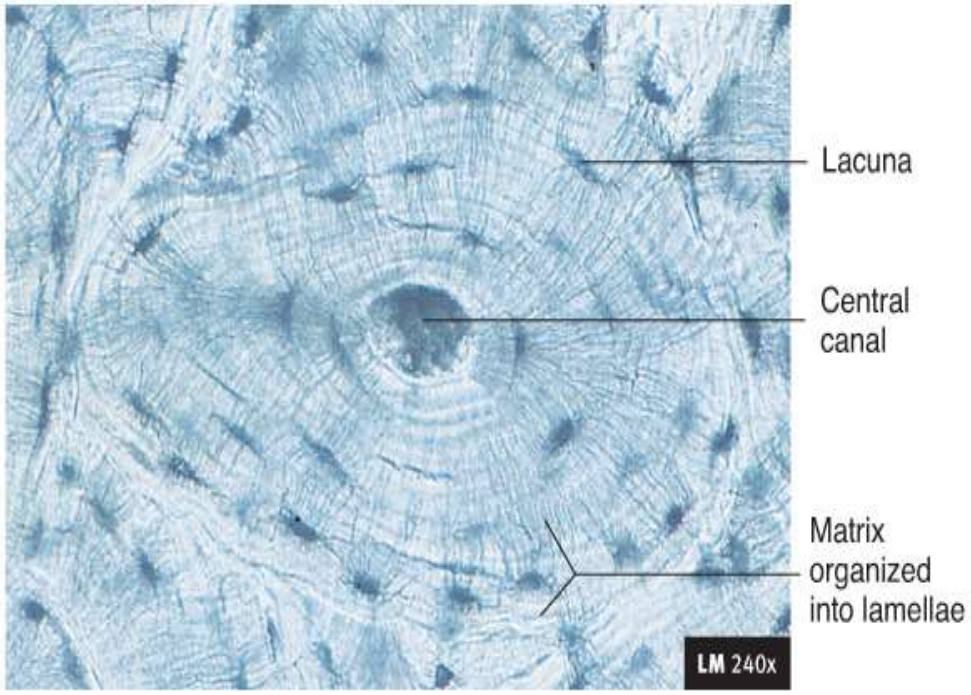
- Hard connective tissue
- 2 types: compact and spongy
- Composed of osteocytes

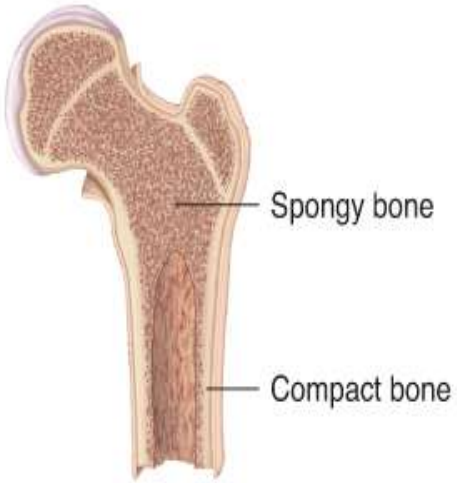
TABLE 4.9 Bone

Structure: Hard, bony matrix predominates; many osteocytes (not seen in this bone preparation) are located within lacunae; the matrix is organized into layers called lamellae

Function: Provides great strength and support and protects internal organs, such as the brain; bone also provides attachment sites for muscles and ligaments; the joints of bones allow movements

Location: All bones of the body





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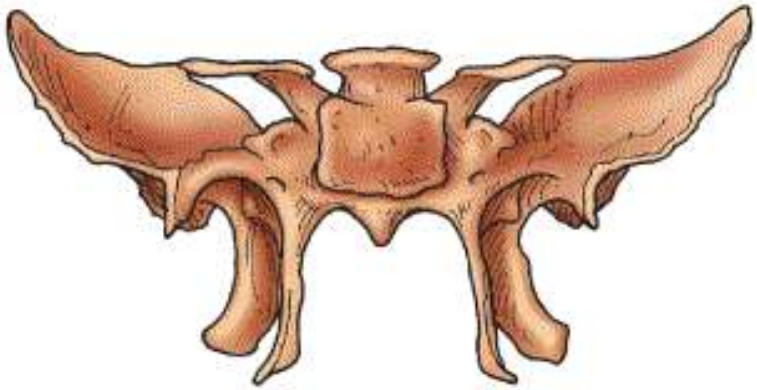
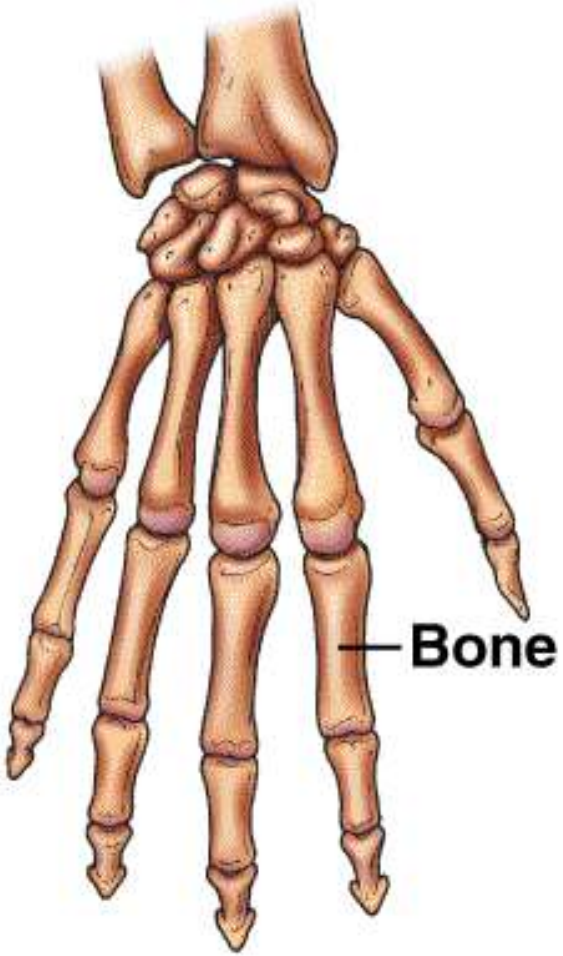
Cancellous and Compact Bone



Irregular bone (sphenoid)

(i)



Blood

- Liquid connective tissue
- Erythrocytes, leukocytes, platelets
- Transport food, oxygen, waste, hormones

Table Art 4.10

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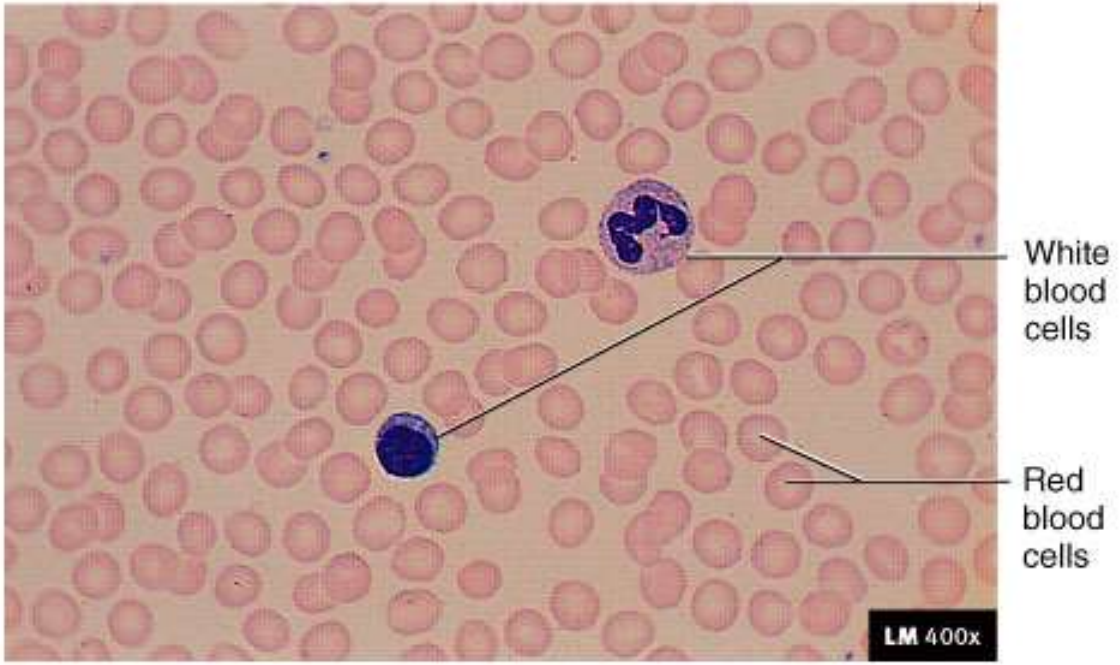

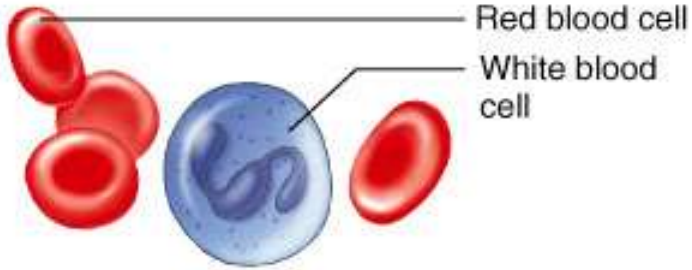

Muscular Tissue

Muscle type	Nucleus/i	Nucleus/i location	Striated
Skeletal (most muscle)	many	peripheral	Y
Cardiac (heart)	1	centrally	Y
Smooth (organs)	1	centrally	N

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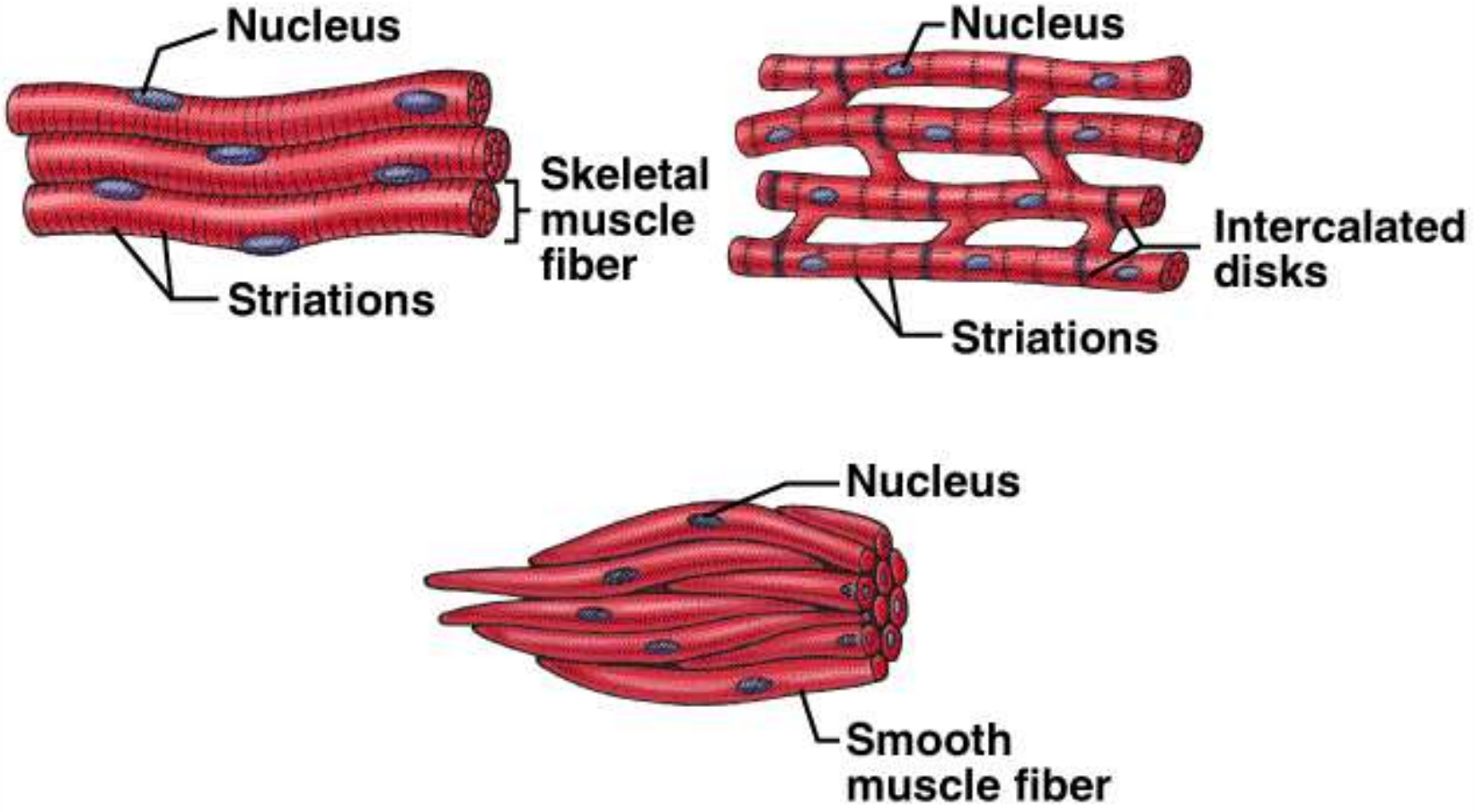
Muscle Tissue



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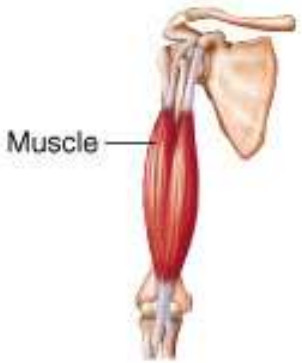

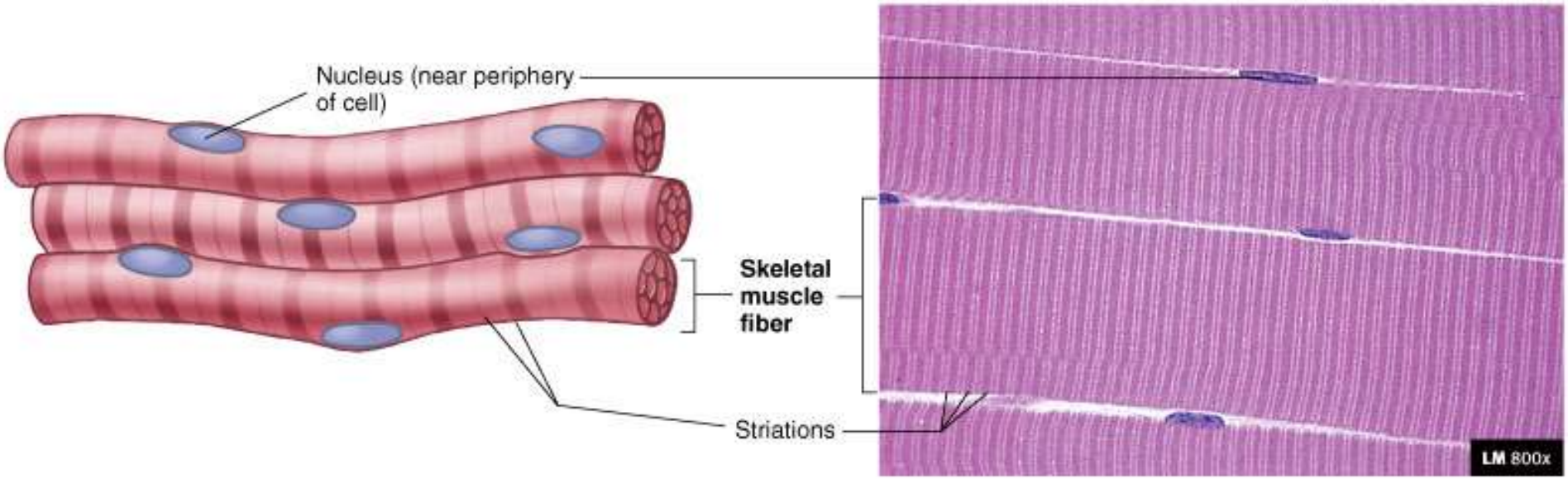

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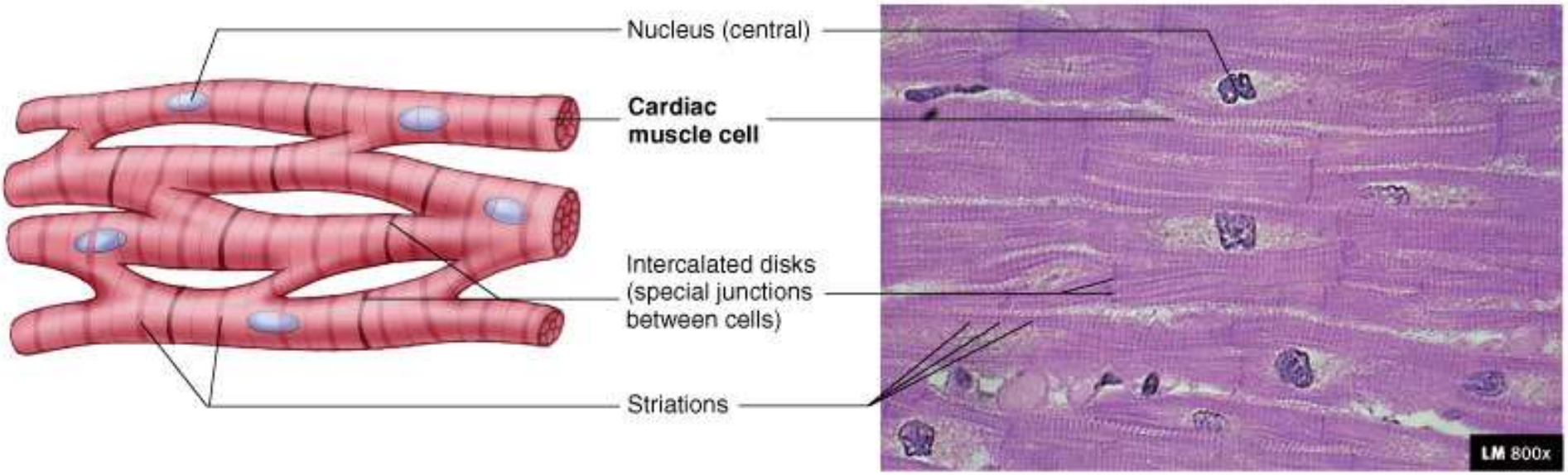

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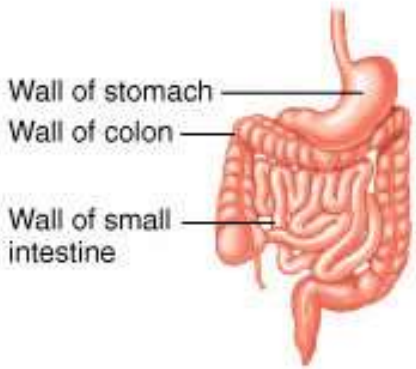

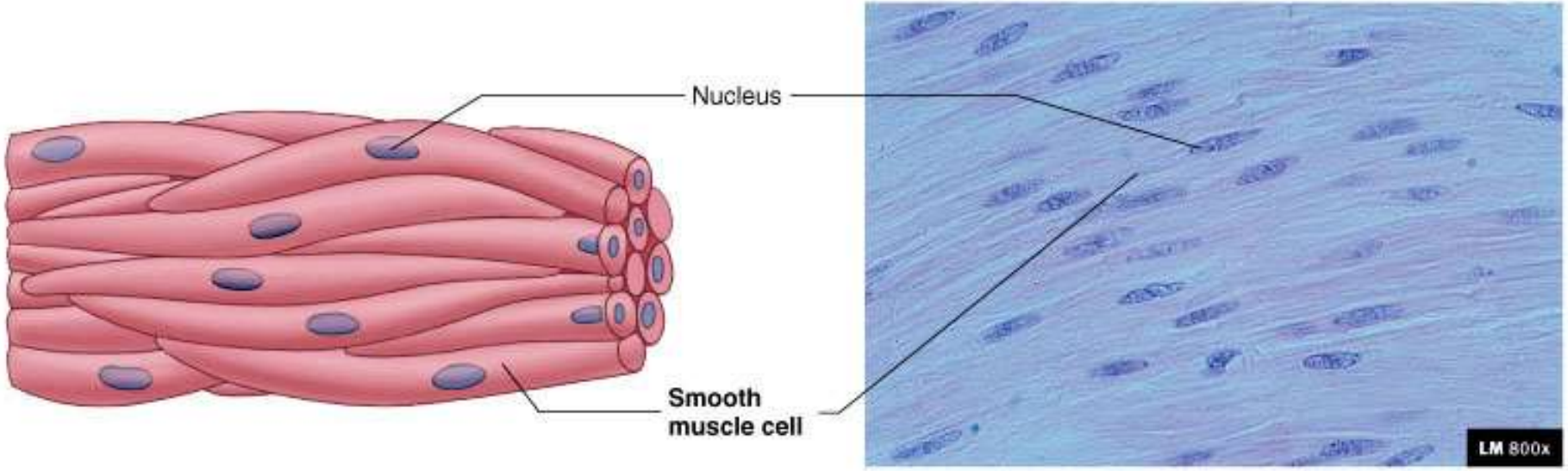

Nervous Tissue

- Consist of neurons or nerve cells
- Found in brain, spinal cord, and peripheral nerves
- Controls and coordinates body movements
- Includes axons, dendrites, cell bodies

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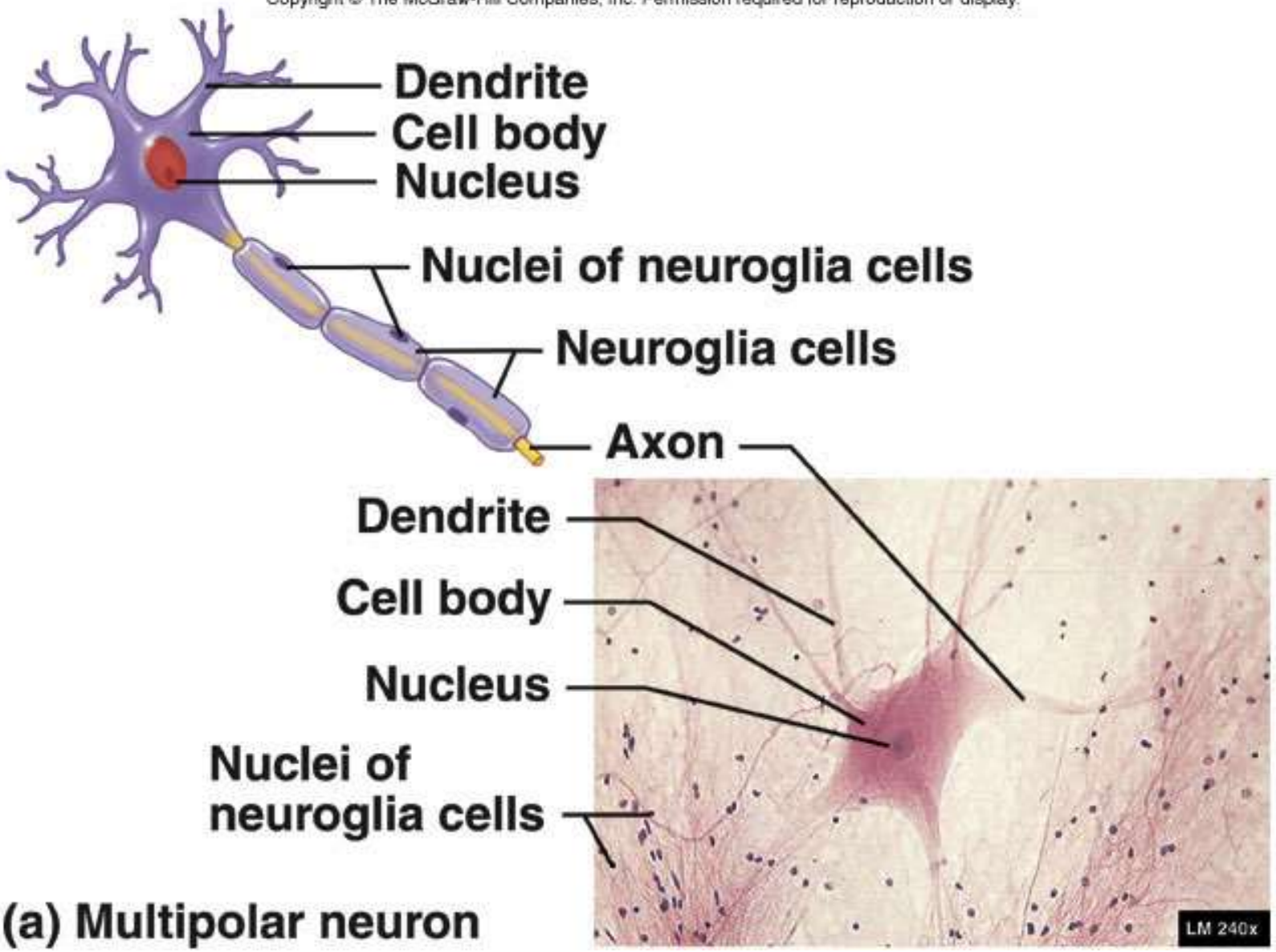


(a) Multipolar neuron

Tissue Repair

- **What is it?**

  substitution of dead cells for viable cells

- **Regeneration:**

  cells of same type develop (no scar)

- **Replacement:**

  cells of a different type develop (scar)

Inflammation

- Occurs when tissues are damaged

- Signals the body's defenses (white blood cells) to destroy foreign materials and damaged cells so repair can occur.

- **Chemical mediators:**
  - released after injury
  - cause dilation of blood vessels

Symptoms of Inflammation

1. **Redness:** blood vessels dilate

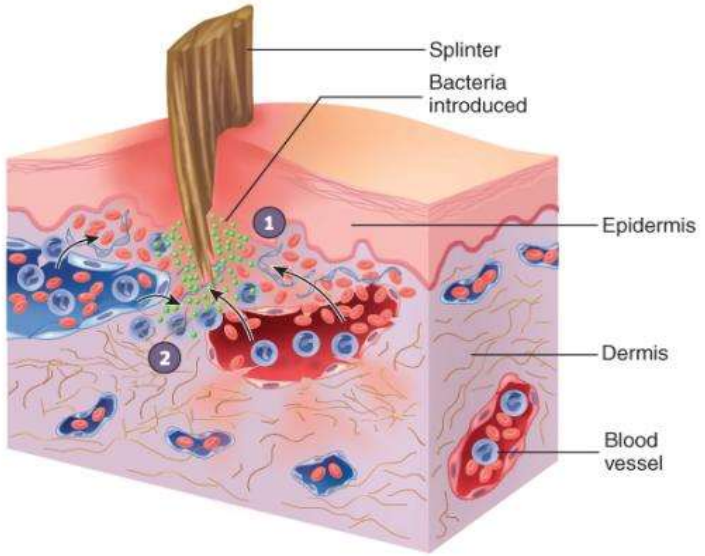
2. **Heat:** due to increased blood flow

3. **Swelling:** from water and proteins

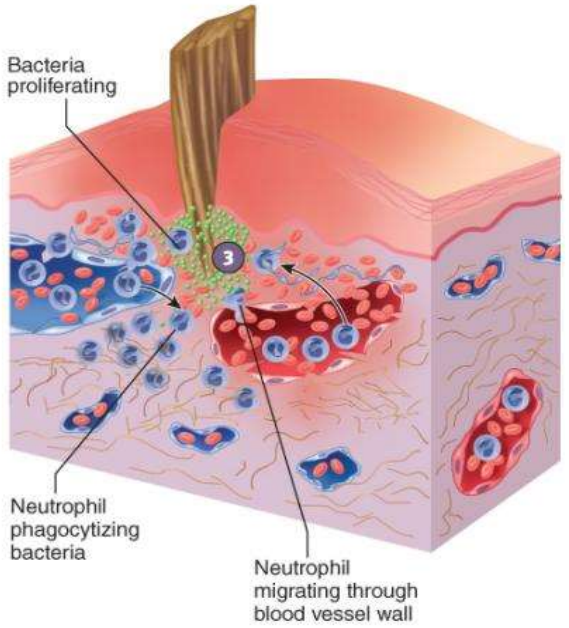
4. **Pain:** nerve endings are stimulated by damage and swelling

1. A splinter in the skin causes damage and introduces bacteria. Chemical mediators of inflammation are released or activated in injured tissues and adjacent blood vessels. Some blood vessels rupture, causing bleeding.

2. Chemical mediators cause capillaries to dilate and the skin to become red. Chemical mediators also increase capillary permeability, and fluid leaves the capillaries, producing swelling *(arrows)*.



3. White blood cells (e.g., neutrophils) leave the dilated blood vessels and move to the site of bacterial infection, where they begin to phagocytize bacteria and other debris.



PROCESS Figure 4.5 Inflammation

Pune District Education Associations Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre, Kharadi, Pune-411014

Tablets

Presented By :-

Mrs. Trusha P. Shangrapawar

Tablets

A **tablet** is a pharmaceutical dosage form. It comprises a mixture of active substances and excipients, usually in powder form, pressed or compacted into a solid.

The excipients can include binders, glidants (flow aids) and lubricants to ensure efficient tabletting; disintegrants to promote tablet break-up in the digestive tract; sweeteners or flavours to enhance taste; and pigments to make the tablets visually attractive.

A polymer coating is often applied to make the tablet smoother and easier to swallow, to control the release rate of the active ingredient, to make it more resistant to the environment (extending its shelf life), or to enhance the tablet's appearance.

Types of tablets

Tablets can be classified according to their drug-release characteristics into:

I. According to drug release rate from the tablet (USP classification):

a- Immediate-release tablet:

The tablet is intended to be released rapidly after administration, or the tablet is dissolved and administered as solution. It is the most common type and includes

1- Disintegrating tablet (conventional or plain tablet)
2- Chewable tablets
3- Effervescent tablets
4- Sublingual and Buccal tablets
5- Lozenges
6- Soluble tablets

b- Modified-release tablet:

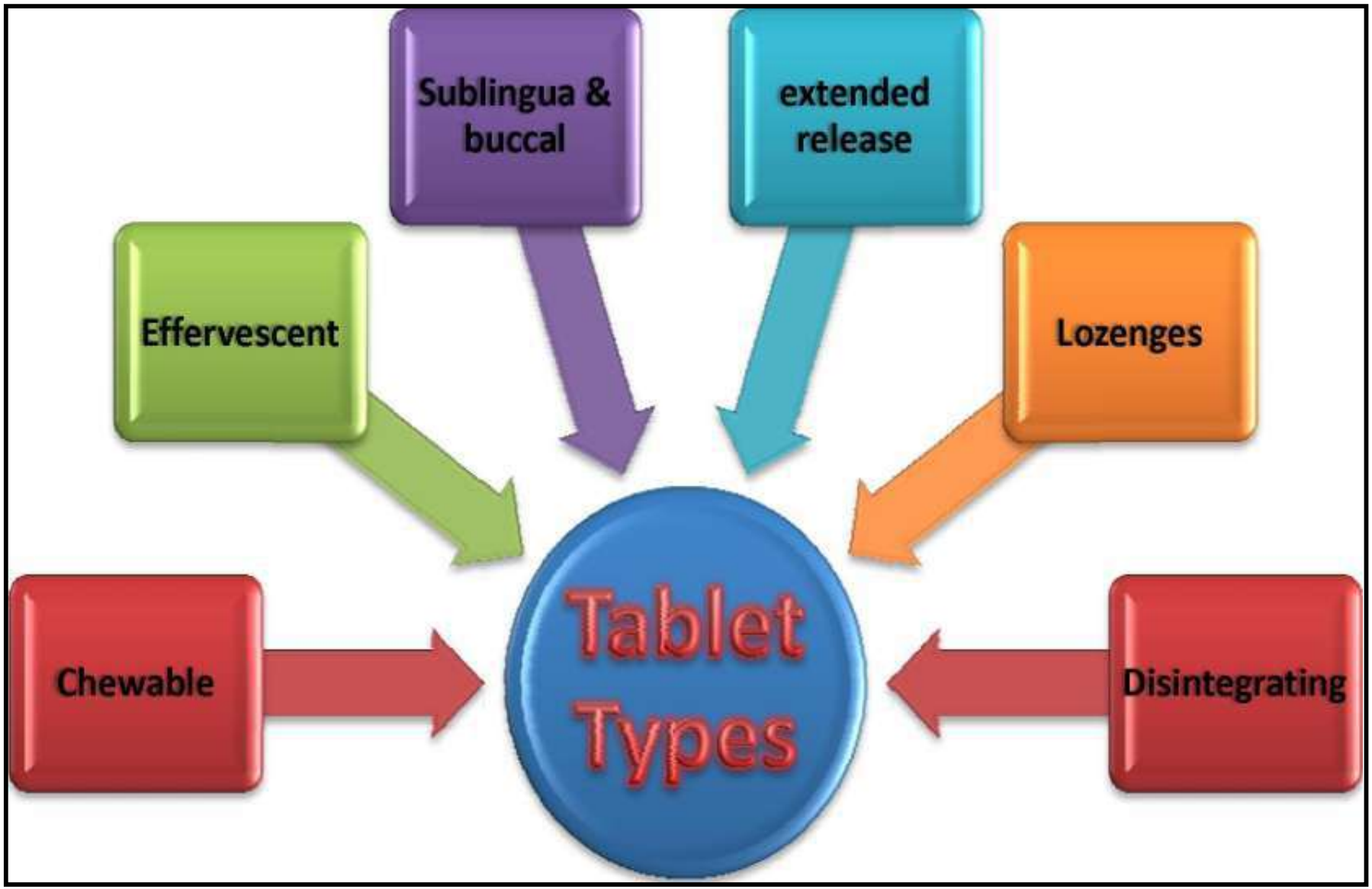
They have release features based on; time, course or location.
-They should normally be swallowed intact.
-Different excipients than immediate release tablets.
-The drug is released from an extended-release tablet slowly at a nearly constant rate.

Delayed-release tablets

The drug is liberated from the tablet some time after administration. After this period has elapsed, the release is normally rapid.
e.g. **Enteric tablet**, for which the drug is released in the upper part of the small intestine after the preparation has passed the stomach.

Multiple compressed tablets

- Tablet within a tablets: core and shell
- Multilayer tablet


Sublingua & buccal
extended release
Effervescent
Lozenges
Chewable
Tablet Types
Disintegrating

Immediate-release tablet:

1- Disintegrating tablet (conventional or plain tablet)

- Disintegrating tablet is the most common type of tablets that is intended to be swallowed and to release the drug in a relatively short time thereafter, by disintegration and dissolution (fast and complete drug release in vivo).
- It includes normally the following type of excipients; filler (with low dose drug), disintegrant, binder, glidant, lubricant and antiadherent.

Bilayer tablets

- Tablet disintegration may be affected by;
1- Choice of the excipients.
2- Production conditions during manufacture.
- Conventional tablet may be single layer or multilayer.
Multilayer tablets are prepared by repeated compression of powders and are made primarily to separate incompatible drugs from each other.

Steps of drug release from disintegrating tablets

2- Chewable tablets

Chewable tablets are to be chewed and thus mechanically disintegrated in the mouth, so NO DISINTEGRANT IS INCLUDED IN ITS COMPOSITION.
Flavoring, sweetening and coloring agents are important.
Sorbitol and mannitol are common examples of fillers in chewable tablets, (mannitol has negative heat of solution which results in cooling effect and also has sweetening action)

Advantages of chewable tablets:
- Provide quick and complete disintegration of the tablet and thus obtain a rapid drug effect after swallowing and dissolution.
- Easy administration, especially for infants and elderly people.
- Could be administered when water is not available.

Examples for chewable tablets are;
- Chewable Aspirin tablets (for children in the treatment of rheumatoid and to prevent clot formations in adults)
-Chewable Antacid tablets

3- Effervescent tablets:

Effervescent tablets are uncoated tablets generally containing acid substances and carbonates or hydrogen carbonates which react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

Effervescent tablets are dropped into a glass of water before administration during which CO2 is liberated. This facilitates tablet disintegration and drug dissolution; the tablet disintegration
should be complete within few minutes.
(Effervescence is a special mechanism for disintegration)
CO2 is created by the reaction between carbonate or bicarbonate and a weak acid such as citric acid or tartaric acid.

Effervescent tablets are dropped into a glass of water before administration, during which carbon dioxide is liberated.

facilitates tablet disintegration and drug dissolution; the dissolution of the tablet should be complete within a few minutes.

Uses of effervescent tablets:

1. Rapid drug action, e.g. analgesic drugs .
2. Facilitate the intake of the drug, e.g. vitamins.

After Dissolution of tablets → buffered water solution will be obtained → Temporarily increases the pH of the stomach

↓

Rapid emptying of the stomach and shortening the residence time

↓

Drug-induced gastric irritation can be avoided (short residence time) e.g. aspirin tablets

As absorption of aspirin in the stomach can cause irritation

← fast drug bioavailability (As drugs are absorbed more effectively in the small intestine than in the stomach)
e.g. analgesics

Advantages of effervescent tablets:

1. To obtain rapid drug action, for example analgesics and antacids.
2. To facilitate drug intake, for example vitamins.
3. They are convenient, easy to use, premeasured dosage form as compared with the powdered dosage forms.
4. They cannot spill as the powdered preparations can.
5. They can be individually packaged to exclude moisture, thereby avoiding the problem of product instability of the unused contents during storage.

Effervescent tablets package →

6. Effervescent tablets often include a flavor and a colorant.
7. Effervescent tablets are prepared by direct compression or dry granulation.
8. Effervescent tablets should be protected from moisture, so that a special package is needed; each tablet is completely covered with aluminum foil and kept in a water-proof container, often including a desiccant. Effervescent tablets may be packed in blister packs.

Effervescent tablets usually contain

Carbonate or bicarbonate and a weak acid such as citric or tartaric

The amount of sodium bicarbonate in an effervescent tablet is often quit high (about 1 gram)

Flavor and a colourant

N.B. Binder and disintegrant are normally not included in the composition

A water-soluble lubricant is preferable in order to avoid a film of hydrophobic lubricant on the surface of the water after tablet dissolution

4- Sublingual and Buccal tablets:

✦ They are used for drug release in mouth followed by systemic uptake of the drug.

✦ A rapid systemic drug effect can thus be obtained without first-pass liver metabolism, because the drug diffuses into the blood, directly through tissues under the tongue in case of sublingual tablets and through oral mucosa in case of buccal tablets.

✦ They are often small and porous, the latter facilitating fast disintegration and drug release.

Sublingual tablets are placed under the tongue.

Ex. **Nitroglycerin sublingual tablet**; it exerts its action within **two minutes** for rapid relief of "Angina pectoris" attack, because the sublingual area is rich in blood supply. Nitroglycerine suffers from first-pass metabolism if taken orally. Also other cardiovascular drug, barbiturates, and vitamins are prepared as sublingual tablet dosage form.

Disadvantage

1) High dose can not be administered
2) Less area is available for absorption
3) Not suitable for bitter and irritating drugs
4) Less patient compliance
5) No eating, Drinking and smoking is allowed
6) Highly ionic drugs can not be administered

Advantages

1) Rapid absorption
2) Dose reduction
3) Fast on set of action
4) Increase B.A.
5) Reduction in side effects
6) Suitable in disease like nausea, vomiting
7) Not required water

Buccal Tablets

- Buccal tablets are small, flat, and oval shaped dosage form and unlike conventional tablets allow for drinking and speaking without major discomfort.

- They soften, adhere to the mucosa and are retained in position until dissolution and/or release is complete

- Can be used for both local and systemic drug delivery

Buccal tablets are placed in the side of the cheek for absorption through oral mucosa.
N.B. Buccal tablets may be also prepared for their local application.

5-Lozenges

They are tablets that dissolve slowly in the mouth and so release the drug dissolved in the saliva.

Lozenges may be used for;

- Local medication for mouth or throat, e.g. local anesthetics, antiseptics and antibiotics.
- Systemic drug uptake.

Compressed lozenges:

are made by using tablet machine with large and flat punches, with high pressure is applied to produce hard tablets, so that they dissolve slowly in mouth.

Bradoral® compressed loyenges
for treatment of sore throat

NO DISINTEGRANT IS INCLUDED IN COMPRESSED LOZENGES COMPOSITION

Other additives (binder and filler) must have pleasant taste or feeling during dissolution. High concentration of fillers which are mainly sugars as glucose, sorbitol or mannitol.
High concentration of binder is used. Common binder used in compressed lozenges is gelatin.

6- Soluble tablets

Soluble tablets are uncoated or film-coated tablets.
They are intended to be dissolved in water before administration.
The solution produced may be slightly opalescent due to the added excipients used in the manufacture of tablets.

7-ODT

- Orally disintegrating tablets are defined as solid oral preparations that disintegrate rapidly in the oral cavity with an in vitro disintegration time of less than 30 seconds, according to FDA guidelines (guidance for industry; 2008).

Modified-release tablet

Allowing the reduction in dosing frequency.

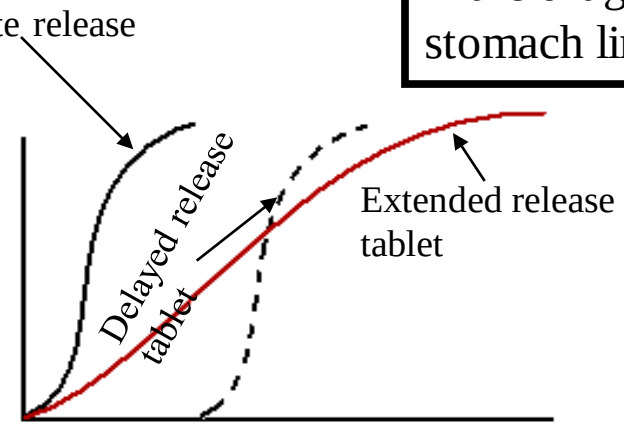
1-Extended-release tablet

Slowly release the drug in the GIT at a constant rate
Prolonged release and sustained release (12-24 hours)

- The aim is to increase the time period during which a therapeutic concentration level in the blood is maintained
- To increase the release time for drugs that can cause local irritation in the stomach or intestine if they are released quickly (iron salts)

2- Delayed-release tablet

Drug release is delayed due to physiological conditions e.g. pH (a lag period followed by normal release).
The best example is enteric coated tablets, the drug is released in the upper part of the small intestine after which the preparation has passed the stomach.
If the drug is sensitive to acid, or is irritant to the stomach lining, an enteric coating can be used.

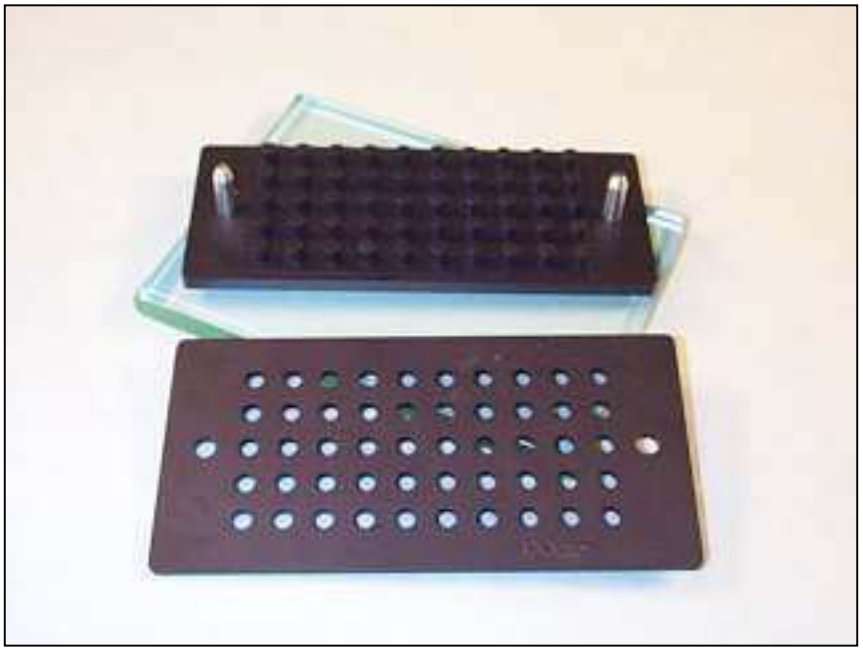


II. According to method of manufacturing:
a- Compressed tablet:

It is obtained by compressing uniform volume of particles using "Tablet compression machine". It's used for large scale production.

b. Molded tablets:

Molding means shaping and hardening of semi- solid mixture of drug and excipients.
It is obtained using ″tablet mold“. It is restricted for small-dose tablet and for small scale production.



Tablet mold

Tablets administered by other routes

These tablets are administered by other routes except for the oral cavity and so the drugs avoided from passing through the gastrointestinal tract.

These tablets may be inserted into other body cavities or directly placed below the skin to be absorbed into systemic circulation from the site of application.

Vaginal tablets

- These tablets undergo slow dissolution and drug release in the vaginal cavity of women. The shape kept ovoid or pear shaped to facilitate retention in vagina.
- These tablets release generally antibacterial, antiseptics or astringents to treat vaginal infections or release steroids for systemic absorption.
- The tablet should be made compatible with plastic tube inserters which are designed to place the tablet in the upper region of vaginal tract.

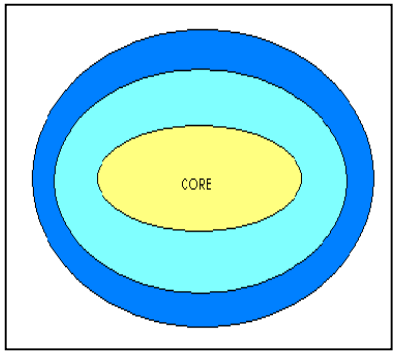
Implants

- These tablets are inserted into subcutaneous tissue by surgical procedures where they are very slowly absorbed over a period of one month or one year.
- A special injector with a hollow needle and plunger is used to administer the rod shaped tablet for other shapes surgery is required. The tablets may be pellets cylindrical or rosette shaped with diameter not more than 8 mm. They are sterile formulation without excipients and made hard with large particle size to achieve gradual drug release. Mainly, these tablets are prepared to deliver growth hormones.

Coated tablets

Definition:

Coated tablets are defined as tablets covered with one or more layers of mixtures of various substances with the intention of (adding) conferring benefits and properties to the dosage form over the uncoated one.



Press-coating (Enteric-coated tablets)

Sugar-coated tablets

Film-coated tablets

✹ Sugar coated tablets:
1- Protect tablets from moisture
2- Mask odor and flavor
3- Elegance

✹ Film coated tablets:
1- Thin film coat
2- Soluble or insoluble polymer film

✹ Enteric coated tablets:
compressed tablets coated with substances that resist solution in gastric fluid but disintegrate in the intestine. It can be used for tablets containing drug substances which are inactivated or destroyed in the stomach, for those which irritate the mucosa or as a means of delayed release of the medication.

Thank you…

Pune District Education Associations
Shankarrao Ursal College of Pharmaceutical Sciences & Research Centre, Kharadi, Pune-411014

Biopharmaceutics of Antisense Molecule and Aptamers

Presented By :-
Mrs. Trusha P. Shangrapawar

CONTENTS

- **ANTISENSE INTRDUCTION**
- ANTISENSE THERAPY
- MECHANISM OF ANTISENSE ACTIVITY
- ROLE OF ANTISENSE, APPLICATION ,CHALLENGES
- ADVANTAGES AND DISADVANTAGES
- **APTAMERS INTRODUCTION**
- CLASSIFICATION
- APTAMERS TARGET BINDING MECHANISM
- PRODUCTION OF APTAMERS (SELEX)
- PROPERTIES AND APPLICATION
- ADVANTAGES AND DISADVANTAGES
- STRATEGIES TO OVERCOME APTAMER LIMITATIONS
- CONCLUSION

ANTISENSE MOLECULE

• Antisense molecules are synthetic segments of DNA or RNA, designed to mirror specific messenger RNA (mRNA) sequences and block protein production.

•Messenger RNA (mRNA) is single-stranded sequence of nucleotides is called "sense" because it results in a gene product (protein).

• One way to target the genetic material is to block mRNA by using “antisense DNA”, which prevents the message from ever becoming a protein.

Antisense therapy

•Antisense therapy is a form of treatment for genetic disorders or infections.

• Antisense drugs blocks abnormal disease related proteins and are being researched to treat a variety of diseases such as cancers, diabetes, asthma, arthritis.

Protein production occurs in two phases

Transcription and **Translation.**

In the transcription phase , the DNA strand is used as a template for manufacturing an mRNA molecule.

mRNA is responsible for communicating the genetic message in the DNA to the cell so that protein production can take place.

In the translation phase , the mRNA travels to the ribosome, and carry out protein synthesis.

BASIC CONCEPT

5' ...A T G G C C T G G A C T T C A... 3' Sense strand of DNA

3' ...T A C C G G A C C T G A A G T... 5' Antisense strand of DNA

↓ Transcription of antisense strand

5' ...A U G G C C U G G A C U U C A... 3' mRNA

↓ Translation of mRNA

Met– Ala– Trp– Thr – Ser – Peptide

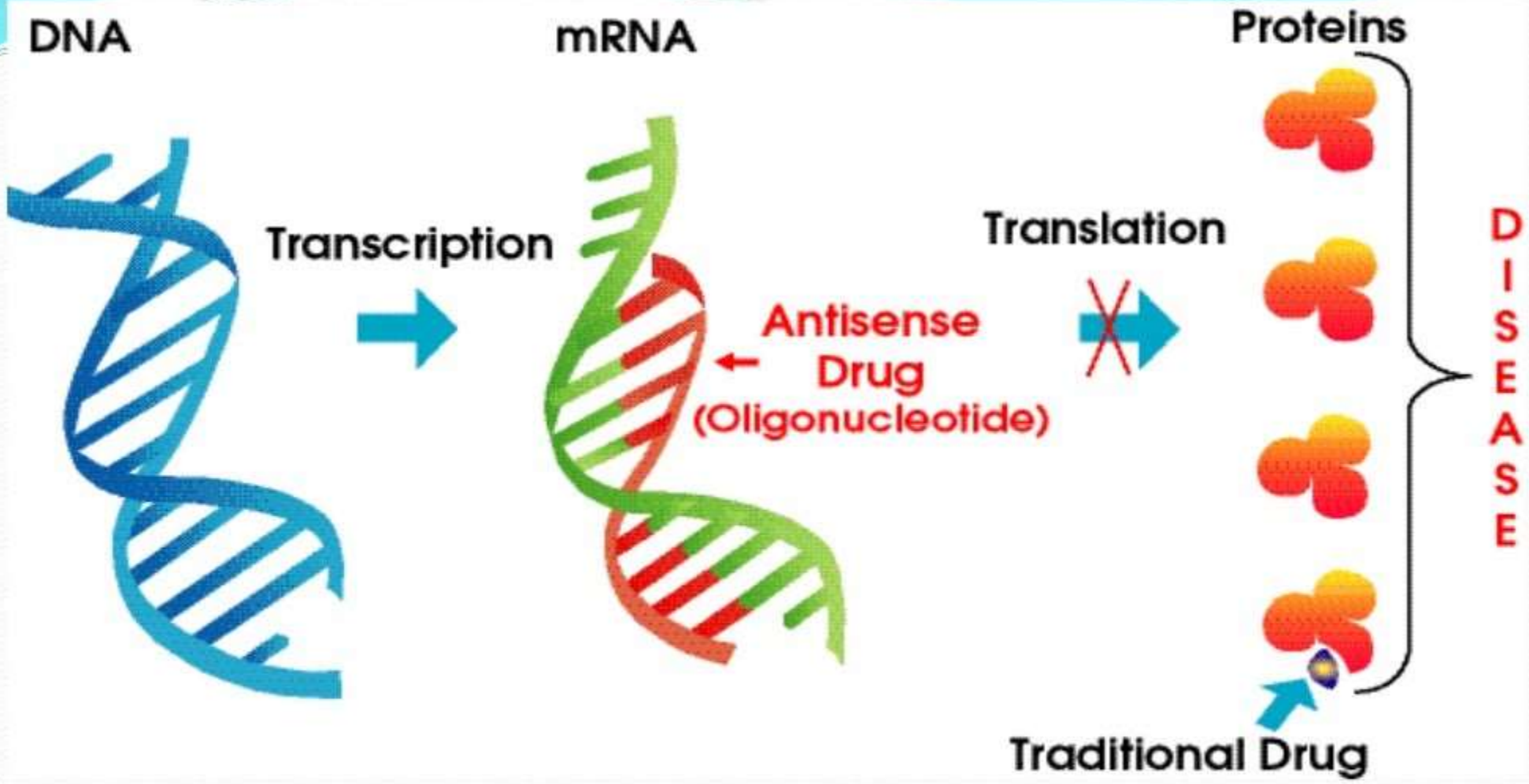
ANTISENSE OLIGONUCLEOTIDES

•Synthetic short segments of DNA or RNA are referred to as oligonucleotides (ODNs).

•An antisense oligonucleotide is a single strand of nucleic acid or nucleic acid analogs. AS-ONs usually consist of 15– 20 nucleotides, which are complementary to target mRNA.

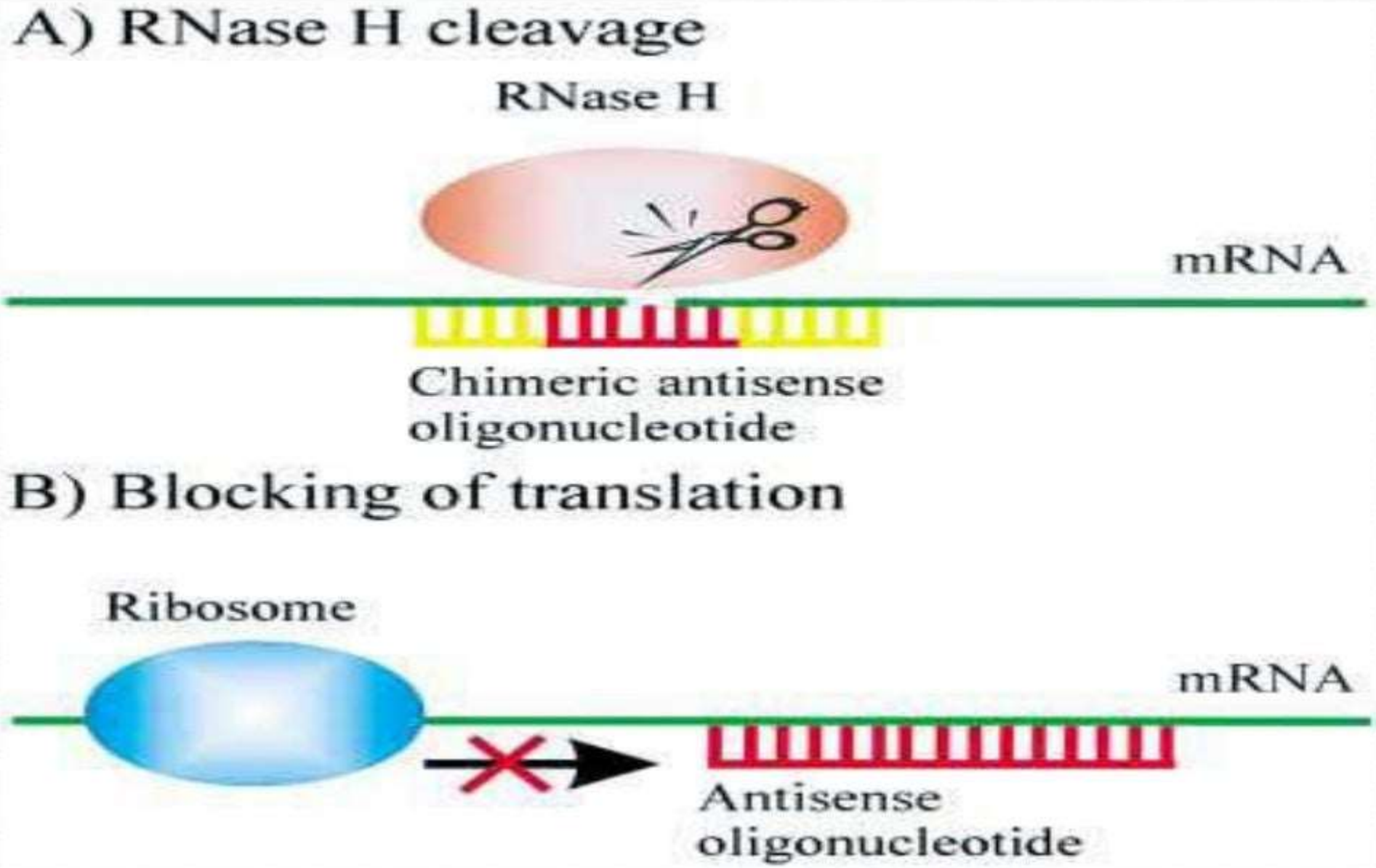
•In this technique Short segments of single stranded DNA called Oligodeoxynucleotides are introduced in to the cell.

•These Oligonucleotides are complementary to the mRNA, and physically bind to it.



•When these oligonucleotides combined with target mRNA, a DNA/RNA hybrid is formed, which is degraded by the enzyme **RNase H**.

MECHANISM OF ANTISENSE ACTIVITY



RNase H

•RNase H is a non-specific endonuclease, catalyzes the cleavage of RNA via hydrolytic mechanism.

•The RNase H-dependent oligonucleotides, which induce the degradation of mRNA .

•The target mRNA is degraded, whereas the antisense oligonucleotide remains intact.

Blocking Translation

•When mRNA forms a duplex with a complementary antisense RNA sequence, translation is blocked.

This may occur because

1. The ribosome cannot gain access to the nucleotides in the mRNA
2. Duplex RNA is quickly degraded by ribonucleases in the cell.

ROLE OF ANTESENSE

- To inactivate ethylene synthesis.
- Reduction of starch content in vegetables.
- Modification of flower colour in various decorative plant.
- Mammalian genomes encode numerous natural antisense transcripts, but the function of these transcripts is not well understood.

APPLICATIONS

- Genetic Disorder
- Cancer
- Renal Transplant Rejection
- Infection (Viral, Protozoal, Fungal)
- Cardiovascular disorder (hypertension)
- Inflammatory and autoimmuno disorders (Asthema)

CHALLENGES

- Need to identify appropiate gene target.
- Need to generate antisense oligonucleotide that is specific for the gene of interest
- Molecules need to be modified to prevent degredation by RNAses and other enzyme.
- Drugs need to be deliverable to the organ of interest.

ADVANTAGES

- Sensitivity of therapy can easily measured
- Potential to produce long lasting responsrs
- Oligonucleotides are manufactured quickly i.e, within a week.

DISADVANTAGES

- Completely artificial molecule, so no vectors possible
- Not always highly effects.
- Side effects for high dosage.
- Delivery usually only via microinjection or electropration.

APTAMERS

In, Latin ***aptus/apto – to fit,*** *and*
In, Greek ***meros – part (Smallest Unit Of Repeating Structure)***

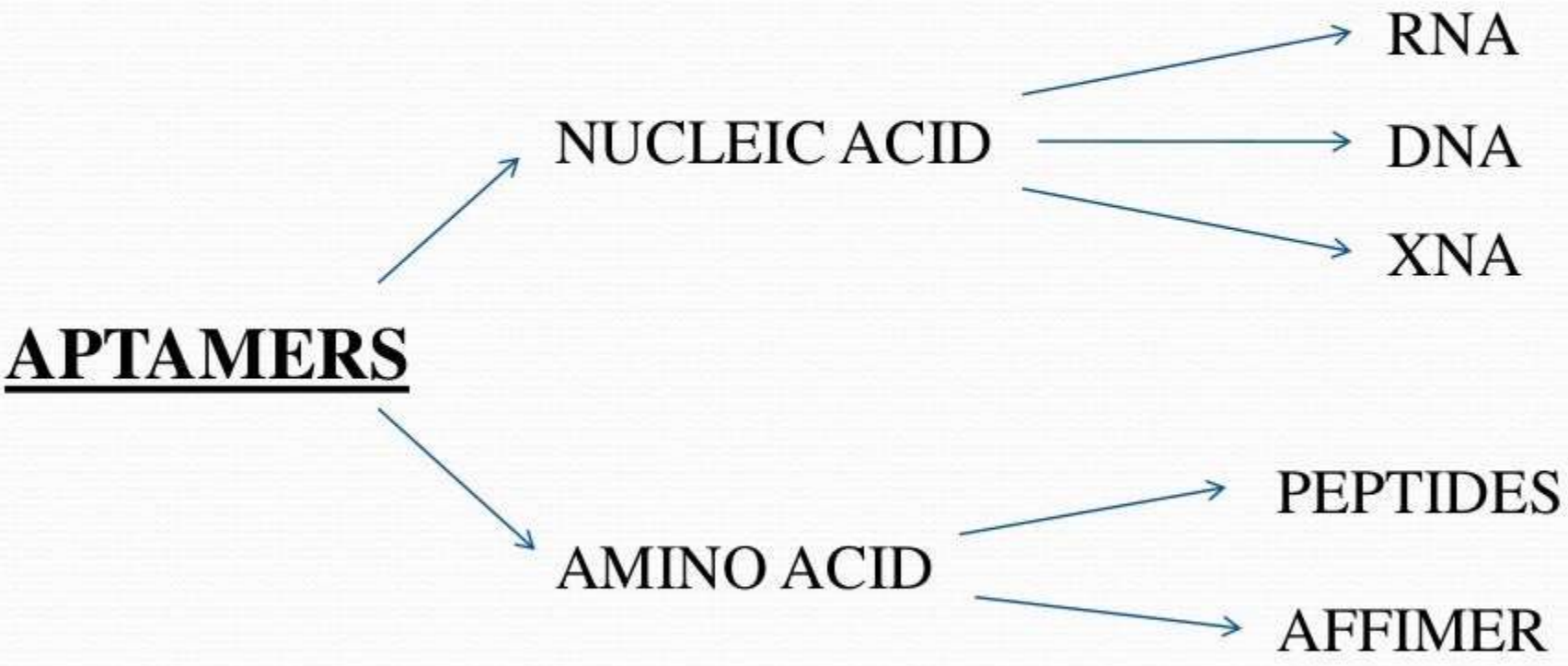
• Single stranded folded oligonucleotides and peptide that bind to molecular (protein) targets with high affinity and specificity

• Provide opportunities for structure-based drug design strategies relevant to therapeutic intervention.

•Can be prepared using SELEX.

•They are similar to monoclonal antibodies.

CLASSIFICATION OF APTAMERS BASED ON STRUCTURE



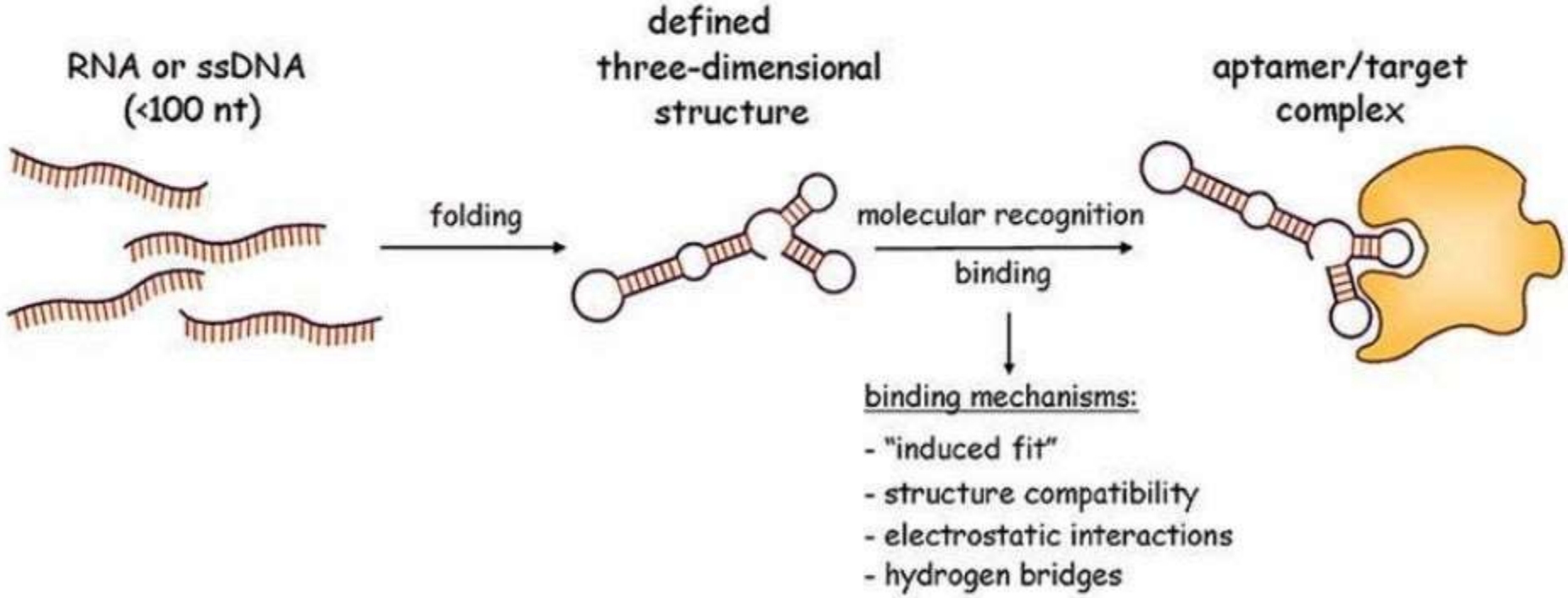
COMPARISON OF RNA, DNA AND PEPTIDE APTAMERS:

RNA aptamer	DNA aptamer	Peptide aptamer
From complex secondary and Tertiary structure	From complex secondary and Tertiary structure	Structure constrains by Scaffoled (temporary structure
From diverse 3d structure	Less diverse 3d structure than RNA aptamer	3d structure constraints by Scaffold
Bind target with the entire Sequence	Bind target with the entire Sequence	Bind target variable region only
Biosenseor, diagonostic , Therapeutics applications	Biosenseor, diagonostic, Therapeutics applications	Biosenseor, diagonostic, Therapeutics applications

Based on their three-dimensional structures, aptamers can well-fittingly bind to a wide variety of targets from single molecules to complex target mixtures or whole organisms.

- They range in size from 20 to 80 bases.
- Aptamers can binds to nucleic acid, proteins and small organic compounds

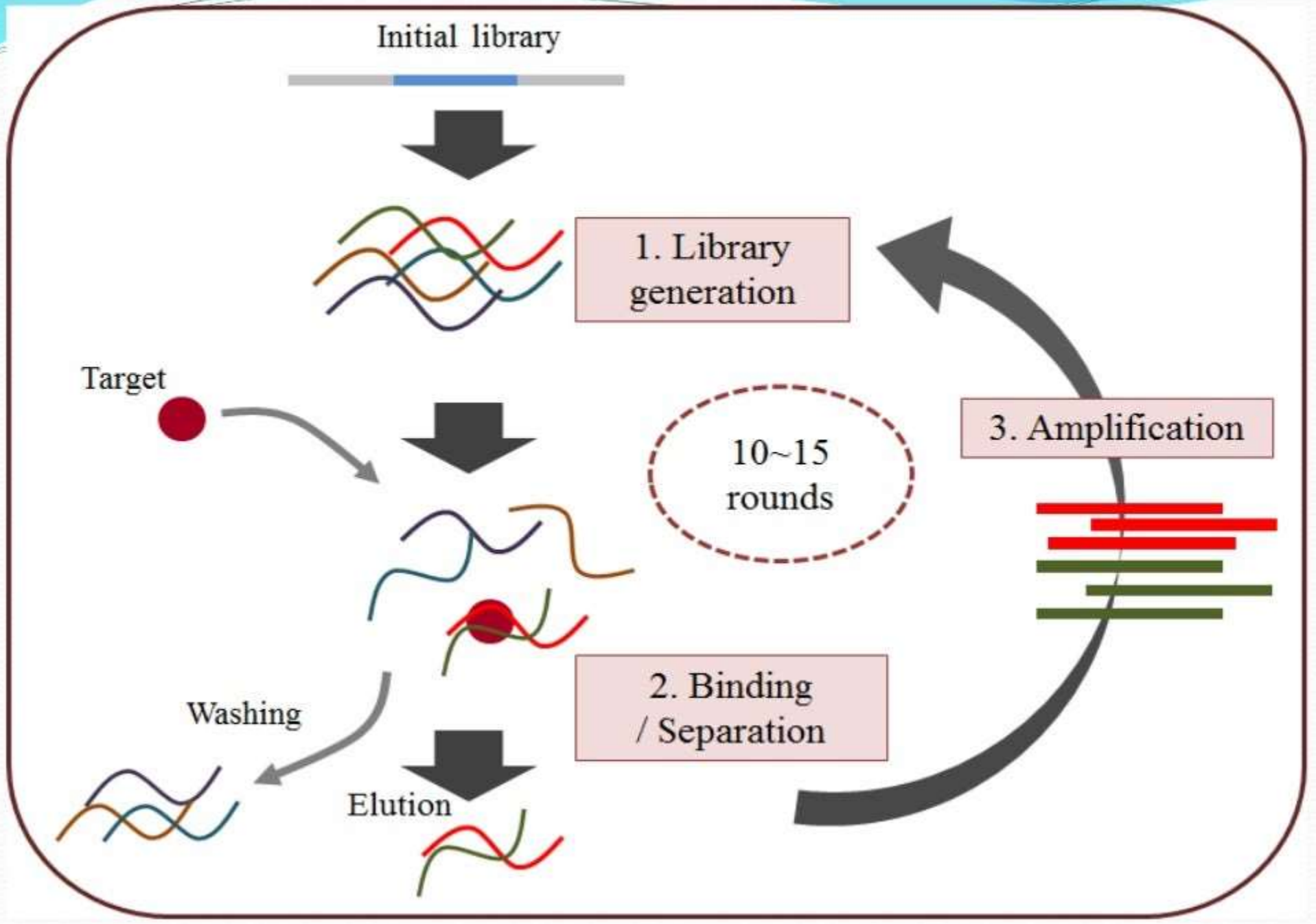
APTAMERS – TARGET BINDING MECHANISM



APTAMER PRODUCTION

SELEX

- Systematic Evolution of Ligands by Exponential Enrichment
- Aptamer are produced by an in vitro selection or systematic evolution of ligand by exponential enrichment (SELEX).
- SELEX produces ssRNA with specific binding target.
- This is an iterative process of binding, partitioning, amplifying novel nucleic acids & regeneration.


Initial library
1. Library generation
Target
10~15 rounds
3. Amplification
2. Binding / Separation
Washing
Elution

In the first step (library generation) :

• A library, except the initial compound in the library, is converted into single-strand nucleotides that consist of random sequence regions, usually 30–40 meters, flanked by the primer binding site.

In the second step (binding and separation) :

• The target-bound library components are separated from the unbound components. This step is generally coupled with several other methods to make selection of the target or the library easy and rapid.

Finally, in the third step (amplification) :

• The target-bound library component is amplified by the PCR to create a new library to be used in the next round.

Aptamers are continuously developed through this on-going process, and their characteristics are identified using various biological assays.

APTAMER SELECTION METHOD BASED ON SELEX

1. Nitrocellulose Membrane Filtration - Based SELEX
2. Affinity Chromatography & Magnetic Bead – Based SELEX
3. Capillary Electrophoresis-based SELEX
4. Microfluidic -based SELEX
5. Cell-SELEX

PROPERTIES OF APTAMERS

- Ability to bind to proteins with high affinity and specificity.
- Usually very small in size but can easily distinguish between very closely related protein also.
- Highest specificity and affinity to the target.
- Small size and similarity to endogenous molecules.
- Ability to disrupt interaction between proteins,
- Low or non immunogenic and non toxic molecules.
- Production of aptamers is done in-vitro using automated synthesis.
- Rationally design duration of action.
- Less cost of oligonucleotide synthesis.
- Function at different temperature, Ph and buffers.
- Unlimited shelf life.

APPLICATION OF APTAMERS

- For new drug
- In drug delivery
- As tehrapeutic tool
- In diagnosis of disease
- In food inspection
- In hazard detection
- In bio-imaging
- As analytical reagent

Aptamers have similar affinities

ADVANTAGES

- Produced chemically in a readily scalable process
- Not prone to viral or bacterial contamination
- Non-immunogenic
- more efficient entry into biological compartments
- greater stability
- Can usually be reversibly denatured.
- Dyes or functional groups can be readily introduced during synthesis.
- Aptamers have similar affinities as antibodies to target molecules.
- They are non-toxic and elicit little or no immunogenic response.

DISADVANTAGES

- Pharmacokinetic and other systemic properties are variable and often hard to predict
- Small size makes them susceptible to renal filtration
- have a shorter half-life
- Unmodified aptamers are highly susceptible to serum degradation
- Aptamer technologies are currently largely covered by a single intellectual property portfolio.

STRATEGIES TO OVERCOME APTAMER LIMITATIONS

- Aptamers can be optimized for activity and persistence under physiological conditions during selection or during structure–activity relationship and medicinal chemistry studies conducted after discovery.
- Addition of conjugation partners such as polyethylene glycol or cholesterol can increase circulating half-life.
- Chemical modifications incorporated into the sugars or internucleotide phosphodiester linkages enhance nuclease resistance.

CONCLUSION

Aptamers provide opportunities for structure-based drug design strategies relevant to therapeutic intervention.

Aptamers' entire selection process can be carried out in a test tube.

They have high binding affinities, selectivity and specificity to their targets making them important for diagnostics and targeted therapy.

They are thermostable and can be structurally modified to enhance specificity and stability, therefore increasing aptamer half-life in in vivo applications.

THANK YOU...

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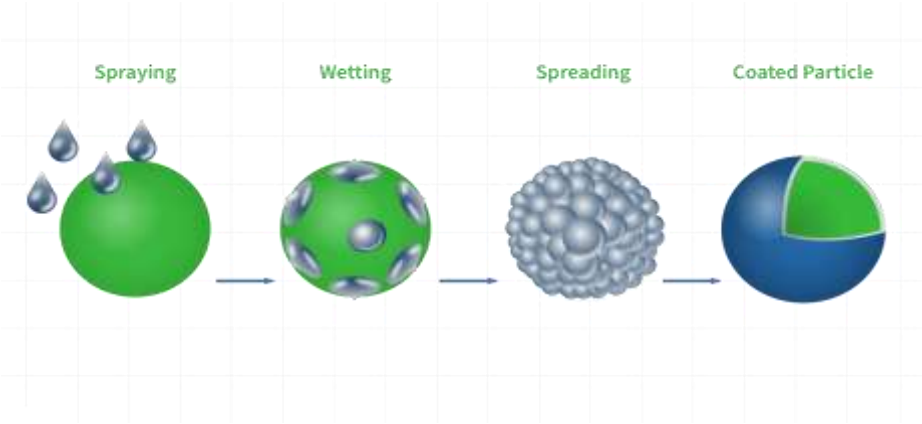
MICROENCAPSULATION



Presented By :-
Mrs. Trusha P. Shangrapawar

INTRODUCTION

✓ **Microencapsulation** is a process by which very tiny droplets or particles of liquid or solid material are surrounded or coated with a continuous film of polymeric material.

✓ The product obtained by this process is called as micro particles, microcapsules.

✓ Particles having diameter between 3 - 800µm are known as micro particles or microcapsules or microspheres.

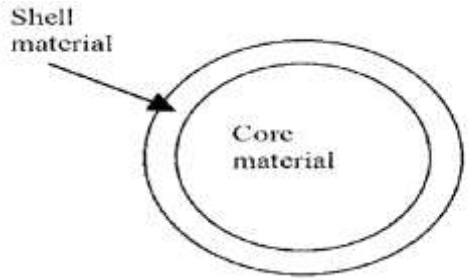
✓ Particles larger than 1000µm are known as Macro particles .

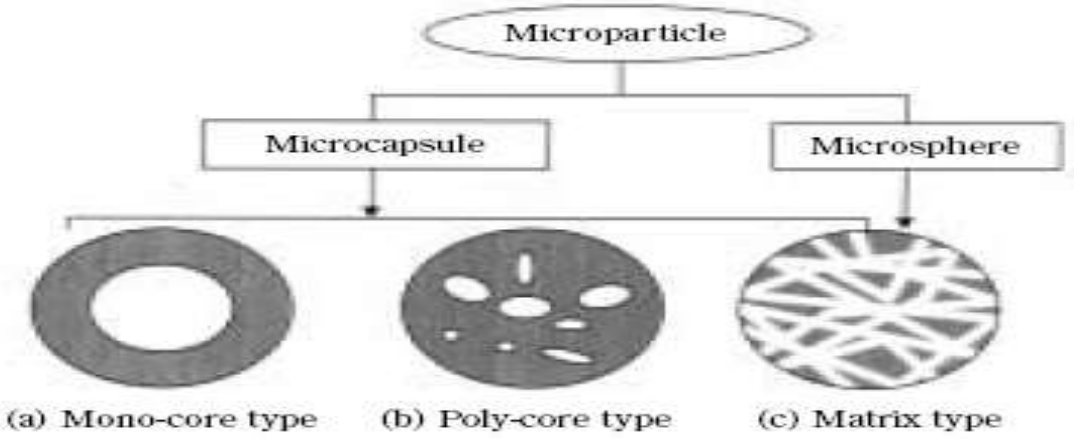
CLASSIFICATION OF MICROPARTICLE

Generally Micro particles consist of two components

a) Core material

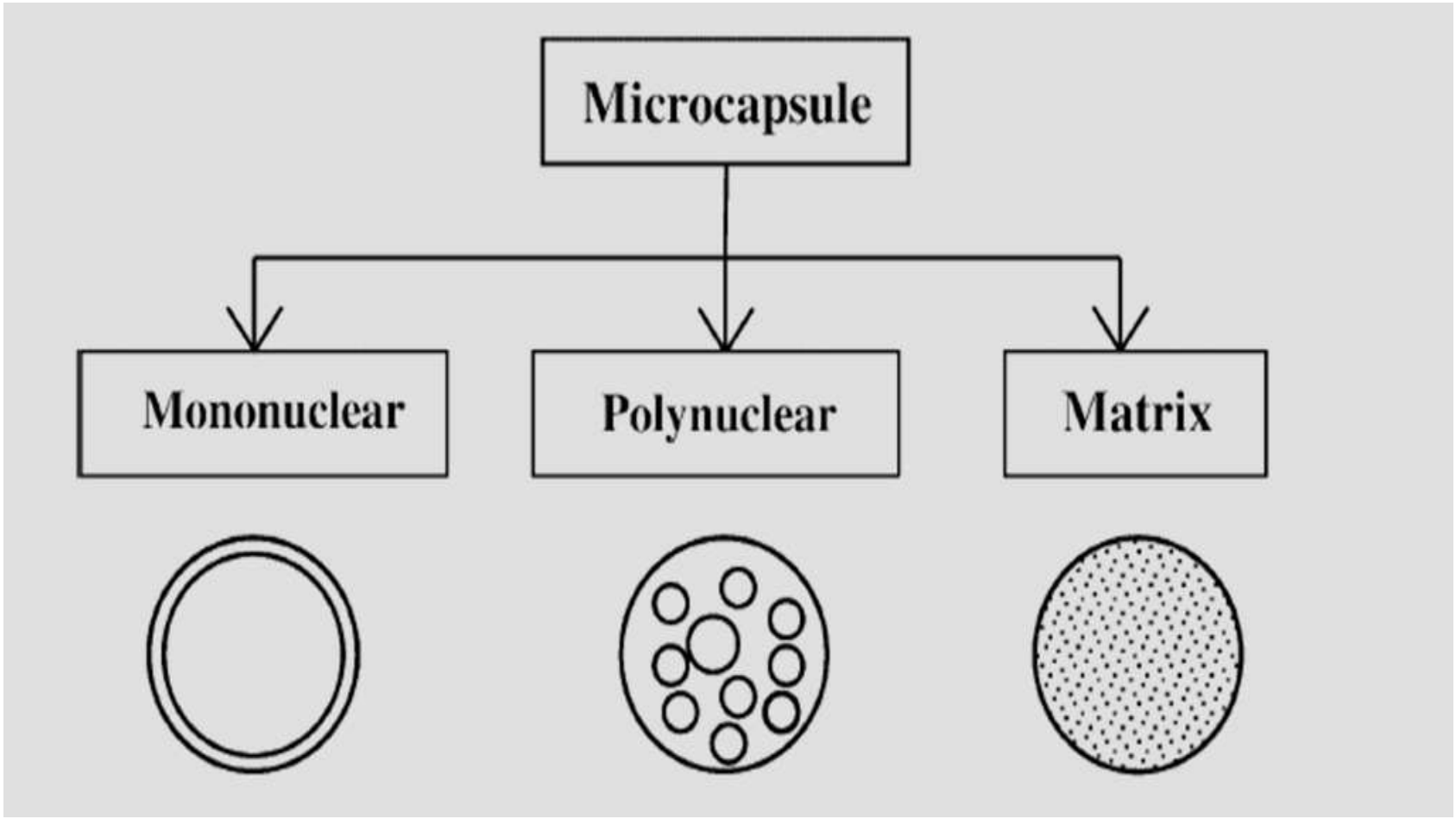
b) Coat or wall or shell material.





1.Microcapsules: The active agent forms a core surrounded by an inert diffusion barrier.

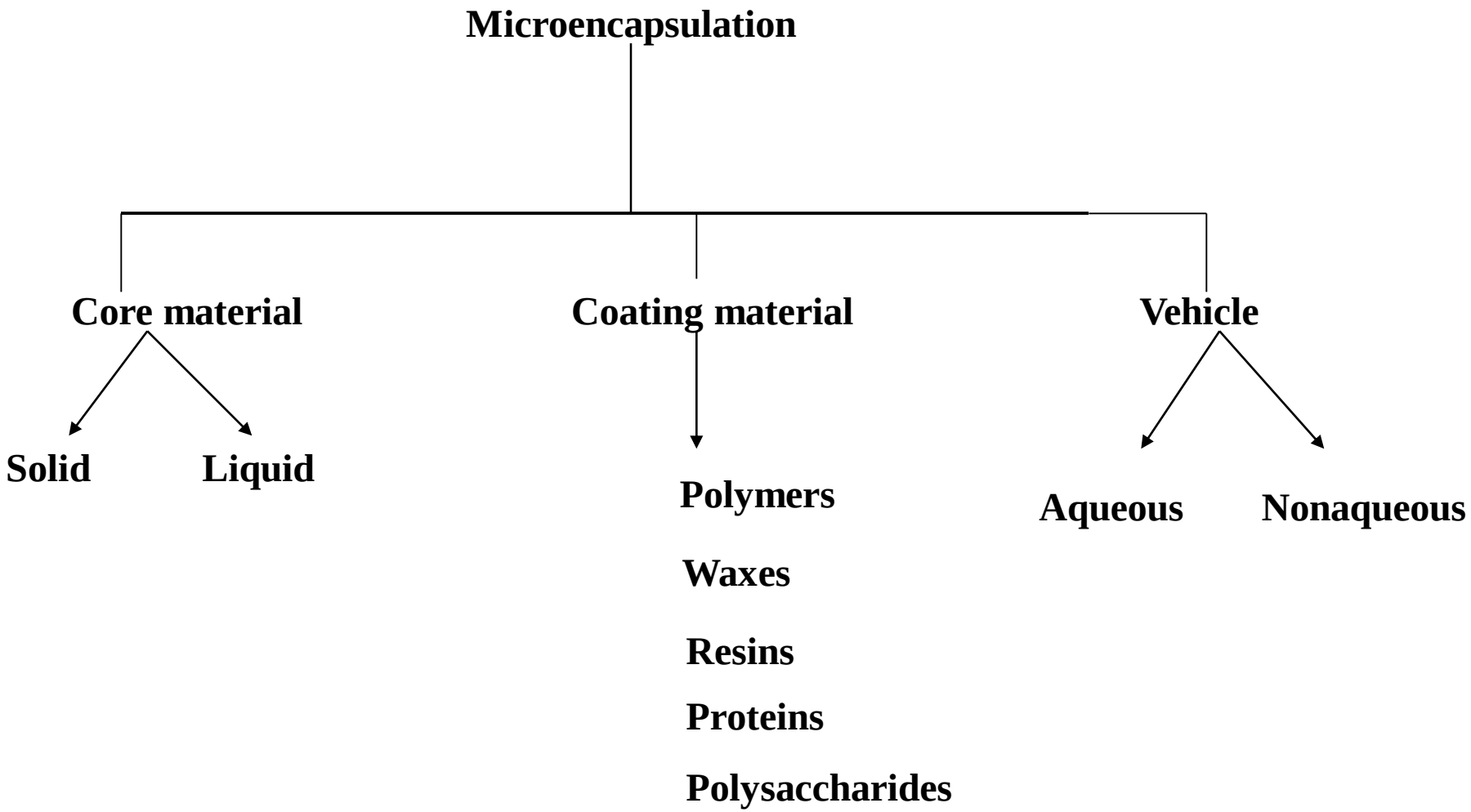
2.Microspheres: The active agent is dispersed or dissolved in an inert polymer.


Microcapsule
Mononuclear
Polynuclear
Matrix

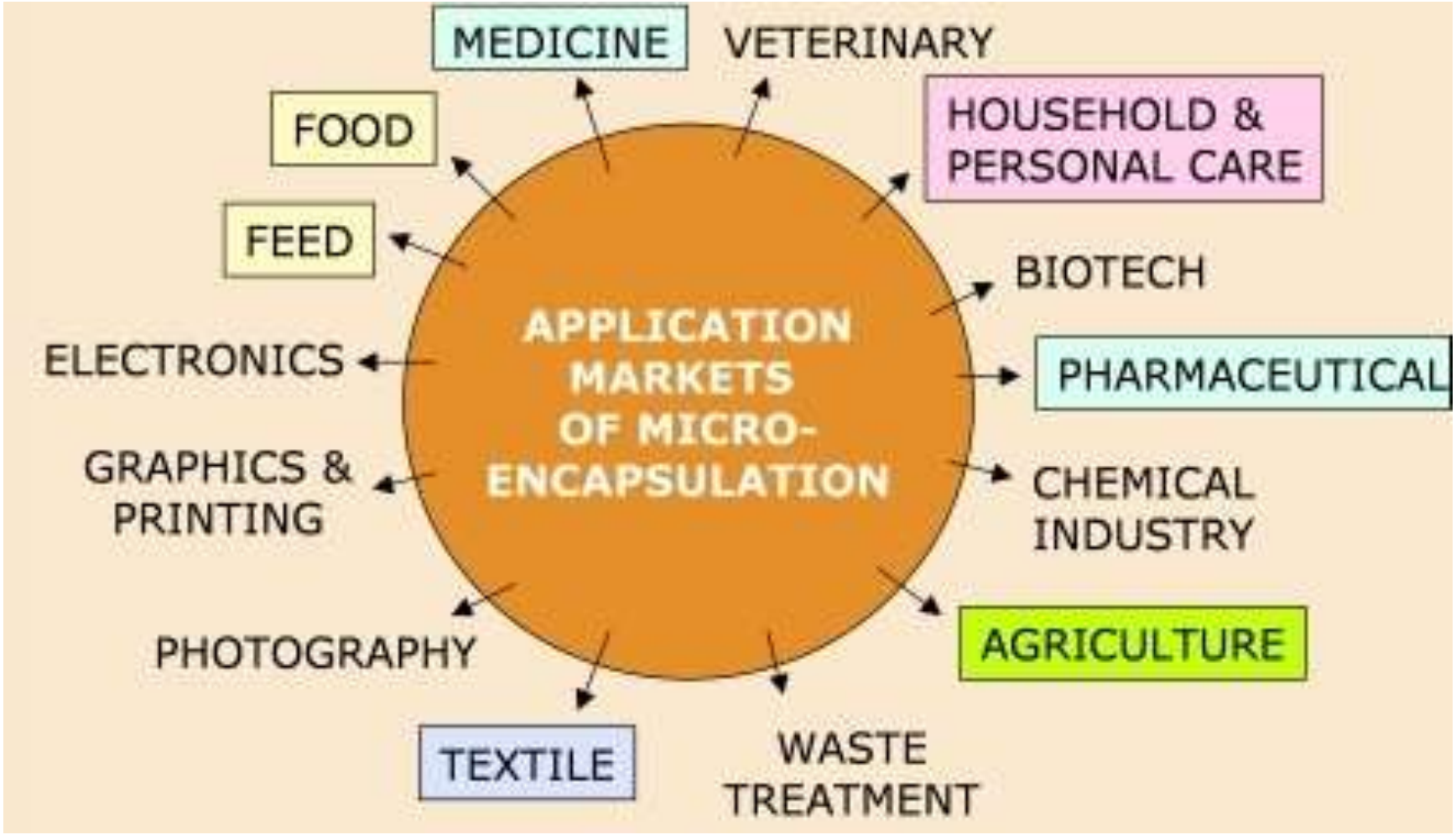
ADVANTAGES:

- ✓ To Increase of bioavailability
- ✓ To alter the drug release
- ✓ To improve the patient’s compliance
- ✓ To produce a targeted drug delivery
- ✓ To reduce the reactivity of the core in relation to the outside environment
- ✓ To decrease evaporation rate of the core material.
- ✓ To convert liquid to solid form & To mask the core taste.

FUNDAMENTAL CONSIDERATION:



APPLICATION OF MICROENCAPSULATION TECHNIQUES:



Microencapsulation : Applications

Chemistry
Printing & recording
Carbonless paper,
Adhesives
Pigments and
Fillers Catalysts

Medicine & Pharmacy & vetinary
Control release, Taste masking
Vectorisation
Artificial organs
single dose treatment

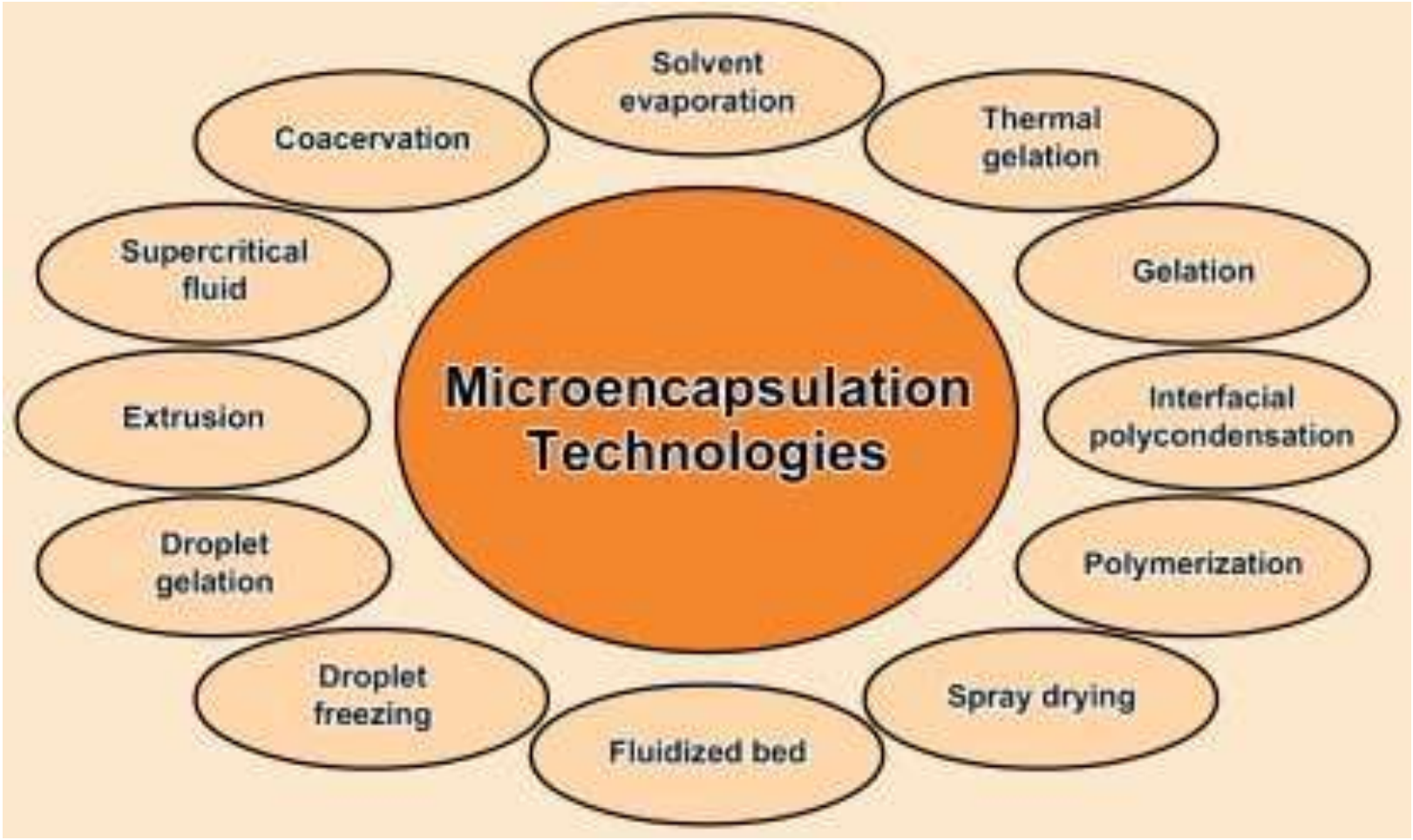
Food & feed
Aromas, Probiotics
Unsaturated oil,
Enzyme food processing
amino acid for cows

Agriculture
Fungicide – herbicide, Insect repellent, Biopesticide
Pigments and fillers
Artificial insemination

Biotechnology & environment
Continuous reactor,
Shear protection,
Reactor oxygenation

Consumer & diversified
Cosmetics,
detergents (enzymes),
sanitary (active, aromas)

MICROENCAPSULATION TECHNIQUES:



MICROENCAPSULATION TECHNIQUES:

1. Air suspension techniques(Wurster)
2. Coacervation process
3. Spray drying & congealing
4. Pan coating
5. Solvent evaporation
6. Polymerization
7. Extrusion
8. Single & double emulsion techniques
9. Supercritical fluid anti solvent method (SAS)
10. Nozzle vibration technology

Thank you…

*Unit: 1

Solubility Of Drug

Subject Teacher:
Dr. Prashant Khade

Phase Rule

1. The phase Rule is an important generalization dealing with the **behavior of heterogeneous systems**. In general the effect of changing pressure, temperature and concentration on a heterogeneous system in equilibrium.
2. This relationship governing heterogeneous equilibria was first discovered as early as **1874 by an American physicist Willard Gibbs.**

It may be stated mathematically as

$$F = C - P + 2$$

PHASE:

A phase may be defined as**: any homogeneous part of a system having all physical and chemical properties the same throughout.** A system may consist of one phase or more than one phases.

(1) A system containing only liquid water is **one-phase or 1-phase system *(P* = 1)**

(2) A system containing liquid water and water vapour (a gas) is a **two-phase or 2-phase system** (P=2).

(3) A system containing liquid water, water vapour and solid ice is a **three-phase or 3-phase system.**

A system consisting of one phase is called a **homogeneous system.**

A system consisting of two or more phases is called a **heterogeneous system.**

Examples:

1. **Pure substance:**
2. **Mixtures of gases.**
3. **Miscible liquids.**
4. **Non-miscible liquids.**
5. **Aqueous solutions.**
6. **Mixtures of solids.**

COMPONENTS:

A system ‘C’ in *the Phase Rule equation* stands for the number of components of a system in equilibrium.

The term component may be defined as : **the least number of independent chemical constituents in terms of which the composition of every phase can be expressed by means of a chemical equation.**

Examples:

1. **One Components:**
2. **Two Components.**
3. **Three Components.**

DEGREES OF FREEDOM:

The term Degree **of** Freedom represented by F in the phase Rule equation (F = C - P + 2) is defined as follows **: the least number of variable factors (concentration, pressure and temperature) which must be specified so that the remaining variables are fixed automatically and the system is completely defined**

A system with

F= 0 is known as **nonvariant** or having no degree of freedom.

F= 1 is known as **univariant** or having one degree of freedom.

F= 2 is known as **bivariant** or having two degrees of freedom.

F= 3 is known as **trivariant** or having two degrees of freedom.

Explanation of the Term Degree of Freedom

F= 1

1. For water and water vapour,

F= 2

1. Pure Gas,

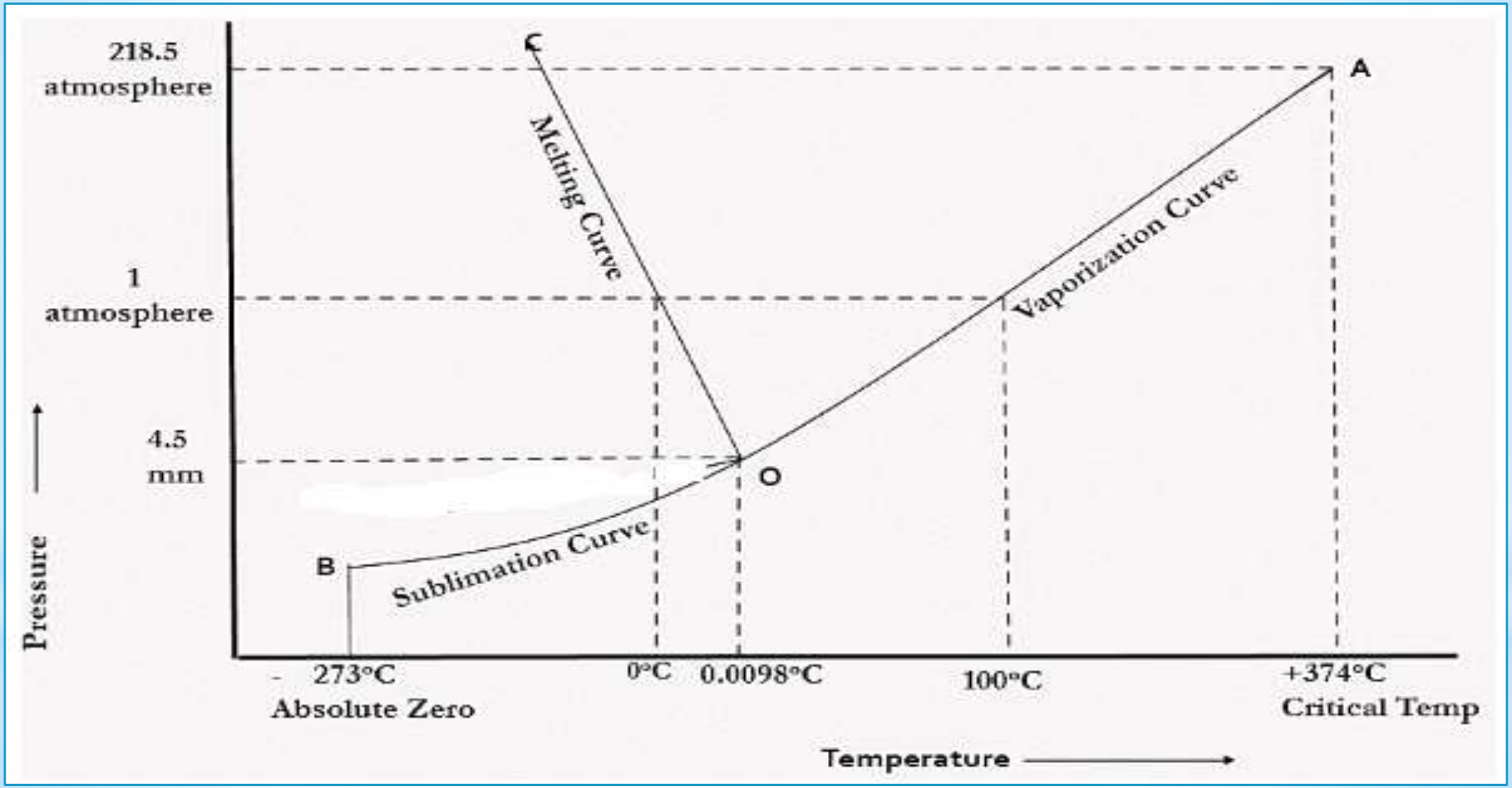
F= 3

1. Mixture of Gas,

F= 0

1. For water, ice and water vapour,

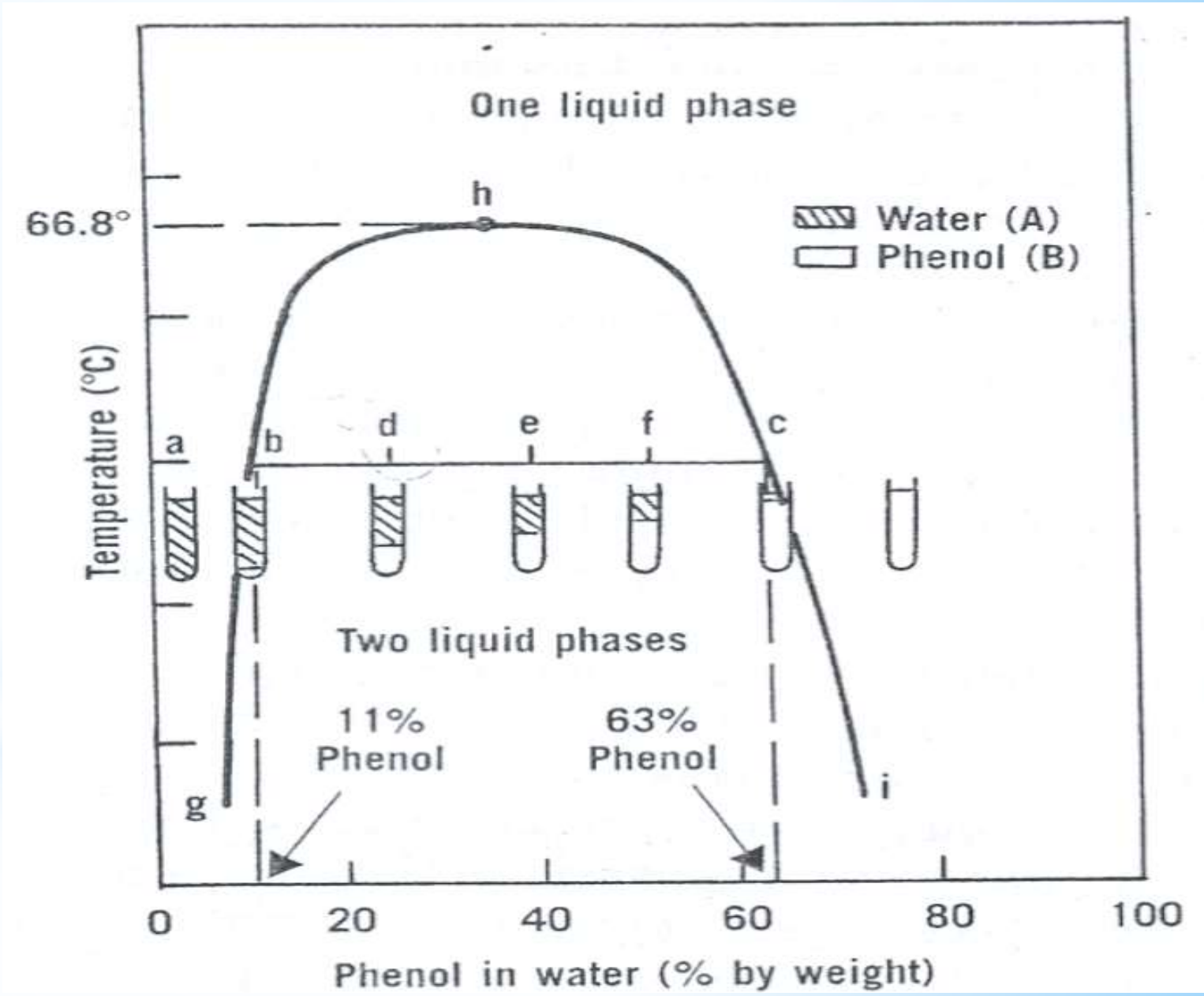
One Component System



https://www.youtube.com/watch?v=si95eubFZdg

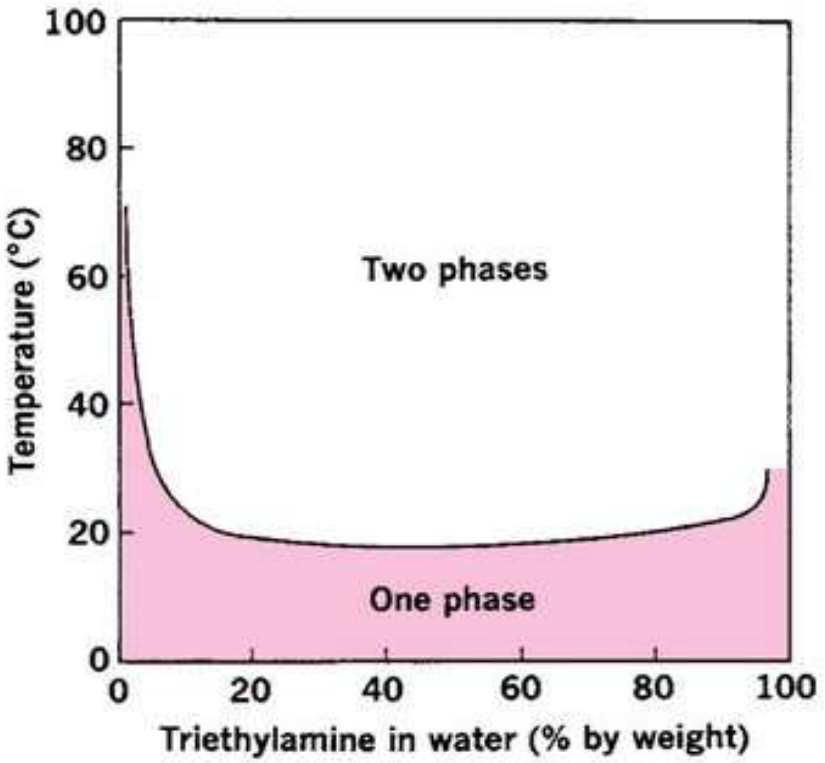
Two Component System

Phenol and water system

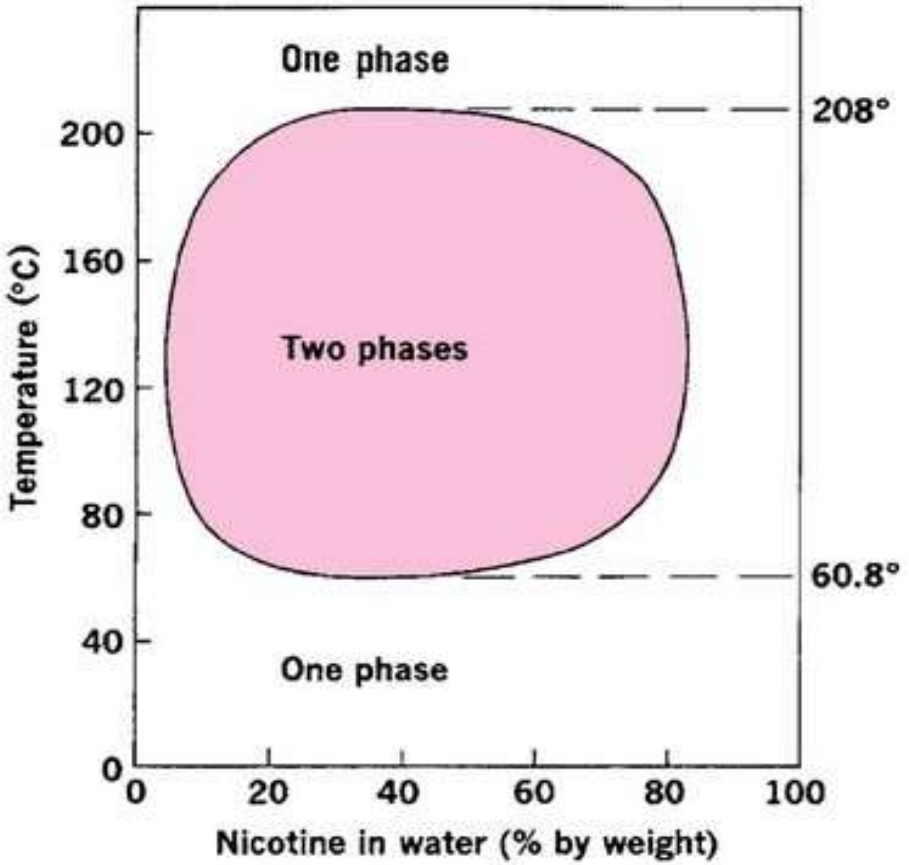


Two Component System

Water vs Triethylamine



Water vs Nicotine



SOLUBILITY OF SOLIDS IN LIQUIDS

Ideal Solutions:

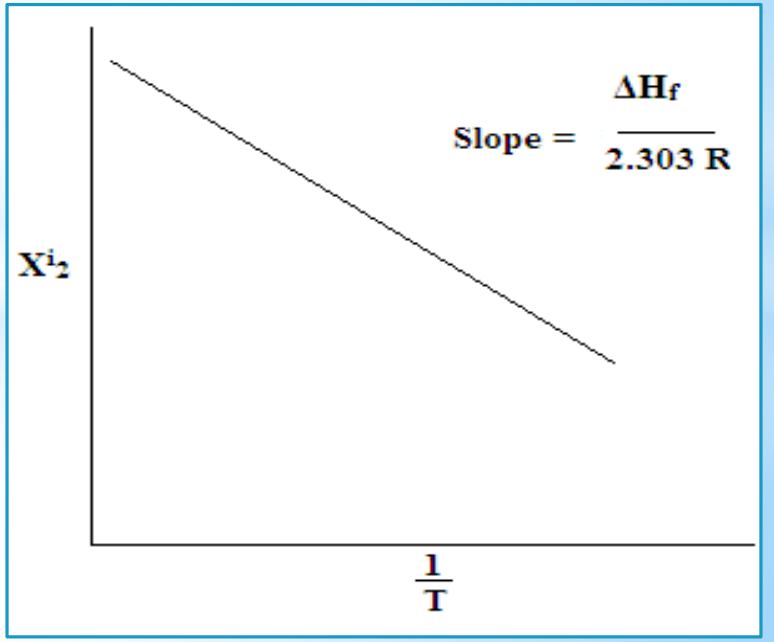
In an ideal solution,

1. The heat of solution is equal to the heat of fusion, ($\Delta H_s = \Delta H_f$), which is assumed to be a constant independent of the temperature.
2. Ideal solubility is **not affected by the nature of the solvent**.
3. In ideal solution, **force of attraction uniform**.

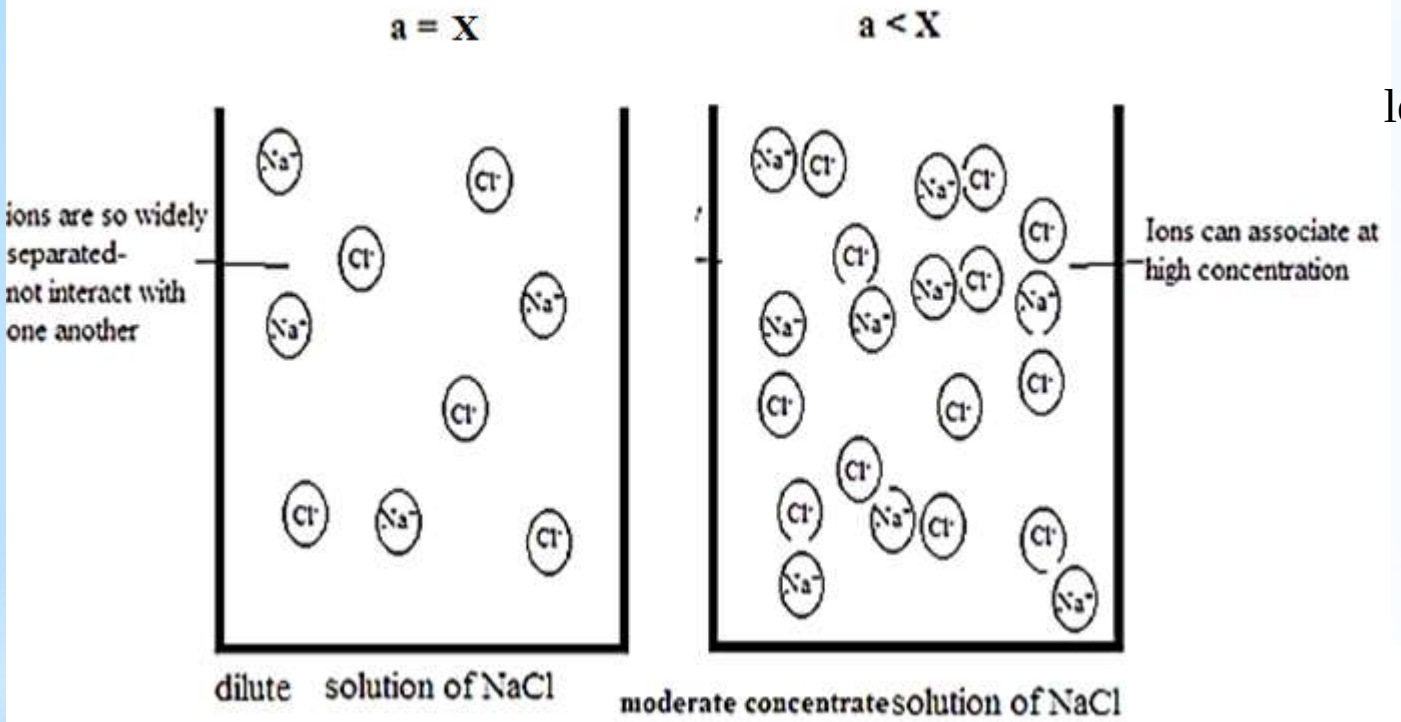
$$-\log X^i_2 = \frac{\Delta H_f}{2.303\ R}\left(\frac{T_0 - T}{T_0\ T}\right)$$

Equation no longer applies when
$T > T_0$;

Equation is adequate, when
T considerably below the melting point T_0



Non-ideal Solutions:



$$a_2 = X_2 \gamma_2$$

$$\log a_2 = \log X_2 + \log \gamma_2 \quad \text{or} \quad -\log X_2 = -\log a_2 + \log \gamma_2$$

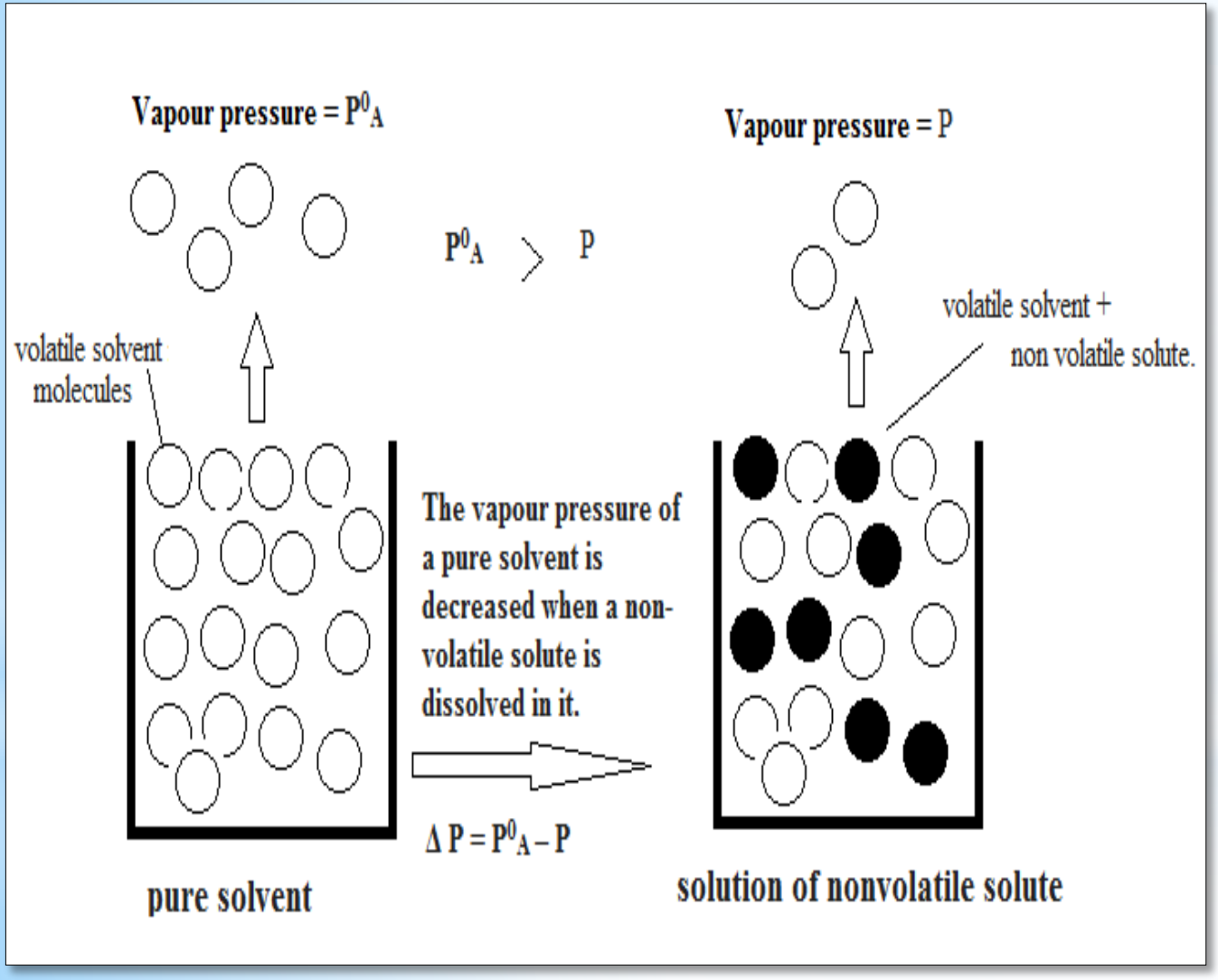
In an ideal solution, $a_2 = X_2^i$

$$-\log a_2 = -\log X_2^i = \frac{\Delta H_f}{2.303\ R}\left(\frac{T_0 - T}{T_0\ T}\right)$$

$$-\log X_2 = \frac{\Delta H_f}{2.303\ R}\left(\frac{T_0 - T}{T_0\ T}\right) + \log \gamma_2$$

COLLIGATIVE PROPERTIES:

Lowering of Vapor pressure:



$$\Delta P = P^0_A - P$$

$$P = P_A + P_B$$

But solute is non-volatile in nature, therefore,

$$P_B = 0$$

$$P = P_A$$

But according to Raoult's Law

$$P_A = P^0_A X_A$$

$$P = P^0_A X_A$$

$$\Delta P = P^0_A - P^0_A X_A$$

$$\Delta P = P^0_A (1 - X_A)$$

Where, $X_A + X_B = 1$ therefore, $X^B = 1 - X_A$

$$\Delta P = P^0_A X_B$$

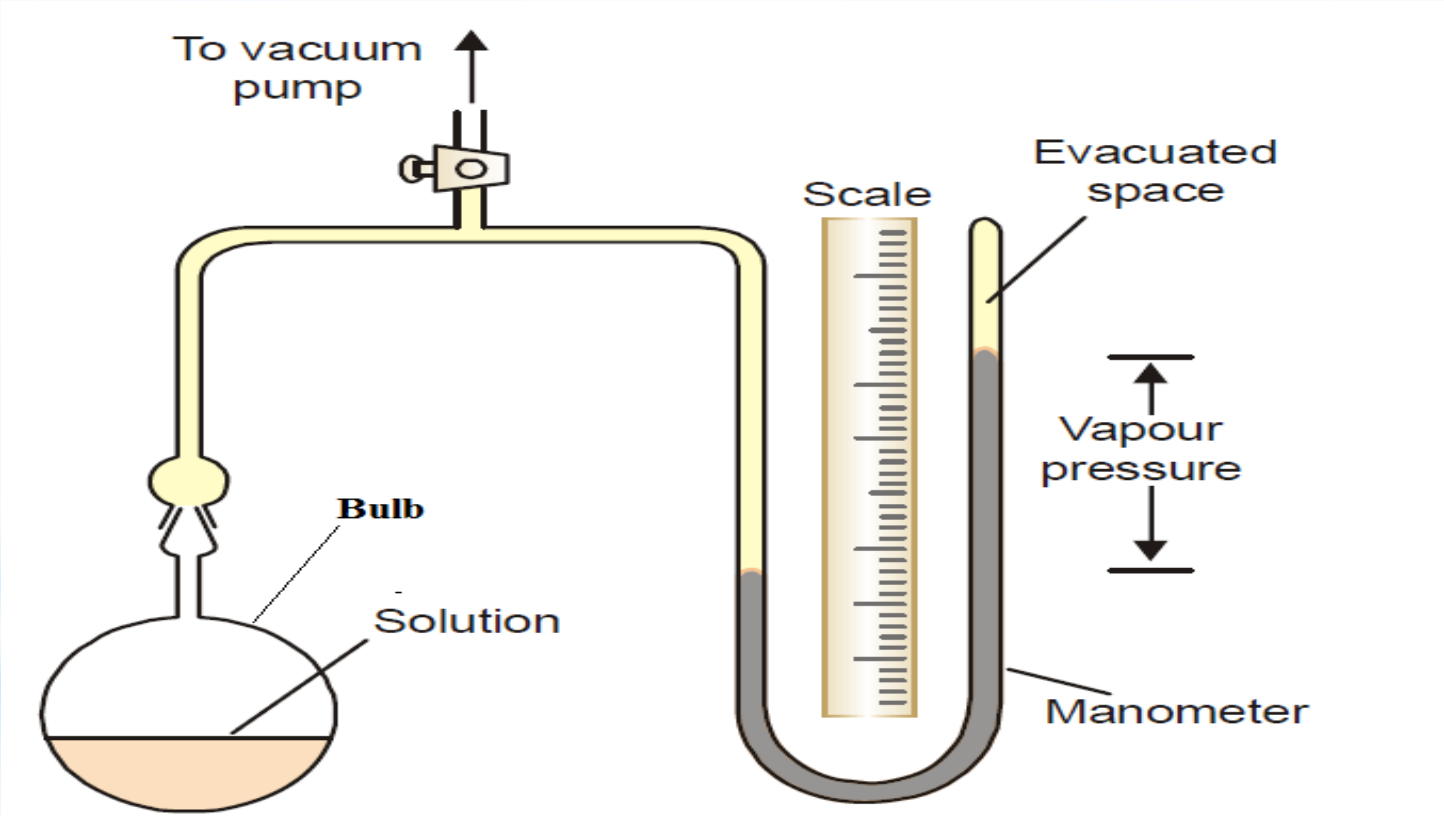
$$\frac{P^0_A - P}{P^0_A} = X_B$$

$$\frac{P^0_A - P}{P^0_A} = \frac{n_B}{n_A} = \frac{W_B / M_B}{W_A / M_A} = \frac{W_B M_A}{W_A M_B}$$

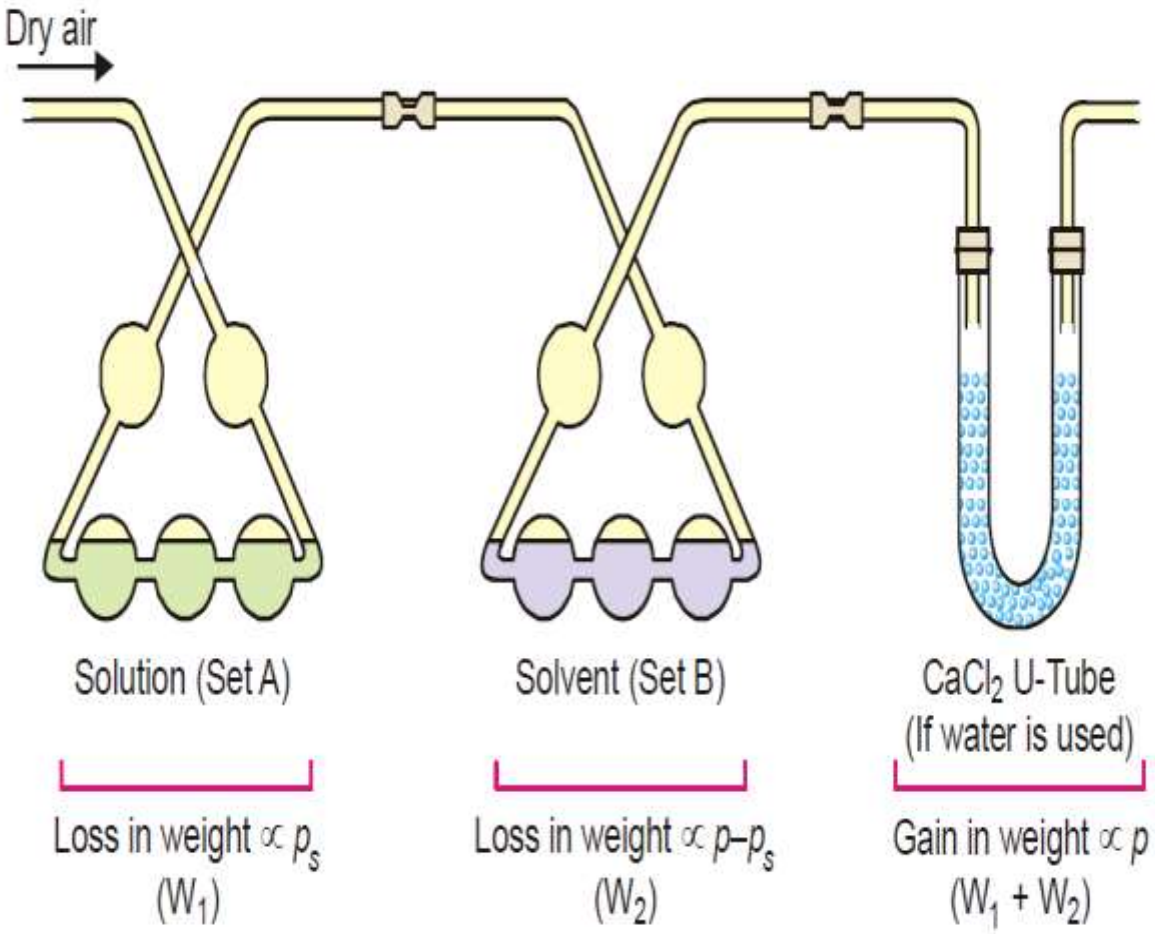
Measurement Of Lowering Of Vapour Pressure

Manometric Method

$$\frac{P_A^0 - P}{P_A^0} = \frac{n_B}{n_A} = \frac{W_B / M_B}{W_A / M_A} = \frac{W_B M_A}{W_A M_B}$$



Ostwald and Walker's Dynamic Method (Gas Saturation Method)



$$w_1 \alpha p_s$$
$$w_2 \alpha p - p_s$$

$$w1 + w2 \alpha ps + p - ps$$

$$w1 + w2 \alpha p$$

$$\frac{p - p_s}{p} = \frac{w_2}{w_1 + w_2}$$

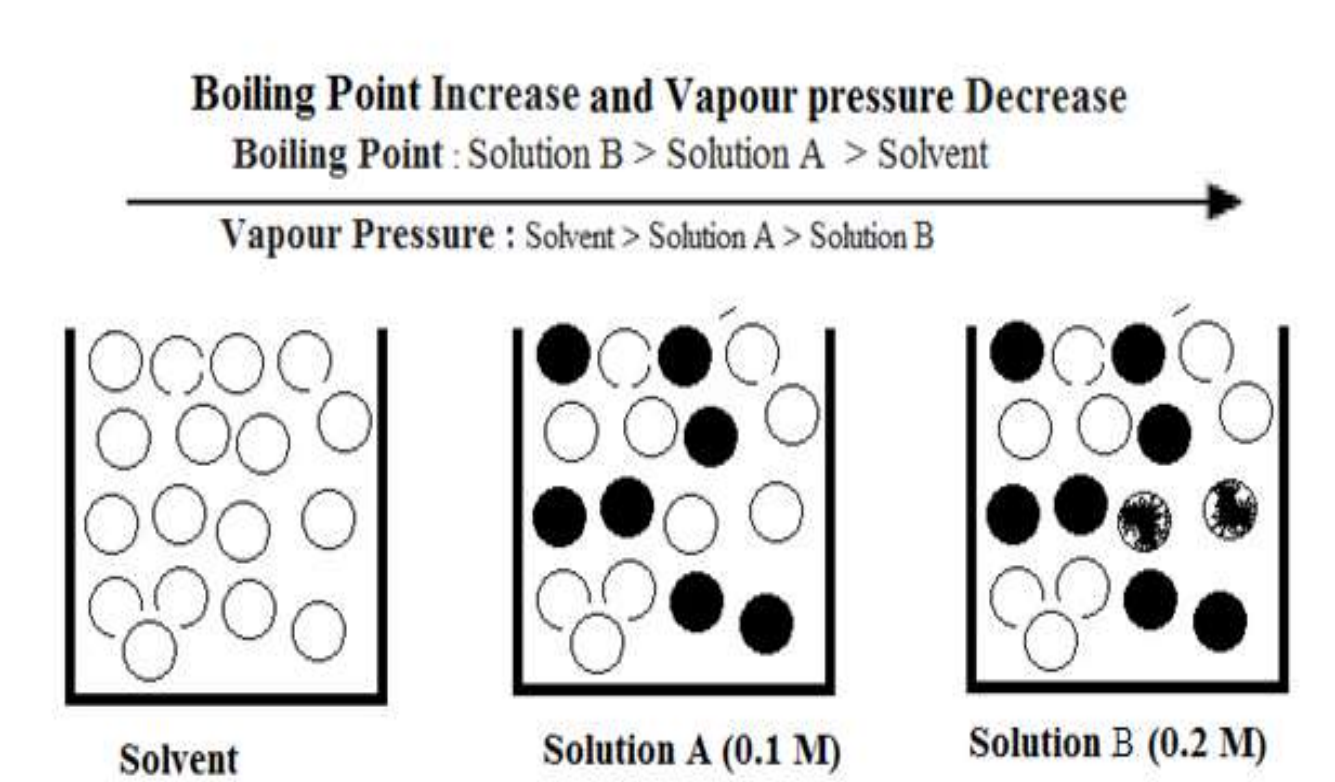
Solved Problem :

The vapour pressure of ether (mol mass = 74) is 442 mm Hg at 293 K. If 3g of a compound B are dissolved in 50 g of ether at this temperature, the vapour pressure falls to 426 mm Hg. Calculate the molecular mass of B. Assume that the solution of A in ether is very dilute.

BOILING POINT ELEVATION

Boiling point:

- Boiling point is defined as the temperature at which the vapour pressure of liquid becomes equal to the atmospheric pressure.
- Boiling point is a characteristic property of liquids and to check the purity of liquid. Liquids having greater intermolecular forces have high boiling points.



If

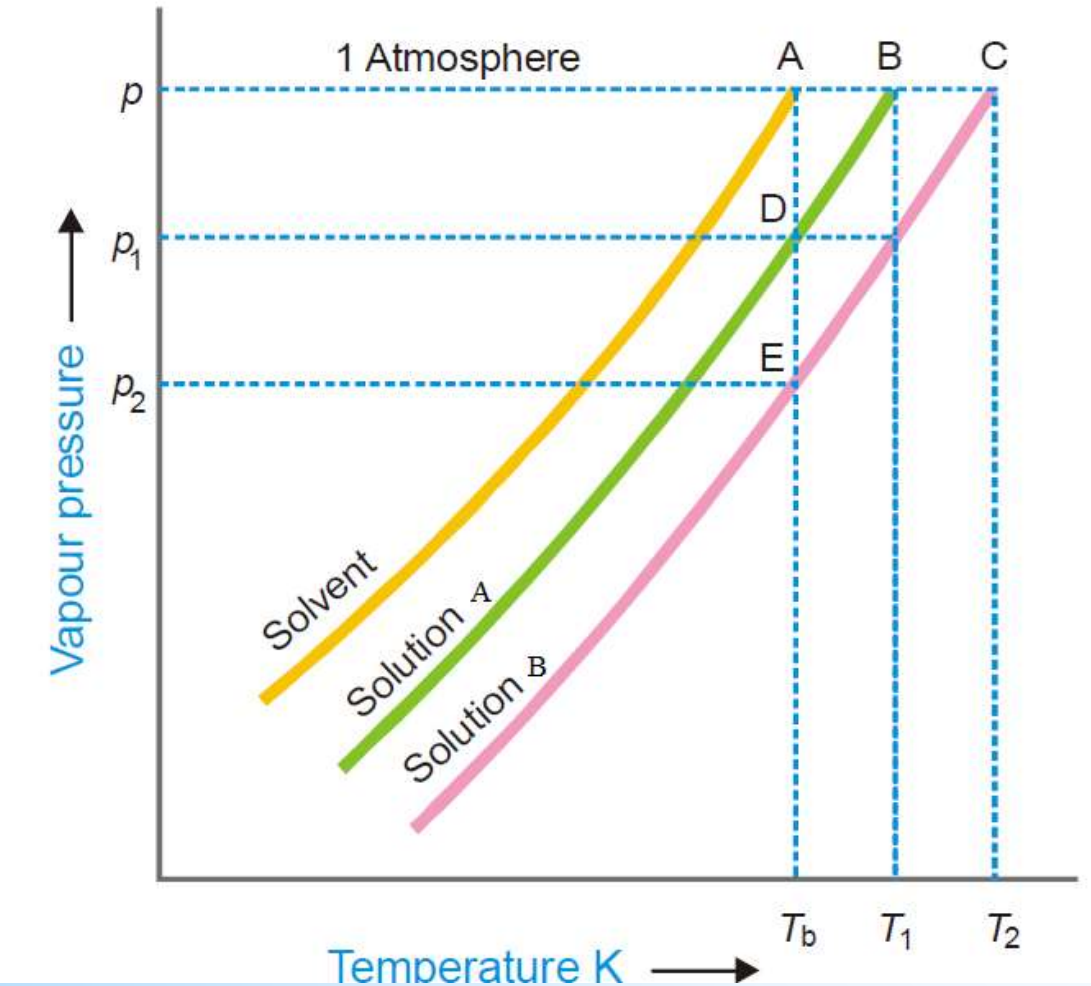
T_b is the boiling point of the solvent

T is the boiling point of the solution,

The difference in the boiling points (ΔT) is called the

elevation of boiling point

$$\Delta T = T - T_b$$



Therefore for similar triangles, ACE and ABD, we have

$$\frac{AB}{AC} = \frac{AD}{AE}$$

$$\frac{T_1 - T_b}{T_2 - T_b} = \frac{p - p_1}{p - p_2}$$

---------- **4.31**

Hence the elevation of boiling point is directly proportional to the lowering of vapour pressure.

Therefore,

$$\Delta T \alpha p - p_s \qquad \text{---------- 4.32}$$

Where,

p is vapour pressure of pure solvent.

p_s is vapour pressure of solution.

Since p is constant for the same solvent at a fixed temperature, form equation (4.32) can write,

$\Delta T \alpha p - p_s / p$ …… 4.33

$$p - p_s \ / \ p = X_B$$

Therefore, equation 4.33 become

$$\Delta T \ \alpha \ X_B \qquad \text{----------} \quad \text{----- 4.34}$$

$$\Delta T = AX_B \qquad \text{---------- 4.35}$$

Where,

A: proportionality constant

X_B : Mole fraction of solute

4.9.2.1. Determination of molecular weight:

Equation (4.35)

$$\Delta T = AX_B$$

Again from equation (4.23)

$$X_B = n_B / n_A - \frac{W_B / M_B}{W_A / M_A + W_B / M_B}$$

For dilute solutions n_A » n_B. Hence n_B may be neglected in comparison with n_A,

$$X_B = \frac{n_B}{n_A} = \frac{W_B / M_B}{W_A / M_A} = \frac{W_B M_A}{W_A M_B} \qquad \text{----4.36}$$

equation (4.36) substitute in equation (4.35)

$$\Delta T = A \frac{W_B M_A}{W_A M_B} \qquad \text{---------- 4.37}$$

Since M_A is constant for solvent, both constant, A and M_A are consider single constant, K_b,

$$\Delta T = K_b \frac{W_B}{M_B} \frac{1}{W_A} \qquad \text{---------- 4.38}$$

where, K_b is a constant called **Boiling point constant or Ebulioscopic constant of molal elevation constant.**

If $W_B / M_B = 1$, $W_A = 1$ $K_b = \Delta T$

Thus,

Molal elevation constant may be defined as the boiling-point elevation produced when 1 mole of solute is dissolved in one kg (1000 g) of the solvent.

If the mass of the solvent (W_A) is given in grams, it has to be converted into kilograms. Thus the expression (26) assumes the form

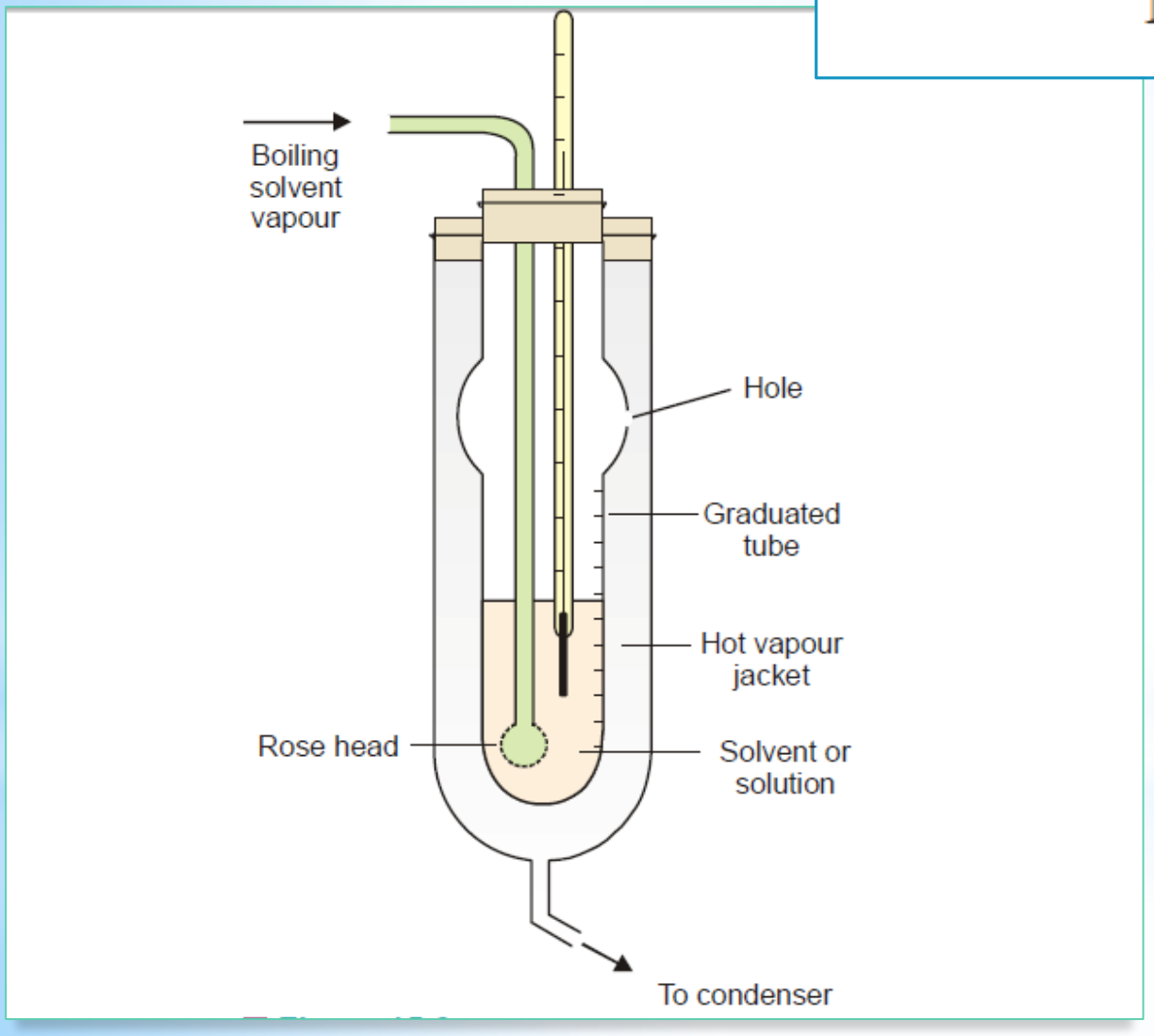
$$\Delta T = K_b \frac{W_B}{M_B} \frac{1}{W_A} 1000 \qquad \text{----------4.39}$$

The quantity of $\frac{W_B}{M_B} \frac{1}{W_A} 1000$ is known as the molality of solution.

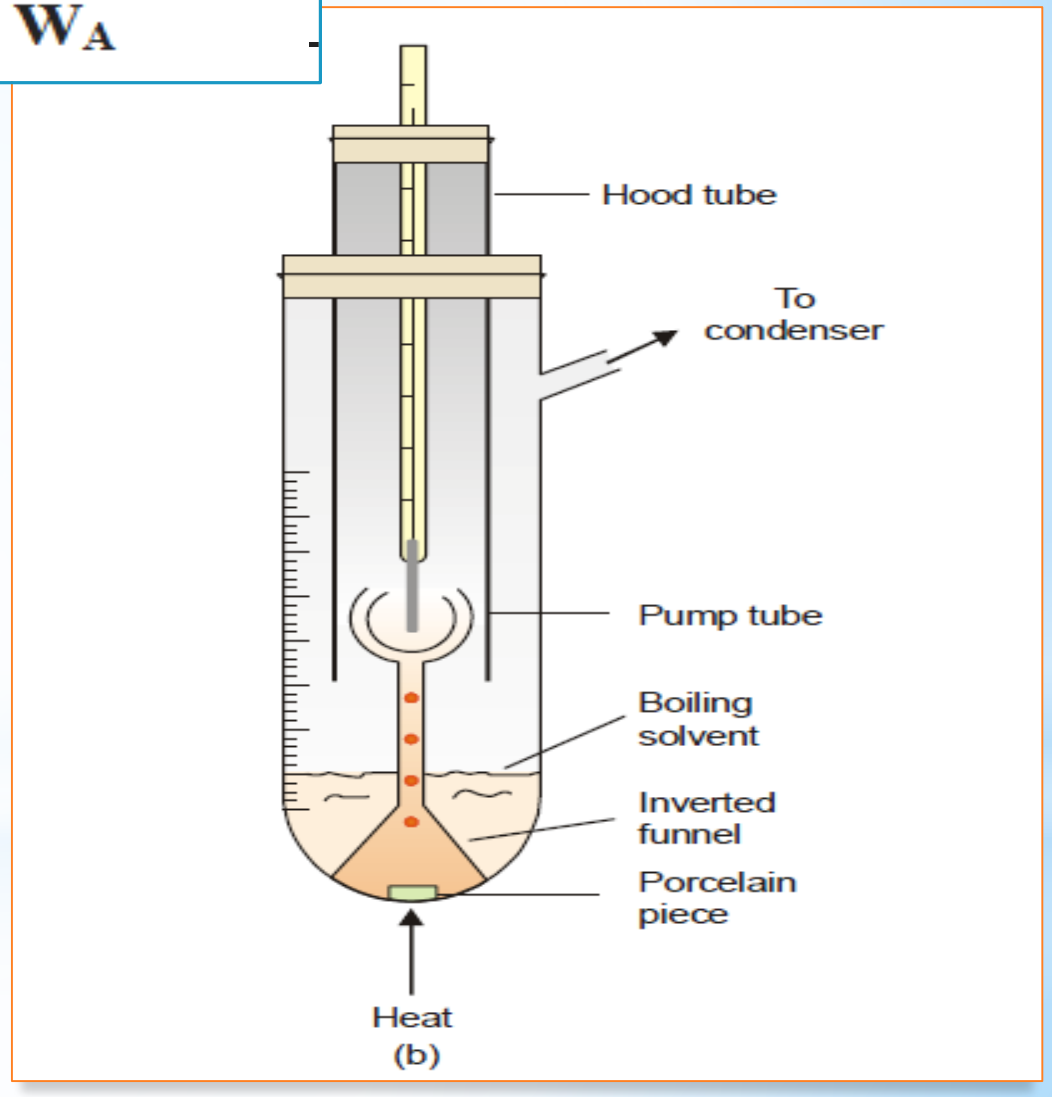
MEASUREMENT OF BOILING-POINT ELEVATION

$$\Delta T = K_b \frac{W_B}{M_B} \frac{1}{W_A} 1000$$

(1) Landsberger-Walker Method



(2) Cottrell's Method



Solved problem 1:

The boiling point of a solution containing 0.20 g of a substance X in 20.00 g of ether is 0.17 K higher than that of pure ether. Calculate the molecular mass of X. Boiling point constant of ether per 1 Kg is 2.16 K.

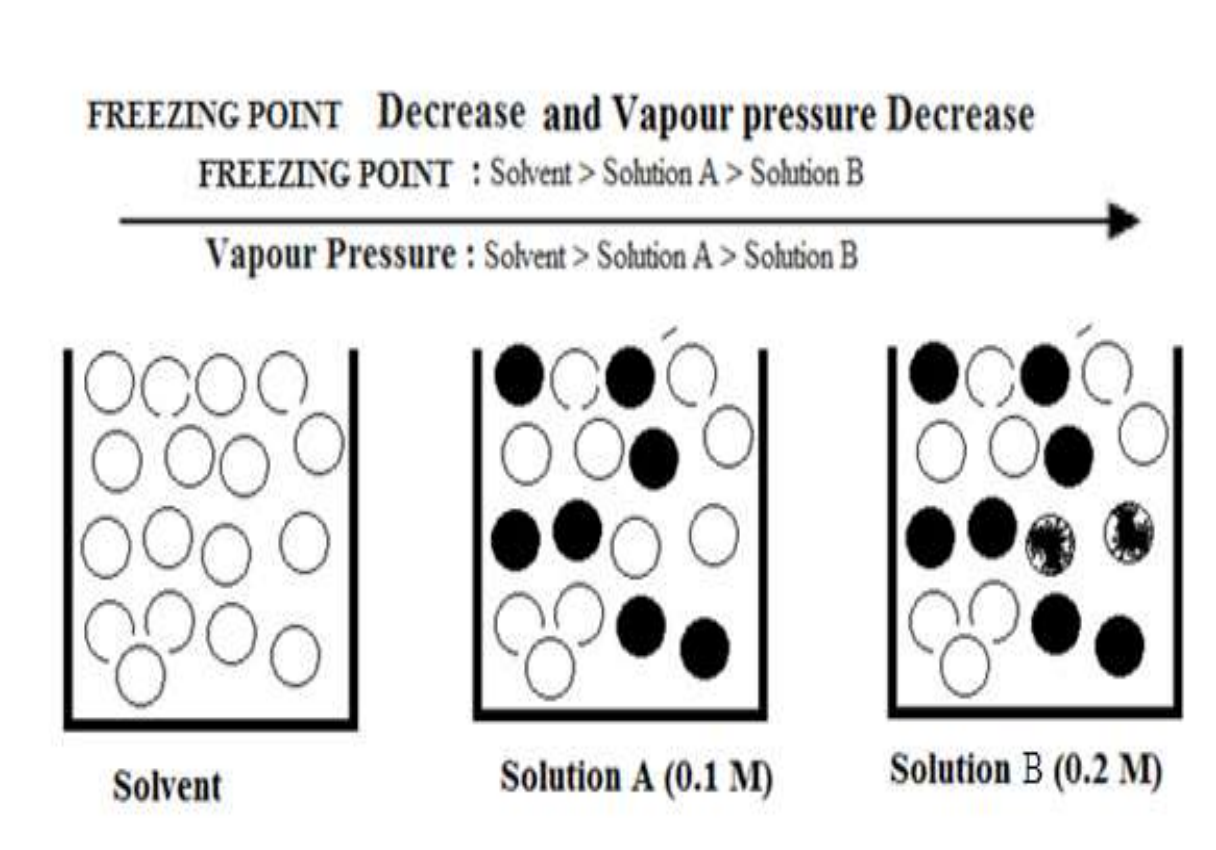
Solved problem 2:

Acetone boils at 56.38°C and a solution of 1.41 grams of an organic solid in 20 grams of acetone boils at 56.88°C. If K for acetone per 100 g is 16.7. Calculate the mass of one mole of the organic solid.

FREEZING POINT DEPRESSION

Freezing point:

- A liquid freezes or solidifies at a temperature at which the liquid and its solid coexist in equilibrium at 1 atmosphere.
- Such a condition is obtained when the vapour pressures of solid and liquid are equal.



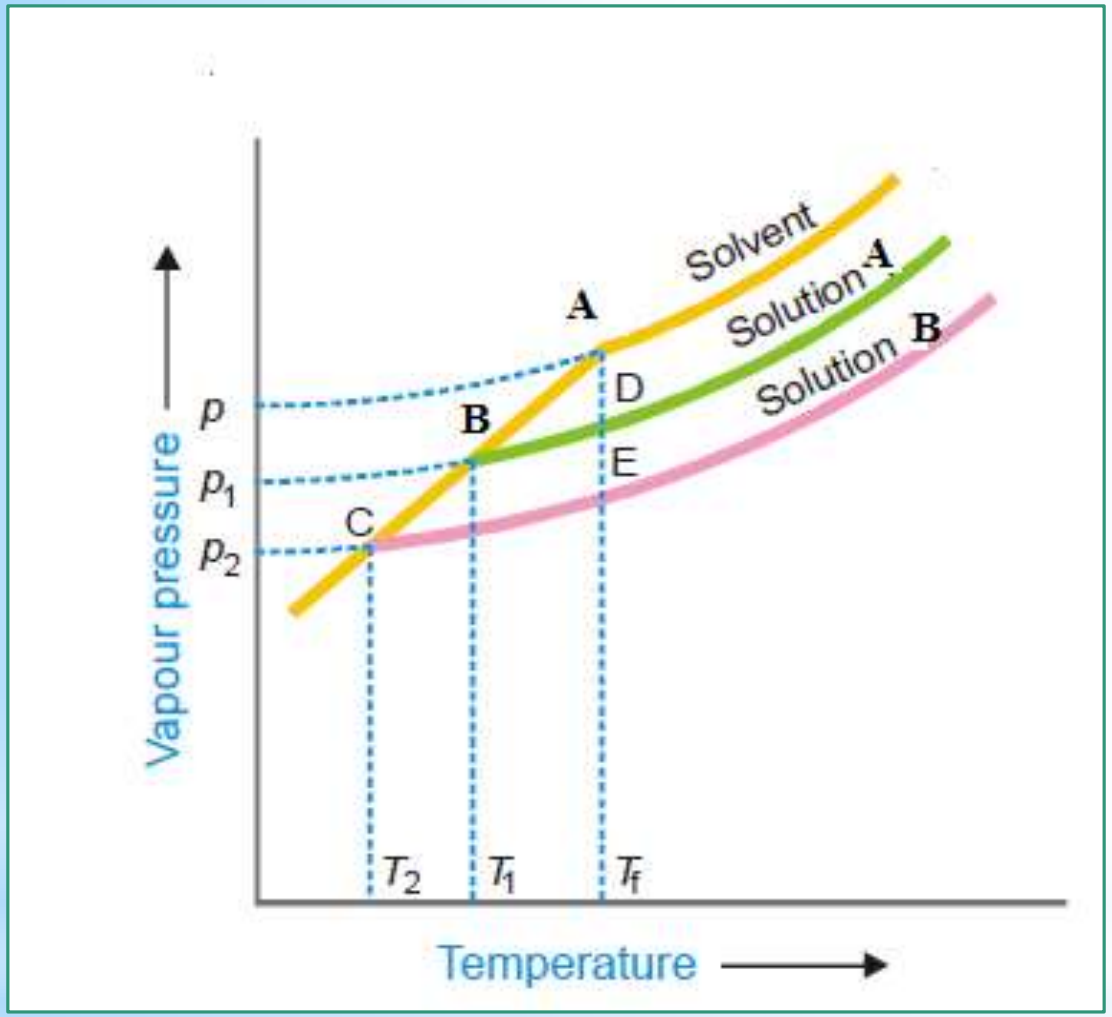
If,

T_f is freezing point of pure solvent

T is freezing point of solution.

The freezing point depression is (ΔT)

$\Delta T = T_f - T$



Therefore for similar triangles, ACE and ABD, we have

$$\frac{AB}{AC} = \frac{AD}{AE}$$

$$\frac{T_f - T_1}{T_f - T_2} = \frac{p - p_1}{p - p_2} \qquad \text{---------- 4.41}$$

Hence the depression of freezing point is directly proportional to the lowering of vapour pressure.

Therefore,

$$\Delta T_f \alpha\ p - p_s \qquad \text{---------- 4.42}$$

Where,

p is vapour pressure of pure solvent.

p_s is vapour pressure of solution.

Since p is constant for the same solvent at a fixed temperature, form equation (4.42) can write,

$$\Delta T_f \alpha\ p - p_s\ /\ p \qquad \text{..... 4.43}$$

This equation indicates that depression of freezing point is directly proportional to the relative lowering of vapor pressure in dilute solution.

According to equation (4.22)

$$p - p_s\ /\ p = X_B$$

substitute equation 4.22 in equation 4.43,

$$\Delta T_f \alpha X_B \quad \text{----------}$$

---------- 4.44

$$\Delta T_f = AX_B \quad \text{---------- 4.45}$$

Where,

A: proportionality constant

X_B : Mole fraction of solute

4.9.3.1. Determination of molecular weight:

$$\Delta T_f = AX_B$$

according to equation (4.23) X_B is,

$X_B = n_B / n_A$ - $\frac{W_B / M_B}{W_A / M_A + W_B / M_B}$

For dilute solutions n_A » n_B. Hence n_B may be neglected in comparison with n_A,

$$X_B = \frac{n_B}{n_A} = \frac{W_B / M_B}{W_A / M_A} = \frac{W_B M_A}{W_A M_B} \quad \text{……4.46}$$

equation (4.46) substitute in equation (4.45)

$$\Delta T_f = A \frac{W_B M_A}{W_A M_B} \quad \text{---------- 4.47}$$

Since M_A is constant for solvent, both constant, A and M_A are consider single constant, K_b,

$$\Delta T_f = K_f \frac{W_B}{M_B} \frac{1}{W_A} \quad \text{---------- 4.48}$$

where, K_f is a constant called **freezing point constant or molal depression constant.**

If $W_B / M_B = 1$, $W_A = 1$ $K_b = \Delta T$

Thus,

molal depression constant may be defined as the freezing point depression produced when 1 mole of solute is dissolved in one kg (1000 g) of the solvent.

If the mass of the solvent (W_A) is given in grams, it has to be converted into kilograms. Thus the expression (4.48) assumes the form

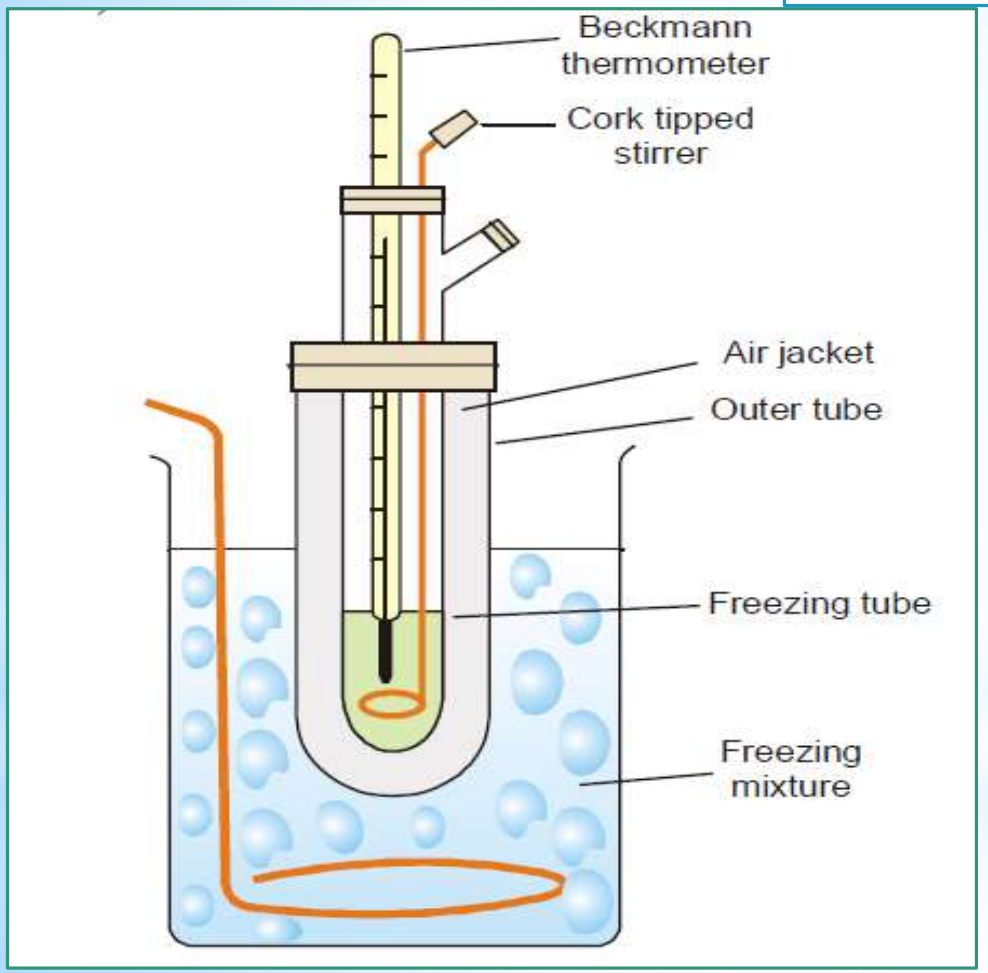
$$\Delta T_f = K_f \frac{W_B}{M_B} \frac{1}{W_A} 1000 \quad \text{----------4.49}$$

The quantity of $\frac{W_B}{M_B} \frac{1}{W_A} 1000$ is known as the molality of solution

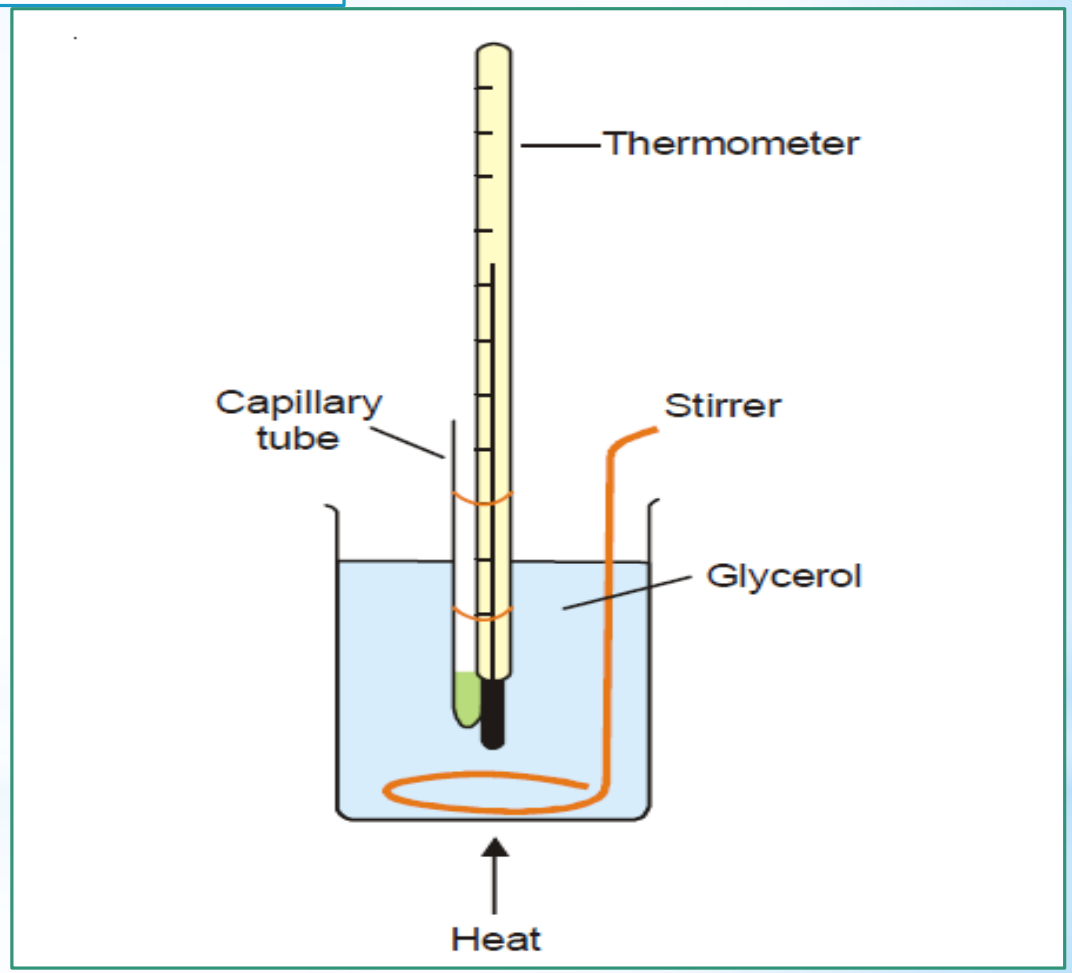
MEASUREMENT OF FREEZING-POINT DEPRESSION

(1) Beckmann's Method (1903)

$$\Delta T_f = K_f \frac{W_B}{M_B} \frac{1}{W_A} 1000$$



(2) Rast's Camphor Method



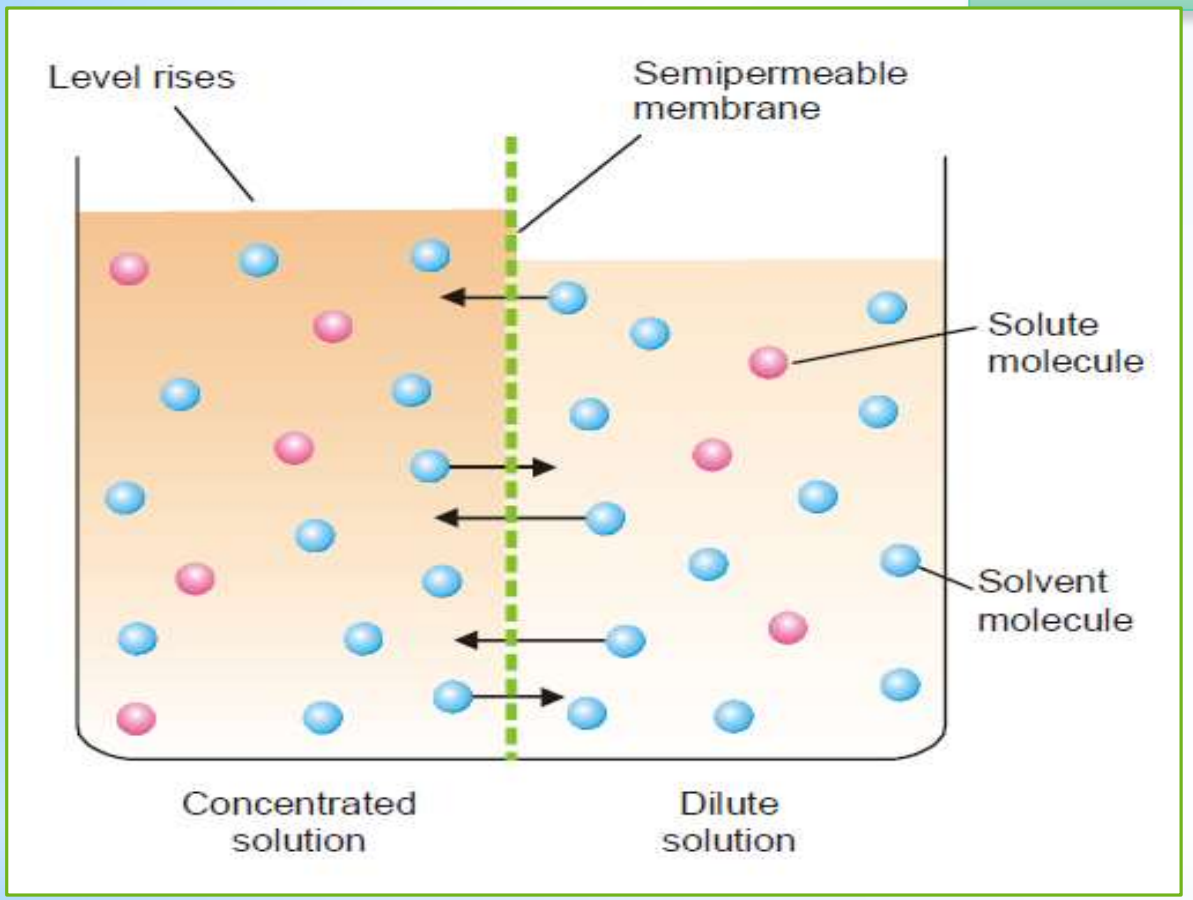
Solved problem 1:

0.440 g of a substance dissolved in 22.2 g of benzene lowered the freezing point of benzene by 0.567°C. Calculate the molecular mass of the substance. (K_f = 5.12°C mol $^{-}$1).

Solved problem 2:

1.250 g of naphthalene was dissolved in 60 cm3 of benzene and freezing point of the solution was found to be 277.515 K, while that of benzene 278.495 K. Density of benzene =0.880 g cm -3, Kf = 5.1 Kg per 1000 g benzene. Calculate the molecular mass of naphthalene.

Osmotic pressure:



Semipermeable membrane:

It is a membrane which allows the solvent molecules but not the solute molecules, to pass through it.

Eg. Cellulose, cellulose nitrate, animal bladder, skin round white of an egg, etc,

LAWS OF OSMOTIC PRESSURE

Boyle-Van't Hoff Law for Solutions

$$\pi \alpha C \quad \ldots\ldots\ldots 4.50$$

Where,

π: osmotic pressure

C: concentration in mol L^{-1}

if n mol of solute dissolved in V liters then,

$$\pi \alpha n/V \text{ or } \pi \alpha 1/V \text{ (if n is one)} \quad \ldots\ldots\ldots 4.51$$

This relation is similar to the Boyl's law for gas and is known as the Boyle-van't Hoff law.

Charles-Van't Hoff Law for Solutions:

The law is stated as, the concentration remaining constant; the osmotic pressure of a solution of a given concentration is directly proportional to the absolute temperature.

$$\pi \alpha T \quad \ldots\ldots\ldots 4.52$$

where;

T is absolute temperature.

This relation is similar to the Charles law for gas and is known as the Charles-van't Hoff Law.

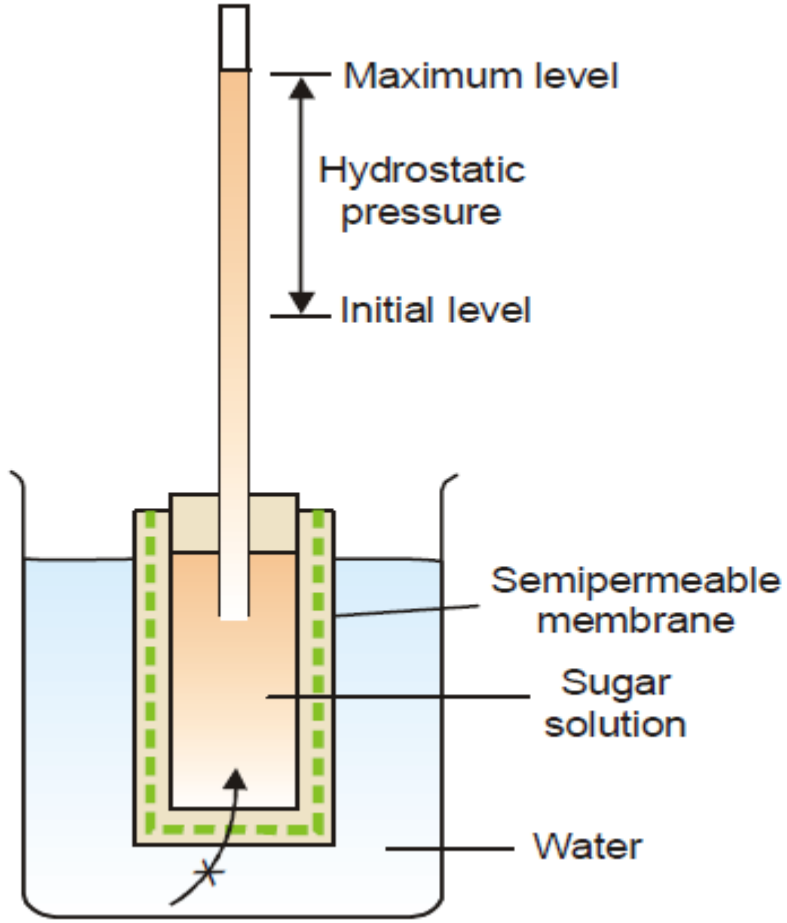
Combining Boyle-Van't Hoff law and Charles-Van't Hoff law deduced following equation

$$\pi \alpha \frac{nT}{V} \quad \ldots\ldots\ldots 4.53$$

$$\pi V \alpha nT \quad \ldots\ldots\ldots 4.54$$

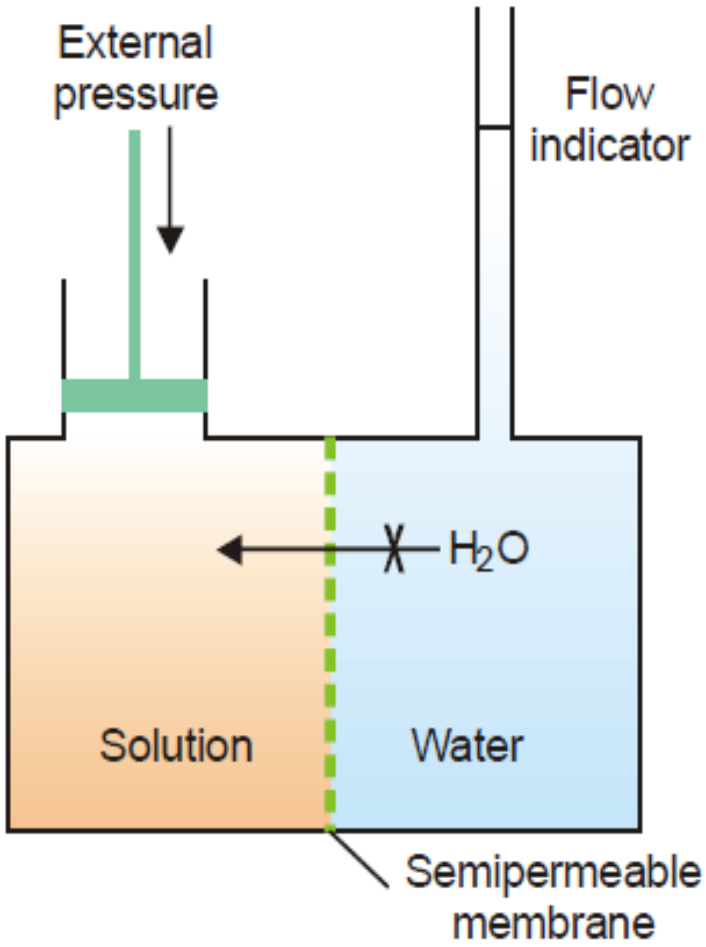
$$\pi V = nRT \quad \ldots\ldots\ldots 4.55$$

Osmotic pressure and Hydrostatic pressure:



The hydrostatic pressure built up or the solution which just stops the osmosis of pure solvent into the solution through a semipermeable membrane is called Osmotic Pressure.

Osmotic pressure and External pressure:



Osmotic pressure may be defined as the external pressure applied to the solution in order to stop the osmosis of solvent into solution separated by a semipermeable membrane

DETERMINATION OF MOLECULAR WEIGHT FROM OSMOTIC PRESSURE

DETERMINATION OF OSMOTIC PRESSURE

Berkeley and Hartley's Method

$\pi V = n RT$

but, $n = w/M$ 4.56

Where,

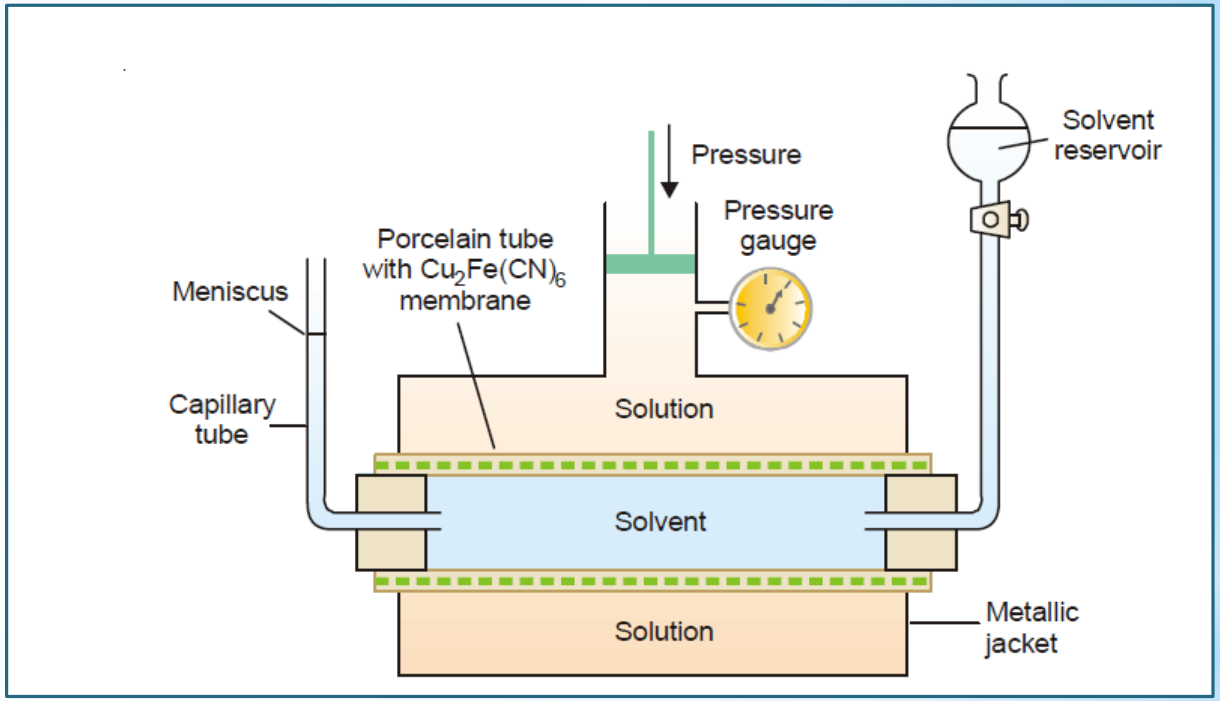
w is weight of solute in solution

M is molecular weight of solute.

Therefore equation 4.56 become

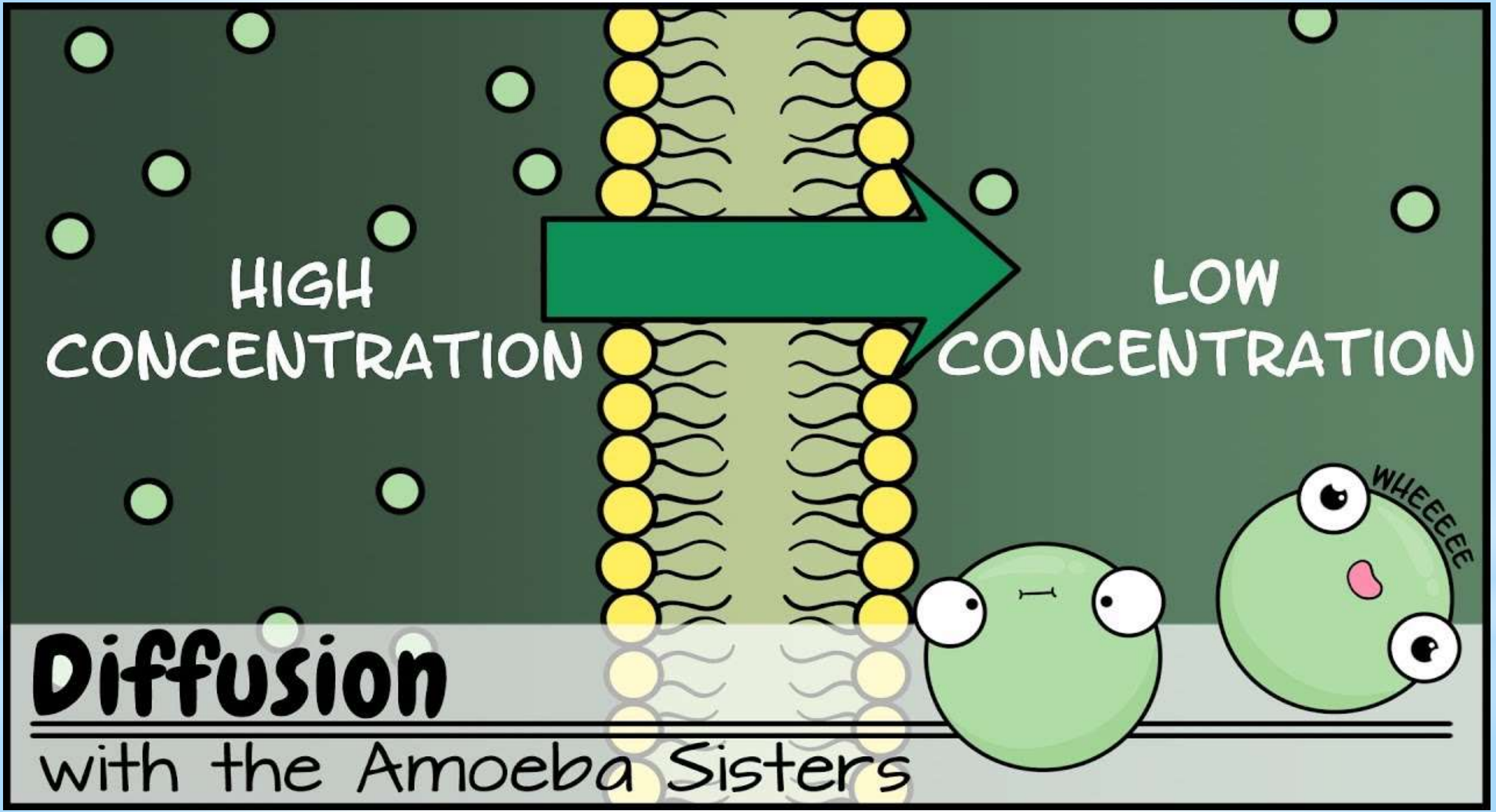
$\pi = wRT/MV$ 4.57

$M = wRT/\pi V$ 4.58



Solved problem:

1. Morse and Frazer observed that a solution of glucose containing 18 grams per liter had an osmotic pressure of 2.39 atmospheres at 23°C. Find the molecular weight of glucose.

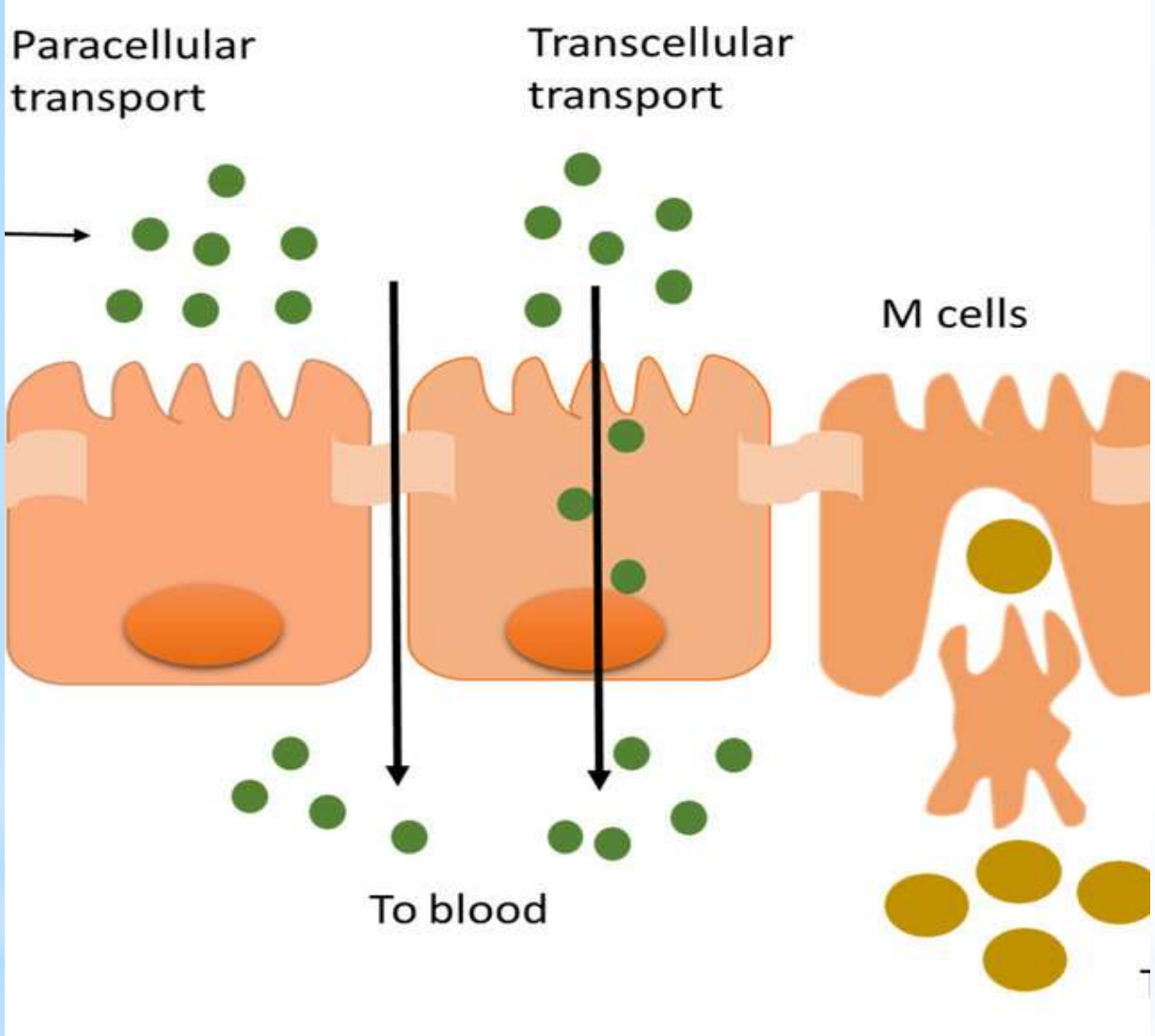

HIGH CONCENTRATION
LOW CONCENTRATION
WHEEEEEE
Diffusion
with the Amoeba Sisters

Diffusion

- Diffusion is a process of mass transfer of molecules of a substance from higher concentration to lower concentration.
- Driving force of diffusion is concentration gradient.
- This process allows every molecule of a substance having higher concentration.
- Semi permeable membrane is absent.

Osmosis

- Osmosis is a process of mass transfer of molecules of a substance from lower concentration to higher concentration through semi permeable membrane.
- Driving force of osmosis is osmotic pressure
- This process doesn't allows every molecule of a substance.
- Semi permeable membrane is present and has an orifice for drug release.



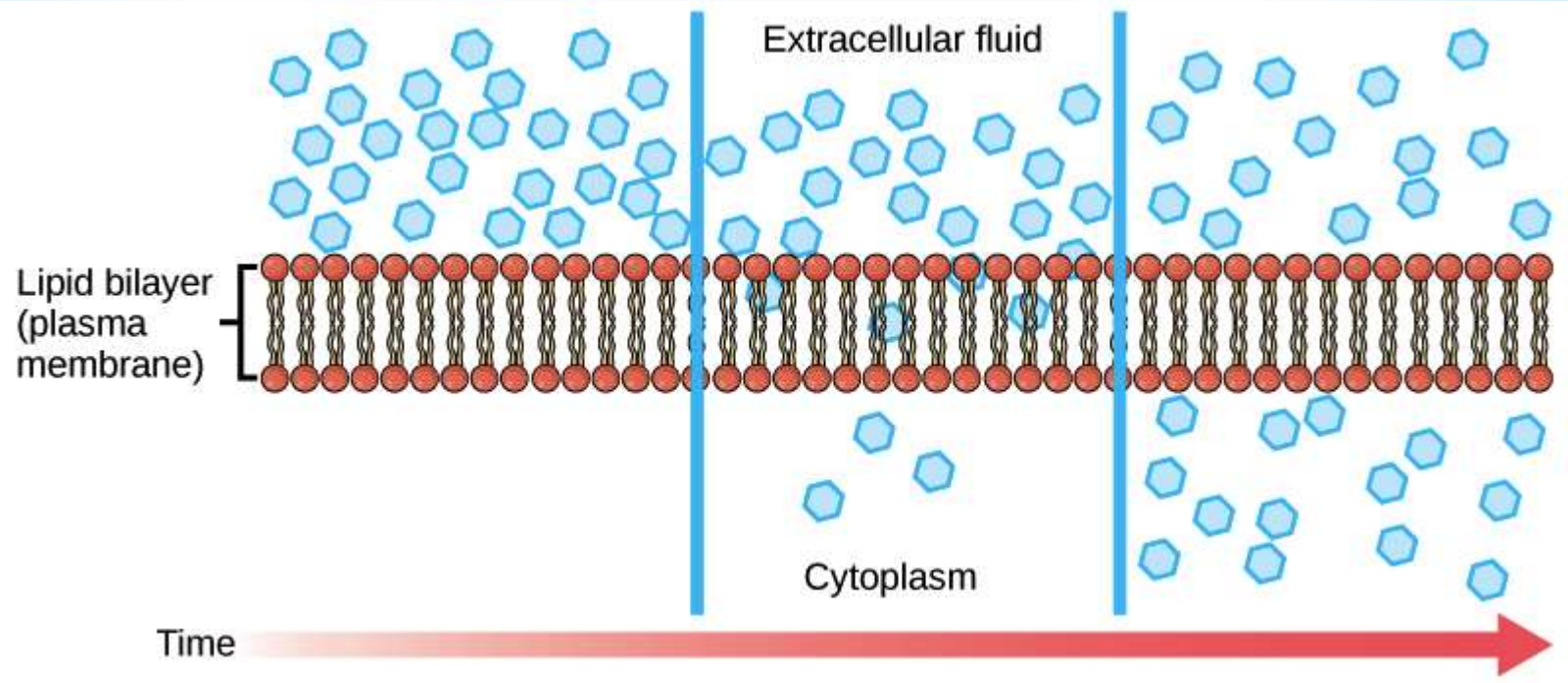
A. Passive Transport

1. **Passive Diffusion**
2. **Pore Transport**
3. **Ion Pair Transport**
4. **Facilitated diffusion**

B. Active Transport

C. Endocytosis

1. **Phagocytosis (Cell Eating- Solid)**
2. **Pinocytosis (Cell drinking- Fluid)**



Passive diffusion:

1. The process of spontaneous passive transport
2. Not directly require chemical energy from ATP
3. molecules and ions move down their **concentration gradient**

3. Ion Pair Transport

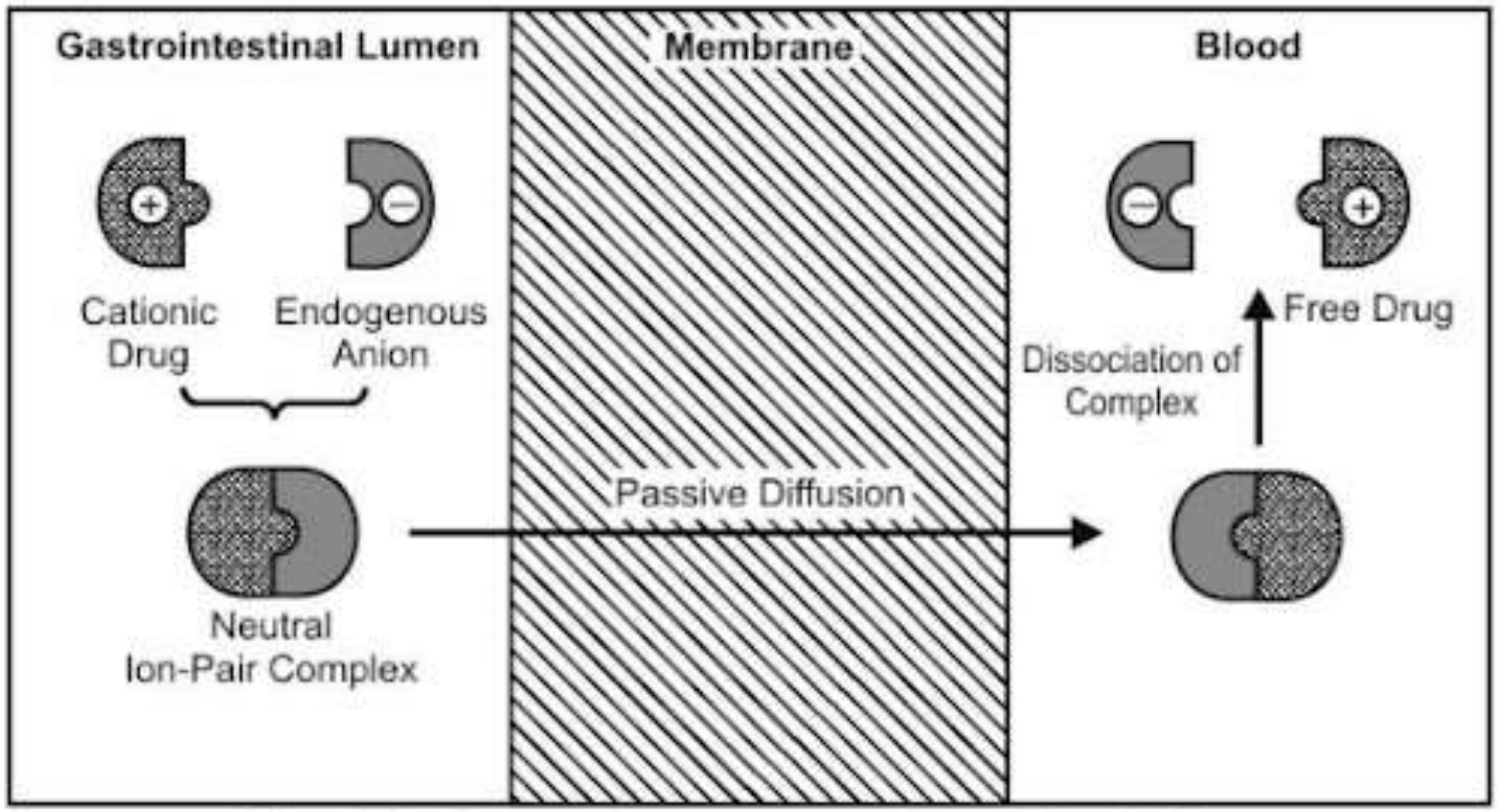


Fig. 2 5. Ion-pair transport of a cationic drug

Facilitated diffusion:

1. The process of spontaneous passive transport
2. **Via specific transmembrane integral proteins.**
3. Not directly require chemical energy from ATP
4. molecules and ions move down their **concentration gradient**

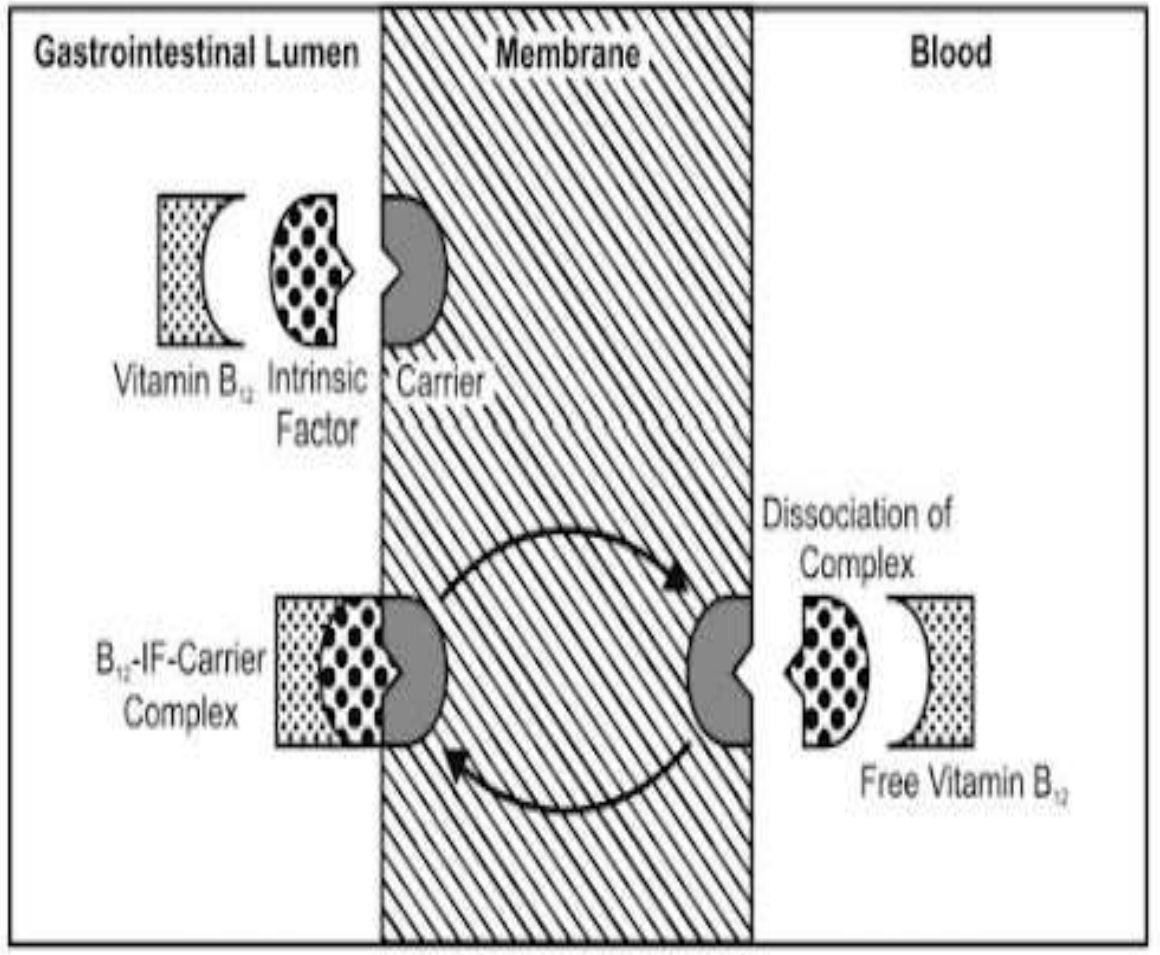


Fig. 2.7. Facilitated diffusion of vitamin B12

Active Transport

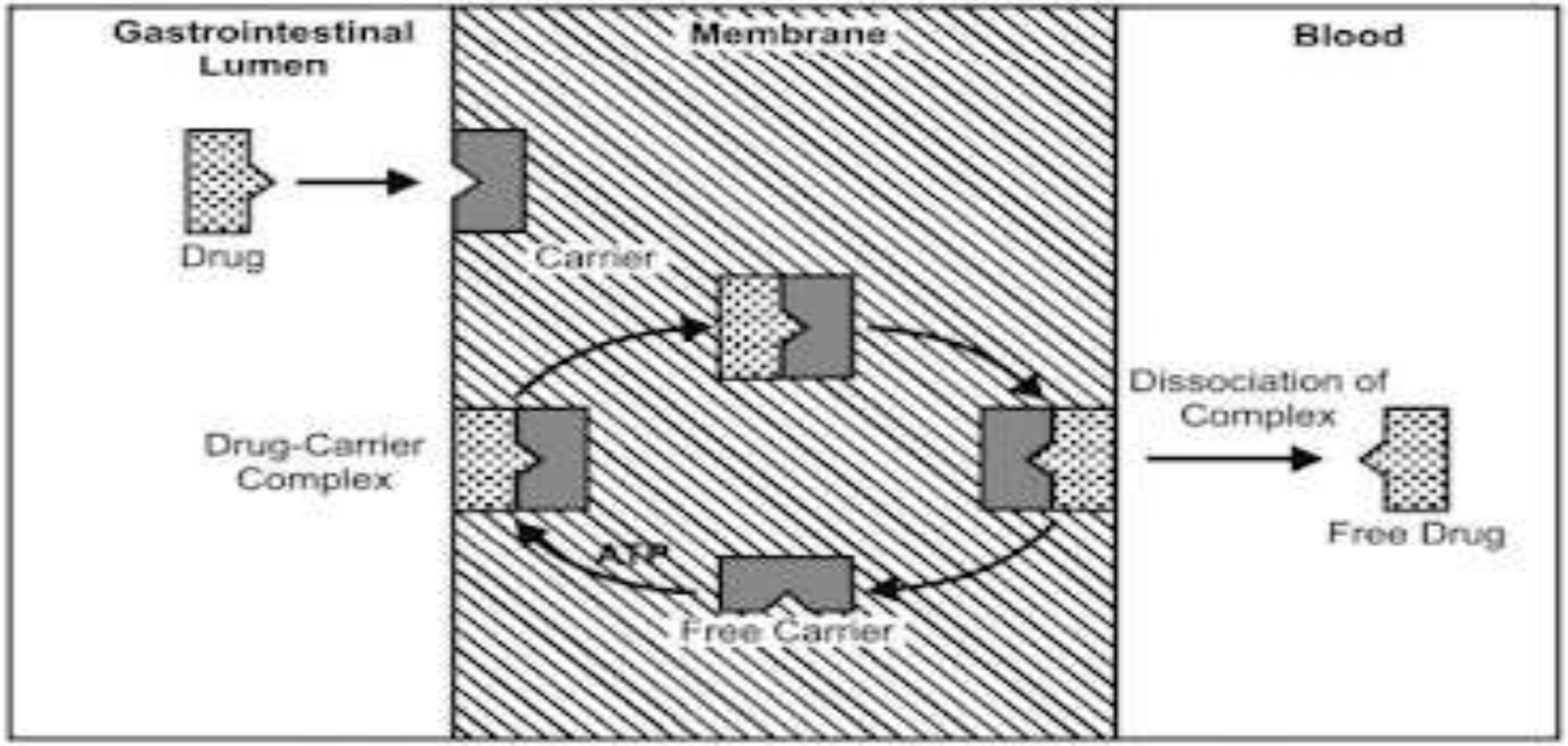
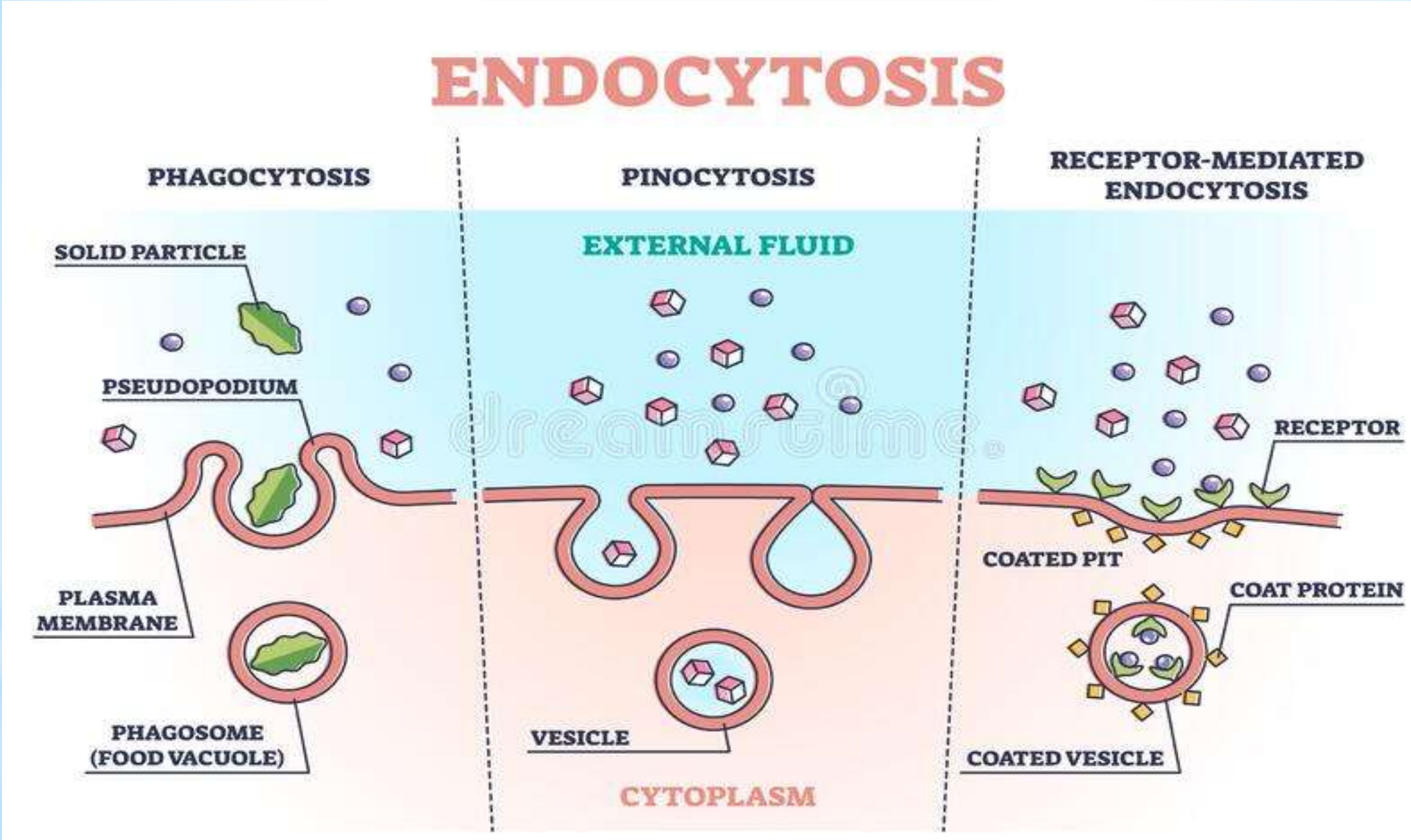
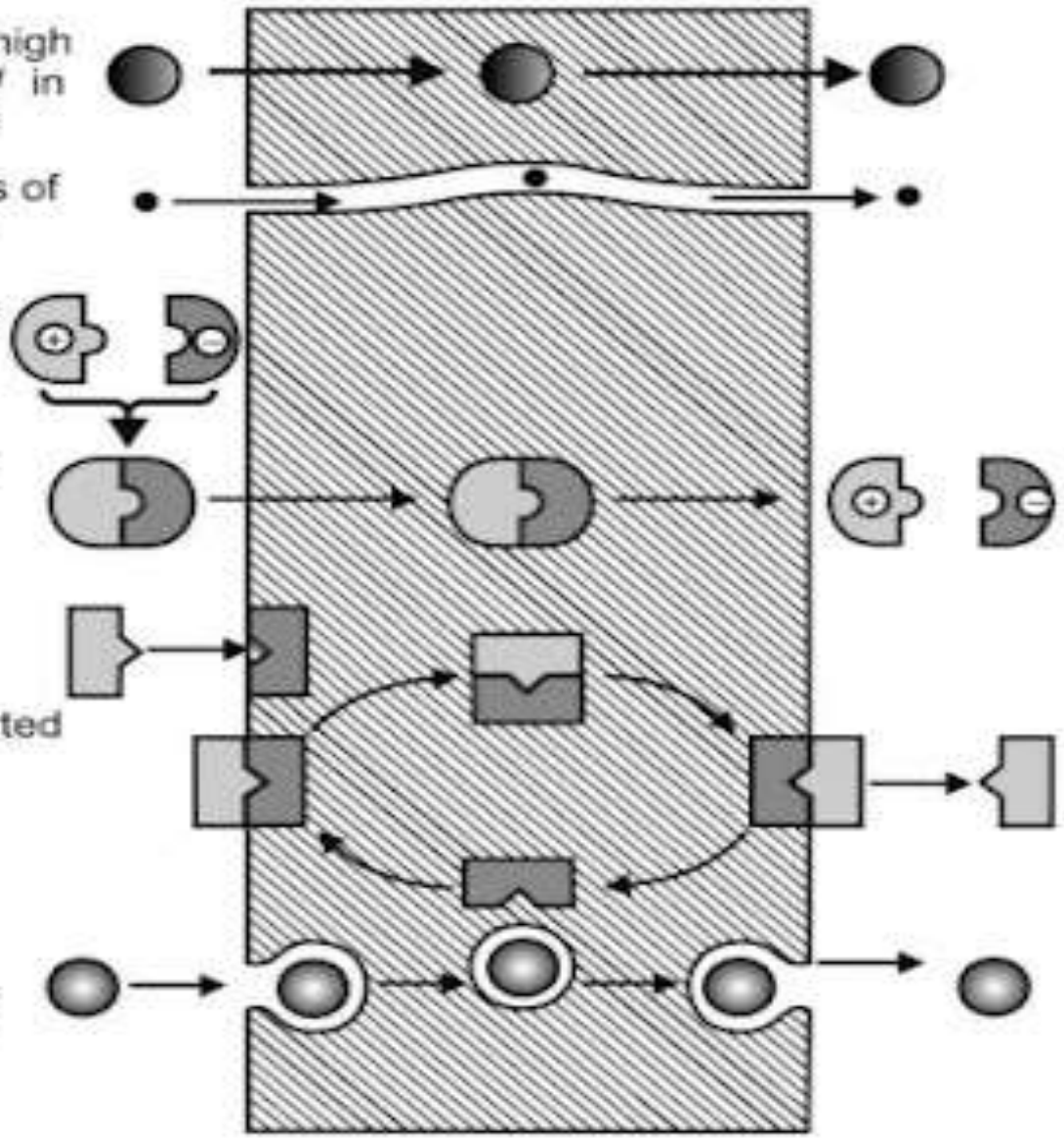


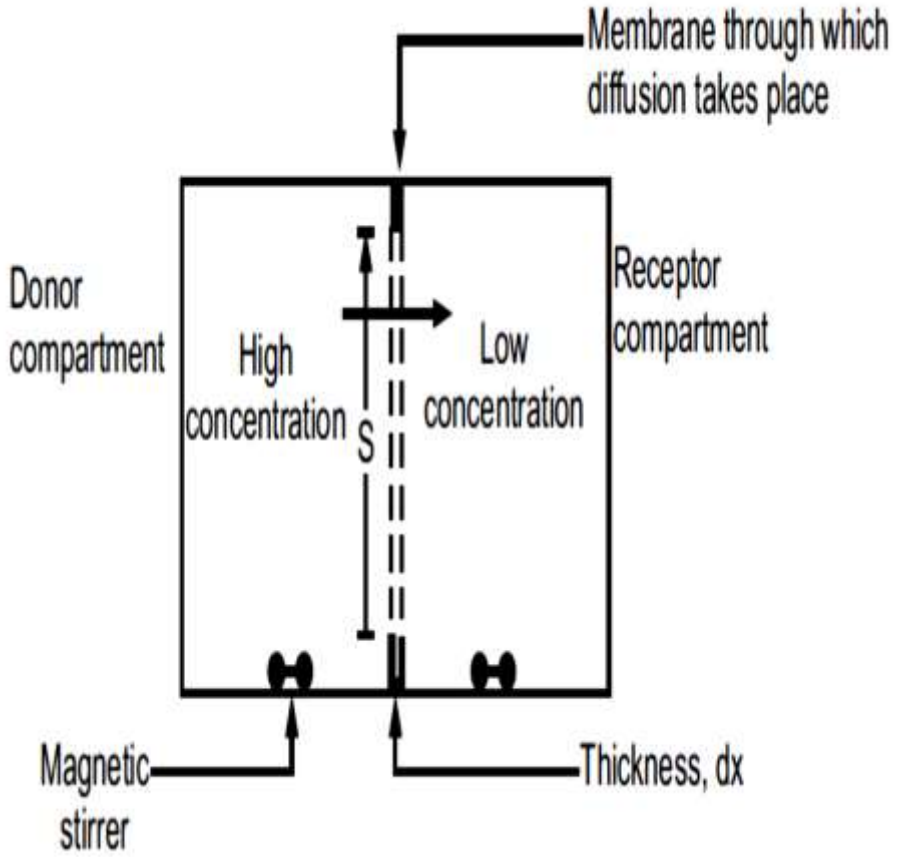
Fig. 2.8. Active absorption of a drug


ENDOCYTOSIS
PHAGOCYTOSIS
PINOCYTOSIS
RECEPTOR-MEDIATED ENDOCYTOSIS
SOLID PARTICLE
EXTERNAL FLUID
PSEUDOPODIUM
RECEPTOR
COATED PIT
COAT PROTEIN
PLASMA MEMBRANE
PHAGOSOME (FOOD VACUOLE)
VESICLE
COATED VESICLE
CYTOPLASM

Absorption Mechanism	Drugs Absorbed
Passive Diffusion	Most drugs having high lipophilicity and MW in the range 100 - 400
Pore Transport	Water-soluble drugs of MW less than 100
Ion-Pair Transport	Drugs that ionise at all pH conditions absorbed after complexing with oppositely charged ions
Carrier-Mediated Transport	Structure-specific drugs with affinity for carriers transported from specific sites
Endocytosis	Macromolecular nutrients and drugs as solid particles or oily droplets



Fick's First Law for Diffusion



According to **Fick's first law,** the amount d_q of substance diffusing in time dt across a plane of area S is directly proportional to the change of concentration dc with distance travelled dx.

Fick's law is written as,

$$d_q = -DS\frac{dc}{dx}\,dt \qquad \ldots (5.5)$$

Where,

D : The diffusion coefficient,

$\frac{dc}{dx}$: The amount of material diffusing per unit time across a unit area, called the concentration gradient.

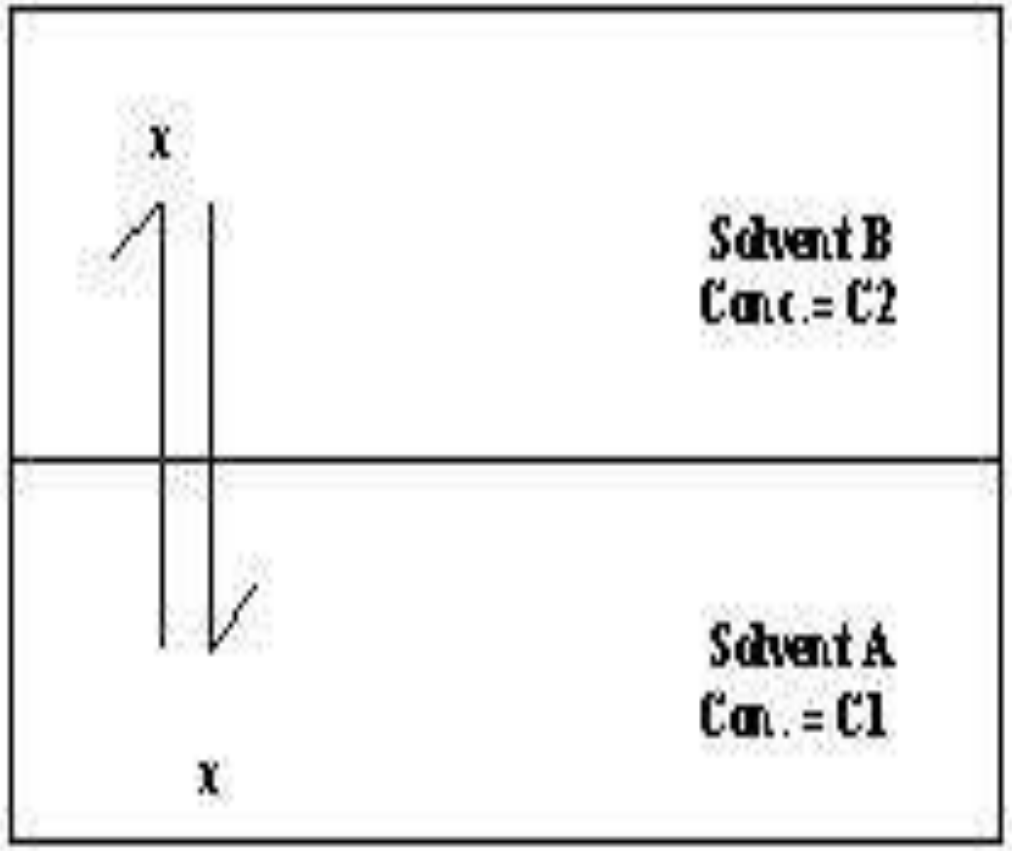
APPLICATIONS OF DIFFUSION

- Absorption, Distribution &Elimination of the drug in biological membranes.
- Release of drug from the dosage form.

e.g.; sustained release, controlled release.

- Estimation of molecular weight of the polymers.

Nernst Distribution Law



$$\frac{\text{Concentration of "X" in solvent 'a' } (C_1)}{\text{Concentration of "X" in solvent 'b' } (C_2)} = K \text{ (constant)}$$

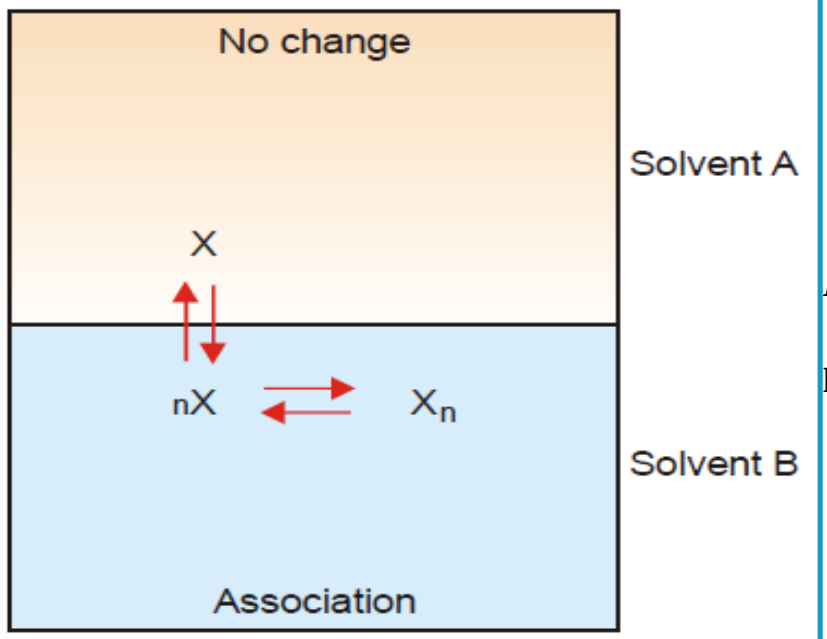
LIMITATIONS OF DISTRIBUTION LAW

The conditions to be satisfied for the application of the Nernst's Distribution law are:

1. **Constant temperature:** The temperature is kept constant throughout the experiment.
2. **Same molecular state**: The molecular state of the solute is the same in the two solvents. The law does not hold if there is association or dissociation of the solute in one of the solvents.
3. **Equilibrium concentrations:** The concentrations of the solute are noted after the equilibrium has been established.
4. **Dilute solutions:** The concentration of the solute in the mo solvents is low. The law does not hold when the concentrations are high.
5. **Non-miscibility of solvents**: The two solvents are non-miscible or only slightly soluble in each other. The extent of mutual solubility of the solvents remains unaltered by the addition of solute to them.

DISTRIBUTION LAW MODIFIED FOR CHANGE IN MOLECULAR STATE

When Solute undergoes Association:



Let,

C_1 be concentration of X in solvent A.

C_3 be concentration of X in solvent B

C_2 be concentration of X_n in solvent B.

Applying distribution law to the equilibrium

X in solvent A = X in solvent B

We have

$$\frac{C_1}{C_3} = K_D$$

……5.22

Applying Mass Law to the chemical equilibrium

nX (Monomer, C_3) = X_n (Associated molecules, C_2)

$$\frac{[X]^n}{[X_n]} = K_c \quad \text{or} \quad \frac{C_3^n}{C_2} = K_c$$

……5.23

Where, K_c is the equilibrium constant.

Taking n^{th} root on both sides in equation 5.23

$$C_3 = \sqrt[n]{K_c C_2}$$

…… 5.24

Substituting value of C_3 in equation 5.22

$$\frac{C_1}{\sqrt[n]{K_c C_2}} = K_D$$

…… 5.25

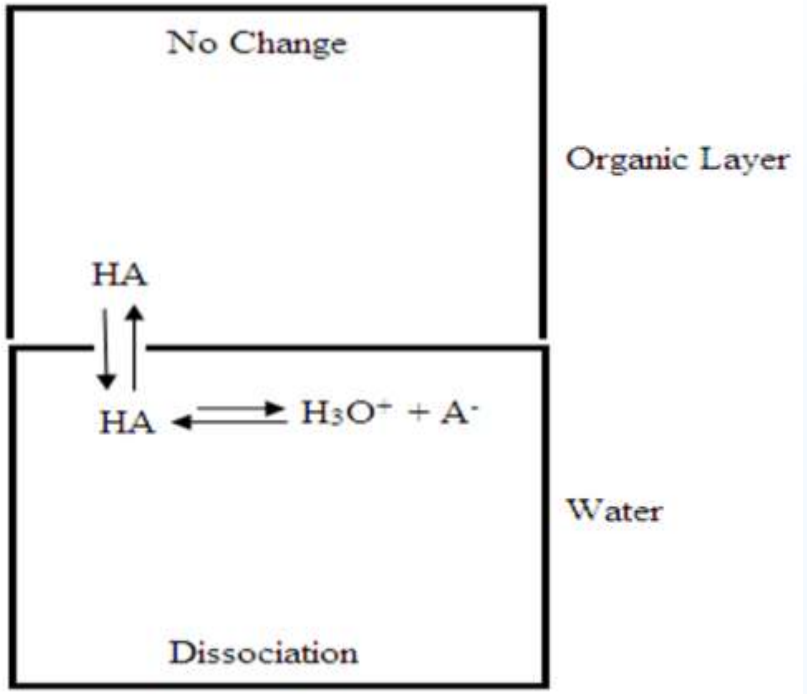
$$\frac{C_1}{\sqrt[n]{C_2}} = K_D \sqrt[n]{K_c} = K \text{ (constant)}$$

……5.26

Thus when association occurs in one solvent, the distribution equation is modified as

$$\frac{C_1}{\sqrt[n]{C_2}} = K_D$$

……5.27



A strong electrolyte (HA) undergoes dissociation in water. The chemical equilibrium can be written as:

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

…… …5.30

Applying the Law of Mass Action to the above chemical equilibrium, dissociation constant (K_a) may be written as:

$$K_a = \frac{[H_3O^+]\,[A^-]}{[HA]} \qquad \text{……..5.31}$$

When Solute undergoes Dissociation:

As per the dissociation equation, $[H_3O^+] = [A^-]$. Substituting this relationship in equation (5.31) gives:

$$K_a = \frac{[H_3O^+]^2}{[HA]} \qquad \text{…….5.32}$$

Equation (5.32) may be rearranged to obtain:

$$[HA] = \frac{[H_3O^+]^2}{K_a} \qquad \text{……5.33}$$

In equation (5.33), HA is the concentration of acid in water, i.e., $[HA]_W$. Let the concentration of undissociated solute in organic layer ($[HA]_0$) be C_1. According to the distribution law, distribution coefficient may be written as:

$$K_d = \frac{[HA]_o}{[HA]_w} \qquad \text{…… 5.34}$$

Substituting equation (5.33) in equation (5.34) for $[HA]_W$ gives

$$K_d = \frac{(C_1)}{[H_3O^+]^2 / K_a} = \frac{(C_1)\,K_a}{[H_3O^+]^2} \qquad \text{……..5.35}$$

Let the concentration of [H3O+] in water be C2. Substituting and rearranging equation (5.35) gives

$$\frac{K_d}{K_a} = \frac{(C_1)}{(C_2)^2} \qquad \text{……..5.36}$$

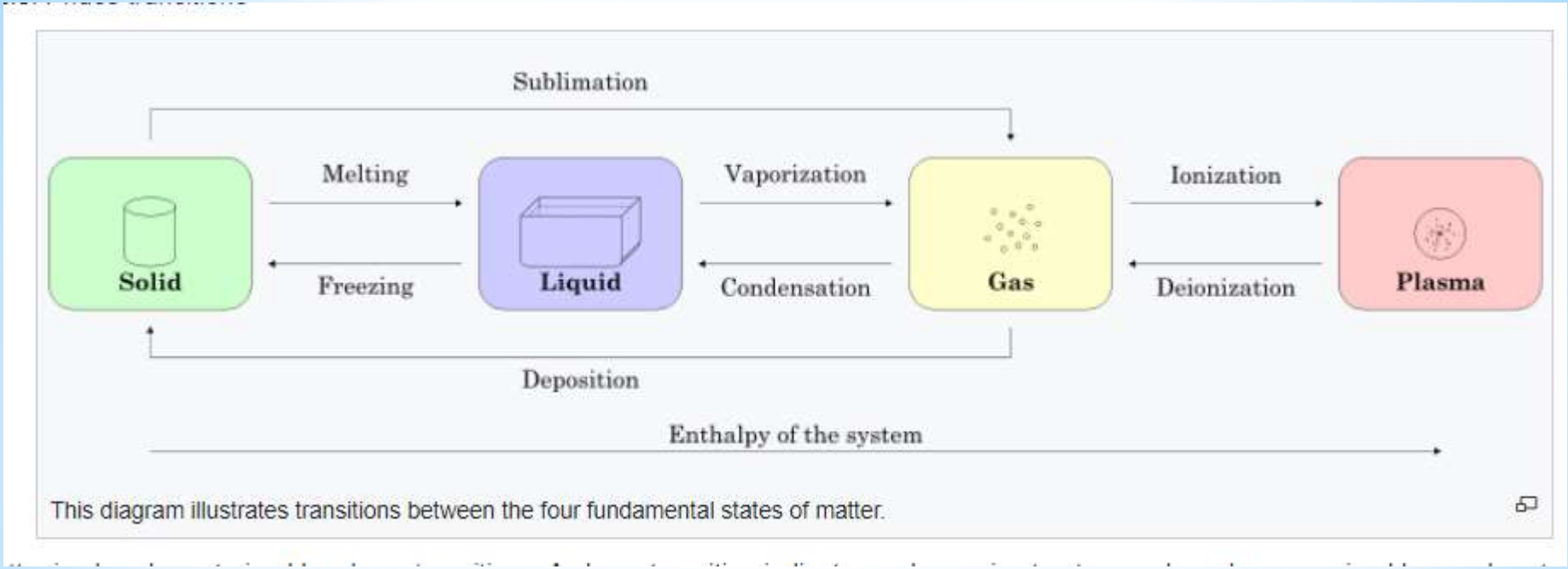
Where K_d / K_a is a constant. This is the modified distribution law.

Unit- II

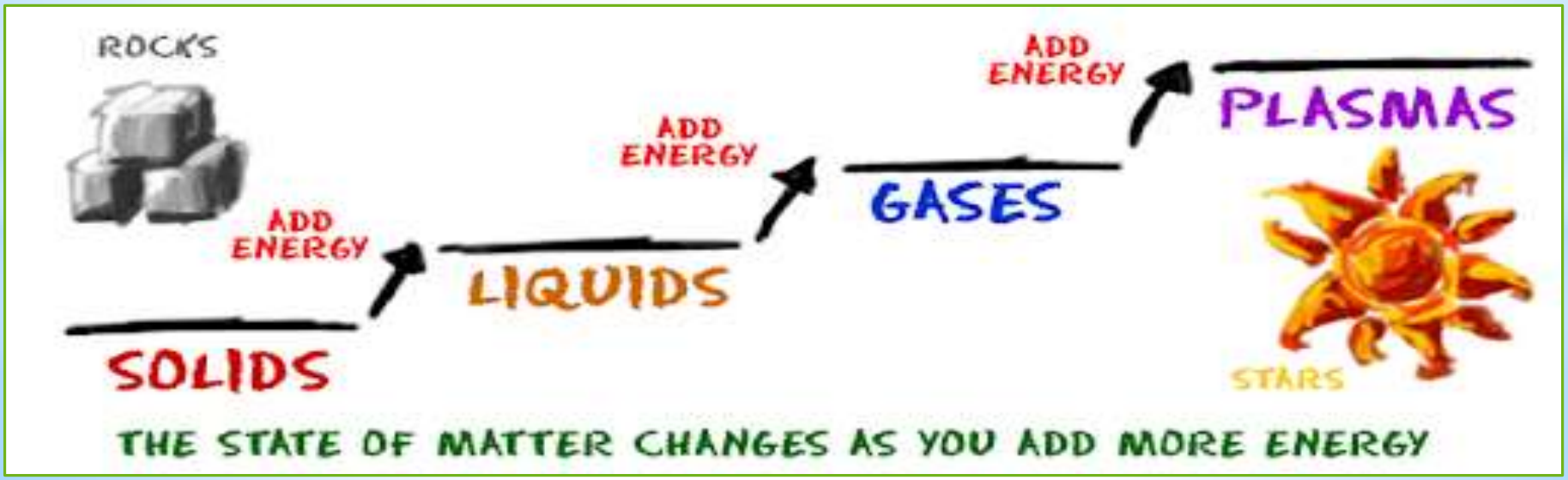
States of Matter and properties of matter

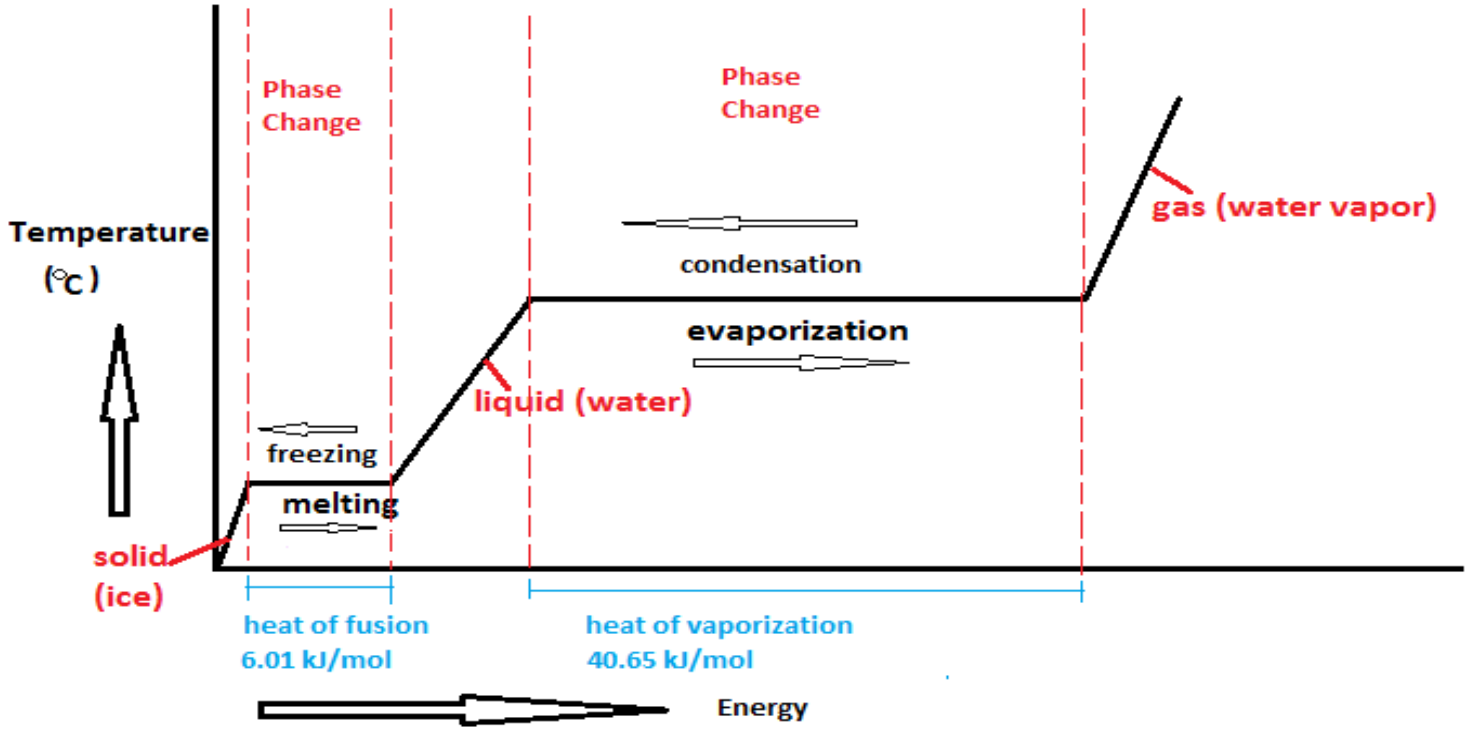
Subject Teacher:
Dr. Prashant Khade

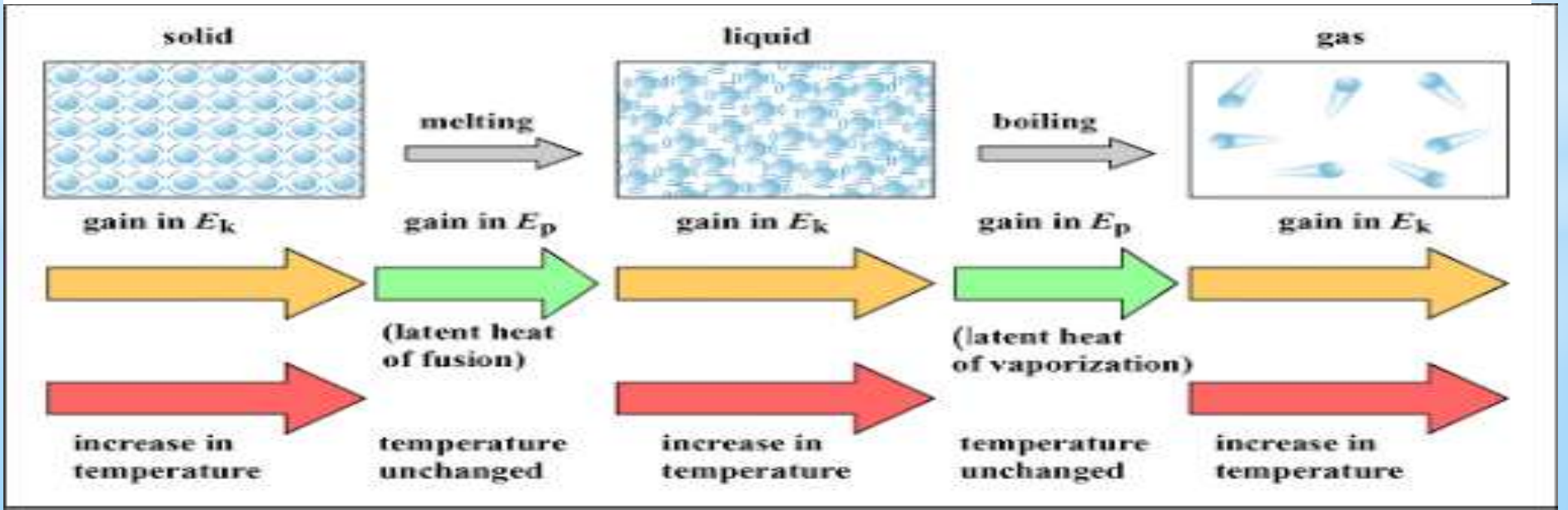
State of matter and changes in the state of matter



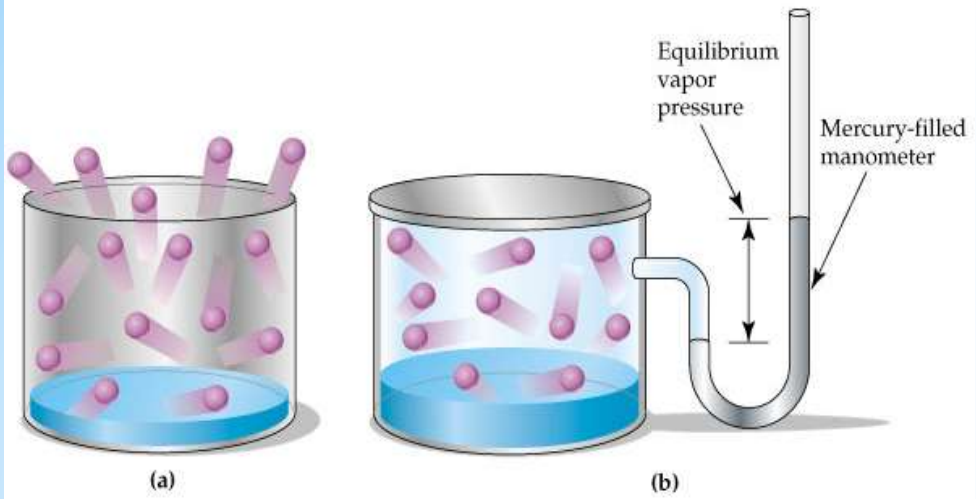
This diagram illustrates transitions between the four fundamental states of matter.

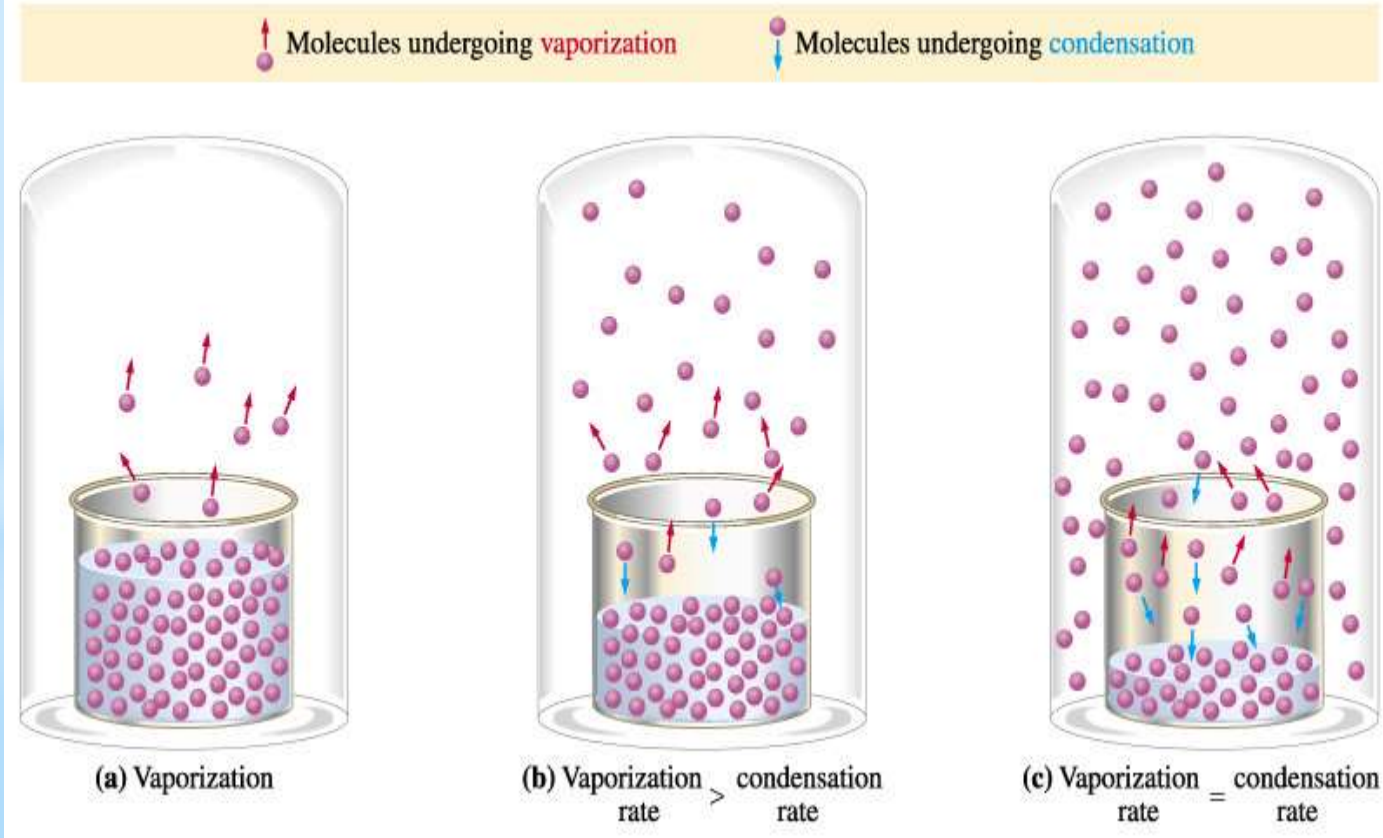



Phase Change
Phase Change
Temperature (°C)
gas (water vapor)
condensation
evaporation
liquid (water)
freezing
melting
solid (ice)
heat of fusion 6.01 kJ/mol
heat of vaporization 40.65 kJ/mol
Energy

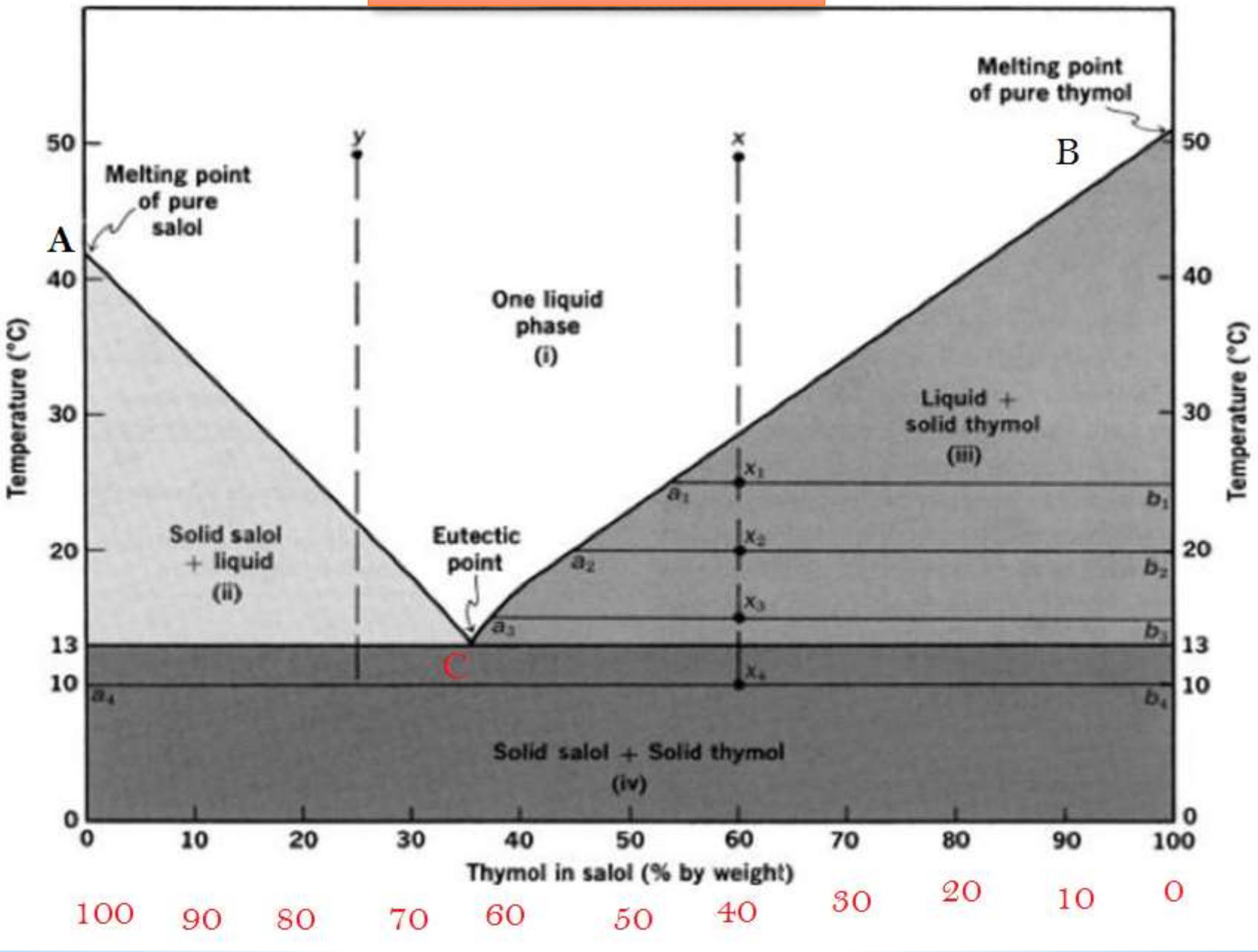

solid
liquid
gas
melting
boiling
gain in E_k
gain in E_p
gain in E_k
gain in E_p
gain in E_k
(latent heat of fusion)
(latent heat of vaporization)
increase in temperature
temperature unchanged
increase in temperature
temperature unchanged
increase in temperature

Vapor Pressure

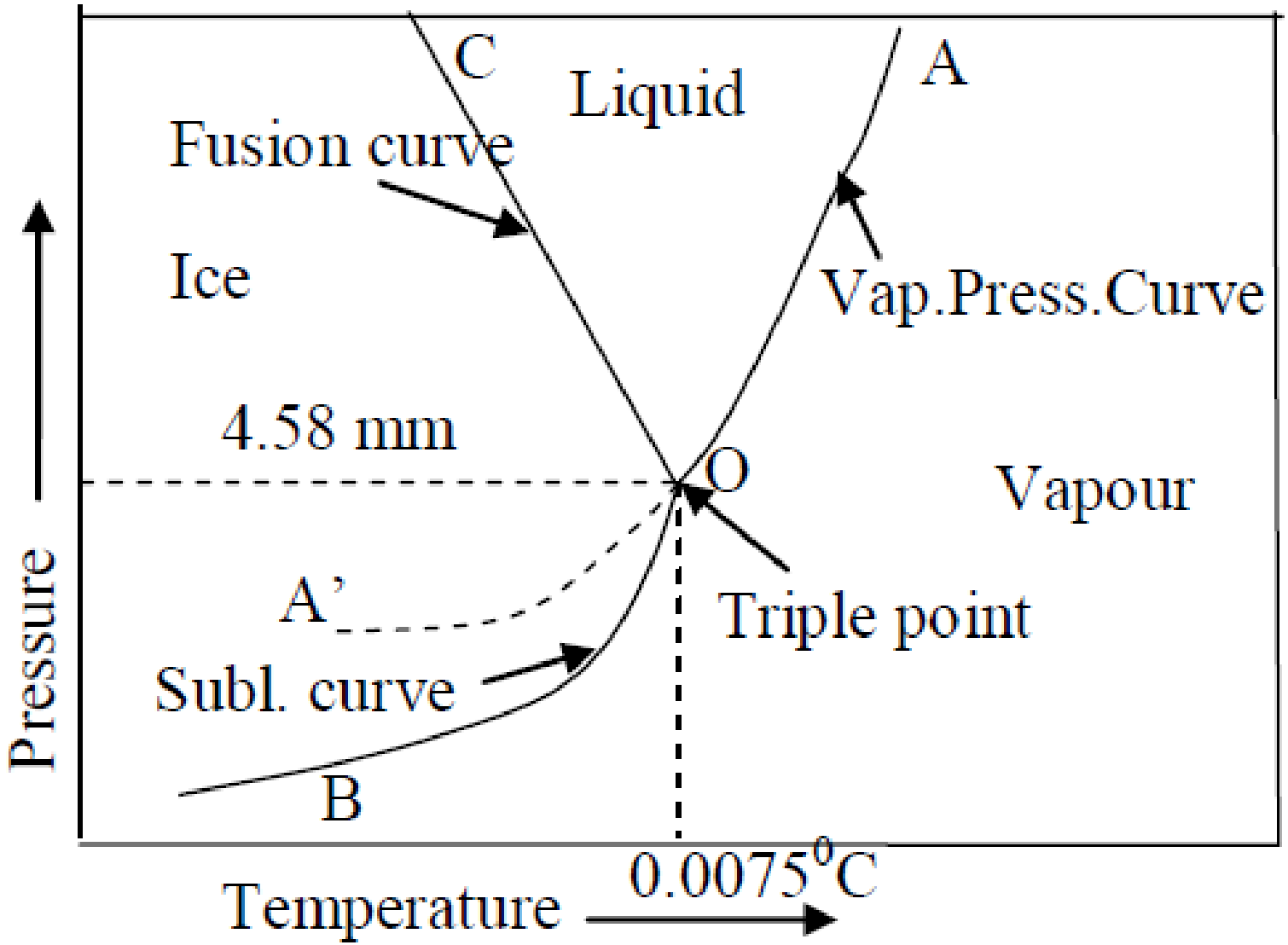




Eutectic Mixtures,



Sublimation Critical Point,



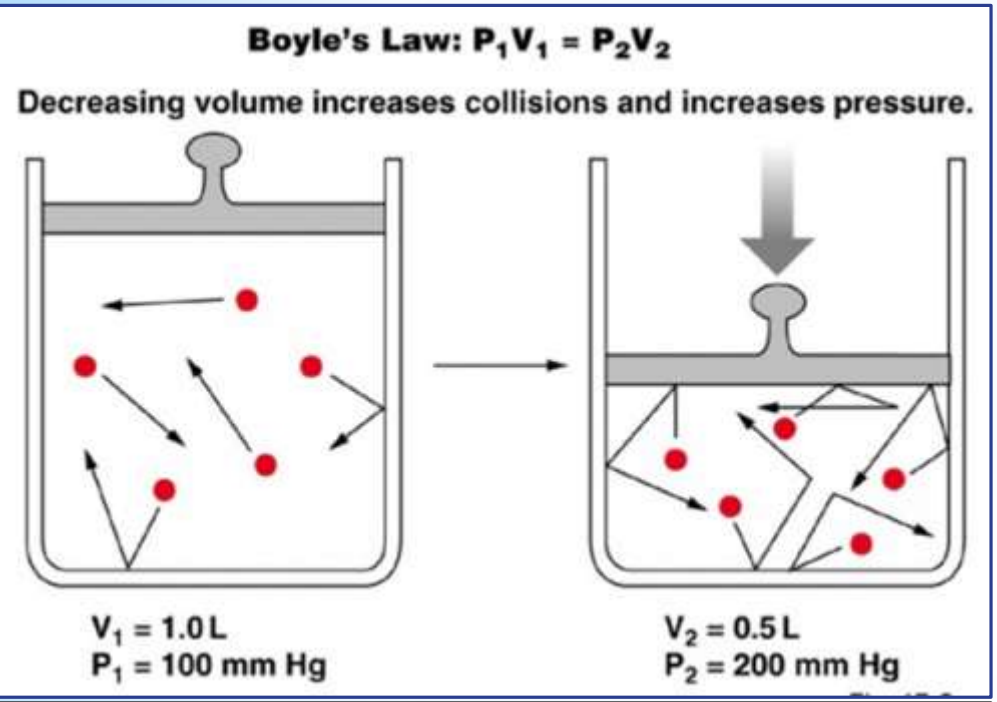
Gas

Properties of Gases:

1. No definite shape or volume:
2. A gas has no surface of its own.
3. A gas is not rigid and is easily compressed.
4. A gas can diffuse into another gas.
5. A gas on cooling changes into liquid state.
6. A gas can flow in all directions. It requires a vessel closed from all sides.

Simple Gas Laws:

Boyle's Law (Effect of pressure)

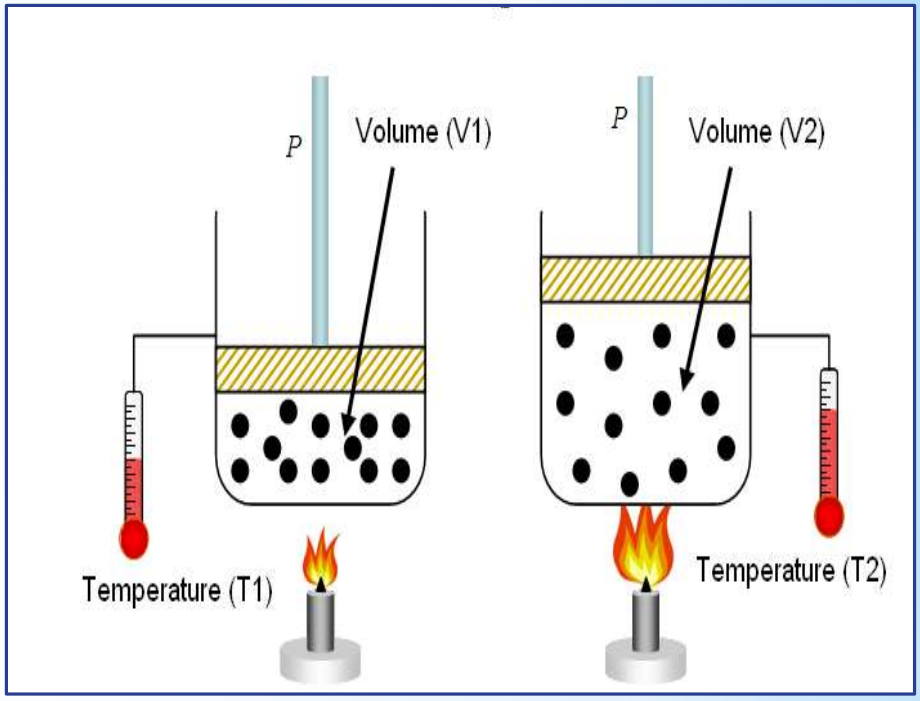


Law expressed mathematically as

$$P \propto \frac{1}{V} \quad \text{or} \quad V \propto \frac{1}{P} \quad \text{(T and n constant)}$$

$$V = k\,\frac{1}{P} \qquad \ldots\ldots\ldots\ldots 2.2$$

Charles's Law (Effect of Temperature)



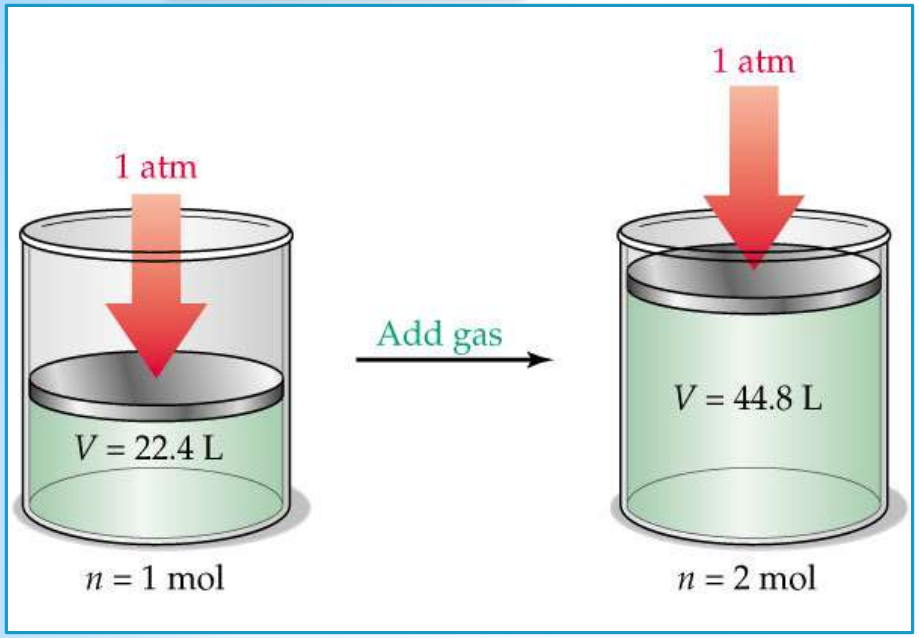
$V \propto T$

or

$V = K\,T$

(P and n constant)

Avogadro's Law



V ∝ n

or

V = K n (T and P constant)

Standard Temperature and Pressure (STP):

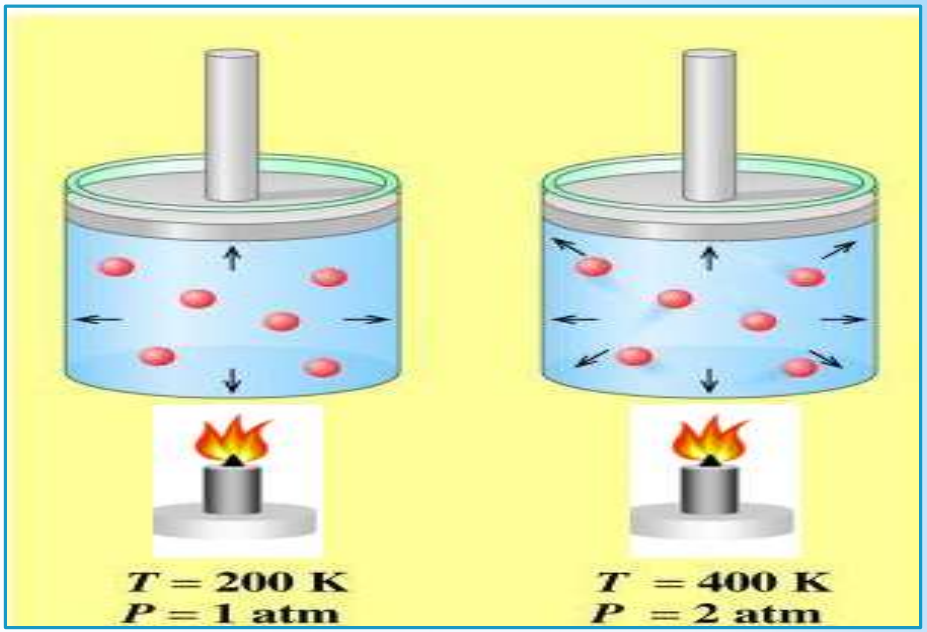
P: 1 atm and

T: 0° C

At STP: 1 mole (n) = 22.4 L

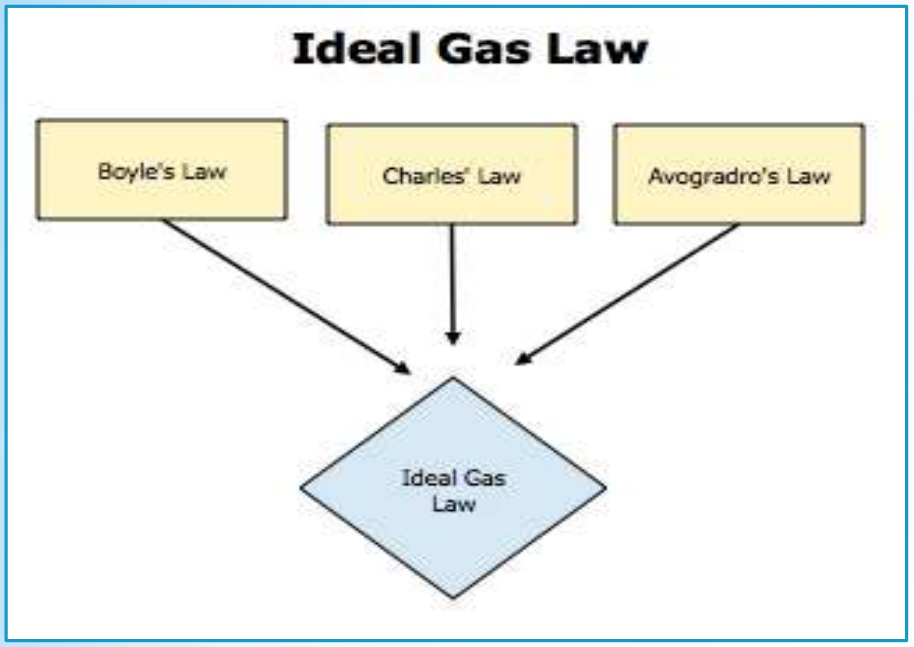
called the molar gas volume or molar volume.

Amontons's Law



P ∝ T (n and V constant)

The Ideal Gas Equation



$$V \propto \frac{nT}{P}$$

$$V = R\frac{nT}{P}$$

$$PV = nRT$$

Boyle's Law, $V \propto \frac{1}{P}$

Charles' Law, $V \propto T$

Avogadro's Law $V \propto n$

Values of R:

1. 0.082057 L atm mol^{-1} K^{-1}
2. 62.364 L Torr mol^{-1} K^{-1}
3. 8.3145 m^3 Pa mol^{-1} K^{-1}
4. 8.3145 J mol^{-1} K^{-1} **(the SI unit for the gas constant)**

Deviation from Ideal Gas Behavior (compressibility factor)

$$PV = nRT$$

$$Z = \frac{PV}{RT}$$

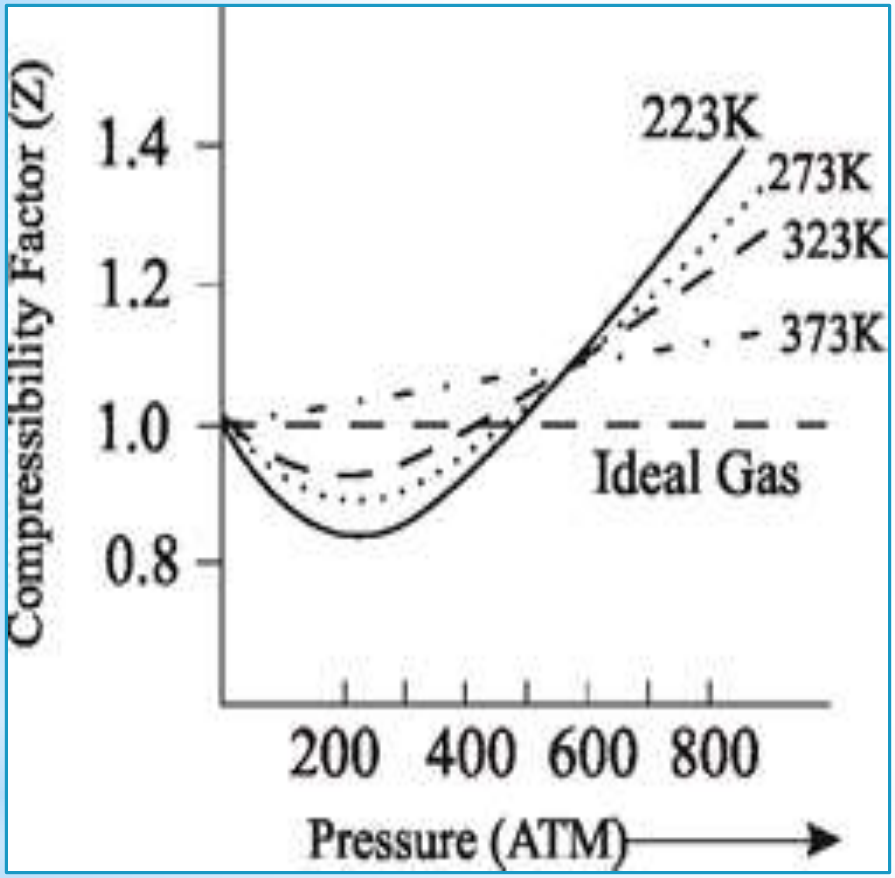
In case of ideal gas, Z = 1 (PV=nRT)
In case of real gas, Z ≠ 1 (PV ≠ nRT)

Thus for, real gas, Z ≠ 1, it can be greater than one or less than one.

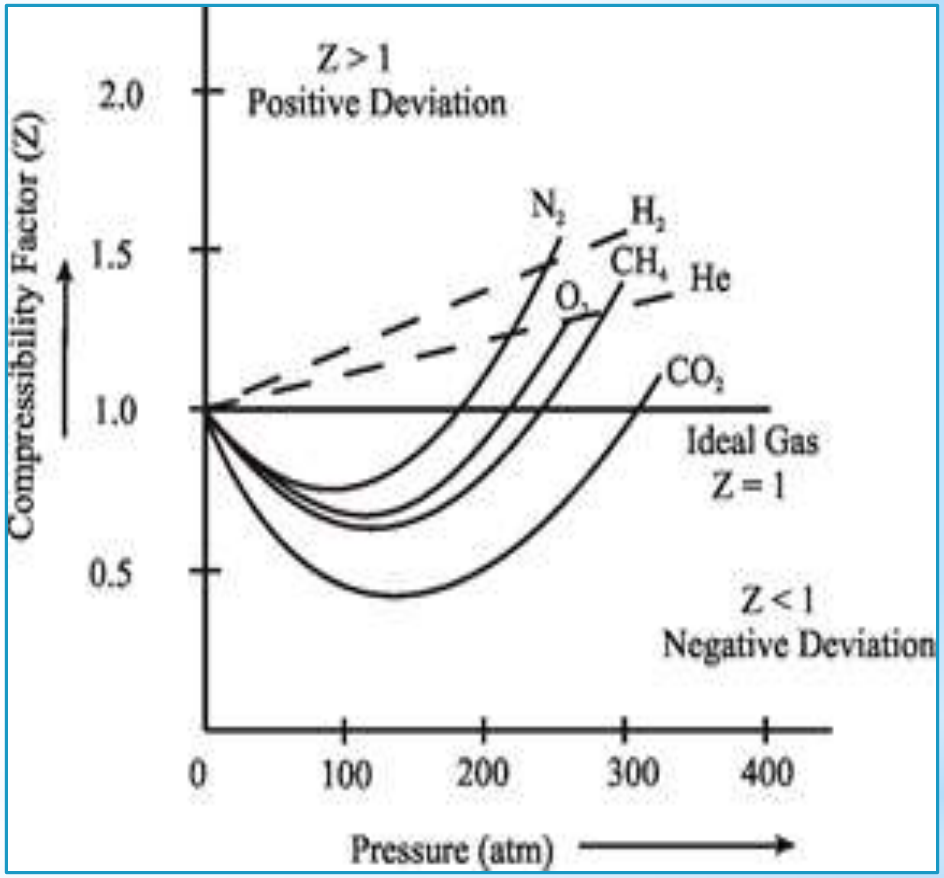
(1)If Z>1, it refers PV is greater than nRT, which implies that gas is less compressible than expected from Ideal behaviour. Such a deviation from Ideal behaviour is known as ***Positive Deviation***. (Difficult to liquefied)

(2)If Z<1, it refers PV is less than nRT, which implies that gas is more compressible than it should if it behaves Ideal gas. Such a deviation is known as ***Negative Deviation***. (Easy to liquefied)

Effect of Temperature:



Effect of Pressure:



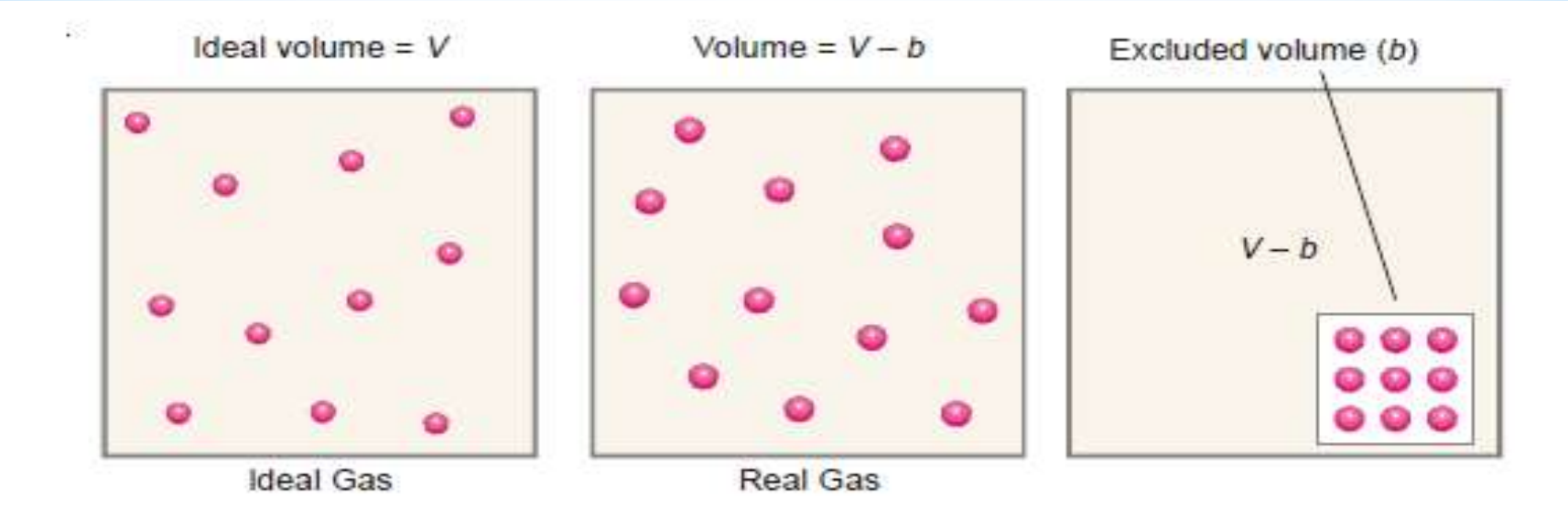
Van der wall's Equation for real gas.

Kinetic Theory for Ideal Gas:
(1) The molecules in a gas are point masses and possesses no volume,
(2) There are no intermolecular attractions in a gas.

Volume Correction:

Real gas volume: Van der Waals assumed that molecules of a real gas are rigid spherical particles which possess a **definite volume.**

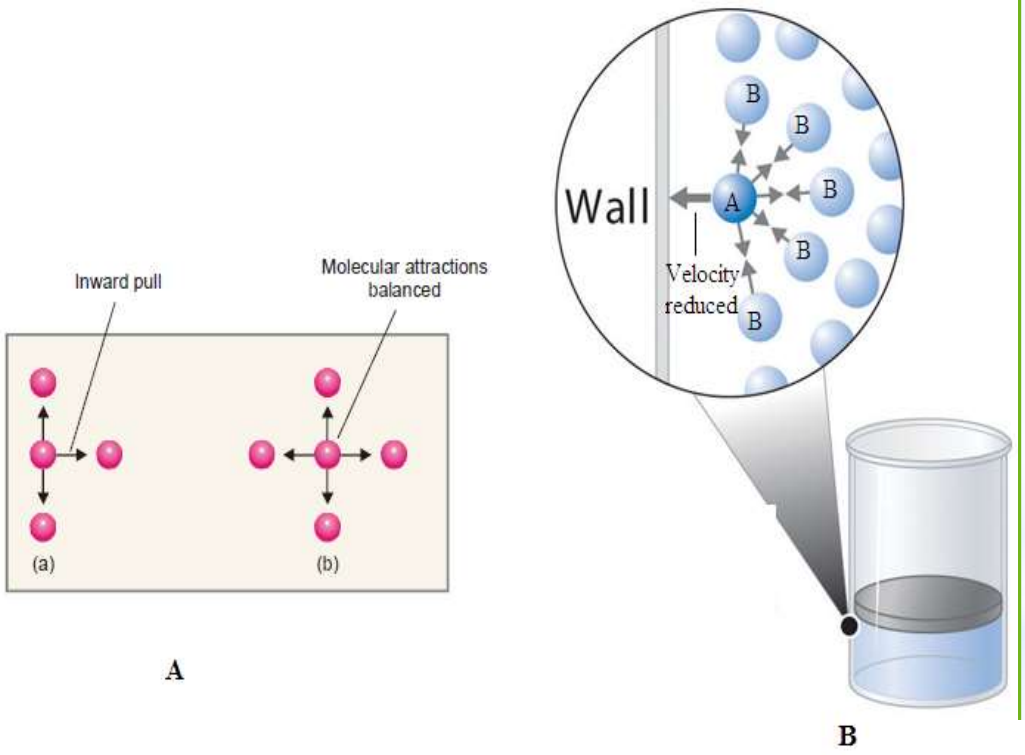
The volume of a real gas = ideal volume (V) - volume occupied by gas molecules (b)



Pressure Correction:

Ideal gas: According to kinetic theory of gas, there are no intermolecular attractions in a gas.

Real gas: Van der Waals assumed that, there are intermolecular attractions in a gas. Therefore, pressure correction in ideal gas equation is necessary



$$P_A = P_{ideal} - P_d \quad \text{or} \quad P_{ideal} = P_A + P_d$$

$$P_d \propto C_A \times C_B$$

$$P_d \propto \frac{n}{V} \times \frac{n}{V}$$

$$P_d = \frac{an^2}{V^2}$$

. Van Der Wall's Equation

$$\left(p + \frac{an^2}{V^2}\right)(V - nb) = nRT$$

LIQUEFACTION OF GASES - CRITICAL PHENOMENON

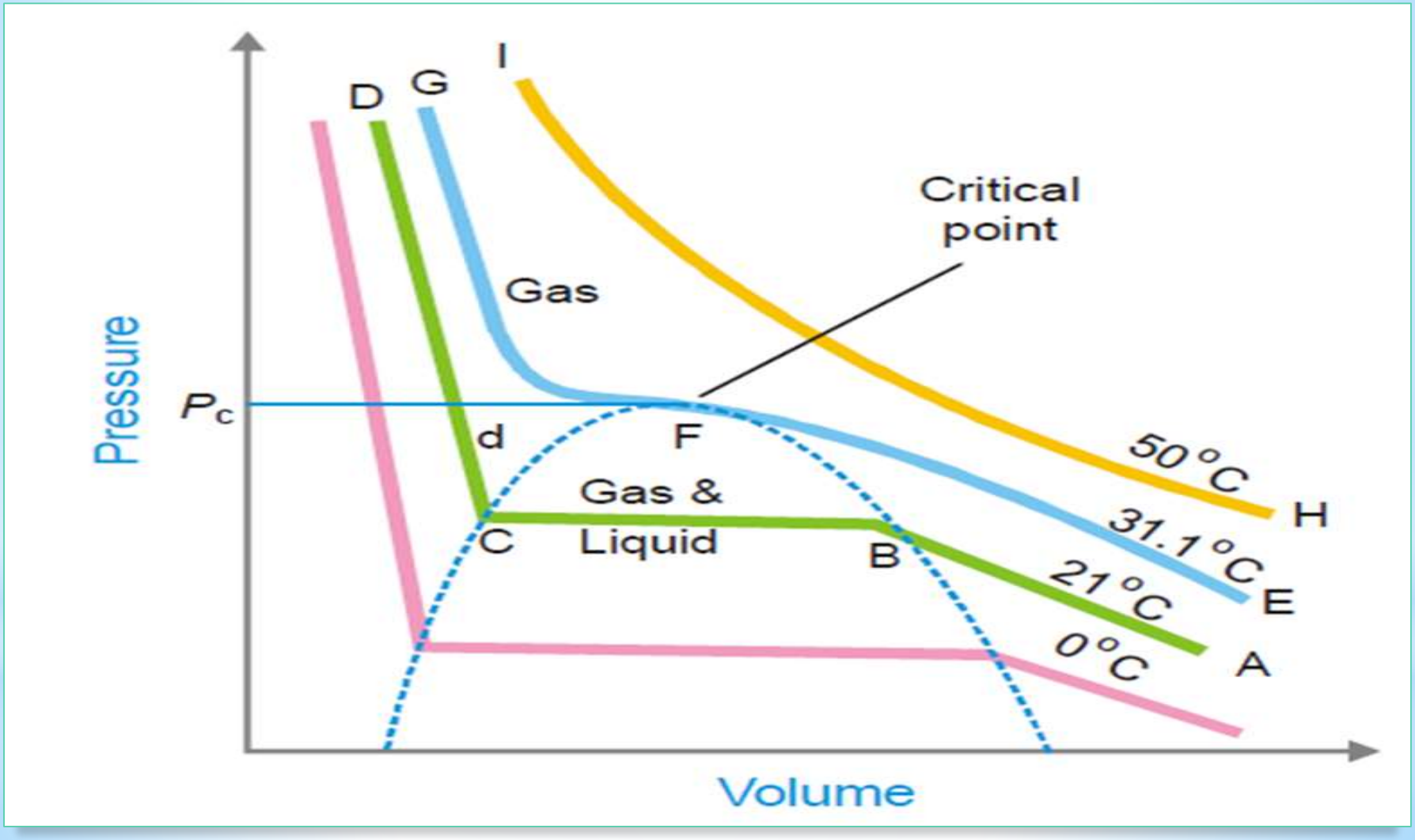
The critical temperature, Tc, of a gas may be defined as that temperature above which it cannot be liquefied no matter how great the pressure applied.

The critical pressure, Pc, is the minimum pressure required to liquefy the gas at its critical temperature.

The critical volume, Vc, is the volume occupied by a mole of the gas at the critical temperature and critical pressure.

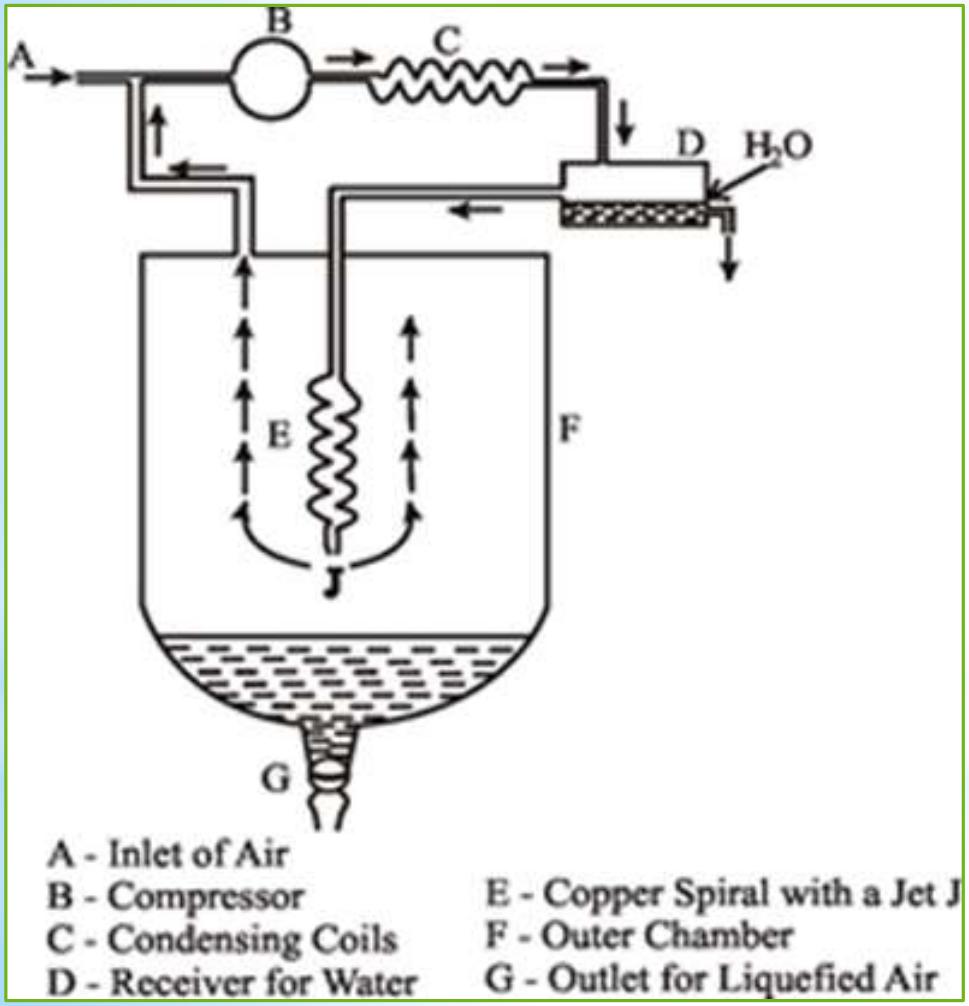
Tc, PC and Vc are collectively called the **critical constants** of the gas.

Andrews Isotherms of Carbon Dioxide



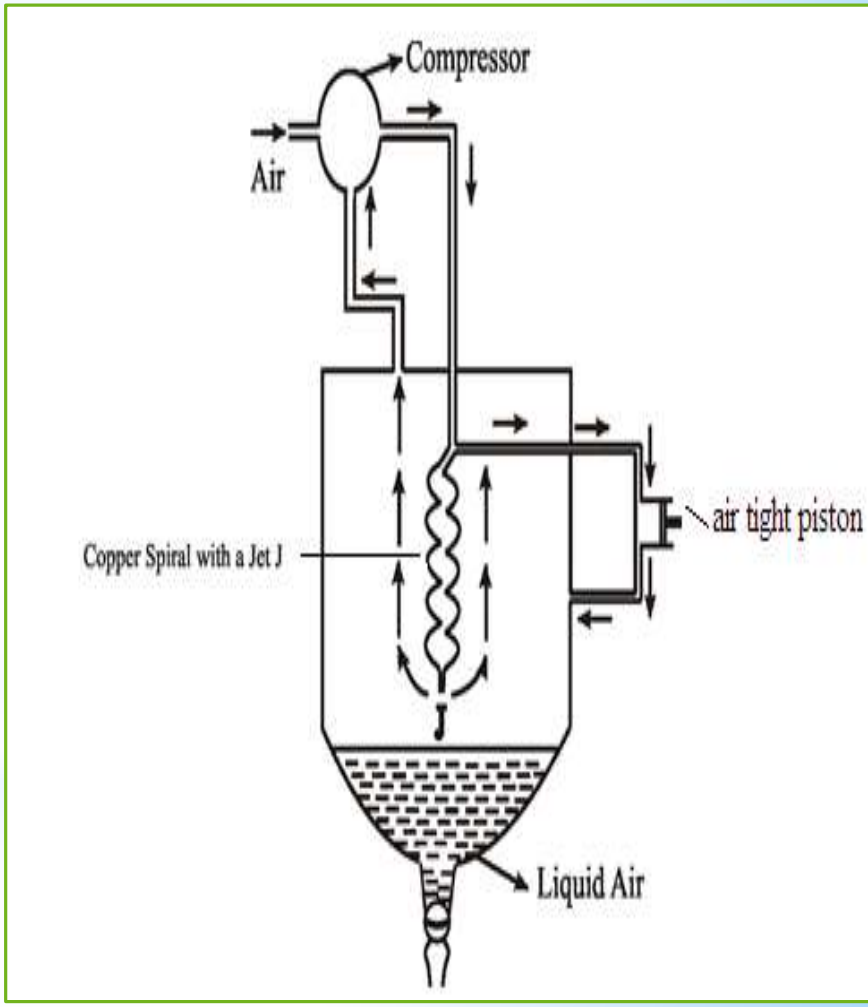
Liquefaction of Gases:

Linde's Method:

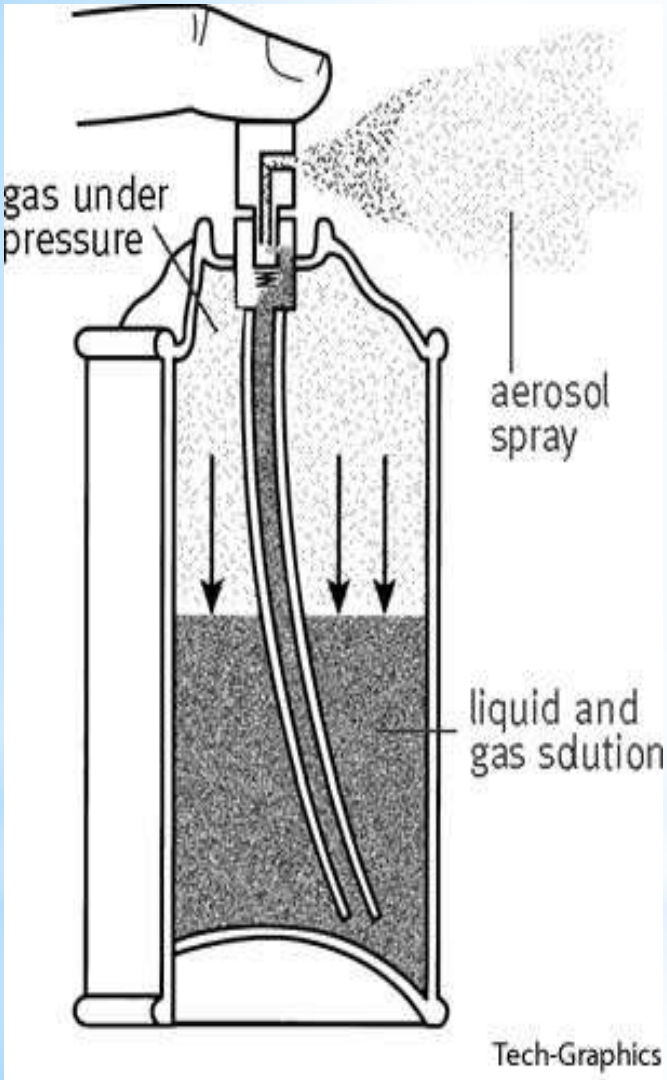


A - Inlet of Air
B - Compressor
C - Condensing Coils
D - Receiver for Water
E - Copper Spiral with a Jet J
F - Outer Chamber
G - Outlet for Liquefied Air

Claude's Method:



Aerosols:

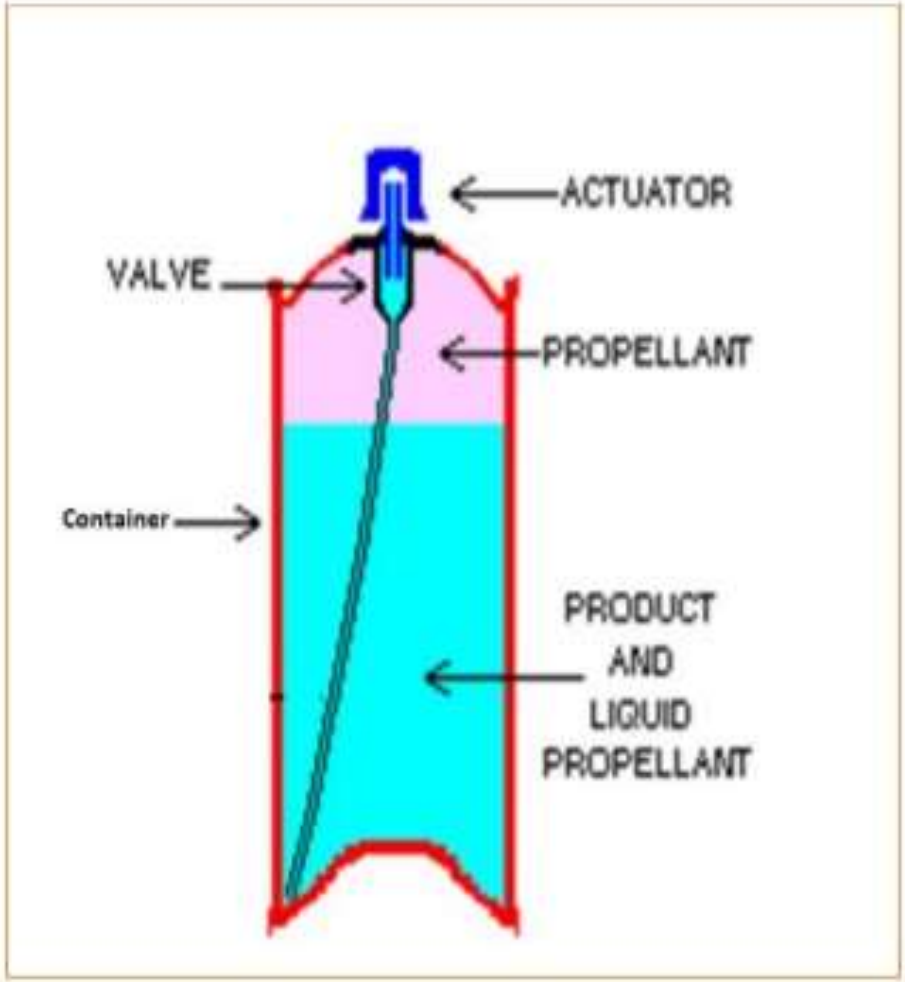


The aerosol container is referred to as a pressurized package in which the therapeutically active drug is dissolved or suspended in compressed or liquefied gas.

The delivery of therapeutically active drug in the form of spray or foam or solid stream is dependent on the ability of the liquefied or compressed gas.

COMPONENTS OF AEROSOLS

- Propellant
- Container
- Valve and actuator
- Product concentrate



Advantage:

- The drug sensitivity to the effect of oxygen or moisture is protected and stability is enhanced.
- The drug can be directly applied to the affected areas.
- Administration of drug by aerosol is a rapid process.
- It protects the drug from gastrointestinal tract degradation.
- Hepatic first pass metabolism is avoided.
- Aerosols are used for both systemic and local application.
- Easy to apply.
- A sterile dose of drug is dispensed and also the contamination of drug is prevented.

PROPELLANT:

The development of pressure within the container by the propellant causes the opening of valve which expels the product by atomization or foam formation

Application	Name of propellant
For oral and inhalation	**Fluorinated hydrocarbons** Di-chloro di-fluro methane (propellant 12) Trichloromonoflouromethane (propellant 11) Di-chloro tetra-fluro ethane (propellant 114)
Topical preparation	Propane, Butane, Isobutane
Compound gases	Nitrogen,Carbon dioxide, Nitrous oxide

Chlorofluorocarbon (CFC) propellants:

A. The basic characteristics of propellants are chemical inertness, lack of toxicity, lack of flammability and explosiveness.

B. Due to the presence of these characteristics, the chlorofluorocarbon (CFC) propellants P-ll, P-12, and P-114 were used in aerosol products for several years.

C. Now a day their uses have been declined as they cause the depletion of ozone layer.

D. But due to their relatively low toxicity and inflammability, they are still use in low amount in the treatment of asthma and chronic obstructive pulmonary disease (COPD).

2. Hydrocarbons Propellants

A. The environmental acceptance, low toxicity and nonreactivity are the characteristics of hydrocarbons propellants allowing them to be used as the propellant.

B. Hydrocarbons are used in the preparation of water based aerosols as they are not susceptible to hydrolysis due to the absence of chlorine.

C. Since they are immiscible with water, so they remain on the top of water.

D. They provide the force to push the contents out of the container.

E. The disadvantage of Hydrocarbon propellant is flammability, explosiveness.

F. Also the use of vapour tap valve reduces flammability.

3. Compressed gas propellants

A. The use of compressed gas like Nitrogen, Nitrogen dioxide and Carbon dioxide as propellant dispenses products in the form of line mists, foams or semisolid.

B. It produces fairly wet sprays and the foams are not as stable as produced by the liquefied gas propellant.

C. Unlike the aerosol prepared with liquefied gas propellant, there is no propellant reservoir.

D. The compressed gas propellant is contained in the headspace of the aerosol container which forces the product concentrate out of the container.

E. So, higher gas pressure is required in this aerosol.

F. This aerosol finds its application to dispense food products, dental creams, hair preparation and ointments.

PRINCIPLE & WORKING PROCESS

➢When the seal opens, the higher pressure inside the can pushes the product up through the dip tube and out of the valve.

➢ A controlled amount of propellant in the product vaporizes as it leaves the can, creating a spray or foam.

➢ A small amount of liquefied gas propellant still in the container also vaporizes, keeping the pressure constant.

➢ The combination of product and propellant is finely tuned to produce just the right concentration, spray pattern and particle size to make the product most effective.

TYPES OF AEROSOL SYSTEMS

Solution system or two phase system

1. Propellant 12 is added alone or in mixture.
2. If propellants having vapour pressure lower than propellant 12 is added to propellant 12, a reduction of vapour pressure is achieved but bigger sized aerosol particles are obtained.
3. Also bigger sized aerosol particles are obtained on addition of cosolvents like ethyl acetate, propylene glycol, ethyl alcohol, glycerin and acetone.
4. No other solvent is required if the drug is soluble in the propellant.
5. The solution system is administered in topical application.
6. Some of the commonly used propellant combinations in solution systems are propellant 12/11 (30:70), propellant 12/114 (45:55), propellant (12/114) (55:45).

WATER BASED SYSTEM

- Large amounts of water can be used to replace all or part of the non aqueous solvents used in aerosols.
- Produce spray or foam.
- To produce spray, formulation must consist of dispersion of active ingredients and other solvents in emulsion system in which the propellant is in the external phase.
- Since propellant and water are not miscible, a three phase aerosol forms (propellant, water and vapor phases).
- Ethanol can be used as cosolvent to solubilize propellant in water. It also reduces surface tension aiding in the production of smaller particles .
- 0.5 to 2% of surfactant is used to produce a homogenous dispersion.

- Surfactants with low water solubility and high solubility in non polar solvents will be most useful eg: Long chain fatty acid esters of polyhydric compounds including glycol, glycerol and sorbitan esters of oleic, stearic, palmitic and lauric acids .
- Propellant concentration varies from about 25 to 60%.
- Aquasol system (Aquasol valve) – dispensing fine mist or spray of active ingredient dissolved in water .
- No chilling effect, since only active ingredient and water are dispensed, propellant is in vapor state.
- Difference between aquasol system and three phase system is aquasol dispenses fairly dry spray with very small particles, non flammability of the product .

METERED DOSE INHALERS

- Used to minimize the number of administration errors.
- To improve the drug delivery of aerosolized particles into the nasal passageways and respiratory tract.

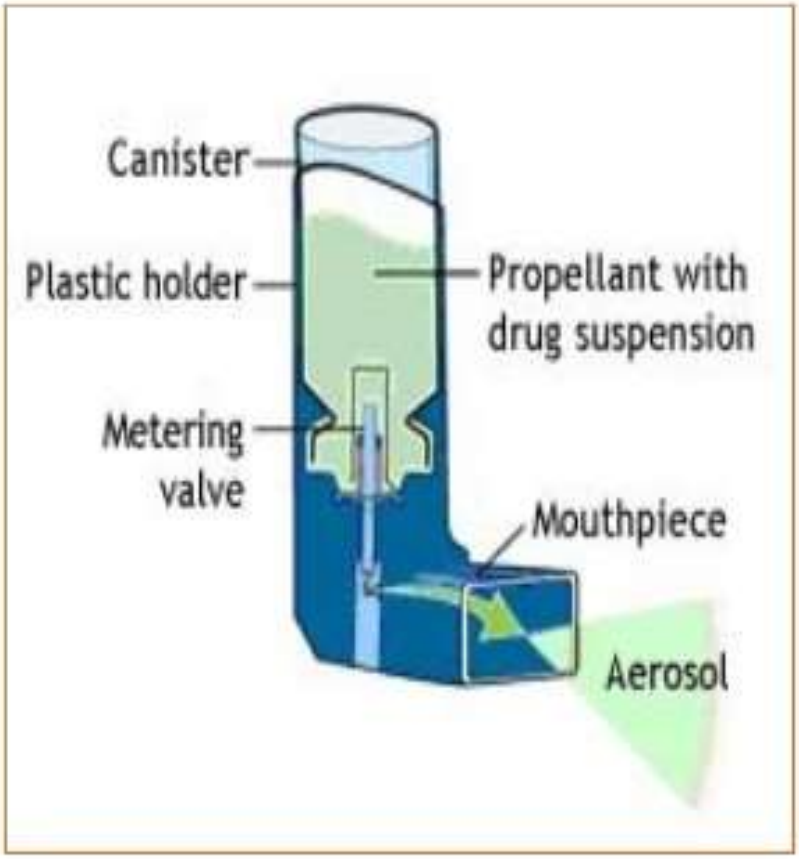
Advantages of MDI:

- It delivers specified amount of dose .
- Portable and compact.
- Quick to use , no contamination of product.
- Dose-dose reproducibility is high.

Disadvantages of MDI :

- Low lung deposition ; high pharyngeal deposition .
- Coordination of MDI actuation and patient inhalation is needed.

Metered Dose Inhalers (MDIs)

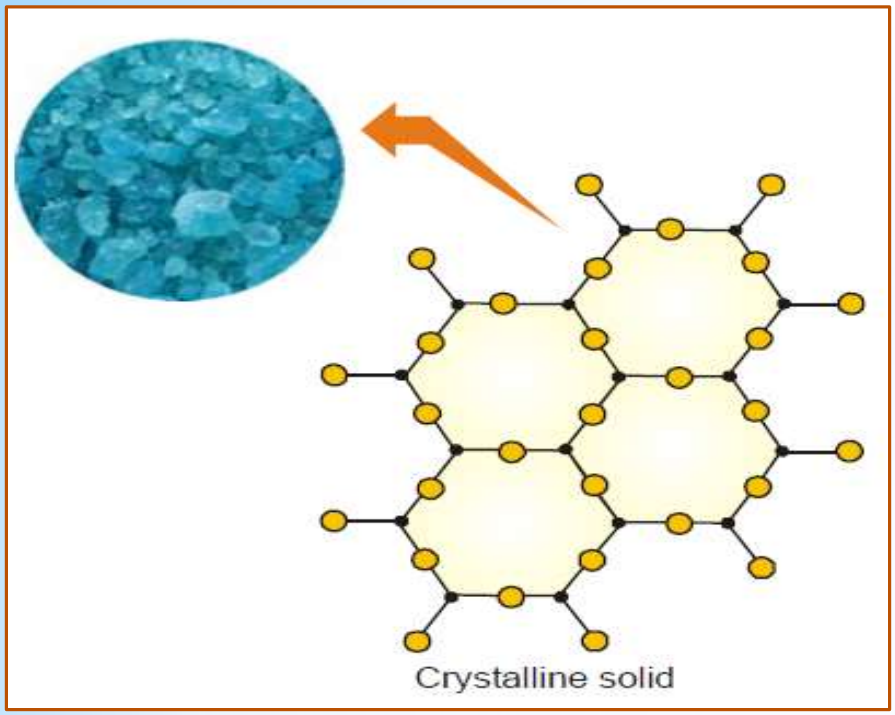


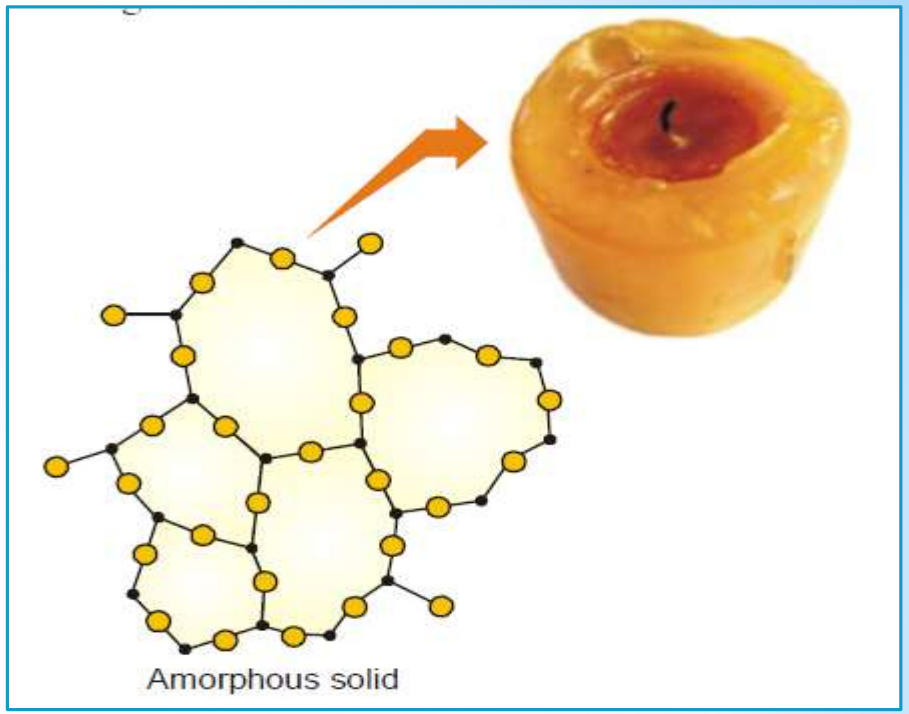
MARKETED PHARMACEUTICAL AEROSOL PRODUCTS

Metered Dose inhalers :

BRAND NAME	DRUG	USE
Flovent Diskus	Fluticasone	Asthma
Advair	Fluticasone and Salmeterol	Asthma
Aerobid	Flunisolide	Asthma
Qvar	Beclomethasone	Asthma
Proventil	Albuterol	Bronchospasm

SOLID STATE





A crystalline solid exists as small crystals, each crystal having a characteristic geometrical shape. In a crystal, the atoms, molecules or ions are arranged in a regular, repeating three-dimensional pattern called the crystal lattice. Sugar and salt are crystalline solids.

1. **Amorphous solid (Gr amorphous = no form)** has atoms, molecules or ions arranged at random and lacks the ordered crystalline lattice. Ex. rubber, plastics and glass.
2. In their disordered **structure- liquids.**
3. Thus glasses - super-cooled or highly viscous liquids.
4. E.g. Very old window panes that have become slightly thicker at the bottom due to gradual downward flow

Isotropy And Anisotropy

Isotropy:

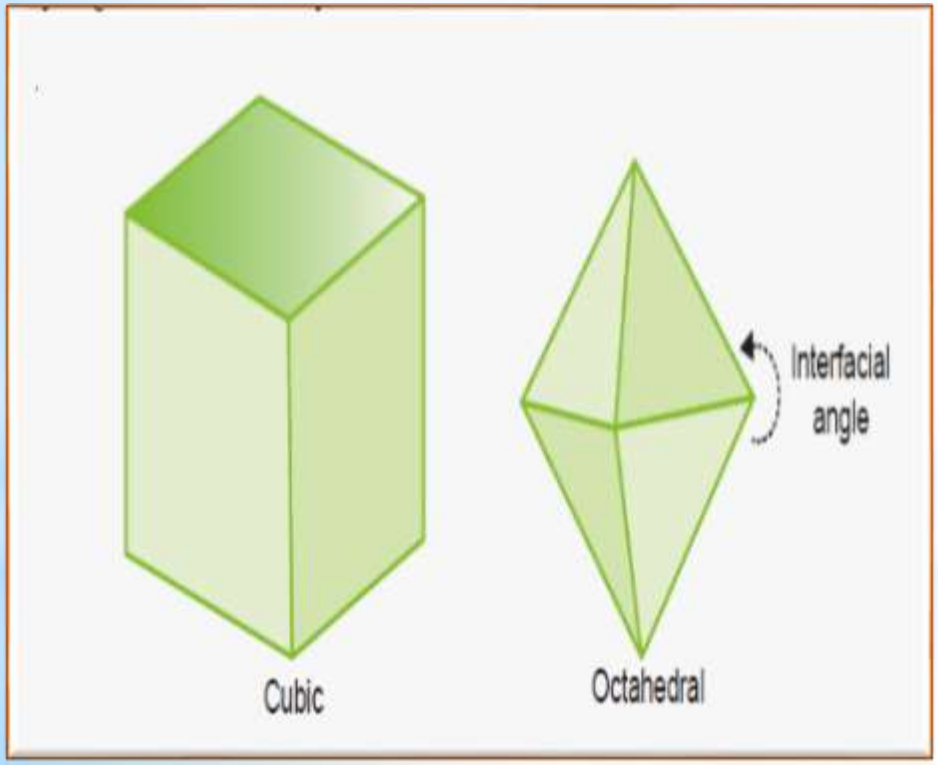
- **Amorphous substances** are said to be isotropic because they exhibit the same value of any property in all directions.
- Thus refractive index, thermal and electrical conductivities, coefficient of thermal expansion in amorphous solids are independent of the direction along which they are measured.

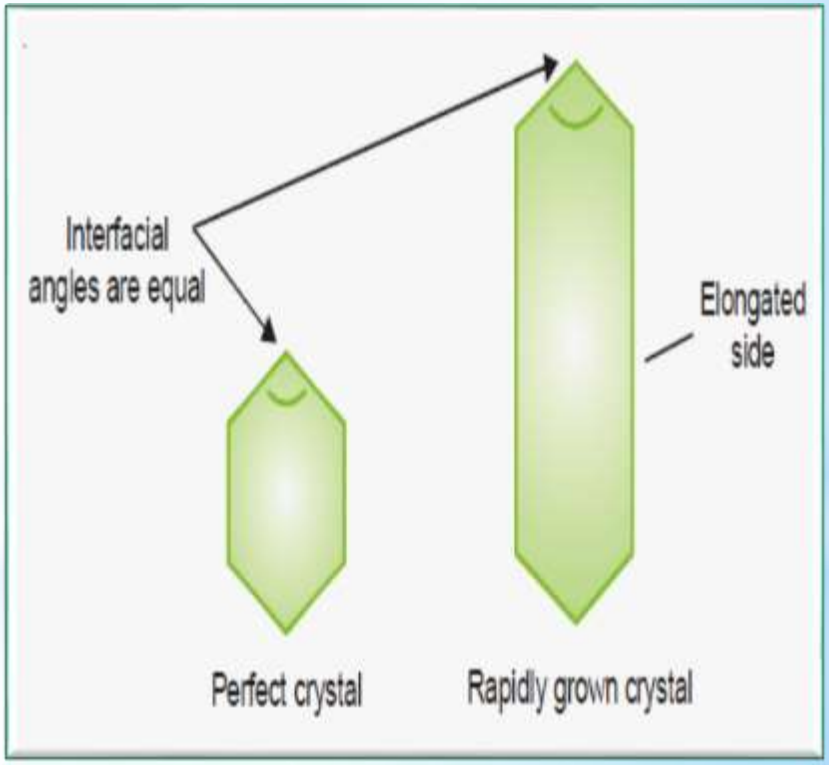
Anisotropy

- Crystalline substances on the other hand, are anisotropic and the magnitude of a physical property varies with directions.
- Crystal of silver iodide, the coefficient of thermal expansion is positive in one direction and negative in the other.
- Velocity of light in a Nicol prism splits up into two components, each travelling with different velocity (double refraction).

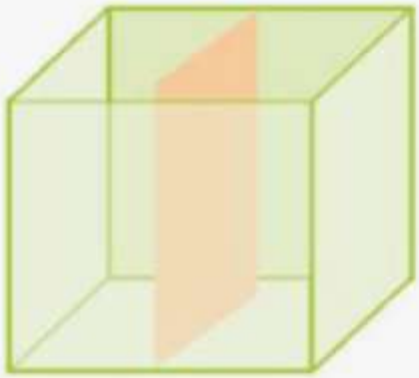
The Habit of a Crystal

- The external shape is called the **habit of the crystal**.
- The plane surfaces of the crystal are called **faces**.
- The angles between the faces are referred to as the **interfacial angles.**
- The interfacial angles for a given crystalline substance are always the same.

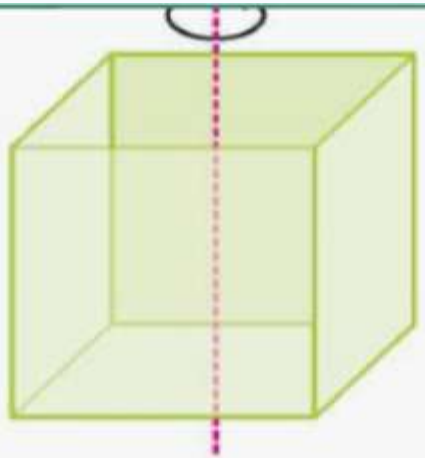




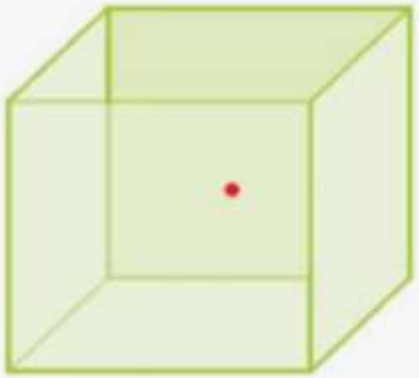
Symmetry of Crystals



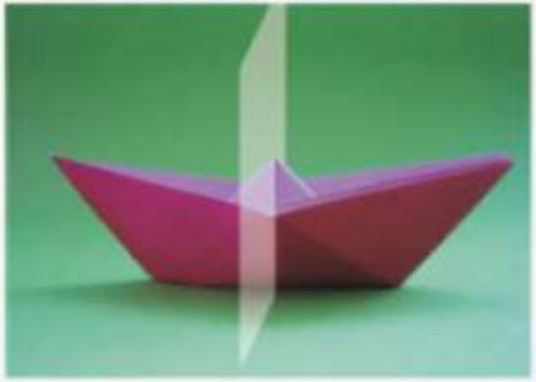
Plane of symmetry

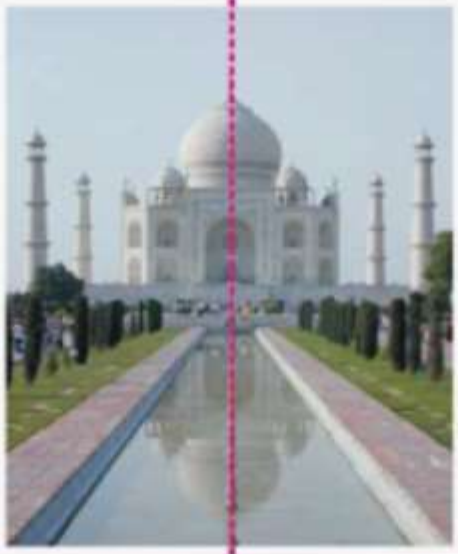


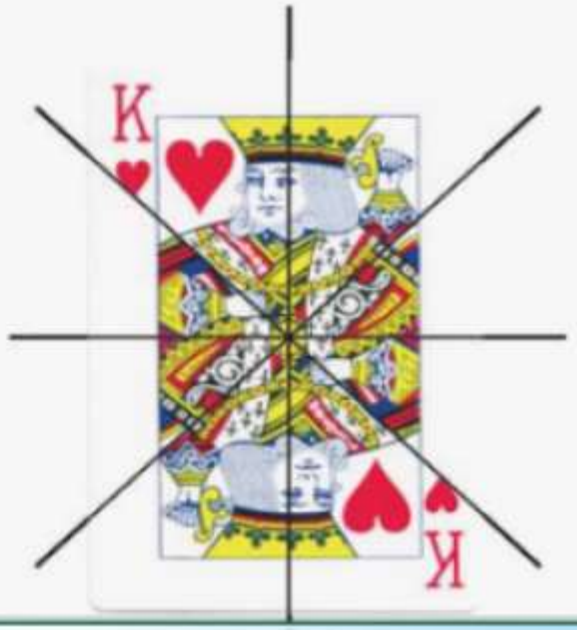
Axis of symmetry



Point of symmetry

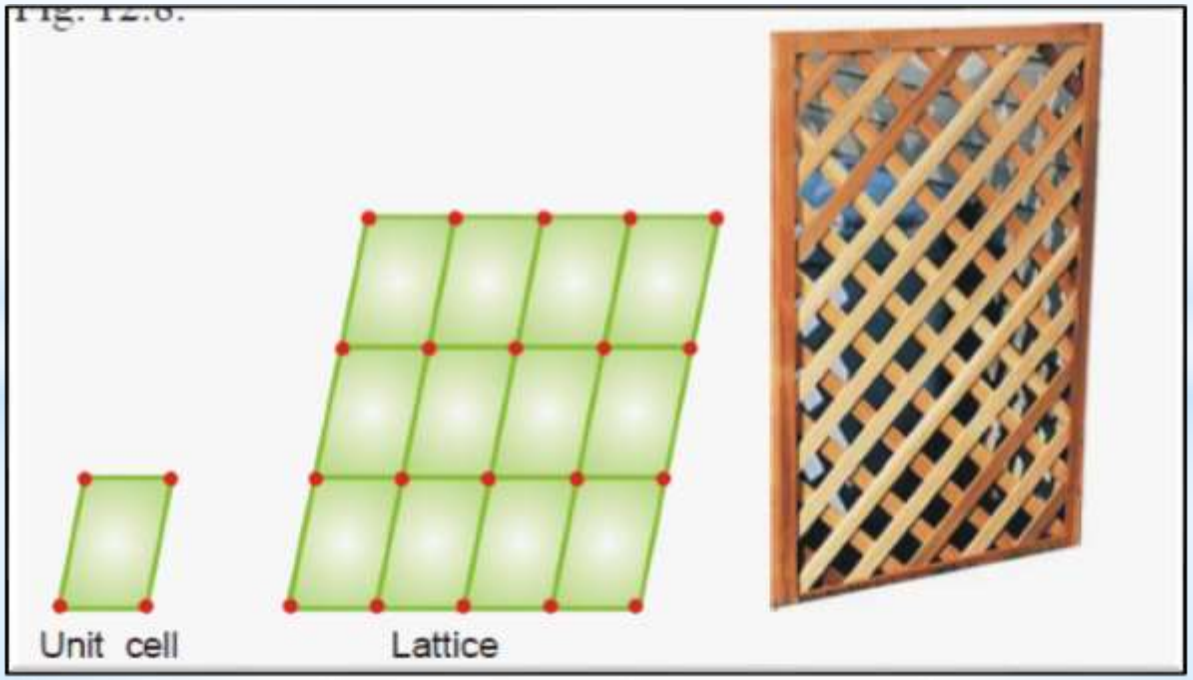






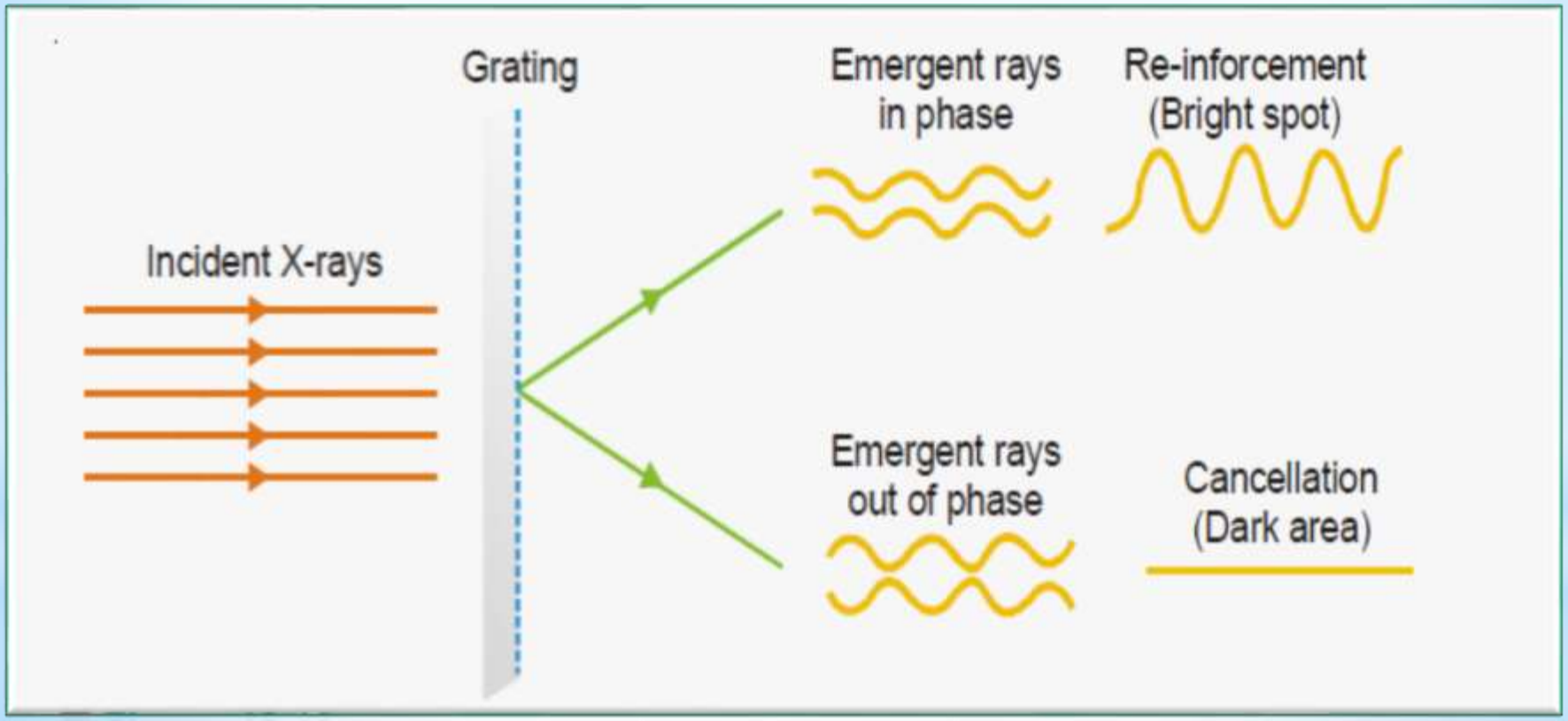
CRYSTAL STRUCTURE

1. The particles (atoms, ions or molecules) in crystals are highly ordered as is evident from their geometrical shapes, smooth surfaces and specific interfacial angles.
2. The particles in crystals are arranged in regular patterns that extend in all directions. The overall arrangement of particles in a crystal is called the **Crystal lattice, Space lattice or Simply lattice.**
3. The simple basic unit or the building block of the crystal lattice is called the **Unit cell.**



Methods of crystal analysis

1. X-Ray Diffraction (X-RAY CRYSTALLOGRAPHY)

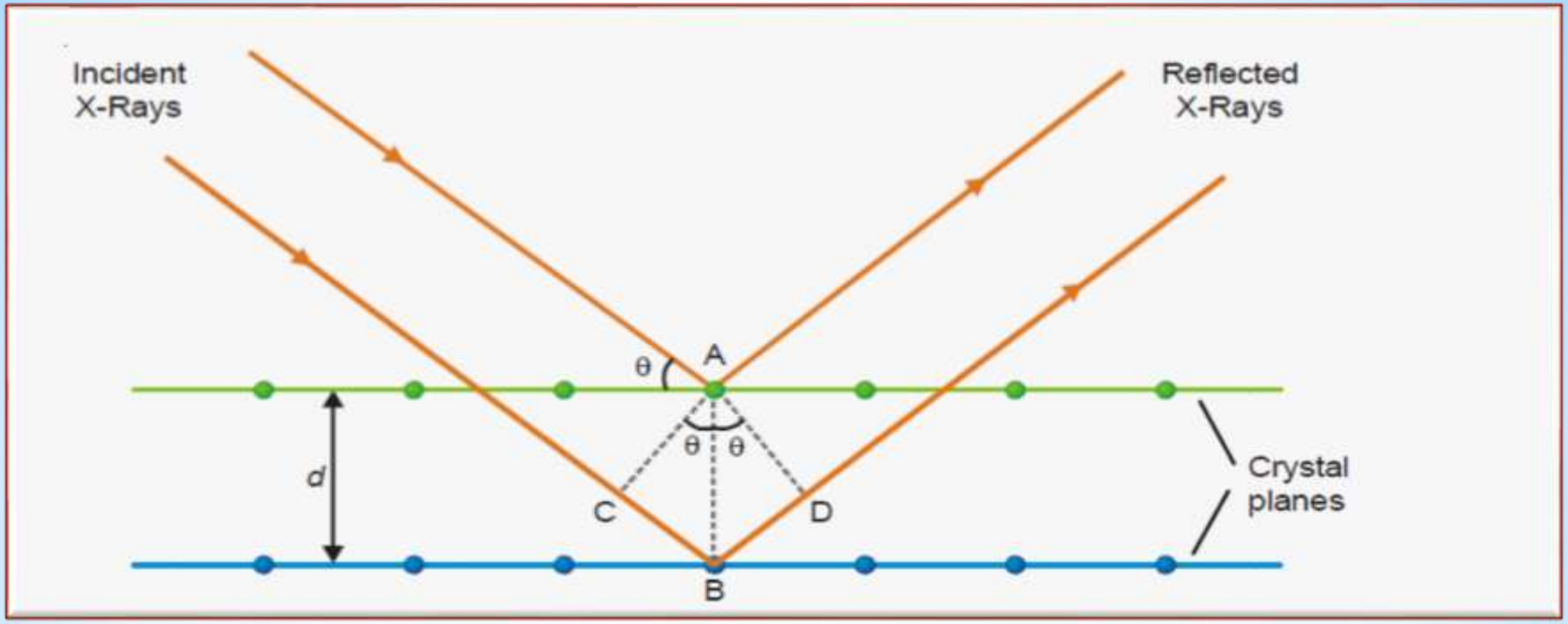


2. Bragg's Equation

In 1913 the father-and-son. W. L. Bragg and W. H. Bragg worked out a mathematical relation to **determine interatomic distances from X-ray diffraction patterns**. This relation is called the Bragg equation.

They showed that:

1. the **X-ray diffracted** from atoms in crystal planes obey the laws of reflection.
2. the two rays reflected by successive planes will be in phase if the extra distance travelled by the **second ray is an integral number of wavelengths**.



penetrate and get reflected from the second plane. These rays will reinforce those reflected from the first plane if the extra distance travelled by them (CB + BD) is equal to integral number, n, of wavelengths. That is,

$$n\lambda = CB + BD \qquad ...(i)$$

Geometry shows that

$$CB = BD = AB \sin\theta \qquad ...(ii)$$

From (i) and (ii) it follows that

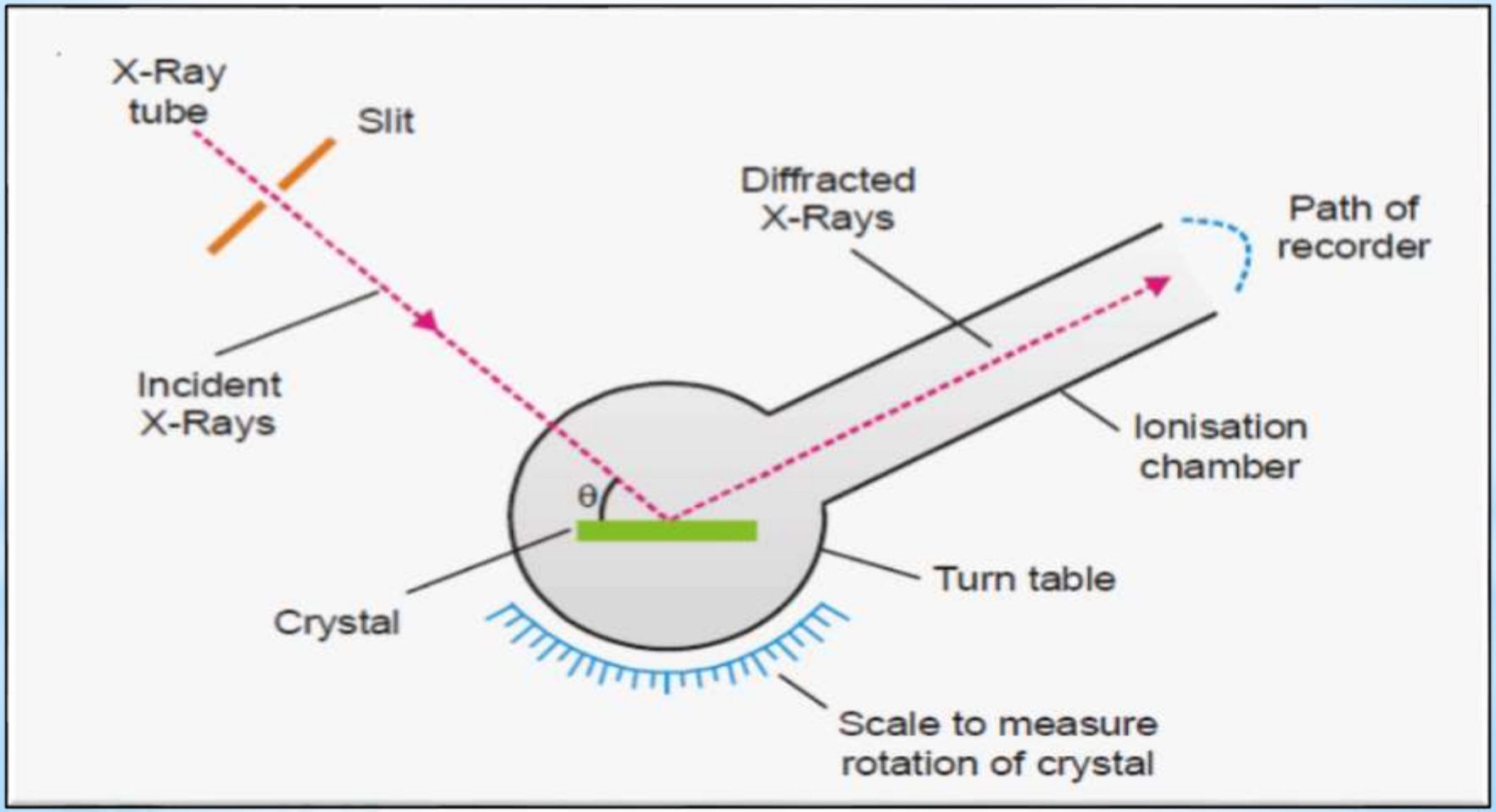
$$n\lambda = 2AB \sin\theta$$

or

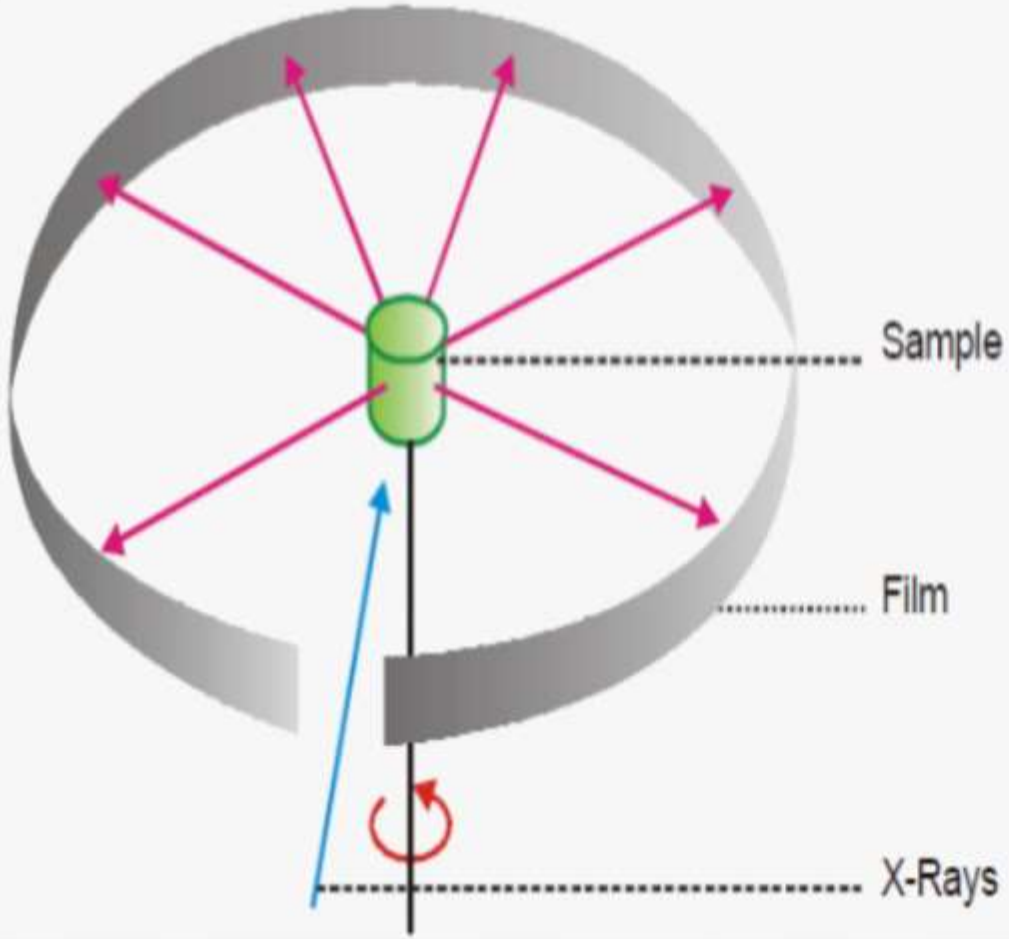
$$n\lambda = 2d \sin\theta$$

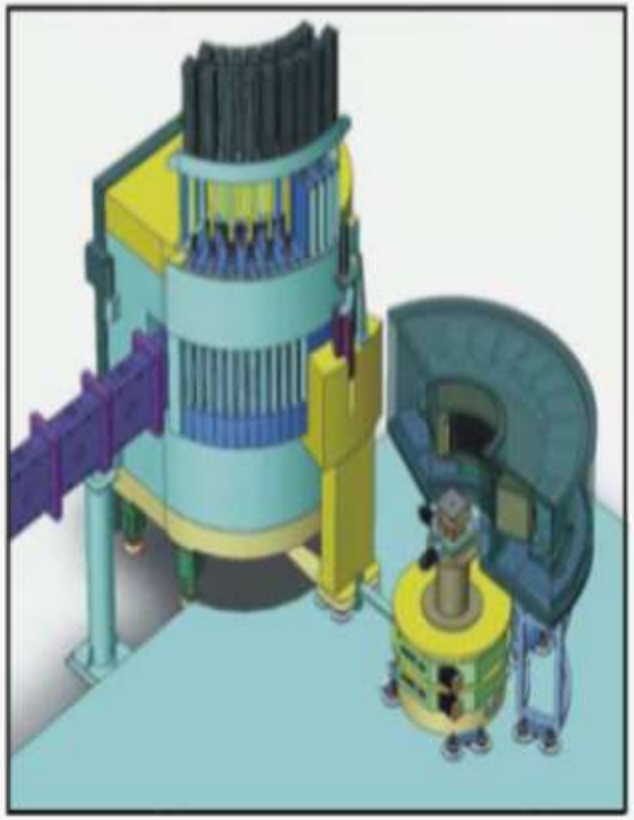
Measurement of Diffraction Angle

1. The rotating crystal method (Braggs. 1913)



2. The powder method (Debye and Scherrer, 1916)





Polymorphism & Allotropy

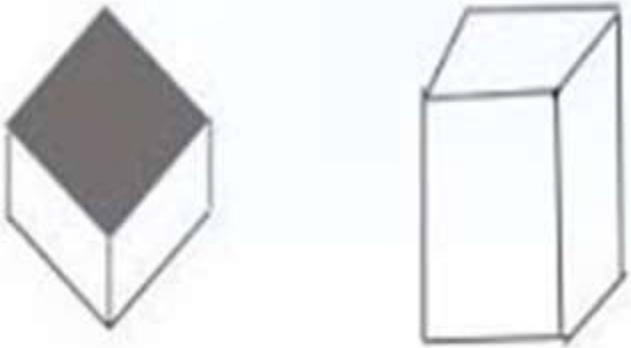
refer to Compound ***refer to an element***

Examples of Polymorphism:

$AgNO_3$

Crystalline form

Rhombohedral **Orthorhombic**



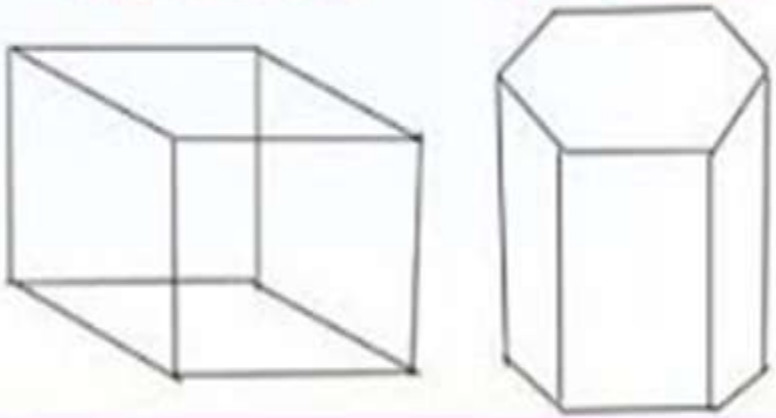
Examples of Allotropy:

Carbon

Crystalline form

Cubic
(Diamond)

Hexagonal
(Graphite)



1. Enantiotropy

$$\alpha\text{-Sulphur} \underset{}{\overset{95.6^{\circ}C}{\rightleftharpoons}} \beta\text{-Sulphur}$$

1. Definite temperature when the two forms have a common vapour pressure.
2. This temperature is known as the **transition temperature.**
3. One form is stable above this temperature and the other form below it.
4. **When the change of one form to the other at the transition temperature is reversible, the phenomenon is called Enantiotropy and the polymorphic forms enantiotropy**
5. For example, rhombic sulphur (a-Sulphur) on heating changes to monoclinic sulphur (p-Sulphur) at 95.6° C (transition **temperature).**

2. Monotropy

White phosphorus ⟶ Red phosphorus

1. It occurs when one form is stable and the other metastable.
2. The metastable changes to the stable form at all temperatures and the change is not reversible.
3. Thus there is no transition temperature as the vapour pressures are never equal.
4. Another example is graphite and diamond, graphite being stable and diamond metastable. although the change is infinitely slow.

3. Dynamic Allotropy

$$S_{\mu} \rightleftharpoons S_{\pi} \rightleftharpoons S_{\lambda}$$

These three forms of sulphur differ in molecular structure. S_{λ} is S_8, S_{π} is S_4 while formula of S_{μ} is ot known. The composition of the equilibrium mixture at 120°C and 444.6°C (b.p. of sulphur) is :

120°C	S_{μ} 0%	S_{π} 3.7%	S_{λ} 96.3%
444.6°C	S_{μ} 37%	S_{π} 4%	S_{λ} 59%

	Form I	Form II	Form III
Name	Stable	Unstable	Metastable
Melting Points	High	In-between	Low
Energy	Less	In-between	High
Solubility	Less	In-between	High
Dissolution	Less	In-between	High
Bioavailability	Less	In-between	High
Conversion	NO	Unstable to Stable Fast	Metastable to Stable Slow

Application:

1. Enhanced solubility

Form	*Melting point, °C*	*Aqueous solubility, mg/ml*
Riboflavin, Form I (stable)	291	60
Riboflavin, Form II	278	80
Riboflavin, Form III	183	1200

2. Improved dissolution

Metastable polymorph of methylprednisolone (Form II) has 1.4 times higher rate of dissolution than stable form (Form I).

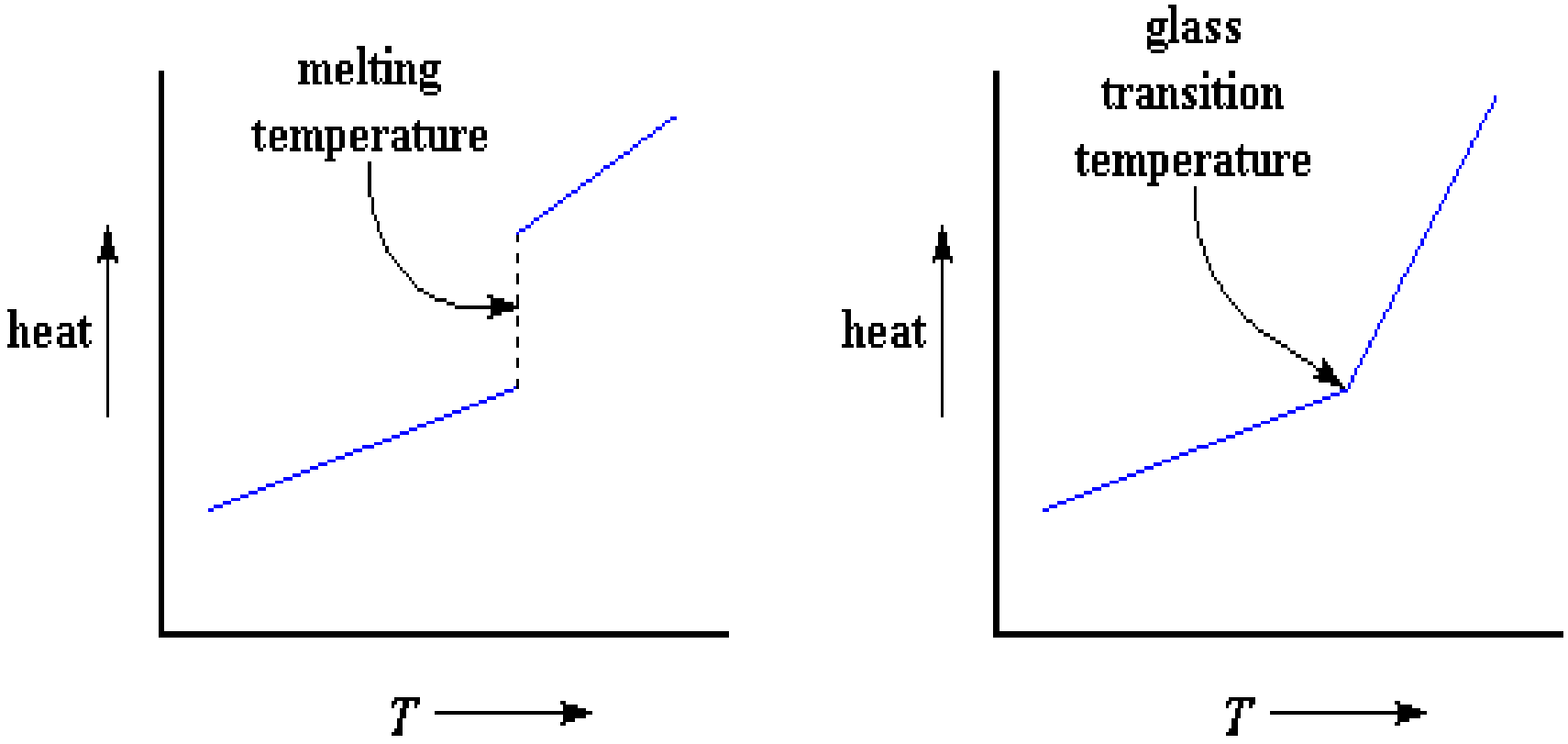
3. Enhanced absorption :

For example, metastable methylprednisolone (Form II) has 1.7 times higher rate of absorption than a stable crystal (Form I).

4. Manufacture of dosage forms :

Gamma (γ) form *Cocoa butter*	*Alpha (α) form* *Cocoa butter*	*Beta prime (β′) form* *Cocoa butter*	*Beta (β) form* *Cocoa butter*
metastable	metastable	metastable	stable
m.p-18 °C	m.p-22 °C	m.p-28 °C	m.p-34.5 °C

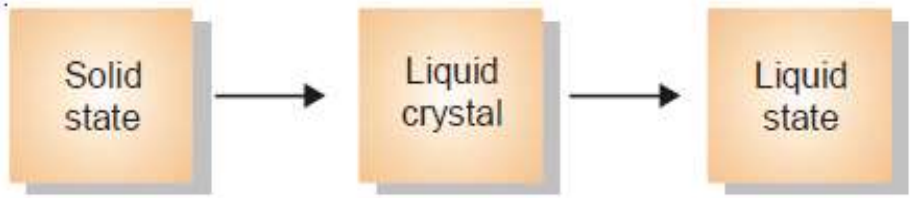
Glass Transition Temperature



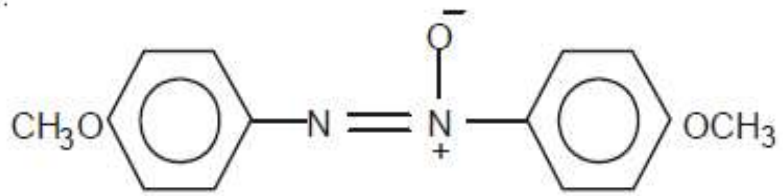
A heat vs. temperature plot for an crystalline polymer, on the left; and a amorphous polymer on the right.

WHAT ARE LIQUID CRYSTALS ?

Some organic solids having long rod-like molecules do not melt to give the liquid substance directly. They, instead, pass through an intermediate state called the **liquid crystal state,** often referred to as the **liquid crystal.** Thus the liquid crystal state is intermediate between the liquid state and the solid state.



One such substance that forms liquid crystal is



p-Ozoxyanisole

The liquid crystals have a structure between that of a liquid and that of a crystalline solid. In a liquid the molecules have a random arrangement and they are able to move past each other. In a solid crystal the molecules have an ordered arrangement and are in fixed positions. In a liquid crystals, however, molecules are arranged parallel to each other and can flow like a liquid. **Thus the liquid crystals have the fluidity of a liquid and optical properties of solid crystals.**

Types of Liquid Crystals

According to their molecular arrangement, the liquid crystals are classified into three types.

Nematic liquid crystals. They have molecules parallel to each other like soda straws but they are free to slide or roll individually.

Smectic liquid crystals. The molecules in this type of crystal are also parallel but these are arranged in layers. The layers can slide past each other.

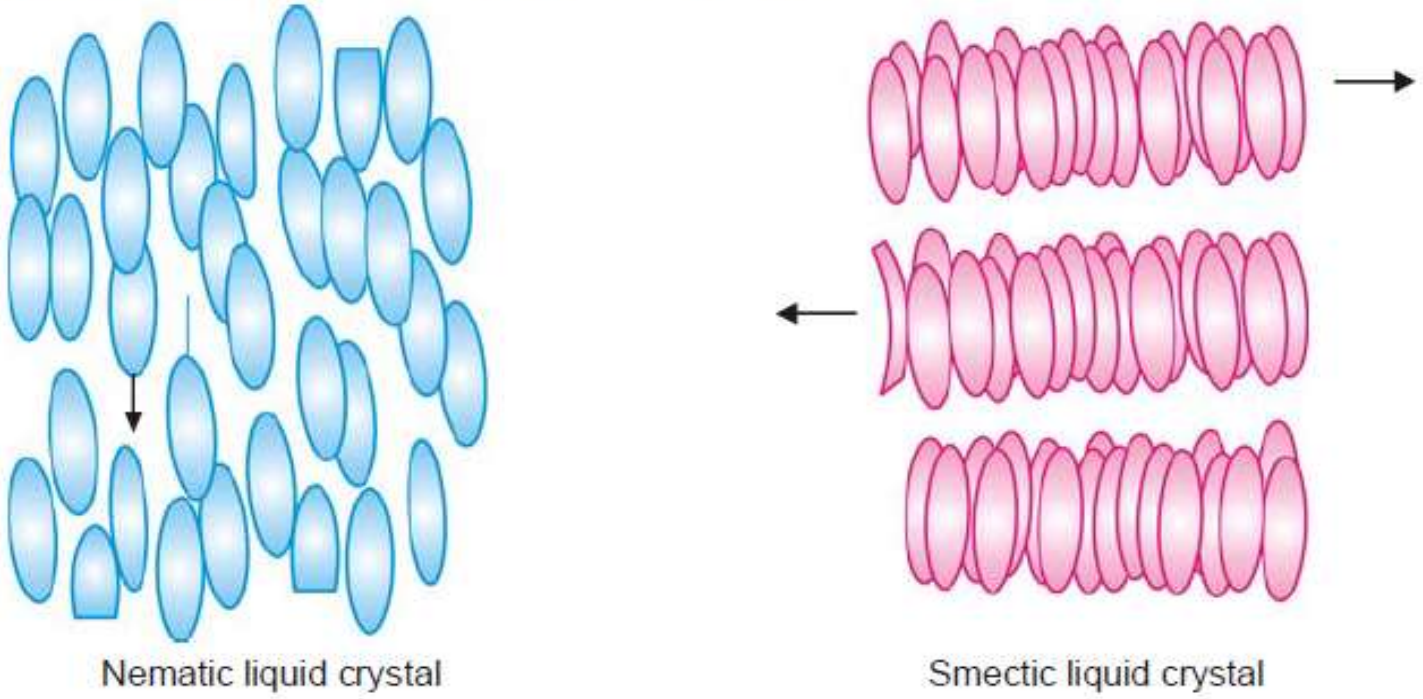
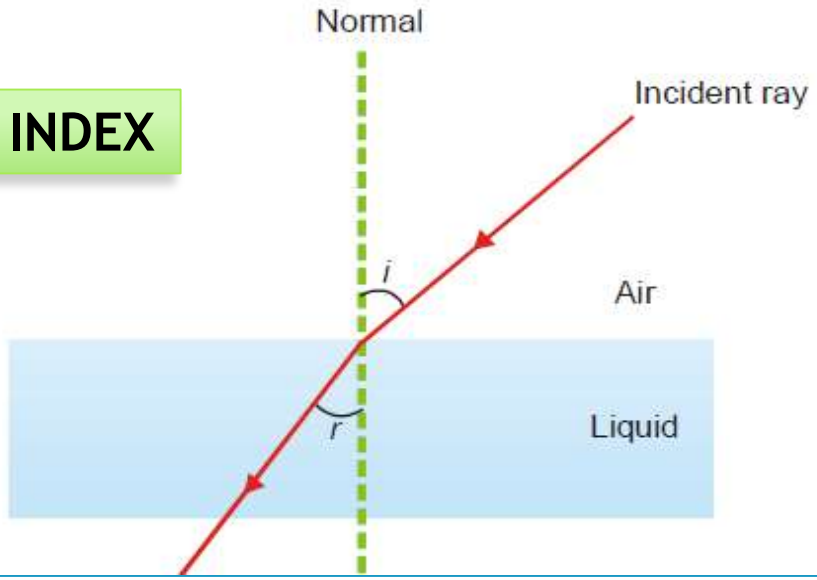


Figure 12.33
Nematic and Smectic liquid crystals.

Chloesteric liquid crystals. As in nematic crystals, in this type of crystal the molecules are parallel but arranged in layers. The molecules in successive layers are slightly rotated with respect to the layers above and below so as to form a spiral structure.

REFRACTIVE INDEX



The refractive index *(n)* of a substance is defined as the ratio of the velocity of light in vacuum or air. to that in the substance :

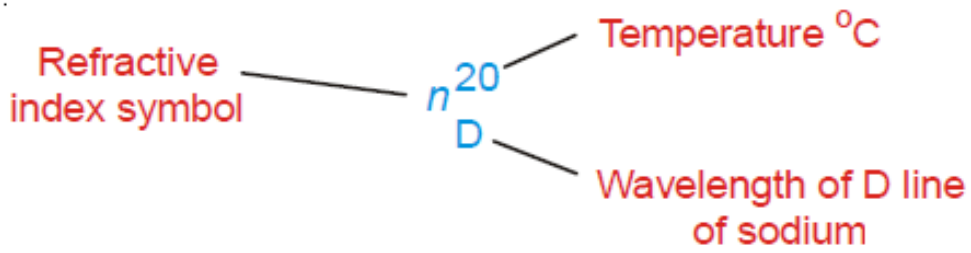
$$n = \frac{\text{Velocity of light in substance}}{\text{Velocity of light in air}}$$

When a ray of light passes from air into a liquid, its direction is changed. This change of direction is called *refraction*. The refractive index of the liquid with respect to air is given by Snelle's Law. According to it.

$$h = \frac{\textbf{Sin i}}{\text{Sin } r}$$

Where i is the angle of incidence and r the angle of refraction.

The wavelength of D-line of the sodium spectrum is generally used for the purpose. If the refractive index of a liquid is measured at 20°C and us ins D-line of sodium, it is represented by the following



Because refractive index is a ratio, it has no units.

SPECIFIC REFRACTION

Lorenz and Lorenz (1880) purely from theoretical considerations derived the following relation for the refractive power of substance.

$$R_s = \frac{n^2 - 1}{n^2 + 2} \cdot \frac{1}{d} \qquad \text{....(1)}$$

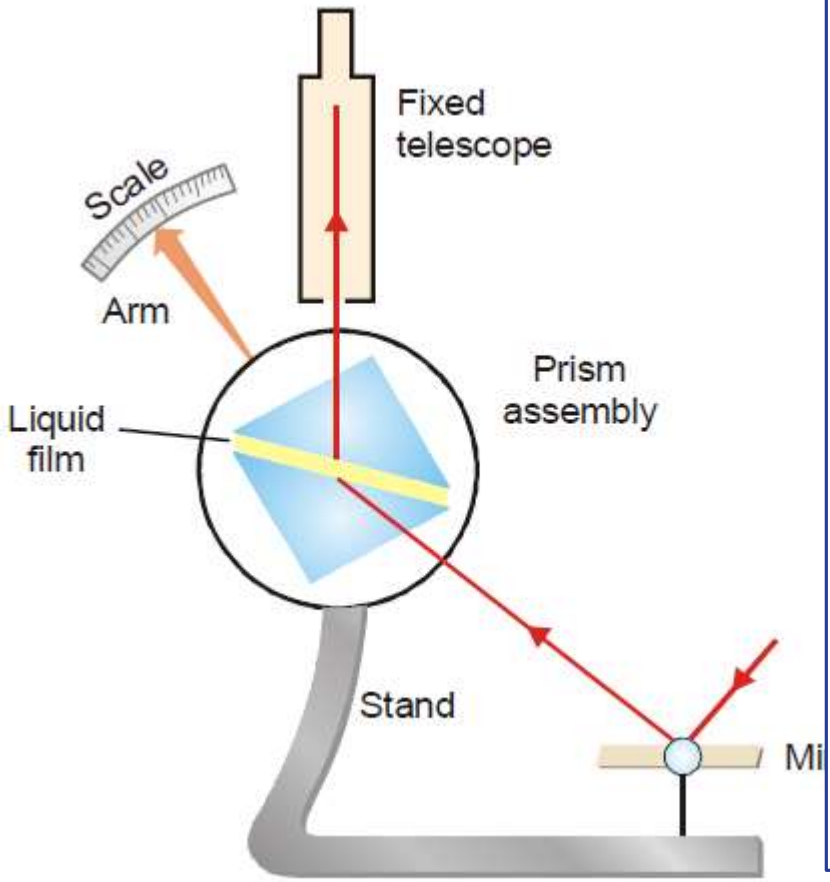
where R_s is the **Specific Refraction,** d the density and n the refractive index. The value of R_s was constant at all temperatures.

MOLAR REFRACTION

It is defined as **the product of specific refraction and molecular mass.** Thus molar refraction of a liquid (R_M) is obtained by multiplying equation (1) by molecular mass (M).

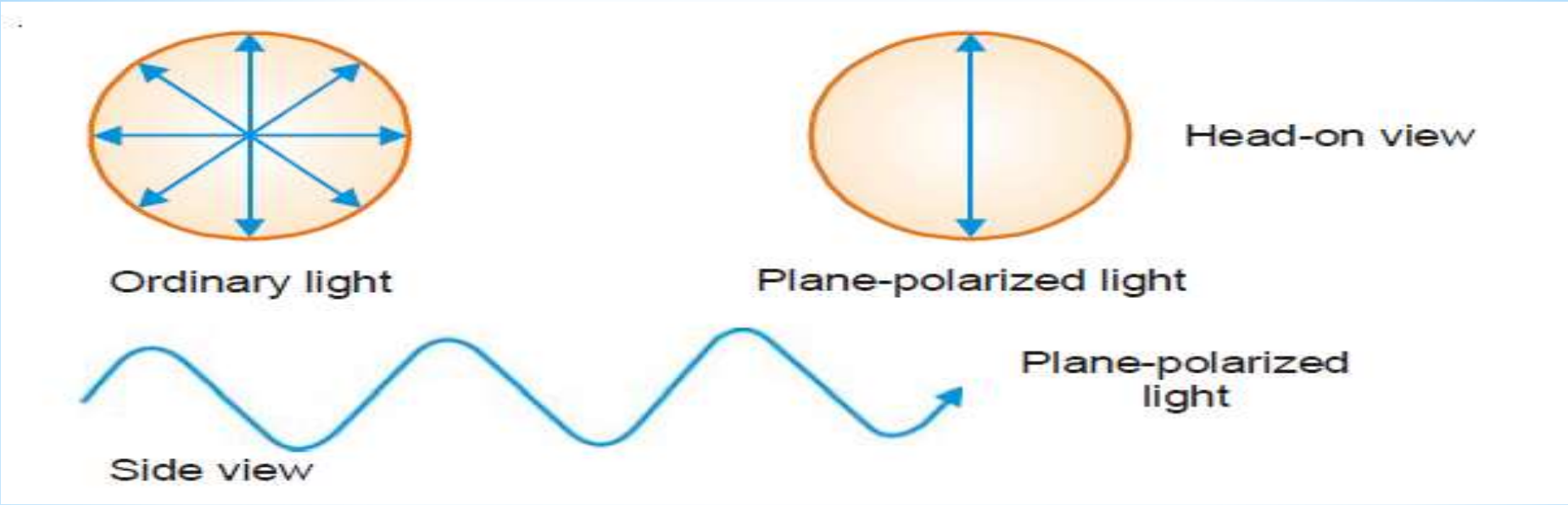
$$R_M = \frac{n^2 - 1}{n^2 + 2} \cdot \frac{M}{d} \qquad \ldots(2)$$

Determination of Refractive Index: **Abbe Refractometer**



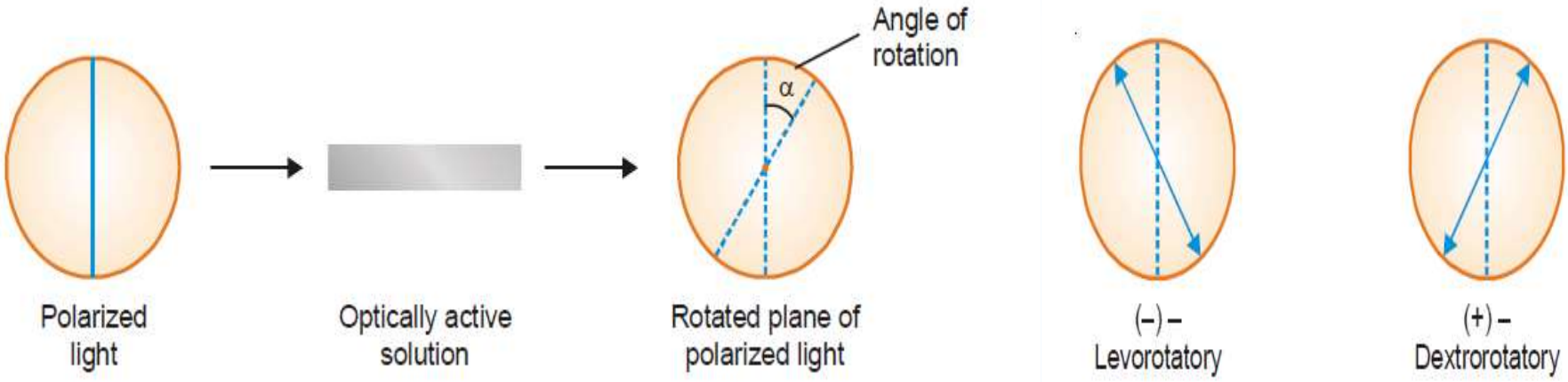
1. A thin film of the liquid is placed between the two prisms.
2. Light from a sodium lamp is made to fall on lower side of the lower prism with the help of a mirror.
3. Thus the view in the telescope appears to be divided into two bands, one bright and one dark.
4. The prism assembly is rotated with the help of a side knob till the cross wire of the telescope coincides with the edge of the bright band.
5. A pointer attached to the prism assembly indicates the refractive index on the scale calibrated to read refractive indices direct.

OPTICAL ACTIVITY



A beam of ordinary light consists of electromagnetic waves oscillating in many planes. When passed through a polarizer *(e.g.*, a Polaroid lens), only waves oscillating in a single plane pass through.

The emerging beam of light having oscillations in a single plane is said to be plane-polarize.



1. When plane-polarized light is passed through certain organic compounds, the plane of polarized light is rotated.
2. A compound that can rotate the plane of polarized light is called **optically active**.
3. A compound which rotates the plane-polarized light to the left (anticlockwise), is said to be **levorotatory.**
4. A compound that rotates the plane-polarized light to the right (clockwise), is said to be **dextrorotatory**.
5. By convention, rotation to the left is given a **minus sign (-)** and rotation to the right is given a **plus sign (+).**
6. For example, (-)-lactic acid is levorotatory and (+)-lactic acid is dextrorotatory.

SPECIFIC ROTATION

The rotation of plane-polarized light is an intrinsic property of optically active molecules. When a polarized beam of light is passed through the solution of an optically active compound, its plane is rotated through an angle α (**angle of rotation**). This rotation depends on the number of optically active molecules encountered. Therefore, α is proportional to both the concentration and the length of the sample solution.

The **specific rotation** which is characteristic of an optically active substance, is expressed as

$$[\alpha] = \frac{\alpha}{l \times c} \qquad \ldots(1)$$

where $[\alpha]$ = specific rotation in degrees

α = observed angle of rotation in degrees

l = length of the sample solution in decimeters (dm; 10 cm)

c = concentration of the sample solution in g/ml

Measurement of Optical Activity: Polarimeter

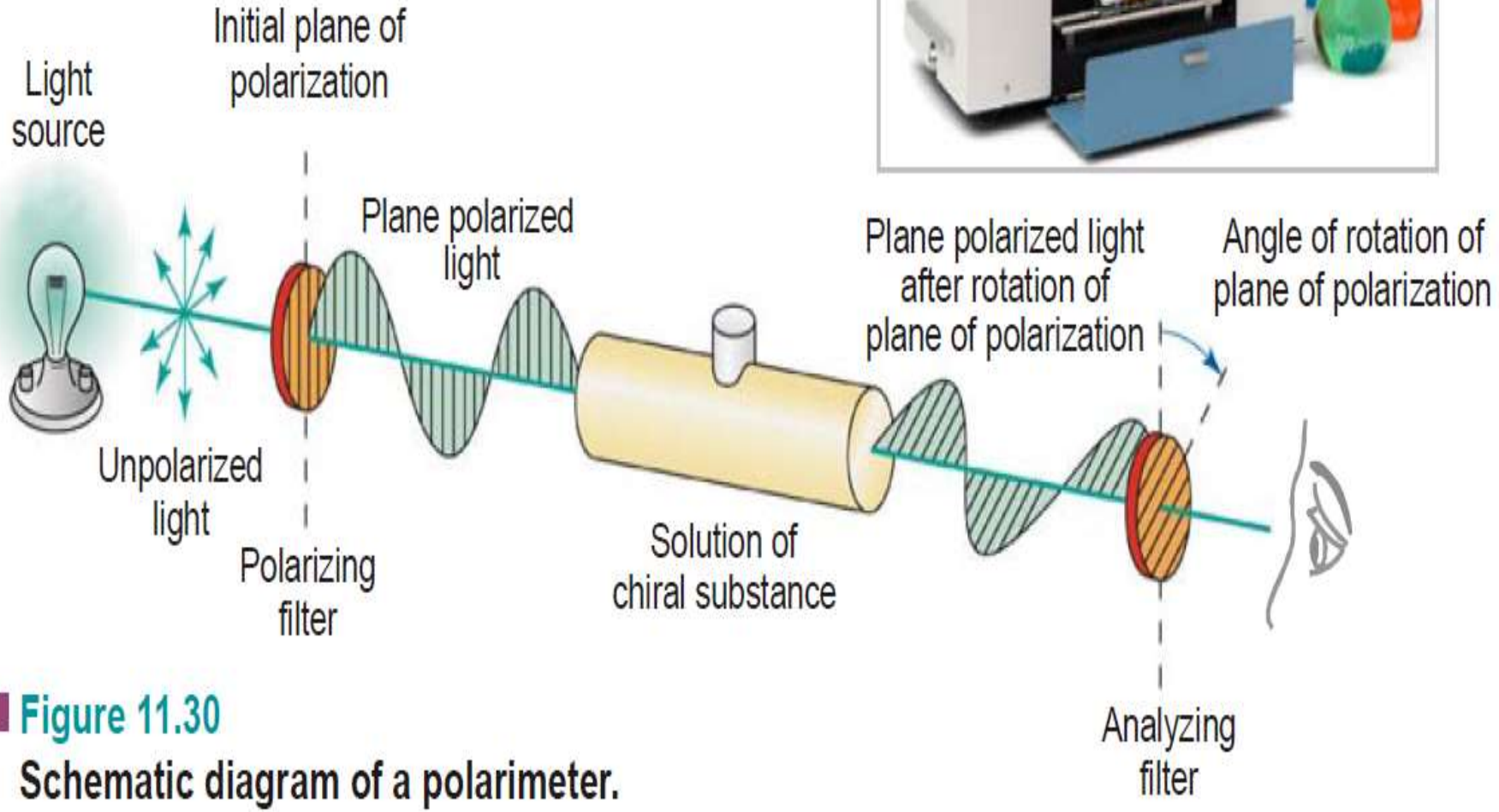
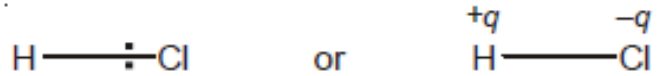


Figure 11.30
Schematic diagram of a polarimeter.

DIPOLE MOMENT

In a molecule such as HCl, the bonding electron pair is not shared equally between the hydrogen atom and the chlorine atom. The chlorine atom with its greater electronegativity, pulls the electron pair closer to it. This gives a slight positive charge ($+q$) to the hydrogen atom and a slight negative charge ($-q$) to the chlorine atom.



Such a molecule with a positive charge at one end and a negative charge at the other end is referred to as an **electric dipole** or simply **dipole.** The degree of polarity of a polar molecule is measured by its dipole moment, μ (Greek *mu*).

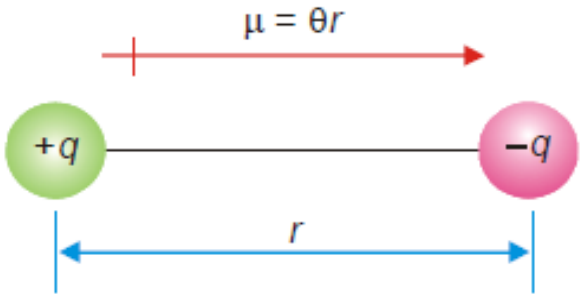


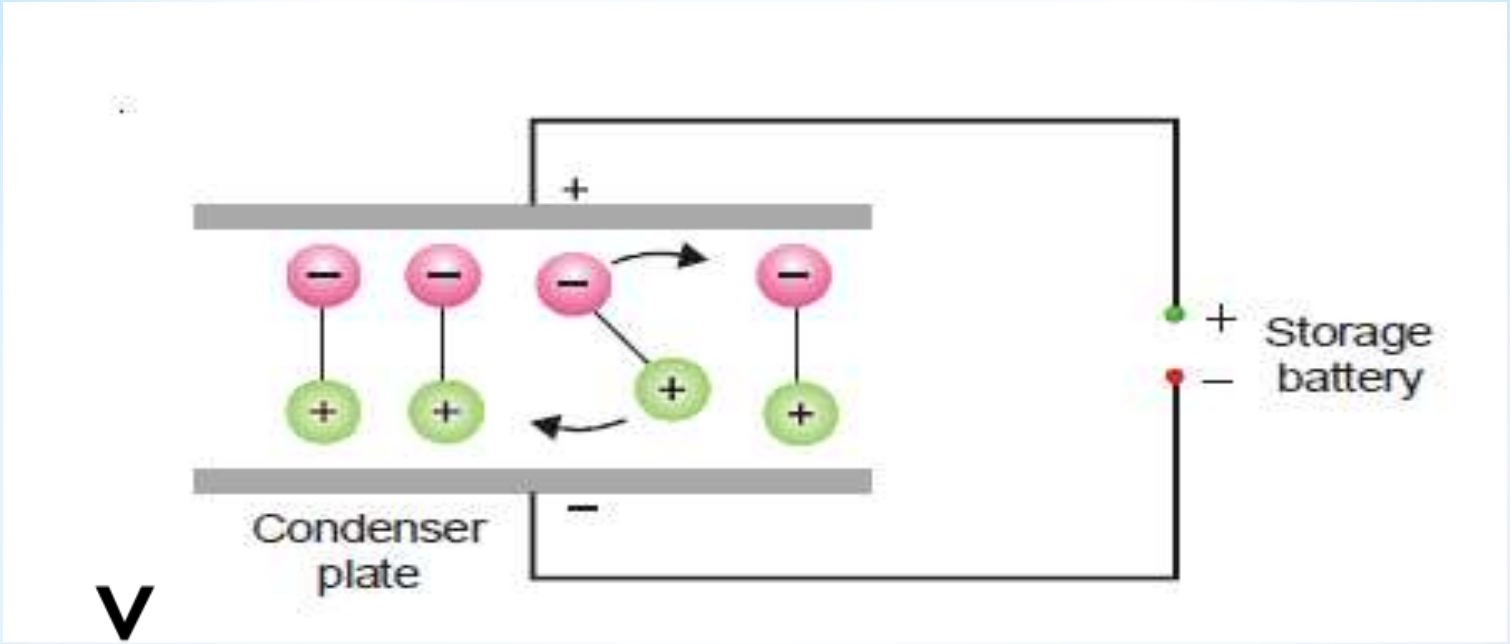
Figure 13.1
An electric dipole of the magnitude μ = θr.

The dipole moment of a polar molecule is given by the product of the charge at one end and the distance between the opposite charges. Thus,

$$\mu = q \times r$$

The dipole moment (μ) is a vector quantity. **It is represented by an arrow with a crossed tail.** The arrow points to the negative charge and its length indicates the magnitude of the dipole moment. Thus a molecule of HCl may be represented as

Determination of Dipole Moment: Electric Condenser



$$\varepsilon = \frac{V}{V_{/}}$$

The dielectric constant is a measure of the amount of electric potential energy, in the form of induced polarization that is stored in a given volume of material under the action of an electric field. It is expressed as the ratio of the dielectric permittivity of the material to that of a vacuum or dry air.

THEORY OF ELECTROLYTIC DISSOCIATION

$$H_2O + Na^+Cl^- \rightarrow Na^+ + Cl^- + H_2O$$

[Ionic compound]

[Strong electrolyte]

$$H_2O + HCl \rightarrow H_3O^+ + Cl^-$$

[Covalent compound]

[Strong electrolyte]

$$H_2O + CH_3COOH \rightleftharpoons H_3O^+ + CH_3COO^-$$

[Covalent compound]

[Weak electrolyte]

Dissociation Constants

- For a generalized acid dissociation,

$$HA(aq) + H_2O(l) \rightleftharpoons A^-(aq) + H_3O^+(aq)$$

the equilibrium expression would be

$$K_c = \frac{[H_3O^+]\,[A^-]}{[HA]}$$

- This equilibrium constant is called the acid-dissociation constant, K_a.

Dissociation constant:

- Since majority of the drugs are either weakly acidic or weakly basic, their solutions in water contain ionised and unionised species.
- In other words the drug undergoes dissociation in their aqueous solutions. The extent of ionisation depends on the pH of the solution. For example a weak acid will ionize mostly in alkaline pH while a weakly basic will ionize in an acidic medium. The unionised drug is more lipid soluble and thus readily absorbed.
- Hence gastrointestinal absorption of a drug can be improved if the extent of ionisation is reduced. The absorption of weakly acidic or basic drugs can be influenced by the following factors:

1. pH at the site of absorption
2. Dissociation constant
3. Lipid solubility of unionised drugs

Dissociation constant:

- At a particular ph the relative concentration of unionised and ionised species in a drug solution can be estimated with the help of Henderson-hasselbalch equation;
- For acidic drugs- pH= pKa + log [ionised drug]/[unionised drug]
- For basic drugs- pH= pKa + log [unionised drug]/[ionised drug]

Dissociation constant

- The contents of the stomach have pH ranging from 1-3.
  - weakly acidic drugs having a pKa value less than 4.3 were absorbed faster than those having pKa value within 2-4.3, strongly acidic drugs were almost not absorbed.
- pH of intestinal fluid vary from 5-8.
  - In case of basic drugs the absorption is faster for those having pKa values less than 8.5 than those having pKa values between9-12.

Dissociation constant

Drugs	pKa value	Ionization and site of absorption
Very weak acids Eg; pentobarbital.	>8	Unionized at all pH values. Can be absorbed throughout any region of the GIT.
Moderately weak acids Eg; aspirin.	2.5-7.5	Unionized in gastric pH and ionised at intestinal ph Mostly absorbed from stomach.
Stronger acids Eg;Disodiumchromoglycate.	<2	Ionized at all pH values. Poorly absorbed from GIT.
Stronger bases Eg; guanethidine.	>11	Ionized at all pH values Poorly absorbed from GIT.
Moderately weak bases Eg;codeine.	5-11	Ionised at gastric pH and unionized at intestinal ph. Mostly absorbed from intestine.
Very weak bases Eg; theophylline.	<5	Unionized at all pH values. Can be absorbed throughout any region of GIT.

Dissociation constant

Uses of Henderson hasselbalch equation:

- To determine pka.
- To predict solubility of any pH provided that the intrinsic solubility (Co) and pka are known.
- To facilitate selection of suitable salt forming compound.
- To predict the solubility and pH properties of the salt.

Methods to determine pka:

- Potentiometric method
- Conductivity method
- Dissolution rate method
- Spectrophotometric method
- Liquid-liquid partition method.

Relative humidity

Absolute Humidity

- Absolute humidity is the total mass of water vapor present in a given volume of air. It does not take temperature into consideration. Absolute humidity in the atmosphere ranges from near zero to roughly 30 grams per cubic meter when the air is saturated at 30 °C (86 °F).
- Absolute humidity is the mass of the water vapor , divided by the volume of the air and water vapor . The absolute humidity changes as air temperature or pressure changes. This makes it unsuitable for chemical engineering .

Relative Humidity

- **Relative humidity** (**RH**) is the ratio of the partial pressure of water vapor to the equilibrium vapor pressure of water at a given temperature. Relative humidity depends on temperature and the pressure of the system of interest. It requires less water vapor to attain high relative humidity at low temperatures; more water vapour is required to attain high relative humidity in warm or hot air. The relative humidity (RH} of an air–water mixture is defined as the ratio of the partial pressure of water vapor (PH_20) in the mixture to the equilibrium vapor pressure of water (P^*_{H20}) a flat surface of pure water at a given temperature :
- $RH = PH_20 / P^*_{H20}$
- Relative humidity is normally expressed as a percentage ; a higher percentage means that the air–water mixture is more humid ; a lower percentage means that the air-water mixture is less humid .

Calculation Of Relative Humidity

- The amount of water vapor in the air at any given time is usually less than that required to saturate the air. The relative humidity is the percent of saturation humidity , generally calculated in relation to saturated vapor density.
- Relative Humidity = Actual Vapor Density / Saturation Vapor Density * 100 %
- The most common units for vapor density are gm/m^3.

Humidity

1. Humidity is the amount of water vapour in the atmosphere .
2. Humidity is the water content of the mixture of water vapour and other element found in the air .
3. Humidity is used to determine the likelihood of precipitation , fog or dew .
4. Determining the humidity of a certain place provides a way to gauge the weather .

Relative Humidity

1. Relative humidity is one type of humidity .
2. Relative humidity is the percentage of water vapour in the air at a given temperature .
3. Relative humidity is used for climate control and how it affects the health, comfort and safety of humans .
4. Relative humidity is also used to ensure of machines, vehicle and buildings.

Determination

https://www.youtube.com/watch?v=2AZWGyoa2HM

Relative Humidity (%)

Dry-Bulb Temperature (°C)	Difference Between Wet-Bulb and Dry-Bulb Temperatures (C°)															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
−20	100	28														
−18	100	40														
−16	100	48														
−14	100	55	11													
−12	100	61	23													
−10	100	66	33													
−8	100	71	41	13												
−6	100	73	48	20												
−4	100	77	54	32	11											
−2	100	79	58	37	20	1										
0	100	81	63	45	28	11										
2	100	83	67	51	36	20	6									
4	100	85	70	56	42	27	14									
6	100	86	72	59	46	35	22	10								
8	100	87	74	62	51	39	28	17	6							
10	100	88	76	65	54	43	33	24	13	4						
12	100	88	78	67	57	48	38	28	19	10	2					
14	100	89	79	69	60	50	41	33	25	16	8	1				
16	100	90	80	71	62	54	45	37	29	21	14	7	1			
18	100	91	81	72	64	56	48	40	33	26	19	12	6			
20	100	91	82	74	66	58	51	44	36	30	23	17	11	5		
22	100	92	83	75	68	60	53	46	40	33	27	21	15	10	4	
24	100	92	84	76	69	62	55	49	42	36	30	25	20	14	9	4
26	100	92	85	77	70	64	57	51	45	39	34	28	23	18	13	9
28	100	93	86	78	71	65	59	53	47	42	36	31	26	21	17	12
30	100	93	86	79	72	66	61	55	49	44	39	34	29	25	20	16

Liquid Complexes (Complex Fluids)

Complex fluids are binary mixtures that have a coexistence between two phases:

- solid-liquid (suspensions or solutions of macromolecules such as polymers),
- Solid-gas (granular),
- liquid-gas (foams) and l
- Iquid-liquid(emulsions).

They exhibit unusual mechanical responses to applied stress or strain due to the geometrical constraints that the phase coexistence imposes.

The mechanical response includes transitions between solid-like and fluid-like behavior as well as fluctuations.

Their mechanical properties can be attributed to characteristics such as high disorder, caging, and clustering on multiple length scales.

Example

Shaving cream is an example of a complex fluid. Without stress, the foam appears to be a solid: it does not flow and can support (very) light loads. However, when adequate stress is applied, shaving cream flows easily like a fluid. On the level of individual bubbles, the flow is due to rearrangements of small collections of bubbles. On this scale, the flow is not smooth, but instead consists of fluctuations due to rearrangements of the bubbles and releases of stress.

Unit: III

Surface and Interfacial Phenomenon

Subject Teacher:
Dr. Prashant Khade

All the liquids have a tendency to assume a **shape having the minimal surface area exposed**. For a drop of a liquid, that shape is the sphere.

The boundary that forms between two phases [e.g. solid and liquid] is **called Interface**.

The **term surface** is normally used to denote interface, when one of the phase is gas [e.g. gas and liquid].

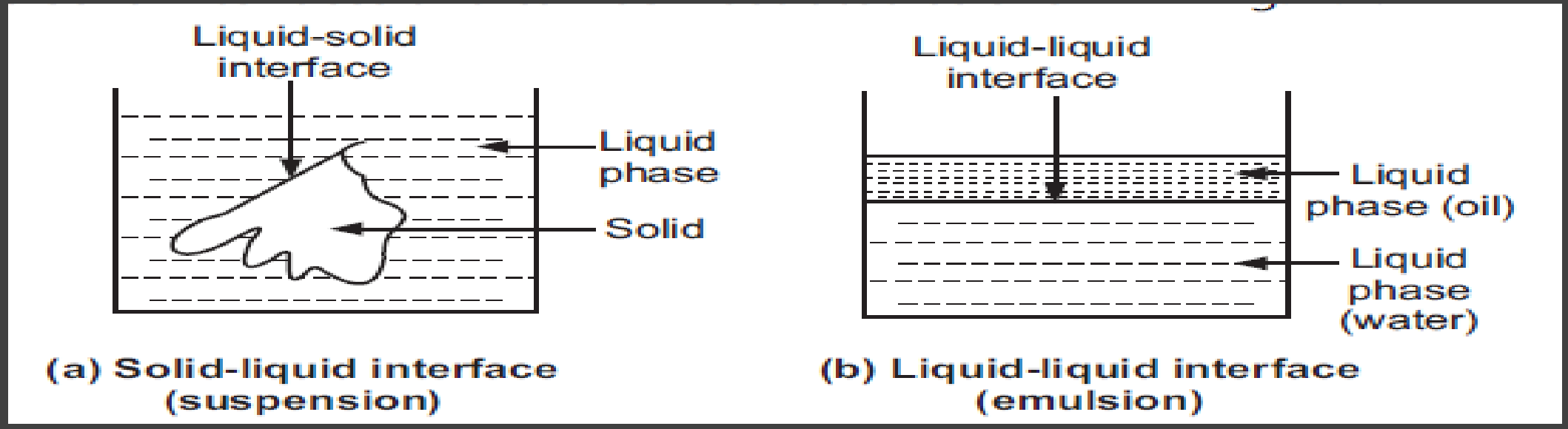
Surface tension is the property of liquids arising from the intermolecular forces of attraction.

Interfacial

phenomena is important property in the formulation of various dispersions like:

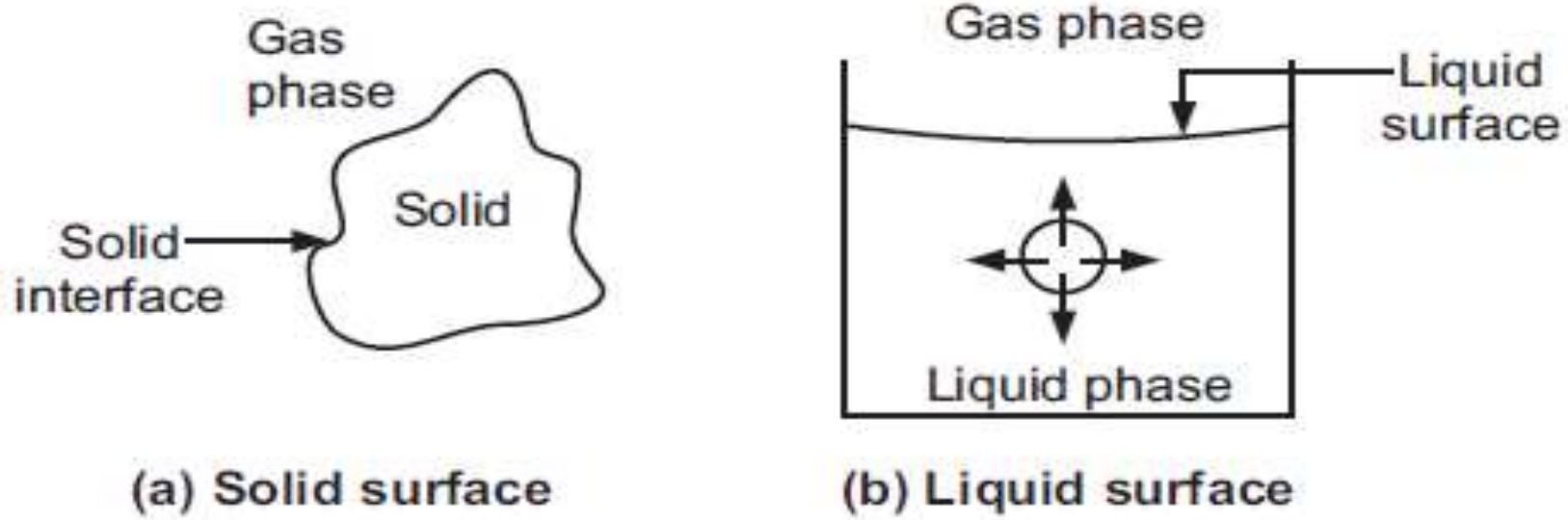
- Suspensions [solid in liquid]
- Emulsions [liquid in liquid]
- Foams [vapour in liquid].

Interfacial phenomena is significant in pharmacy and medicine that affects absorption of drugs from solid dosage forms, penetration of molecules through biologic membranes and stability of emulsion and suspensions.



(a) Solid-liquid interface (suspension)

(b) Liquid-liquid interface (emulsion)



(a) Solid surface

(b) Liquid surface

Representation of (a) solid-gas interface, (b) liquid-gas interface

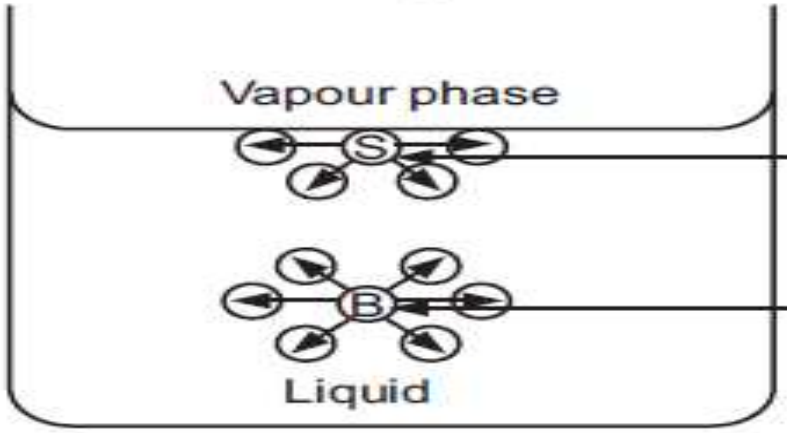
Surface Tension

Surface tension is defined as 'the force, in dynes, acting on the surface of the liquid at right angles to any line of length of surface, 1 centimeter'.

Surface tension of liquids can be explained by considering the inter-molecular forces between the molecules in the neighborhood.

In a liquid, molecules experience greater attraction from the neighboring molecules.

Such intermolecular attractions between like molecules are called **cohesive forces while attractions between unlike molecules are called adhesive forces.**



Liquid (water) phase contained in a vessel

1. Surface molecules. (Say S)
2. It is partially surrounded by other molecules.
3. There are no molecules above it.
4. Downward pull because adhesive forces.
5. Force acting on the surface is called Surface Tension.

1. A molecule in bulk phase of water (say B).
2. Attracted equally from all sides by neighbouring molecules.
3. Force acting on the molecule 'B' is zero.

Interfacial Tension

Interfacial Tension is defined as 'the force per unit length existing at the interface between two immiscible liquids'.

- **Miscibility:** When two liquids are completely miscible, the interface cannot be observed and hence, do not possess interfacial tension. Examples are water and ethyl alcohol.
- **Immiscibility:** When benzene and water are mixed together, these remain immiscible and form a boundary. Hence, benzene and water system has interface and shows interfacial tension.

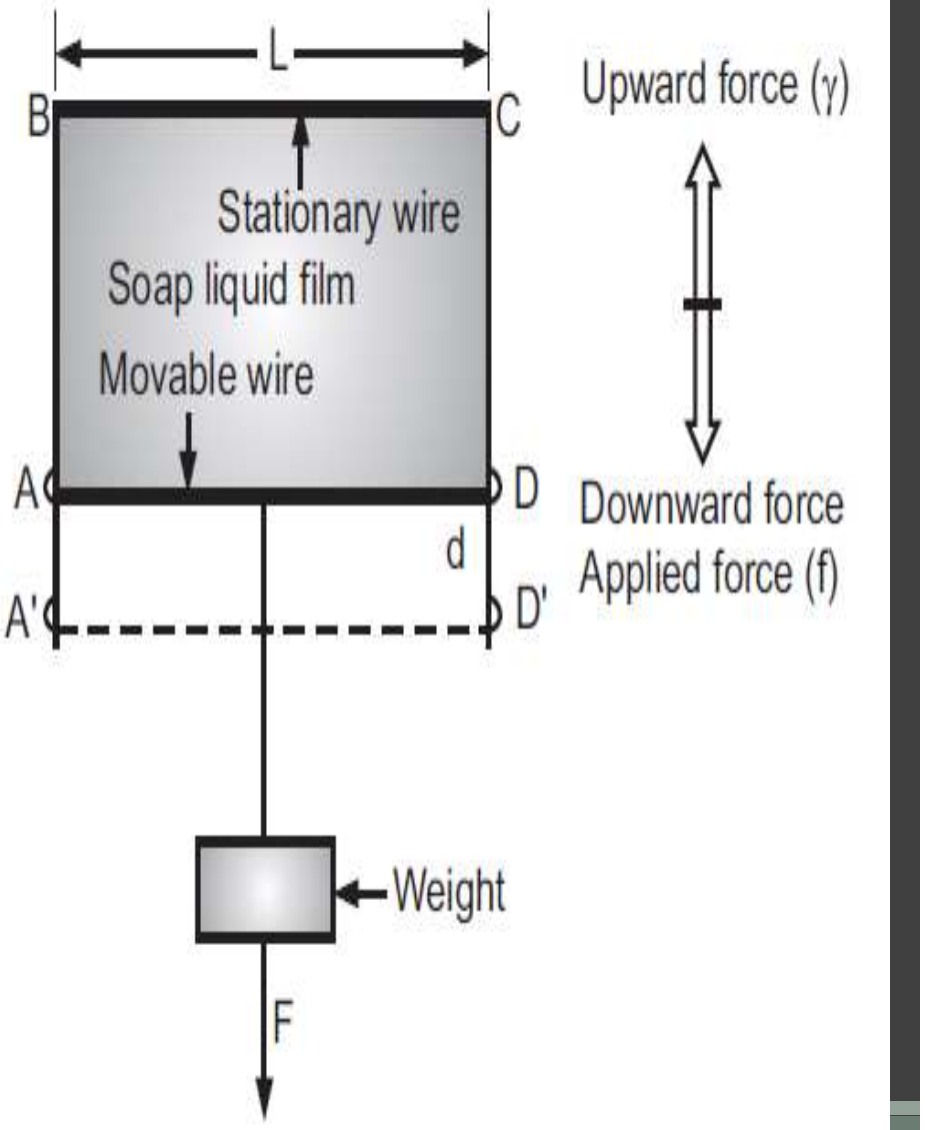
Interfacial tension (γ_{AB}) between two liquids A and B will be equal to the difference between the respective surface tensions (i.e. γ_A and γ_B).

$$\gamma_{AB} = \gamma_A - \gamma_B$$

Therefore, the interfacial tension should be between the surface tensions of the two liquids. This is indeed found to be true for many liquids (e.g., water and carbon tetrachloride). However, for many liquids, this prediction does not hold (e.g., water and cyclohexanol).

SURFACE FREE ENERGY

Surface free energy is defined as 'the work required to increase the area of a liquid by 1 sq. cm.



If force '*f*' is applied on AD (downward component), it shifts the movable wire from AD to A'D' (i.e. it is shifted by distance d). Then, the work done W is given by

$$\text{Work done (W)} = \text{Force} \times \text{Distance moved}$$

$$W = f \times d \quad \ldots (1.1)$$

The above force acts against the surface tension (upward component) 'γ' of the liquid, since surface tension tries to contract the liquid. There are two surfaces of each length 'L' on either side of the wire. The force acting on the surface is:

$$f = \gamma \times 2L \quad \ldots (1.2)$$

Substituting equation (1.2) in equation (1.1) gives

$$W = \gamma \times 2L \times d \quad \ldots (1.3)$$

Since, 2L × d is equal to increase in surface area 'ΔA' produced by extending the soap film, equation (1.3) changes to:

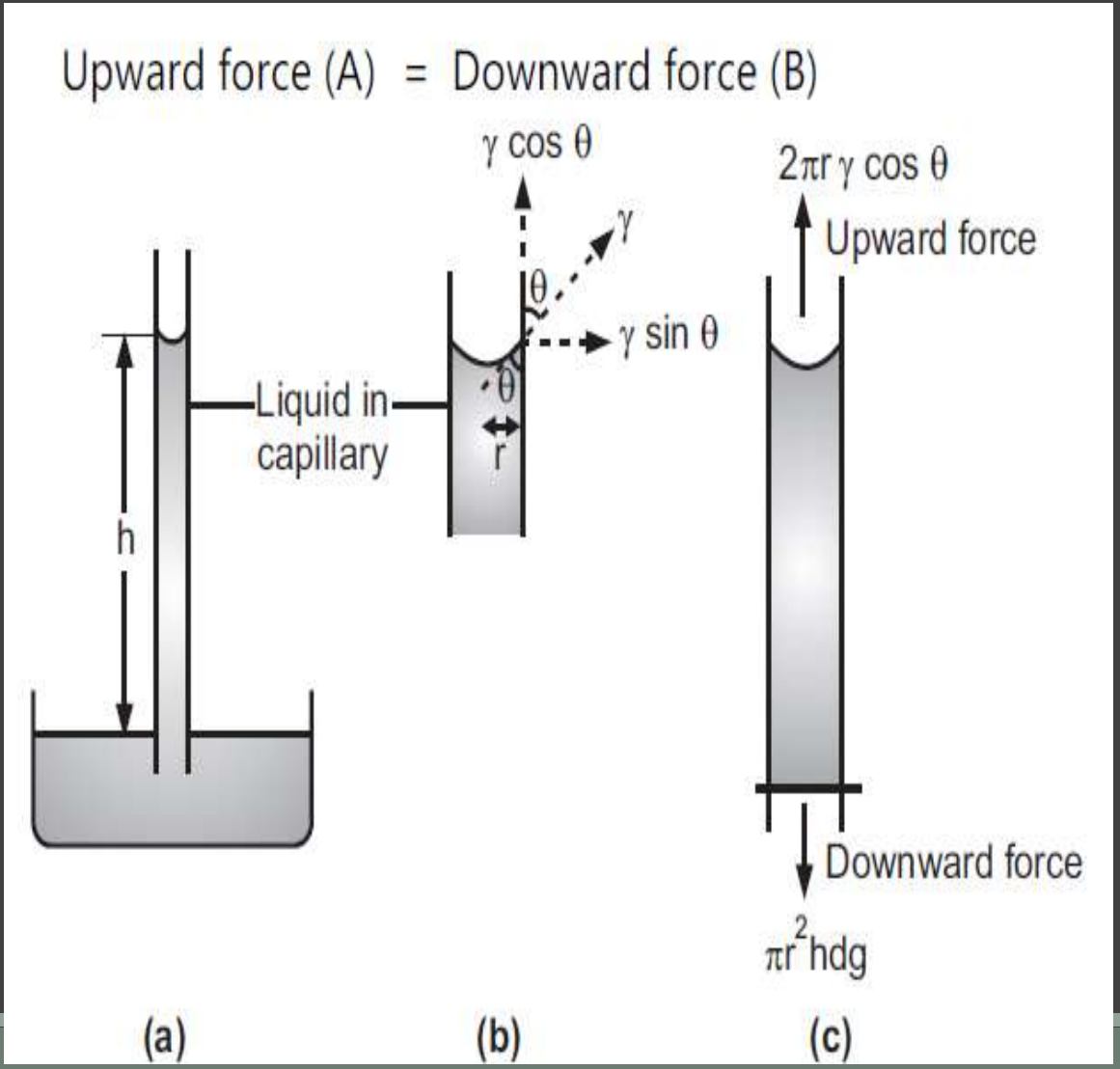
$$W = \gamma \times \Delta A$$

OR

$$\Delta G = \gamma \times \Delta A \quad \ldots (1.4)$$

DETERMINATION OF SURFACE TENSION AND INTERFACIAL TENSION

1. Capillary Rise Method



Upward force (A) = $\gamma \cdot \cos\theta \cdot 2\pi r$

Where,

γ : Surface tension,

θ : Contact angle between liquid and capillary wall,

$2\pi r$: Capillary inside circumference.

But, for water, 'θ' is considered insignificant ($\theta = 0$), because water completely wet capillary wall. The contact angle is very small for liquid used in pharmacy similar to water, Cos θ approaches 1. Then, $\cos\theta = 1$ and equation (1.6) change to

Upward force (A) = $\gamma .2\pi r$ ... (1.7)

Downward force (B):

The downward force is due to gravity and the weight of the liquid in the capillary rise.

$$\text{Downward force (B)} = \text{Mass of liquid in capillary} \times \text{Acceleration}$$

$$= \text{Volume} \times \text{Density} \times \text{Acceleration}$$

$$= \text{Cross-sectional area} \times \text{Height} \times \text{Density} \times \text{Acceleration}$$

$$= \pi r^2 \times h \times \rho \times g \quad \ldots (1.8)$$

Substitute equation (1.7) and (1.8) in (1.5)

$$\gamma \times 2\pi r = \pi r^2 \times h \times \rho \times g \quad \ldots (1.9)$$

$$\gamma = \frac{1}{2} r h \rho g \quad \ldots (1.10)$$

Equation (1.10) is used to calculate surface tension.

2. Drop Formation Method

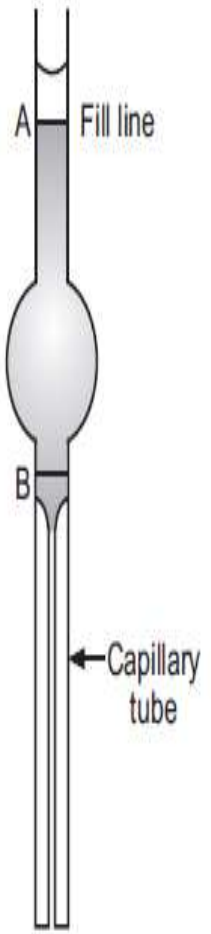


Fig. 1.6: Stalagmometer

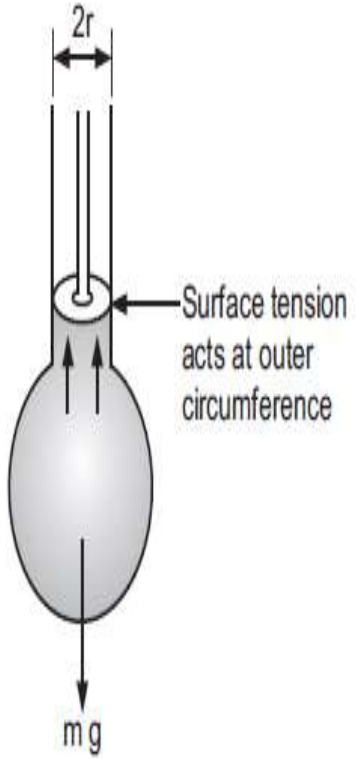


Fig. 1.7: A drop forming a tube having radius r

Upward force (A) = Downward force (B)

i.e. $\gamma 2\pi r = mg$

where,

γ : Surface tension,

$2\pi r$: Outer circumference of the tube,

m : Mass of the drop,

g : Acceleration due to gravity.

2. Drop Formation Method: A. Drop Weight Method

Then from equation (1.11)

$$m_1 g = 2\pi r \gamma_1 \quad \ldots (1.12)$$

$$m_2 g = 2\pi r \gamma_2 \quad \ldots (1.13)$$

Dividing (1.12) by (1.13)

$$\frac{m_1}{m_2} = \frac{\gamma_1}{\gamma_2} \quad \ldots (1.14)$$

2. Drop Formation Method: A. Drop Number Method

Let n_1 and n_2 be the number of drops produced by the same volume V of the two liquids. Thus,

The volume of one drop of liquid 1 = V/n_1

The mass of one drop of liquid 1 = $(V/ n_1)\rho_1$

Similarly,

The mass of one drop of liquid 2 = $(V/ n_2)\rho_2$

Where, ρ_1 and ρ_1 are the densities of two liquids.

Then from equation (1.14)

$$\frac{\gamma_1}{\gamma_2} = \frac{\left(\frac{V}{n_1}\right)\rho_1}{\left(\frac{V}{n_2}\right)\rho_2} = \frac{n_2\,\rho_1}{n_1\,\rho_2} \quad \ldots (1.15)$$

The value of ρ_1 is determined with a pyknometer. Knowing ρ_2 and γ_2 from reference tables, γ_1 can be calculated.

3. Ring-Detachment Method

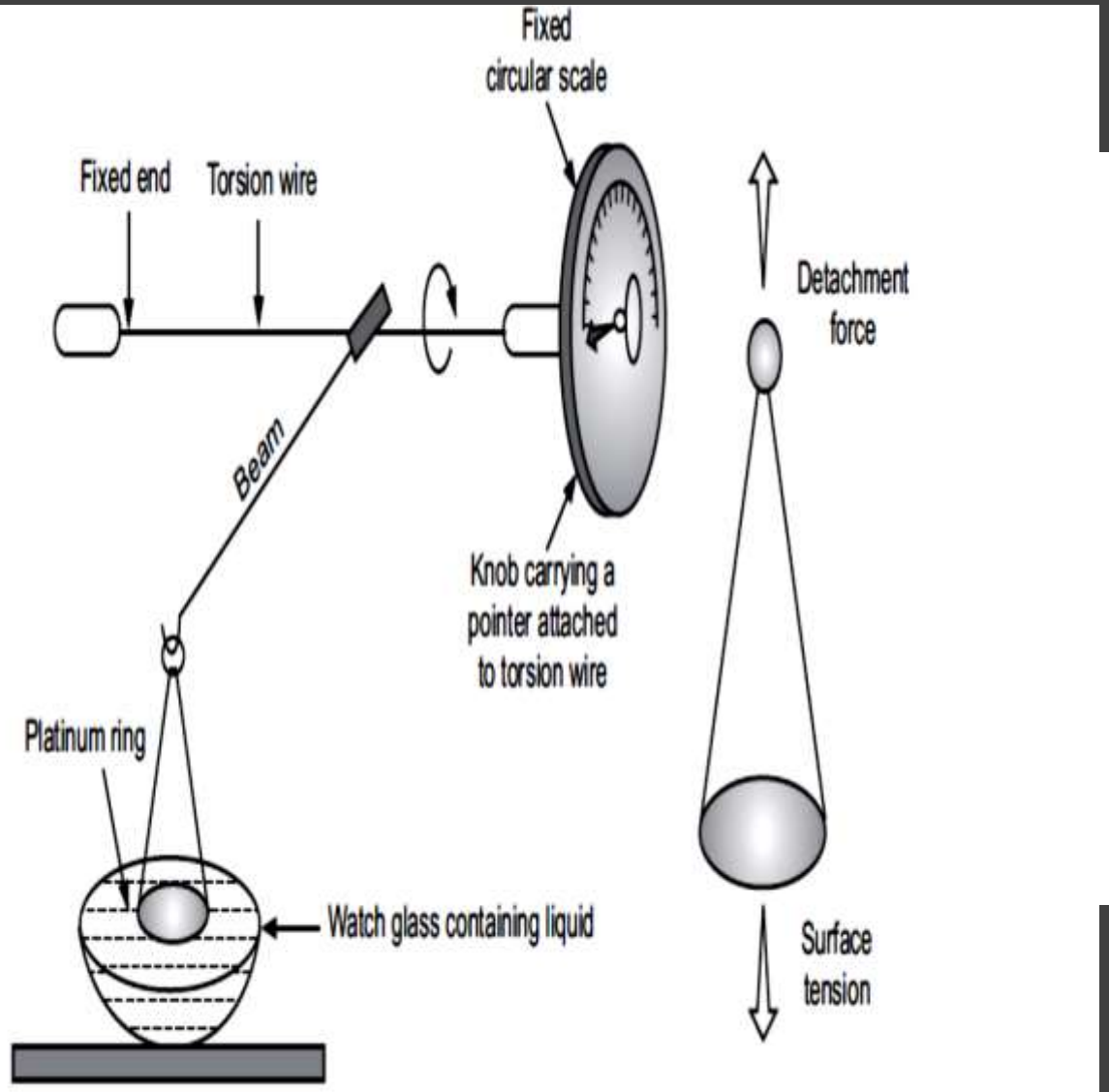


Fig. 1.8: du Nouy ring with a suspending hook

Fig. 1.9: du Nouy tensiometer

$$\text{Downward force} = mg = 2\pi r\,\gamma.2$$

$$= 4\pi r\,\gamma$$

Substitute equation (1.17) and (1.18) in equation (1.16)

$$F(\text{dial reading in dynes}) = 4\pi r\,\gamma$$

or

$$\gamma = \frac{F(\text{dial reading in dynes})}{4\pi r}$$

Surface tension is calculated from equation (1.19).

4. Maximum Bubble Pressure Method

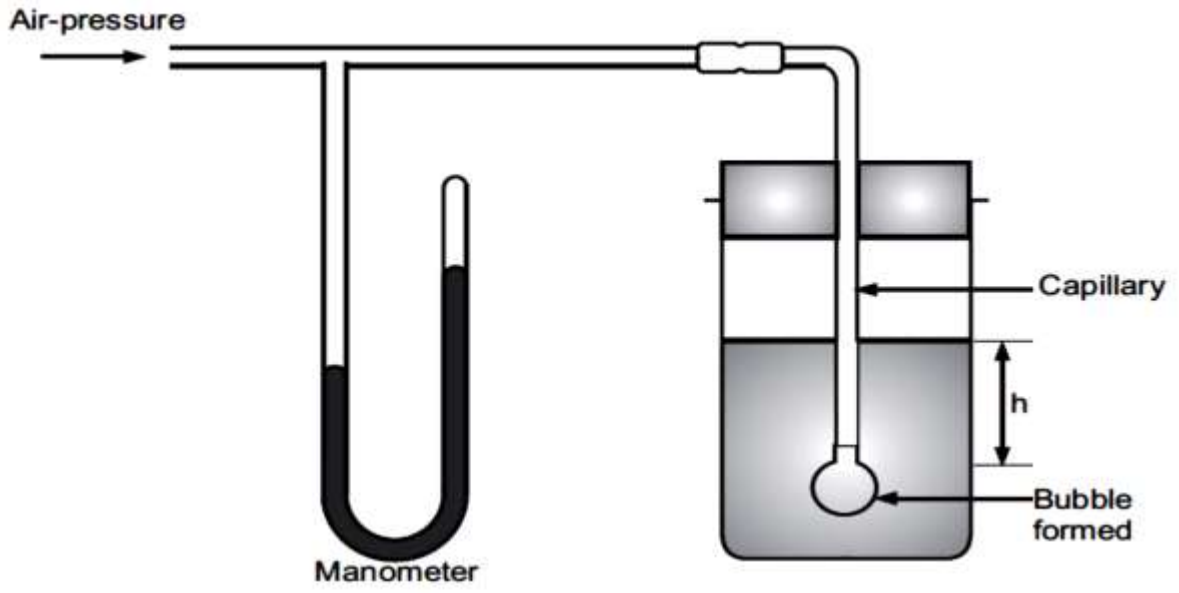
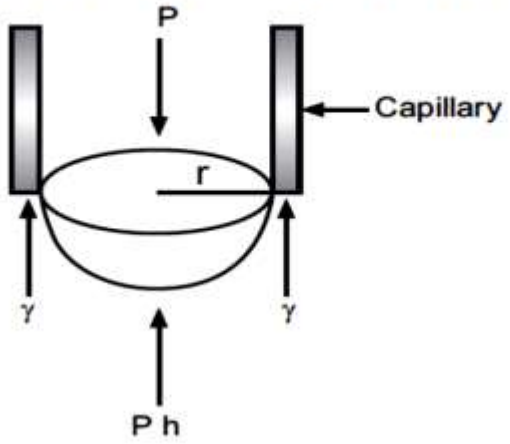


Fig. 1.10: Maximum bubble pressure apparatus



$$P \pi r^2 = P_h \pi r^2 + 2\pi r \gamma$$

$$P = P_h + \frac{2\gamma}{r}$$

$$P = h d g + \frac{2\gamma}{r}$$

SPREADING COEFFICIENT

1. Work of Adhesion:

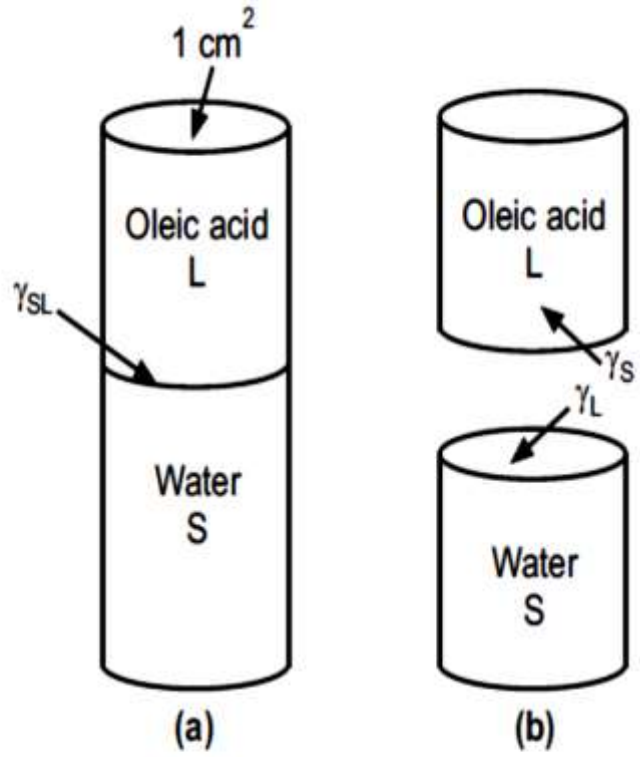


Fig. 1.12: The work of adhesion

The work of adhesion = $W_a = \gamma_L + \gamma_S - \gamma_{LS}$

2. Work of Cohesion:

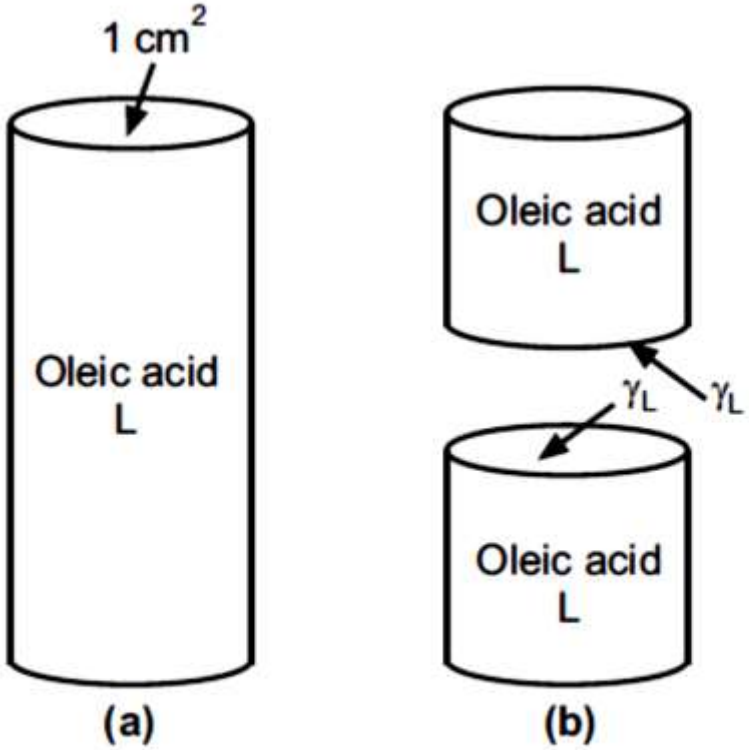


Fig. 1.13: The work of cohesion

The work of cohesion = $W_c = 2\gamma_L$

Equation for Spreading Coefficient:

When liquid (L, oleic acid) is spreading on sublayer liquid (S, water), the work of adhesion (W_a) is greater than the work of cohesion (W_c).

The spreading coefficient is obtained by equation:

$$S = W_a - W_c = (\gamma_L + \gamma_S - \gamma_{LS}) - 2\gamma_L \quad \ldots (1.24)$$

$$S = (\gamma_L + \gamma_{LS}) - \gamma_S \quad \ldots (1.25)$$

If $\gamma_S > (\gamma_L + \gamma_{LS})$, then 'S' is positive, indicate spreading,

If $\gamma_S < (\gamma_L + \gamma_{LS})$, then 'S' is negative, indicate no spreading.

For the organic liquid spread on water, initial spreading coefficient may be positive or negative, but the final spreading coefficient is always negative. Some examples of substances and their spreading coefficients are given in table 1.2.

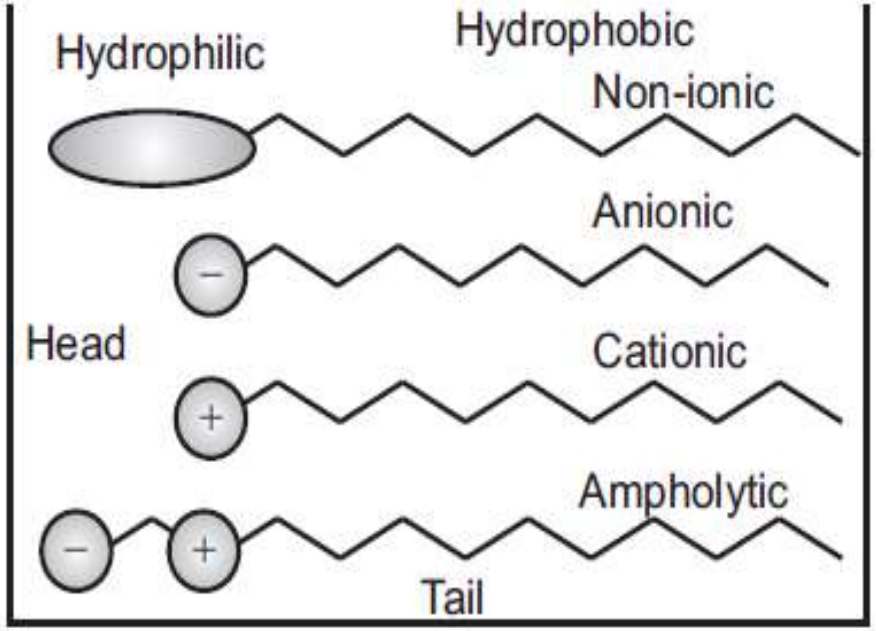
ADSORPTION AT LIQUID INTERFACES

1. Surfactant

Structure and Orientation of Surfactant

All surfactant molecules consist of at least two parts

1. The hydrophilic part: It is soluble in water called as polar head.
2. The hydrophobic part: It is insoluble in water called as non-polar tail.



Reasons for the reduction in the surface tension:

1. When surfactant molecules are adsorbed at the water surface means that the surfactant molecules replace some of the water molecules in the surface.
2. The forces of attraction between surfactant and water molecules are less than those between two water molecules; hence the contraction force is reduced.

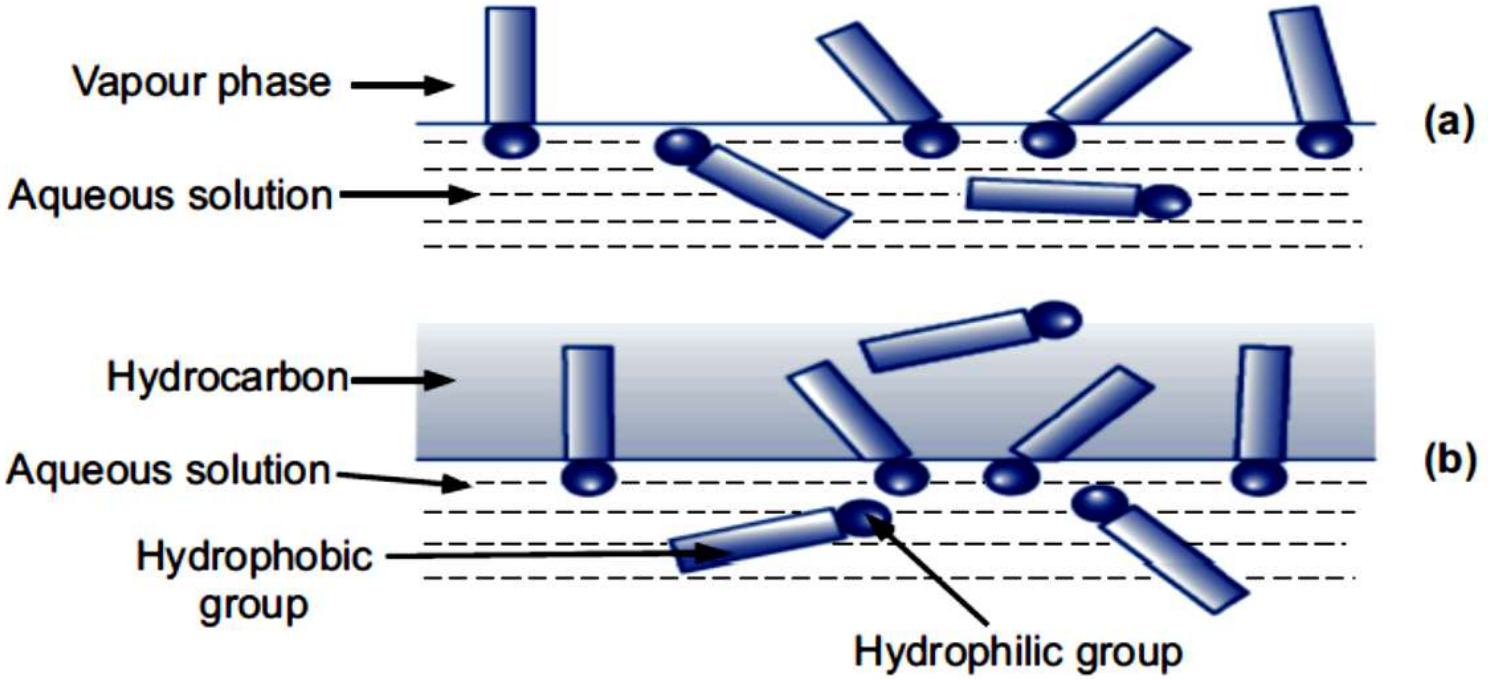
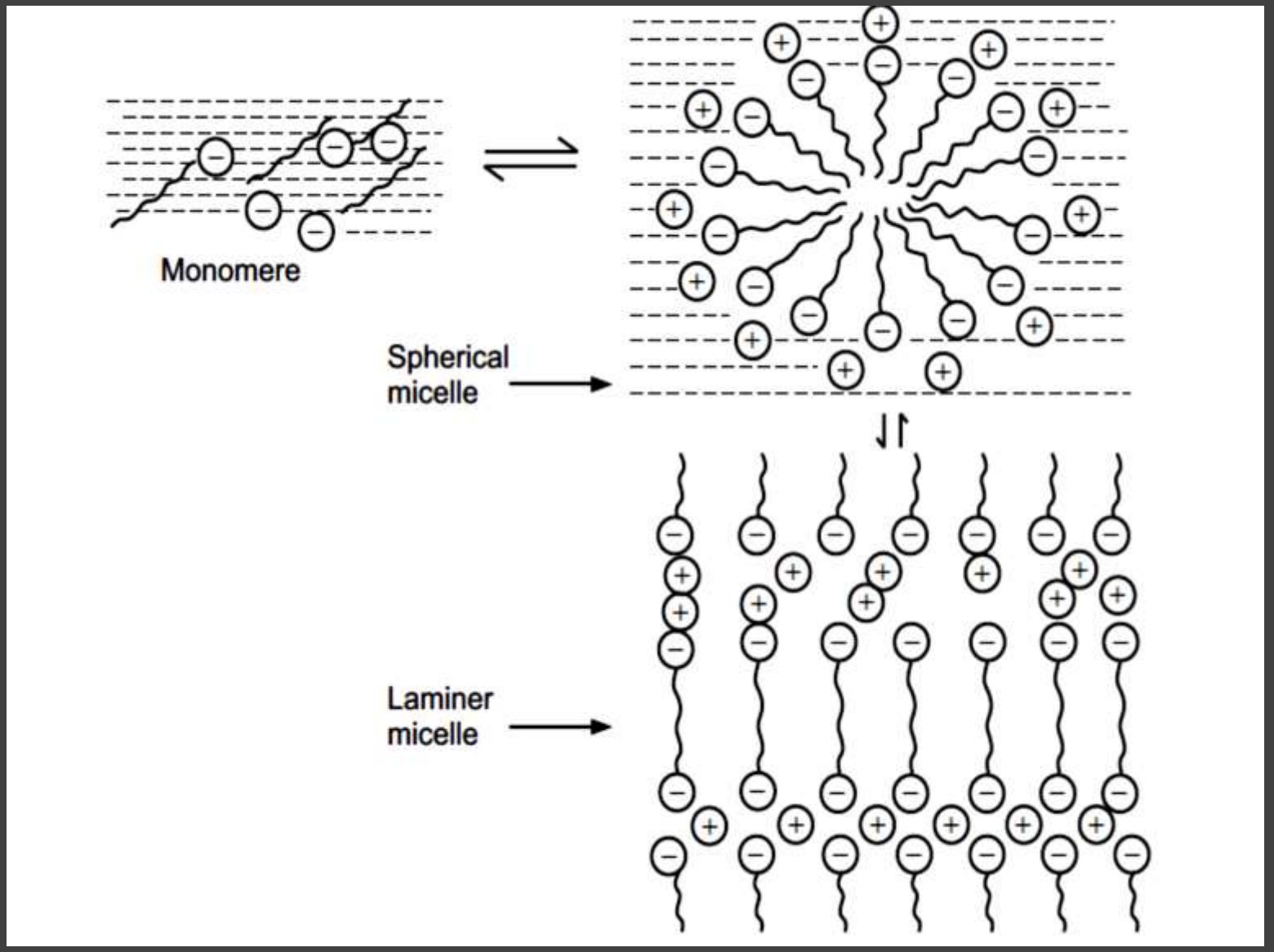
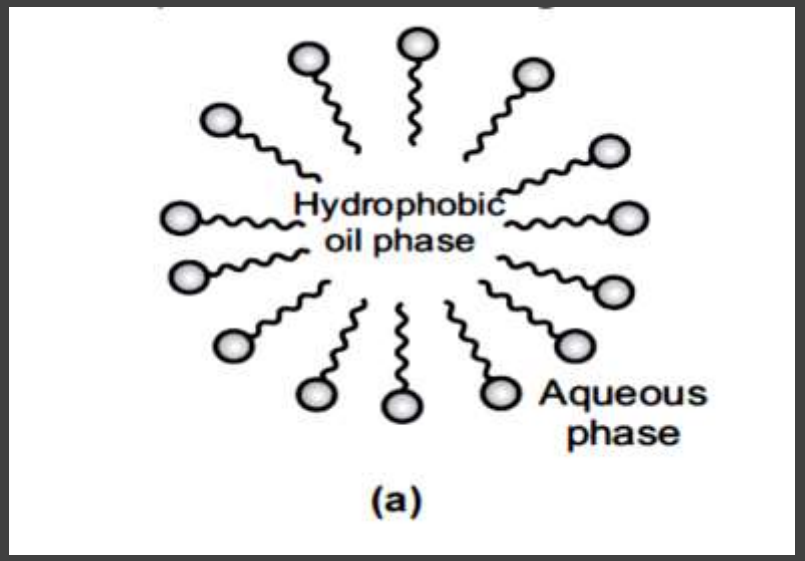
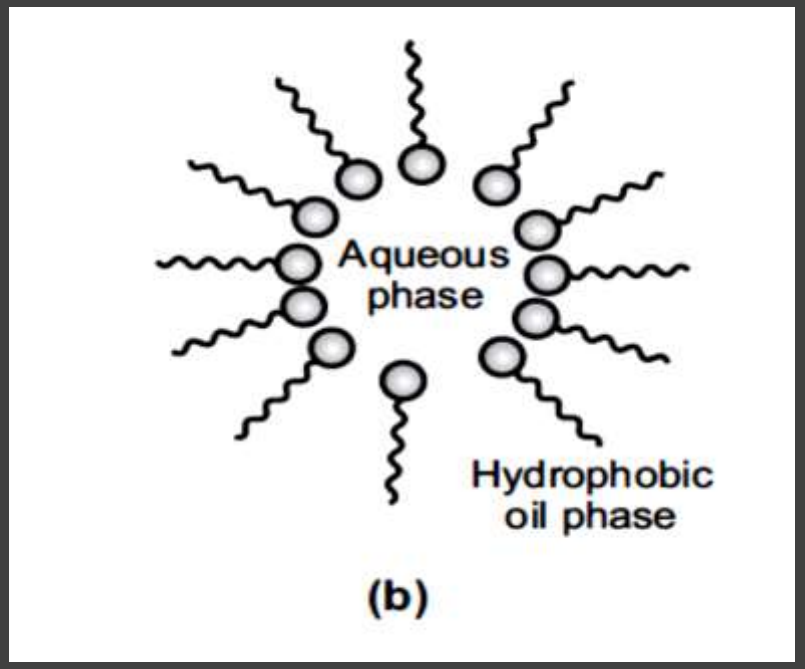


Fig. 1.15: Orientation of amphiphiles at (a) solution–vapour interface and (b) two liquids interface

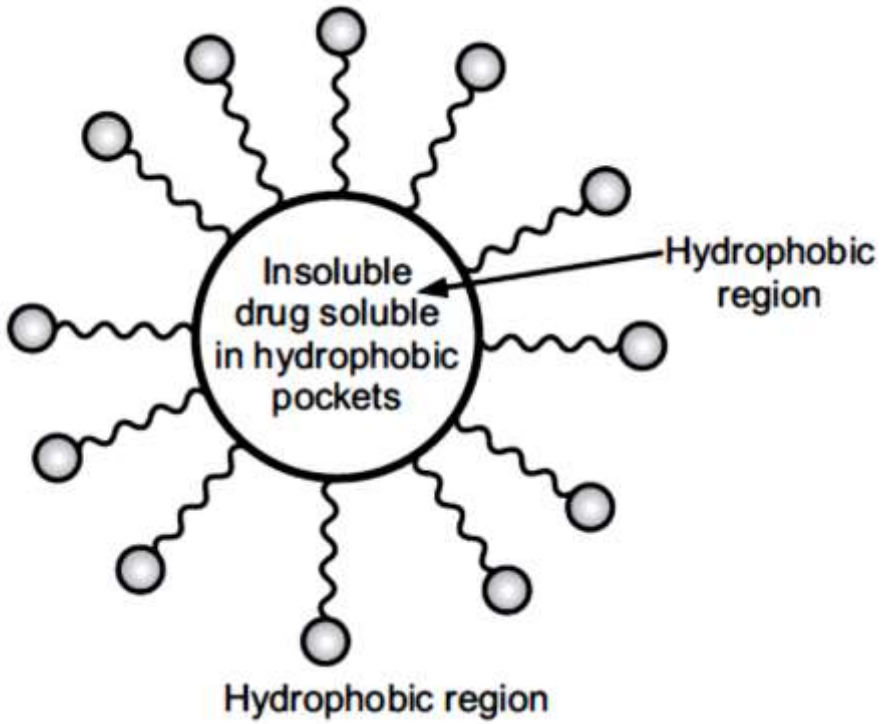
Micelles Formation of Surfactant

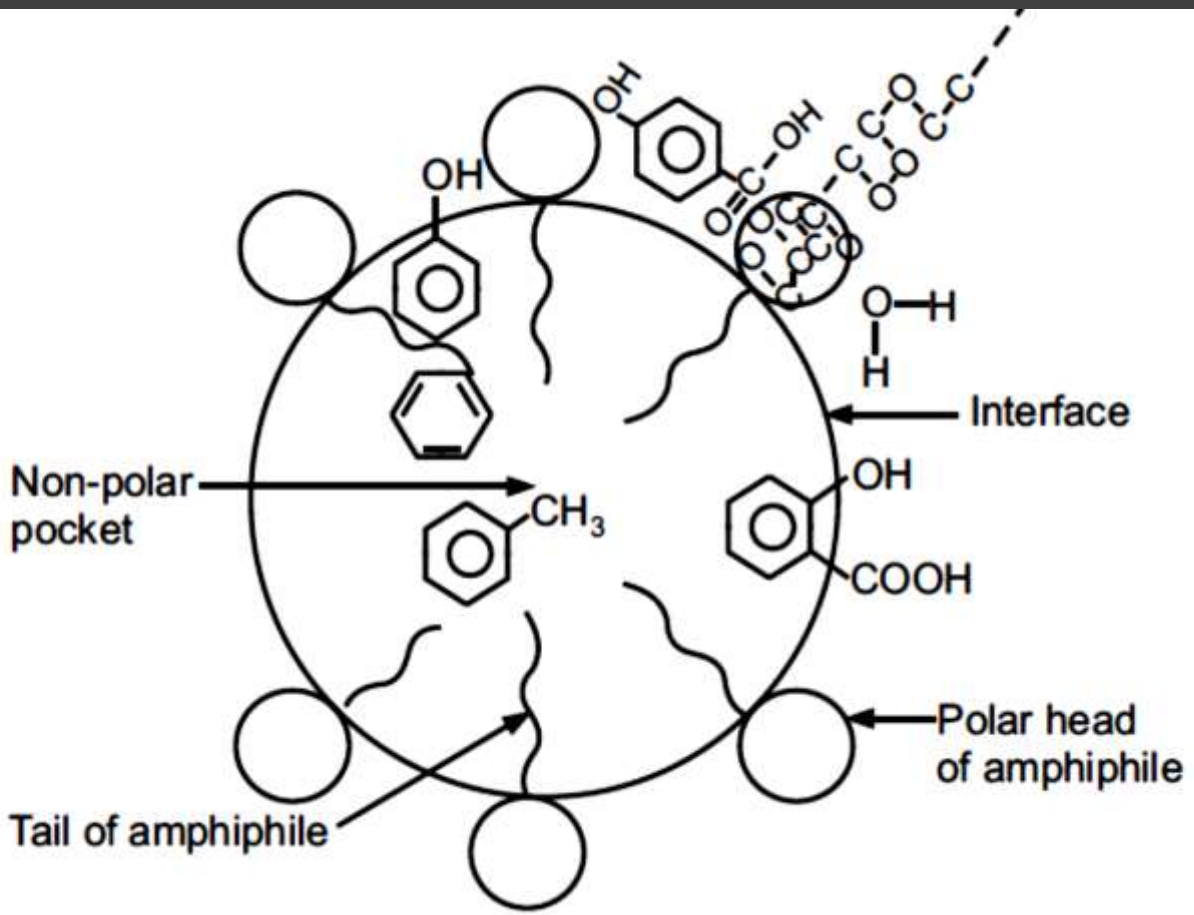






Micellar Solubilisation



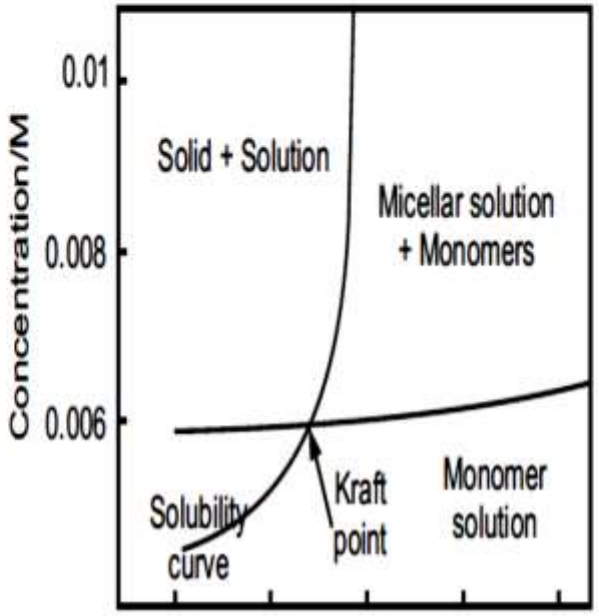


Krafft Point

At the krafft temperature it becomes possible to form micelles and the surfactant becomes much more soluble; there is a break in the solubility curve, as shown in Fig. 1.20, example is sodium decyl sulphonate.

Below krafft point: No formation of micelle and limited solubility of surfactant.

Above krafft point: Formation micelles and the surfactant become much more soluble.



The CMC is primarily determined by the hydrophobic group of surfactant.

The Krafft temperature depends strongly on;

1. The nature of the head group and
2. The length of the hydrocarbon chain.

The hydrocarbon fragments have to be in a liquid state for micelle formation to occur. Solidification of the hydrocarbon chain is favoured by the cohesion between hydrocarbon chains, which increases with their length. Thus, the Krafft temperature increases with chain length, as shown in Table 1.3 for the Krafft temperatures of the sulphonates.

Table 1.3: Krafft temperatures of the sulphonates

$C_{10}SO_3Na$	$C_{12}SO_3Na$	$C_{14}SO_3Na$	$C_{16}SO_3Na$	$C_{18}SO_3Na$
22°C	34°C	43°C	52°C	60°C

Cloud Point

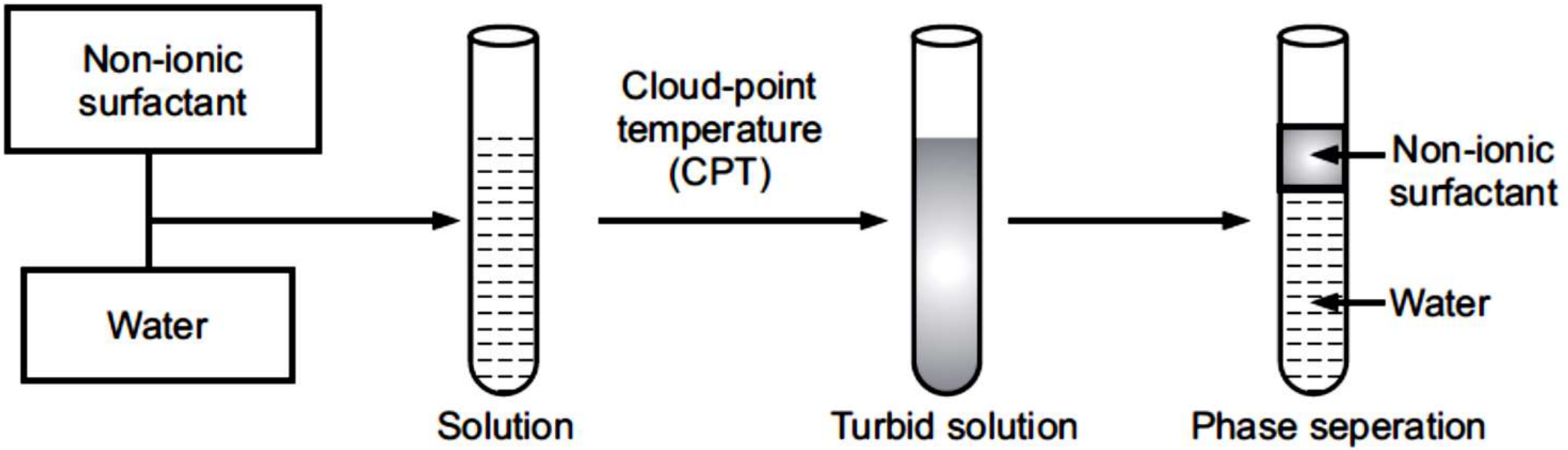
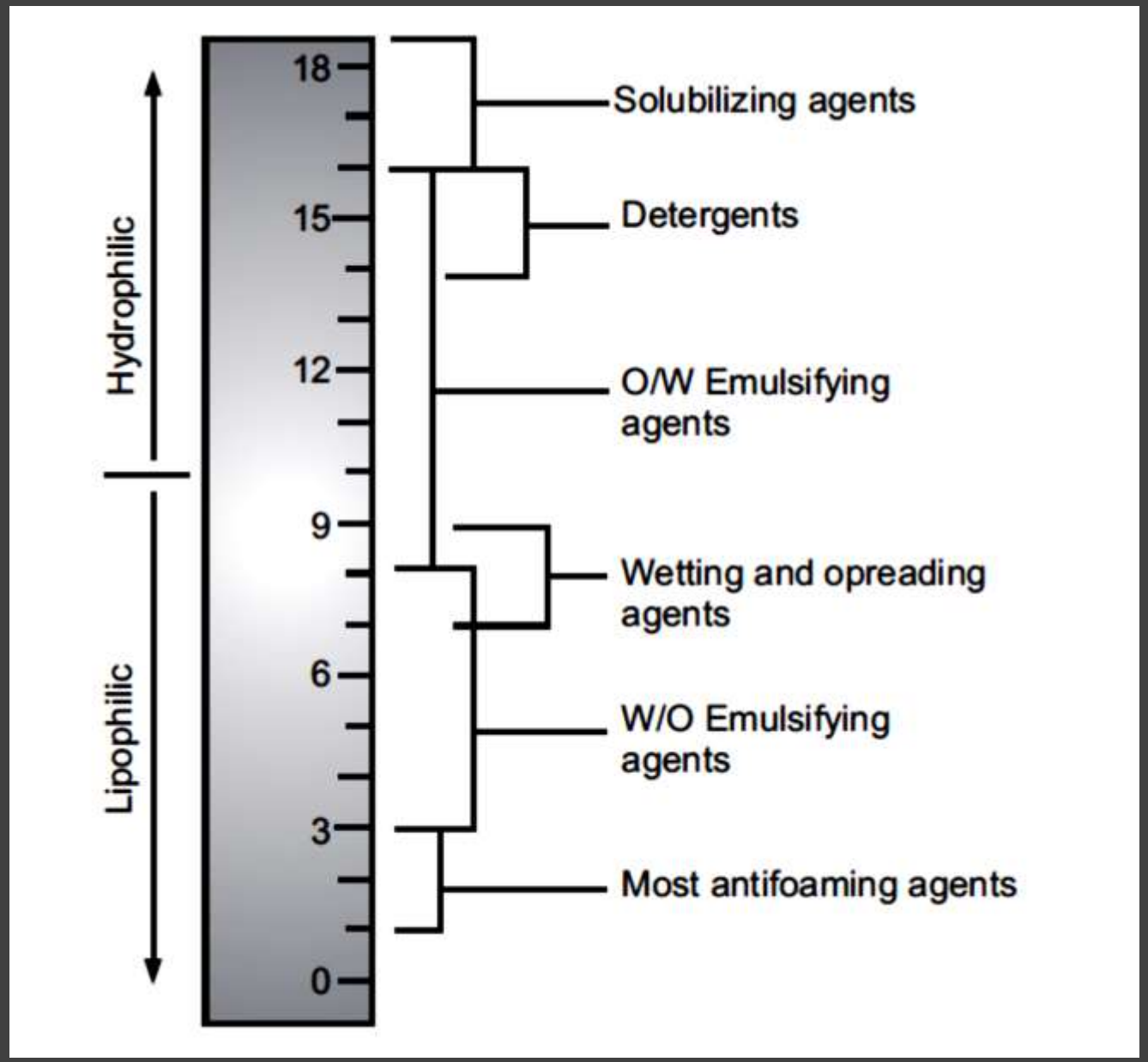


Fig. 1.21: Cloud-point non-ionic surfactant



The Hydrophilic-Lipophilic Balance (HLB Scale)

Method of Estimation:

$$HLB = \sum(\text{Hydrophilic group number}) - \sum(\text{Hydrophobic group number}) + 7$$

Table 1.4: Various groups and group number

Groups	Group number
$SO_4^- Na^+$	38.7
CH_3	0.475
CH_2	0.475

Method II:

$$HLB = 20\left(1 - \frac{S}{A}\right)$$

Method III:

$$HLB = \frac{E + P}{5}$$

Method IV:

$$HLB = \frac{E}{5}$$

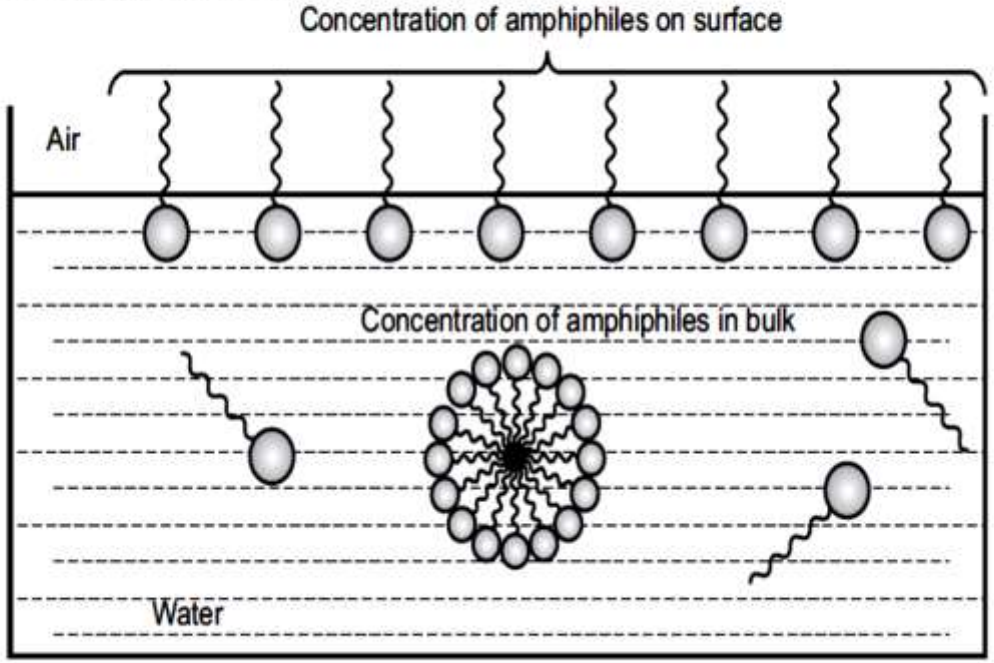
Soluble Monolayer And Gibbs Adsorption Isotherm & Gibbs Adsorption Isotherm

The following parameters are evaluated in this study:

1. Surface excess
2. Surface tension.
3. Concentration of amphiphiles in bulk and surface.

Surface excess is the difference between the surface and bulk concentration. (Fig. 1.23)

Such a direct measurement of surface excess provides number of problems. Hence, Gibbs equation is widely used alternative method.



Surface excess : Concentration of amphiphiles on surface – Concentration of amphiphiles in bulk

The Gibbs equation may be expressed as follows:

$$\Gamma = \frac{-a_2}{RT}\left[\frac{d\gamma}{da_2}\right] \quad \ldots (1.31)$$

Γ = Moles of solute adsorbed/unit area, or surface excess

R = Ideal gas constant

T = Absolute temperature

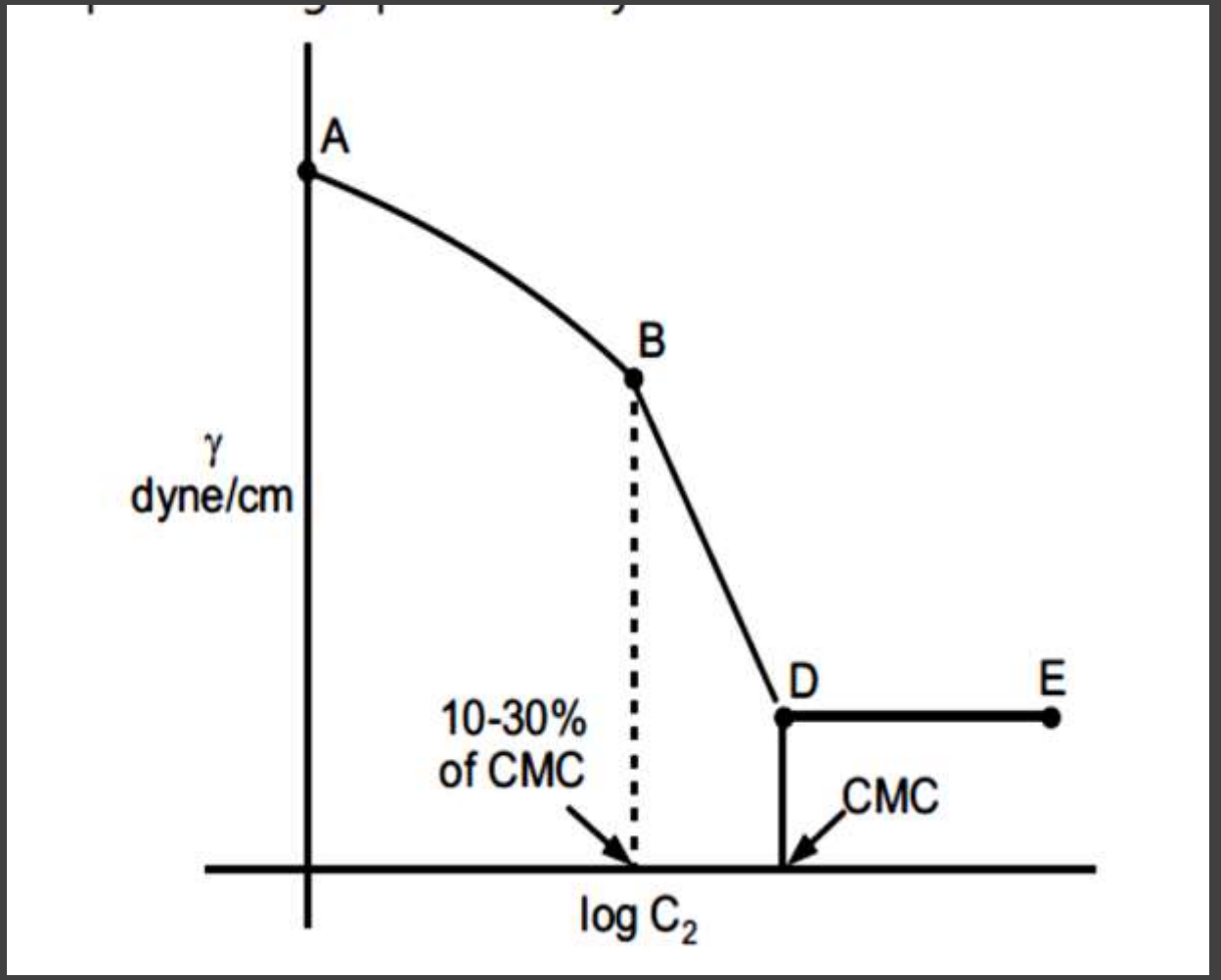
$d\gamma$ = Change in the surface tension

da_2 = Change in the solute activity at activity a_2

$$\Gamma = \frac{-c_2}{RT}\left[\frac{d\gamma}{dc_2}\right]$$

Since, the term $\frac{dc}{c}$ is equal to $d(\ln c)$, the Gibbs equation can be written as

$$\Gamma = \frac{-1}{RT}\left[\frac{d\gamma}{d \ln c_2}\right]$$



INSOLUBLE MONOLAYERS AND THE FILM BALANCE

Cross-Sectional Area of Insoluble Monolayer:

Knowing the area of the film and the volume of the spreading liquid, it should therefore be possible to compute the thickness of such films. The film thickness is equal to the length of the molecules standing in a vertical position of the surface when the molecules are packed in closest arrangement. Furthermore, if the molecular weight and the density of the spreading oil are known, the cross-sectional area available to the molecules can be easily computed.

Area of cross-section per molecule is given by

$$\text{Area of cross-section/molecule} = \frac{MS}{V\rho N} \quad \ldots (1.34)$$

Where, M : Molecular weight of the spreading liquid,
S : The surface area covered by the film,
V : The volume of the spreading liquid,
ρ : Density,
N : Avogadro's number.

Film Balance:

5. The compressive force per unit area on the float is known as the **surface pressure, π.** It is the difference in surface tension between the pure substrate, γ_o (water), and that with a film spread on it, γ, and is written as

$$\pi = (\gamma_o - \gamma) \quad \ldots (1.35)$$

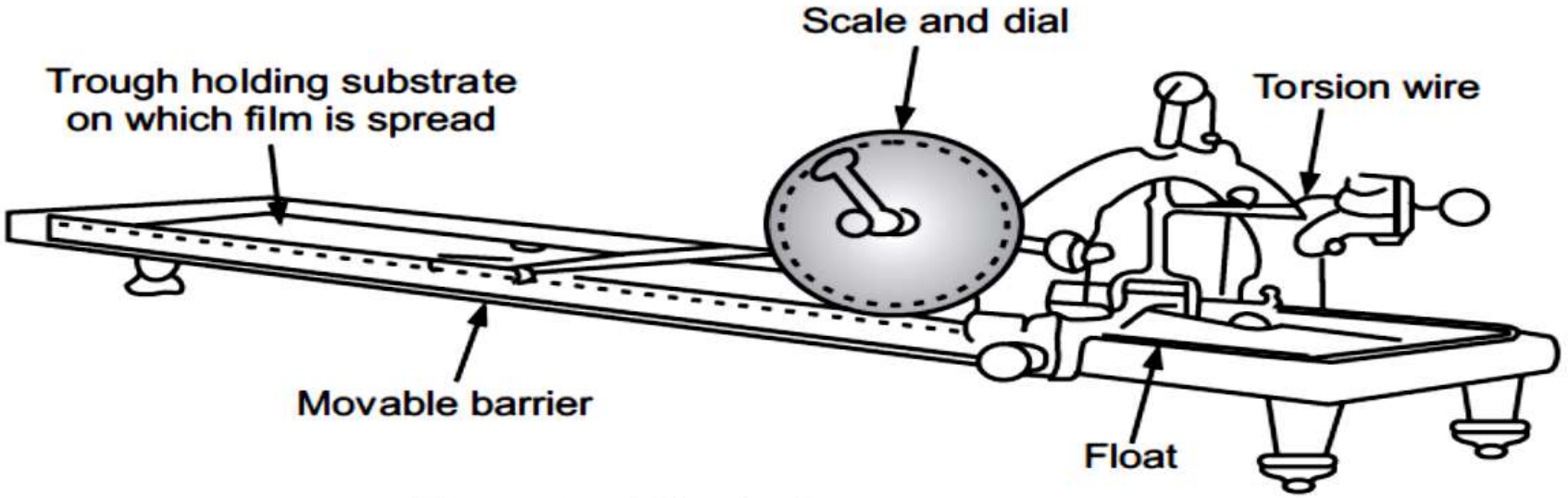


Fig. 1.25: Film balance apparatus

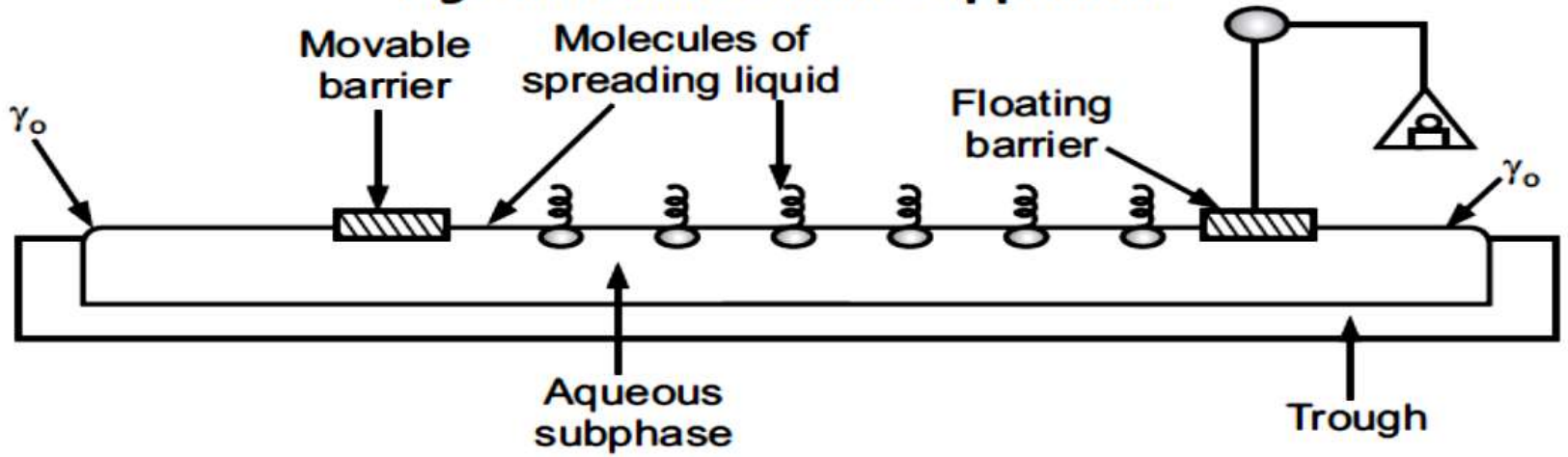
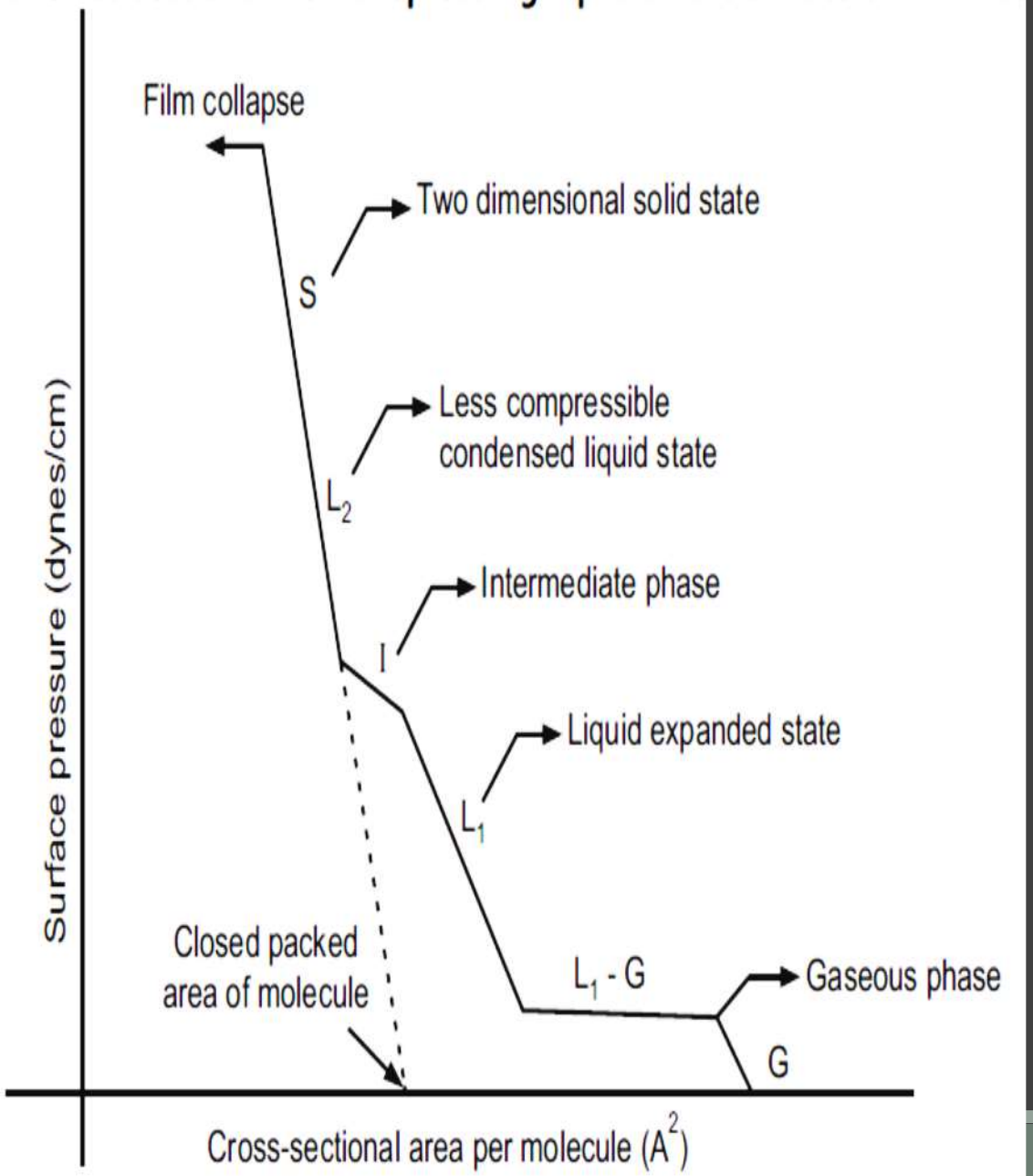


Fig. 1.26: Cross-sectional view of spreading liquid on the surface of a film balance



The film (surface) pressure is then plotted against the area of the film (Fig. 1.27).

Region G: When the film is spread over an area greater than 50 to 60 A^2/molecule, it exerts little pressure on the floating barrier. The film acts like a gas in two dimensions.

Region L_1-G: As the film begins to be compressed, a liquid phase L_1 appears that coexists in equilibrium with the gas phase. This occurs at a low surface pressure (e.g., 0.2 dynes/cm or less.)

Region L_1: The liquid expanded state may be thought of as a bulk liquid state, but in two dimensions.

Region I: Further compression of the film often leads to appearance of an intermediate phase.

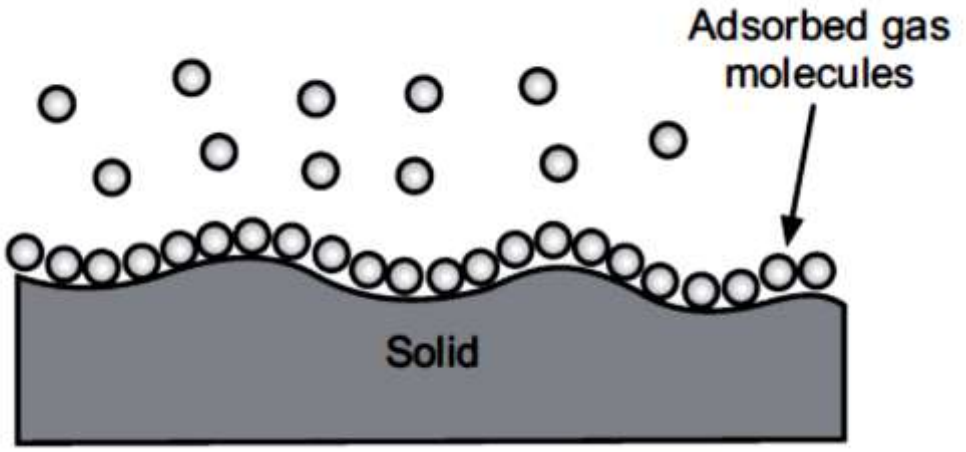
Region L_2: A less compressible condensed liquid state, region L_2.

Region S: Where the film can be regarded as being in a two-dimensional solid state. Finally the molecules slip over one another and the film breaks when it is greatly compressed.

Applications:

1. **The electric potential and viscosity** of monomolecular films may be studied by means of the film balance.
2. **The molecular weight** of high polymers such as proteins can be estimated by its use.
3. **The study of biologic systems:** Some protein molecules unfold from a spherical configuration into a flat film when spread on the surface of the film in trough, and the relationship between unfolding and biologic activity can be studied.
4. **The sizes and shapes of molecules** of steroids, hormones, and enzymes and their interaction with drugs at interfaces can also be investigated by means of the film balance.

ADSORPTION AT SOLID INTERFACE



The phenomenon of concentration of molecules of a gas or liquid at a solid surface is called **adsorption.**

The substance that deposits at the surface is called **Adsorbate** and the solid on whose surface the deposition occurs is called the **Adsorbent.**

Physical Adsorption	Chemisorption
1. Caused by intermolecular van der Waal's forces.	1. Caused by chemical bond formation,
2. Depends on nature of gas. Easily liquefiable gases are adsorbed readily.	2. Much more specific than physical adsorption.
3. Heat of adsorption is small (about 5 kcal/mol)	3. Heat of adsorption is large (20-100 kcal/mol).
4. Reversible.	4. Irreversible.
5. Occurs rapidly at low temperature; decreases with increasing temperature.	5. Increases with increase of temperature.
6. Increase of pressure increases adsorption; decrease of pressure causes desorption.	6. Change of pressure has no such effects.
7. Forms multimolecular layers on adsorbent surface.	7. Forms unimolecular layer.

Adsorption of Gases on Solids

Adsorption Isotherms: 1. Freundlich Adsorption Isotherm

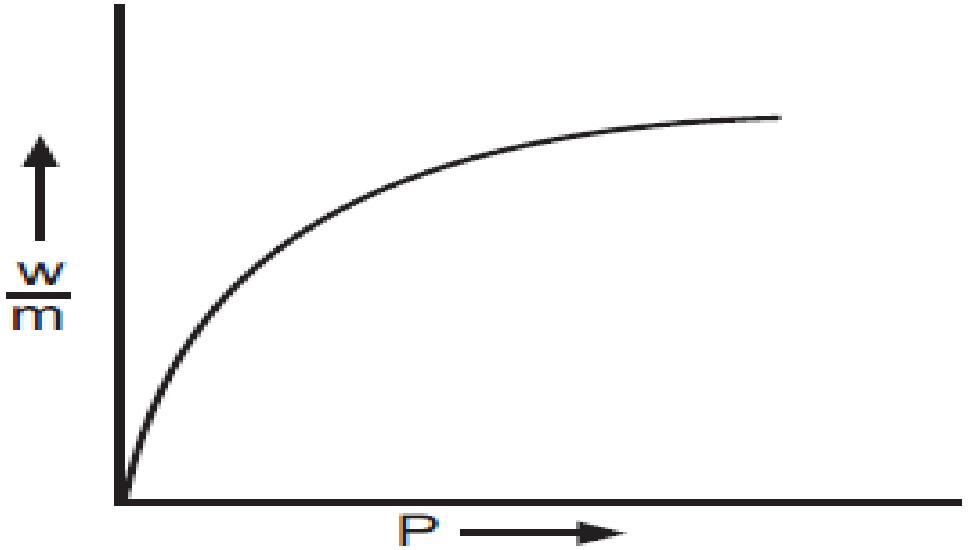
Freundlich suggested relation called the Freundlich adsorption isotherm,

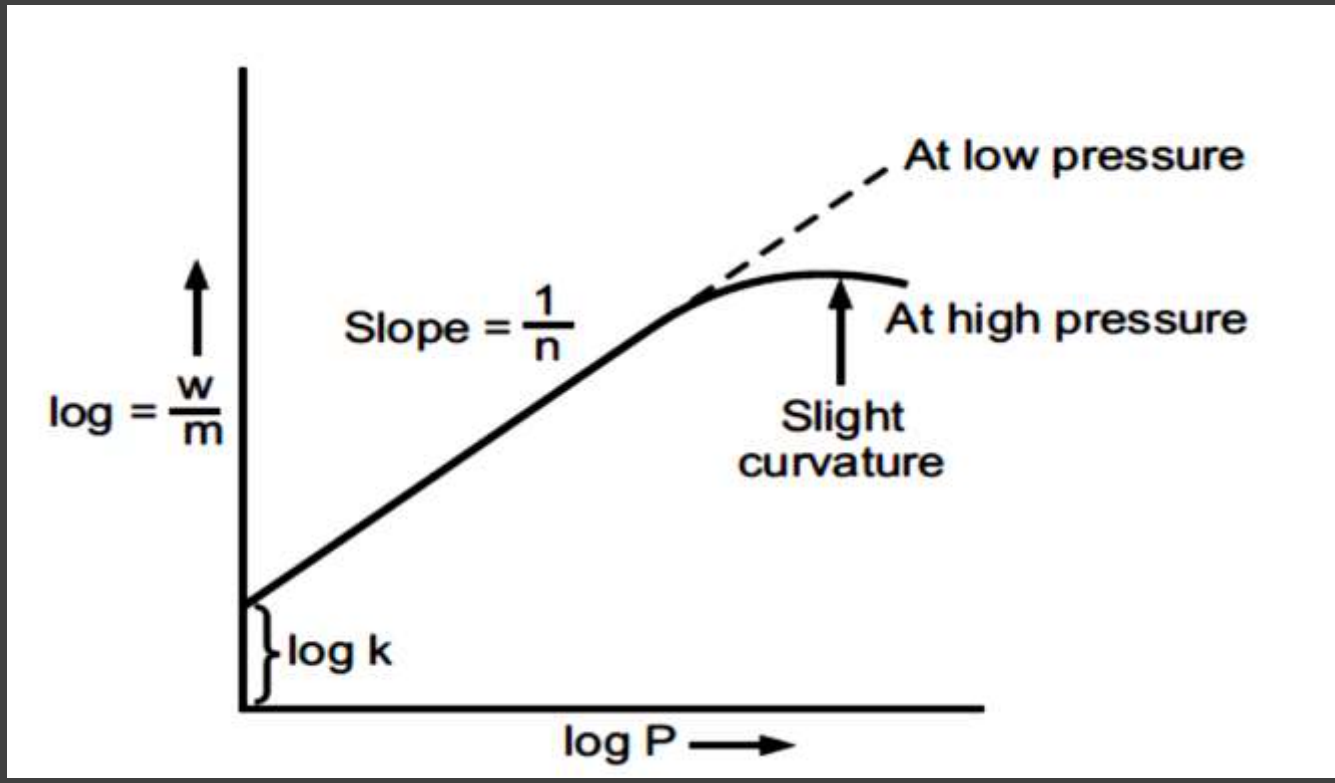
$$y = \frac{w}{m} = kP^{1/n} \qquad \ldots (1.36)$$

Where, y: The mass w of gas adsorbed per unit mass m of solid adsorbent at constant pressure P.

k and n: These are constants depending on the nature of the gas and the adsorbent and on temperature.

This relation is obtained by plotting the mass of the gas adsorbed per unit mass of adsorbent (w/m) against equilibrium pressure (P) [Fig. 1.30].





$$\log\left(\frac{w}{m}\right) = \log k + \frac{1}{n}\log P$$

2. Langmuir Adsorption Isotherm

Assumptions:

Langmuir made the following assumptions.

1. The layer of the gas molecule is adsorbed on active sites of the solid adsorbent.
2. It forms layer one-molecule thick (monolayer).
3. The adsorbed layer is uniform all over the adsorbent.
4. There is no interaction between the adjacent adsorbed molecules.
5. The rate of adsorption (condensation) is proportional to number of unoccupied sites on solid surface.
6. The rate of desorption (evaporation) is proportional to number of occupied sites on solid surface.

Derivation:

The amount of gas adsorbed on solid surface depends on pressure of gas on solid surface. At a particular pressure 'P':

1. θ is the fraction of the total surface covered by the gas molecules,
2. $1 - \theta$ is the fraction of the total surface uncovered by the gas molecules,
3. **The rate of adsorption**, $r_1 = k_1 (1 - \theta) P$
4. **The rate of desorption**, $r_2 = k_2 \theta$

Where, k_1 and k_2 are proportionality constants for adsorption and desorption respectively.

$$r_1 = r_2 \quad \ldots (1.38)$$

$$k_1 (1 - \theta) P = k_2 \theta \quad \ldots (1.39)$$

$$k_1 p - k_1 \theta P = k_2 \theta \quad \ldots (1.40)$$

$$k_1 P = k_2 \theta + k_1 \theta P \quad \ldots (1.41)$$

$$k_1 P = \theta (k_2 + k_1 P) \quad \ldots (1.42)$$

$$\theta = \frac{k_1 P}{(k_2 + k_1 P)} \quad \ldots (1.43)$$

$$\theta = \frac{(k_1/k_2) P}{1 + (k_1/k_2) P} \quad \ldots (1.44)$$

Now, assume that

$$\frac{k_1}{k_2} = b \quad \text{or} \quad \theta = \frac{y}{y_m}$$

$y = \frac{w}{m}$: The mass 'w' of gas adsorbed per gram of solid adsorbent 'm'

y_m : The mass of gas that 1 gram of solid adsorbent can take up when monolayer is completed

$$\frac{y}{y_m} = \frac{bP}{1 + bP}$$

$$y = \frac{y_m bP}{1 + bP} \quad \ldots (1.45)$$

Inverting equation and multiplying by 'P', we get

$$\frac{P}{y} = \frac{1}{y_m b} + \frac{P}{y_m} \quad \ldots (1.46)$$

Multilayer Adsorption:

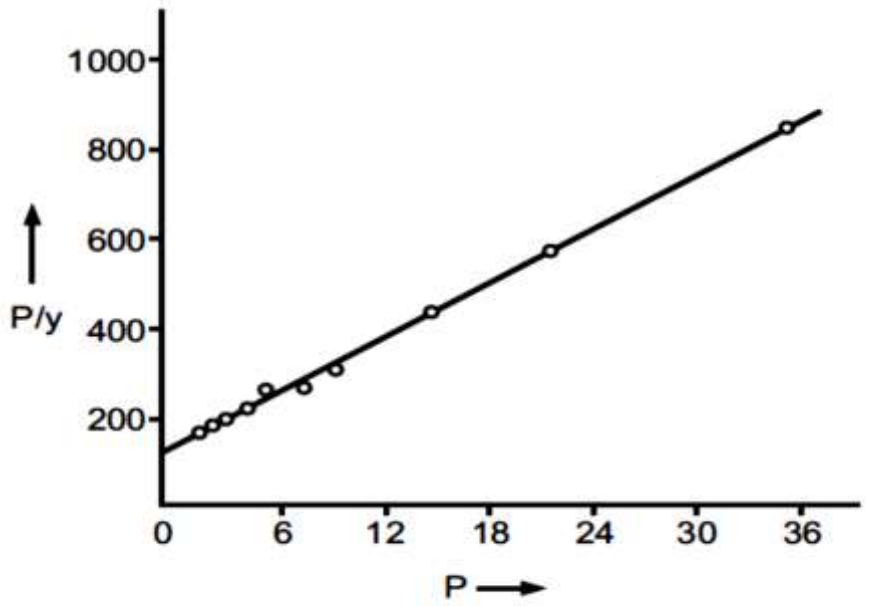
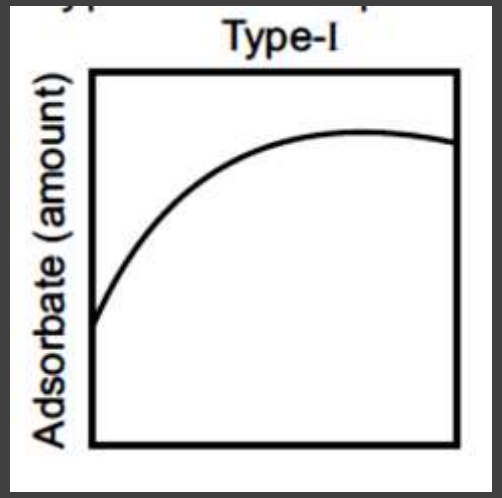


Fig. 1.32: P/y against P

$$\frac{P}{y(P_o - P)} = \frac{1}{y_m b} + \frac{b - P}{y_m b} \cdot \frac{P}{P_o} \qquad \ldots (1.47)$$

- P : Pressure of the adsorbate, in mm Hg,
- y : Mass of the vapour per gram,
- P_o : Vapour pressure at saturation (monolayer),
- y_m : The mass of gas that 1 gram of solid adsorbent can take up when monolayer is completed,
- b : Constant, proportional to heat of adsorption and latent heat of condensation of subsequent layers.

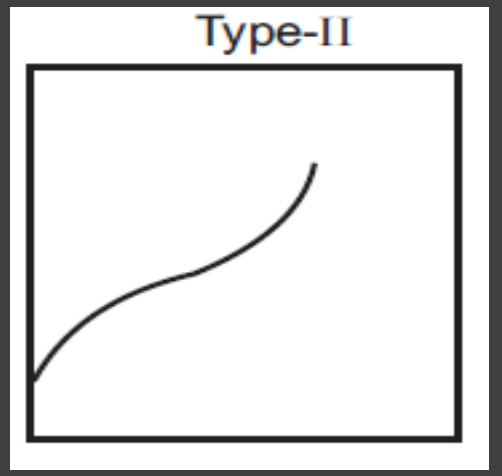
Adsorption Isotherm



Type I:

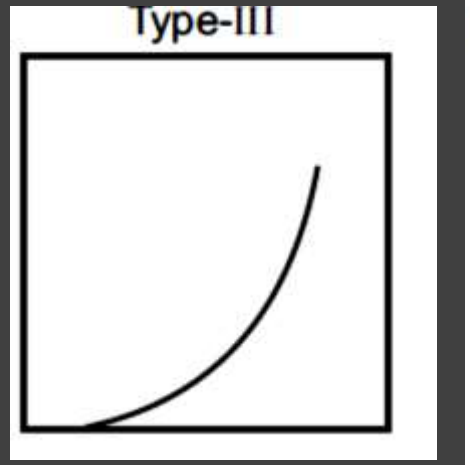
1. This represents increase in adsorption with increasing pressure and followed levelling OFF.
2. Leveling OFF: Due to saturation of gas molecule and formation of monolayer on surface of solid.

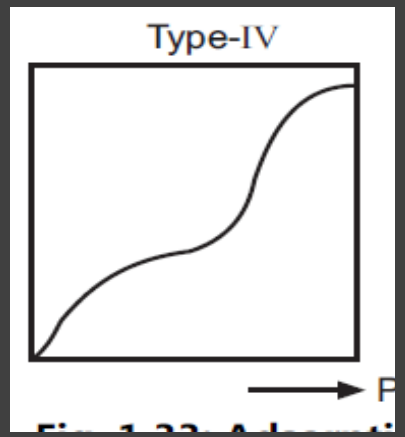
   Example: Freundlich and Langmuir adsorption isotherm.
3. Adsorption of nitrogen gas at 78 K on charcoal.

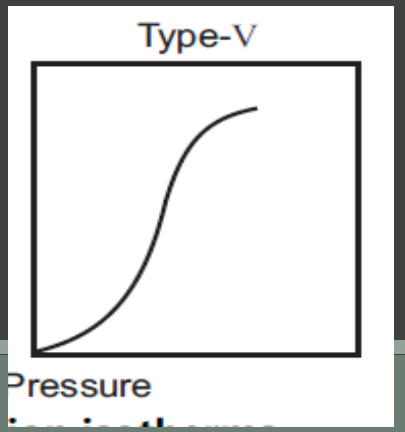


Type II:

1. This is sigmodial in shape and occur when gas undergoes physical adsorption onto non-porous solid to form a monolayer.
2. When the pressure is increased multilayer is formed.
3. Type - II is described by BET equation ($b > 2$) as explained earlier.
4. Example: Adsorption of nitrogen gas at 78 K on iron or platinum catalyst.







Type III:

1. It is not commonly observed.
2. The heat of adsorption of gas in the first layer is less than the latent heat of condensation of successive layers.
3. In the BET equation, the constant 'b' is smaller than 2.
4. Example: Adsorption of bromine gas on silica.

Type IV:

1. This represents the adsorption of gas on porous solids.
2. First point of inflection represents the monomolecular layer adsorption.
3. Condensation within the pores of the solid and the multimolecular layer is represented by further adsorption.
4. Example: Adsorption of benzene on silica gel.

Type V:

1. This is uncommon and shows capillary condensation.
2. Example: Water vapour on charcoal at 100°C.

Pharmaceutical and medicinal importance's of wetting phenomenon:

1. **Suspension:** The displacement of air from the surface of sulfur, charcoal and other powders for the purpose of dispersing these drugs in liquid vehicles.
2. **Granulation:** In granulation process, powders are mixed with liquid binding agent. The success of process depends of good wettability of powder.
3. **Film coating:** In coating process, wetting and spreading of liquid coating mixture over tablet surface occurs.
4. The displacement of air from the matrix of cotton pads and bandages so that medicinal solutions may be absorbed for application to various body areas.
5. The displacement of dirt and debris by the use of detergents in the washing of wounds.

Surfactants are used as wetting agent because:

1. Lowering interfacial tension between solid and liquid.
2. Lowering contact angle between solid and liquid.
3. Displacement of air from solid for intimate contact of solid and liquid.

Contact Angle

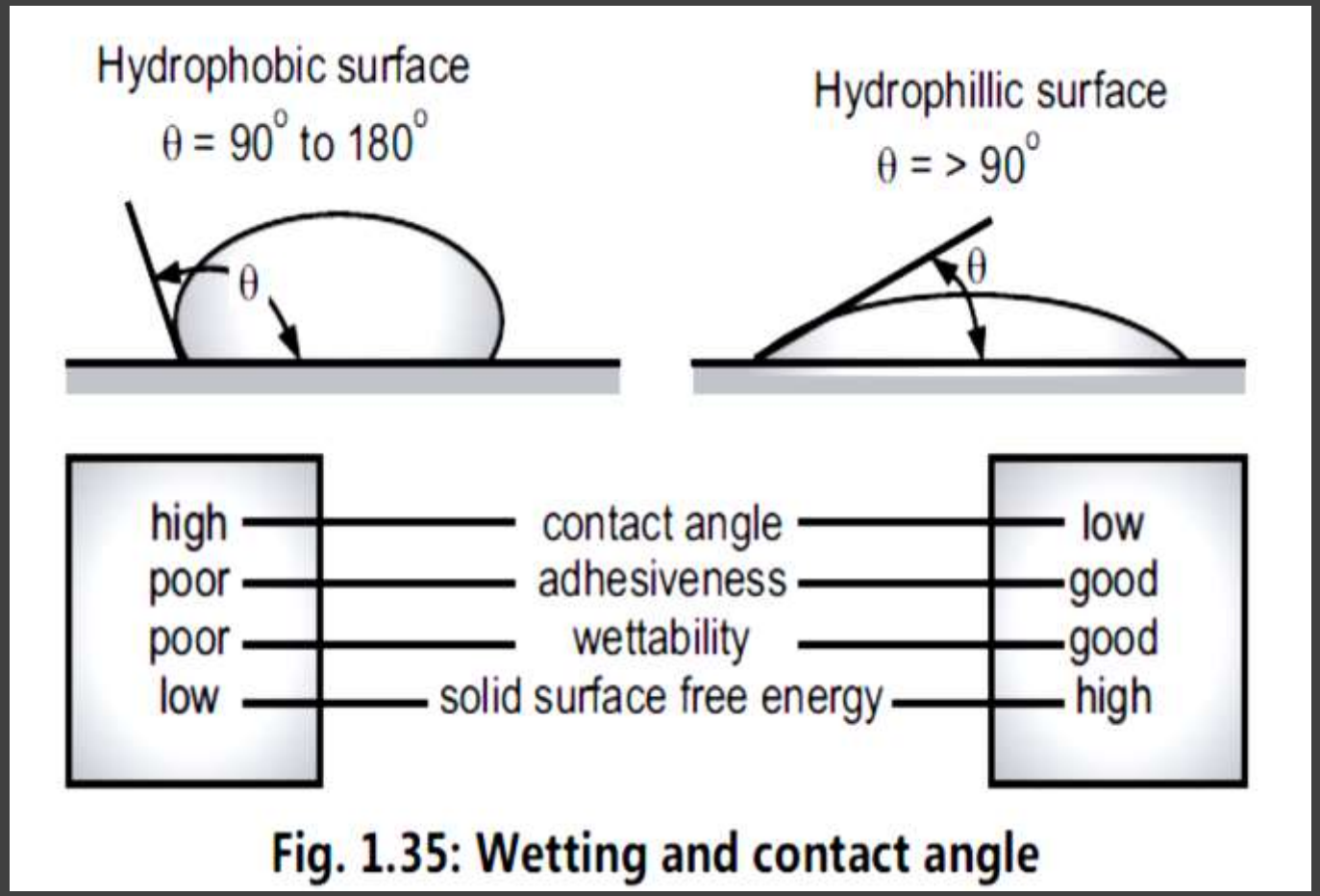
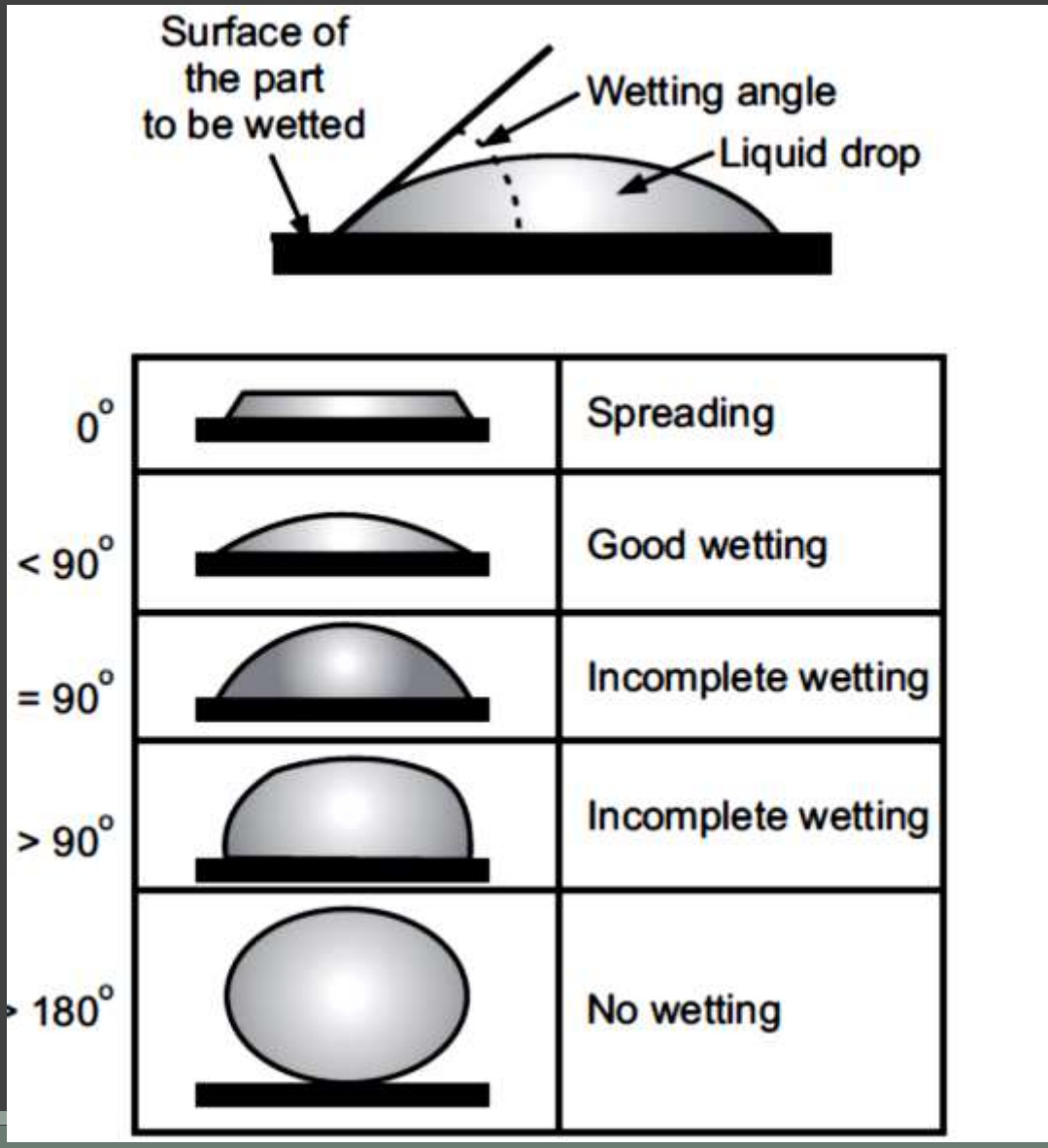


Fig. 1.35: Wetting and contact angle



Young's Equation

γ^{sv} : Interfacial tension between solid and vapour.

γ^{sl} : Interfacial tension between solid and liquid.

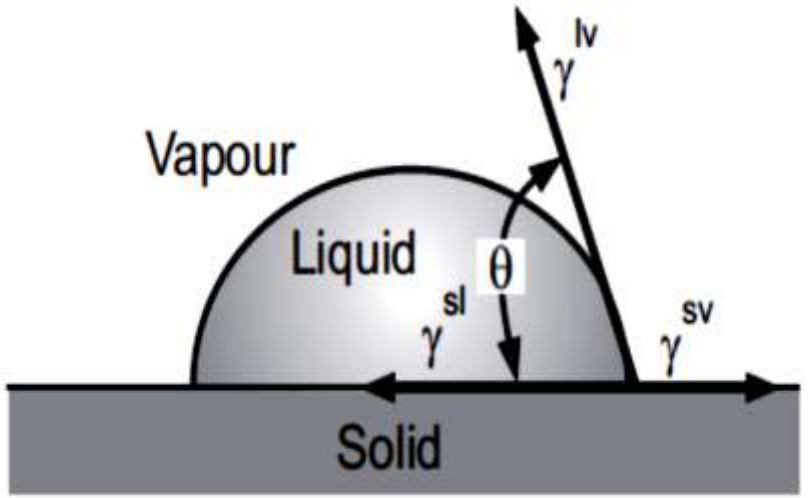
γ^{lv} : Interfacial tension between liquid and vapour.

At equilibrium,

$$\gamma^{sv} = \gamma^{sl} + \gamma^{lv} \cdot \cos\theta$$

Where, θ is the contact angle.

Equation (1.49) is called **Young's Equation.**



The work of adhesion between the solid and liquid interface is given by Dupre's equation (1.22).

$$W^{sl} = \gamma^{sv} + \gamma^{lv} - \gamma^{sl} \quad \ldots (1.50)$$

By combining equation (1.49) and (1.50) we get alternative for Young's equation:

$$\therefore \quad W^{sl} = \gamma^{sl} + \gamma^{lv} \cdot \cos\theta + \gamma^{lv} - \gamma^{sl} \quad \ldots (1.51)$$

$$W^{sl} = \gamma^{lv} (1 + \cos\theta) \quad \ldots (1.52)$$

Wetting process improves till $\cos\theta = 1$ or $\cos\theta = 0$ and this is the ideal wetting.

Wetting Process

(A) Adhesional wetting: (stage a – b)

The first step in the wetting of powder is adhesional wetting in which the surface of solid is brought into contact with the liquid surface.

(B) Immersional wetting: (stage b – c)

The particle is then forced below the surface of the liquid i.e. Immersional wetting. During this step, solid-liquid interface is formed and solid-air interface is lost.

(C) Spreading wetting: (stage c – d)

Finally, the liquid spreads over the entire surface of the solid as spreading wetting occurs. The work of spreading wetting is equal to form new solid-air and liquid-air interface and loss of the solid-air interfaces.

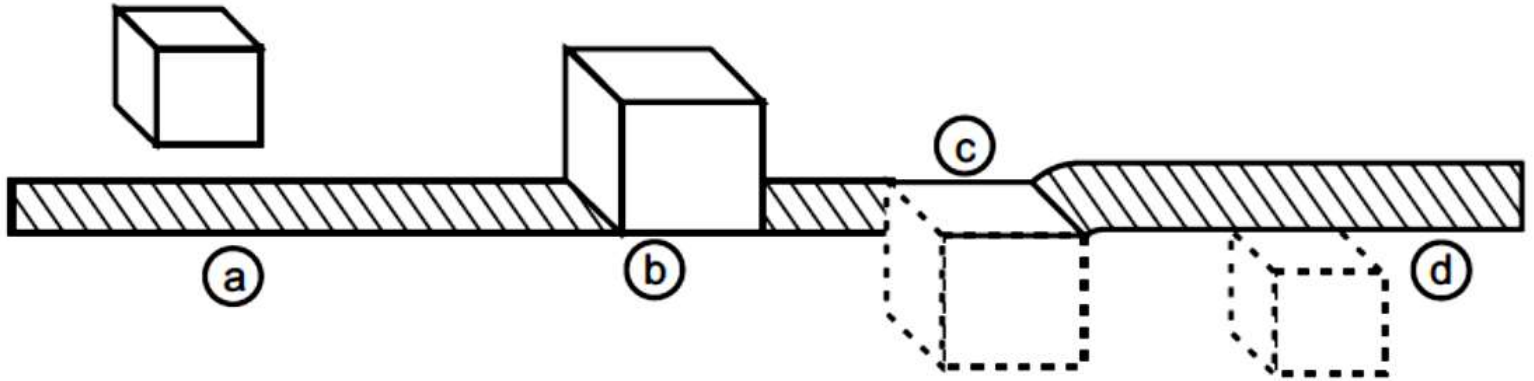


Fig. 1.38: Wetting process

Detergency

Detergency is a complex process and a number of steps are simultaneously involved (Fig. 1.40). These are:

1. Initial wetting of the dirt from the surface.
2. Solubilising of the dirt.
3. Removing the insoluble dirt as deflocculating particles.
4. Suspending the particles in the detergent solution.
5. Removing the oil soluble materials and convert into emulsion.
6. Converting the dirt into foam so as to wash easily.

The HLB requirement for the detergency is about 13 to 16.

Some examples of detergents of ionic type are:

Cationic type: Zephiran (benzyldimethylcetyl ammonium chloride), Cetrimide (cetyltrimethyl ammonium chloride).

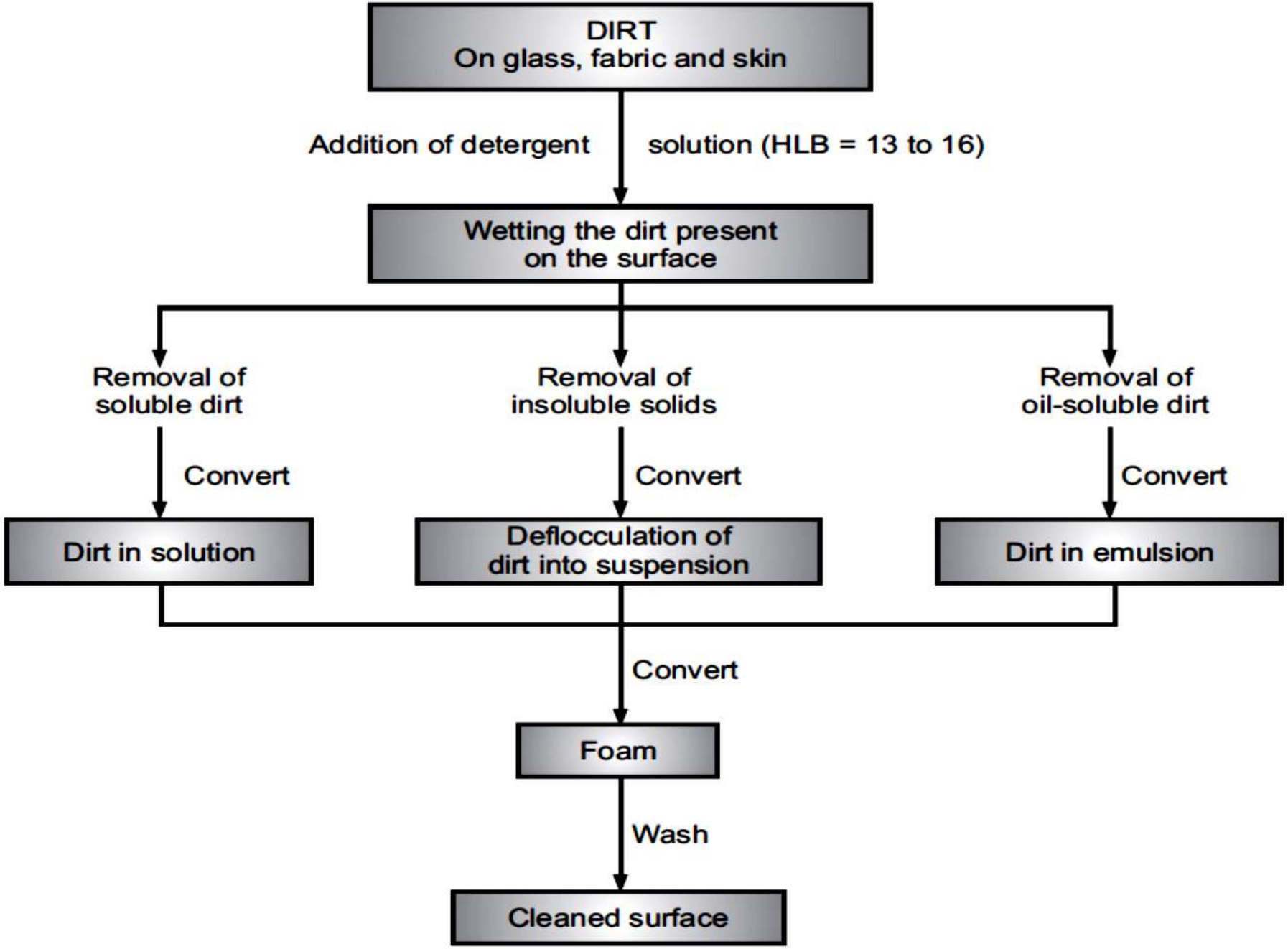


Fig. 1.40: The phenomenon of detergency

BP 403 T. PHYSICAL PHARMACEUTICS-II (Theory)

Unit-II
Rheology & Deformation of Solids

Subject Teacher:
Dr. Prashant Khade

Rheology (a term invented by Bingham and formally adopted in 1929) may be defined, as

'the study of the flow and deformation properties of matter under the influence of stress'.

The term rheology has its origin from the **Greek "rheo" means flow and "logos" means science.**

These systems change their flow behaviour when exposed to different stress conditions in the following situation.

1. Mixing of formulation.
2. Pouring from a bottle.
3. Extrusion of semisolid dosage forms after squeezing the tube.
4. Rubbing the product onto and into the skin.
5. Pumping the product from mixing and storage to filling equipment.
6. Passage through syringe needle.

Thus, flow behaviour has extreme importance in both product development and quality assurance, i.e. the determination that the desired attributes of body and flow are retained for the required shelf-life of the product.

Importance of Rheology

1. Hair shampoos
2. Toothpastes
3. Thixotropic or 'solid' paints
4. Printing inks
5. Ink

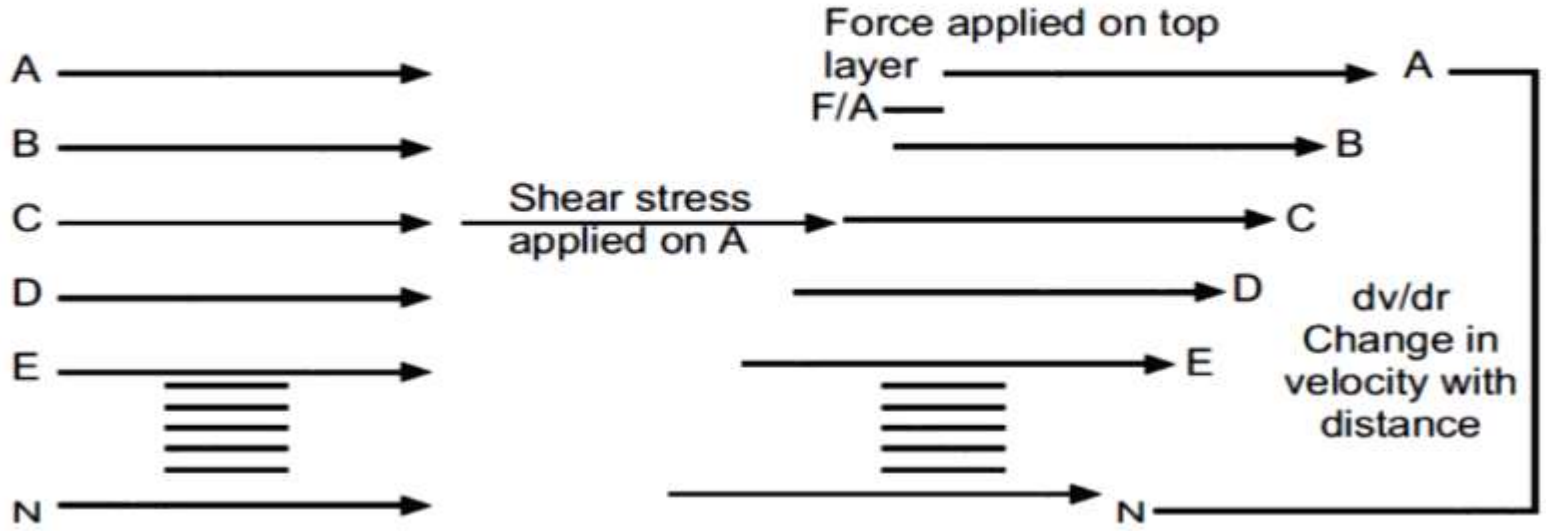
CONCEPT OF VISCOSITY AND NEWTONIAN'S LAW FOR FLOW

Viscosity:

The viscosity of a fluid may be described simply **as its resistance to flow or movement.**

E.g. water, which is easier to flow than syrup, is said to have the lower viscosity.

Thus, liquids resist flow when force is applied. This resistance is estimated and expressed as viscosity.



Newtonian's Law for Flow

1. Velocity Gradient or Rate of Shear, dv/dr:

The difference of velocity **dv** between two planes of liquid separated by an infinitesimal distance **dr** is the **velocity gradient or rate of shear**,**dv/dr**

$$\text{Rate of shear, } G = \frac{dv}{dr}$$

2. Shearing Stress (F):

The force per unit area F'/A required for flow of liquid is called the **shearing stress.**

$$\text{Shearing Stress} = F = \frac{F'}{A}$$

Hence,

The rate of shear should be directly proportional to the shearing stress.

$$\text{Shearing stress} \propto \text{Rate of shear}$$

$$\frac{F'}{A} \propto \frac{dv}{dr}$$

$$\frac{F'}{A} = \eta \frac{dv}{dr} \quad :$$

or

$$F = \eta G$$

$$\eta = \frac{F}{G}$$

Where,

η : viscosity coefficient and also referred as **viscosity.**

A representative flow curve, or **rheogram**, obtained by plotting F versus G, for a Newtonian system is shown in Fig. 2.3. A straight line passing through the origin is obtained.

Unit of Viscosity

The unit of viscosity is the poise (1 centipoise = 0.01 poise).

Poise is defined as 'the shearing force required to produce a velocity of 1 cm/sec between two parallel planes of liquid each 1 cm^2 in area and separated by a distance of 1 cm.'

The cgs units for the poise are dyne sec cm^{-2} (i.e. dyne sec/cm^2) or g cm^{-1} sec^{-1} (i.e. g/cm sec).

Fluidity (ϕ), is defined as 'the reciprocal of viscosity'.

$$\phi = \frac{1}{\eta}$$

Kinematic Viscosity

The U.S. Pharmacopeia; Indian Pharmacopeia, and the National Formulary include an explanation of kinematic viscosity, which is the viscosity (η) as divided by density (ρ) of the liquid.

$$\text{Kinematic viscosity} = \frac{\eta}{\rho}$$

Units of kinematic viscosity are the stok (s) and the centistoke (cs).

Other Terms for Viscosity

Most dispersions does not obey Newtonian flow;

Viscosity of polymeric dispersion increases almost many folds with concentration, i.e., the increase in viscosity with increase in concentration. Example, methylcellulose USP in the concentration of 2.0 % w/w in water shows an apparent viscosity of 80 poise at room temperature. The viscosity of water is 0.01 poise. In other words, the increase in viscosity of water is 8000 fold.

Relative viscosity (η_r): This term is defined as 'the ratio of viscosity of the dispersion (η) to that of the solvent η_o (vehicle)'. It is mathematically expressed as:

$$\text{Relative viscosity, } \eta_r = \frac{\eta}{\eta_o}$$

Specific viscosity: This term is defined as 'the relative increase in the viscosity of the dispersion over that of the solvent (vehicle) alone'. It is mathematically expressed as:

$$\text{Specific viscosity, } \eta_{sp} = \frac{\eta - \eta_o}{\eta_o}$$

Reduced-viscosity: This term is defined as 'the ratio of specific viscosity to the concentration (c)'. It is mathematically expressed as:

$$\text{Reduced viscosity} = \frac{\eta_{sp}}{c}$$

Factors Affecting the Viscosity

The flow behaviour of a material may be affected by a number of external factors. The five most important parameters are:

1. **Substance:** The viscosity of a liquid depends on its physical and chemical properties of the substances in it.
2. **Temperature:** Temperature has a major effect on the viscosity. As the temperature increases, the system acquires thermal energy which is used for braking cohesive forces. The viscosity of liquid decreases means there is increase in flow of liquid.
   The relationship between viscosity and temperature may be expressed as:

$$\eta = Ae^{E_V/RT}$$

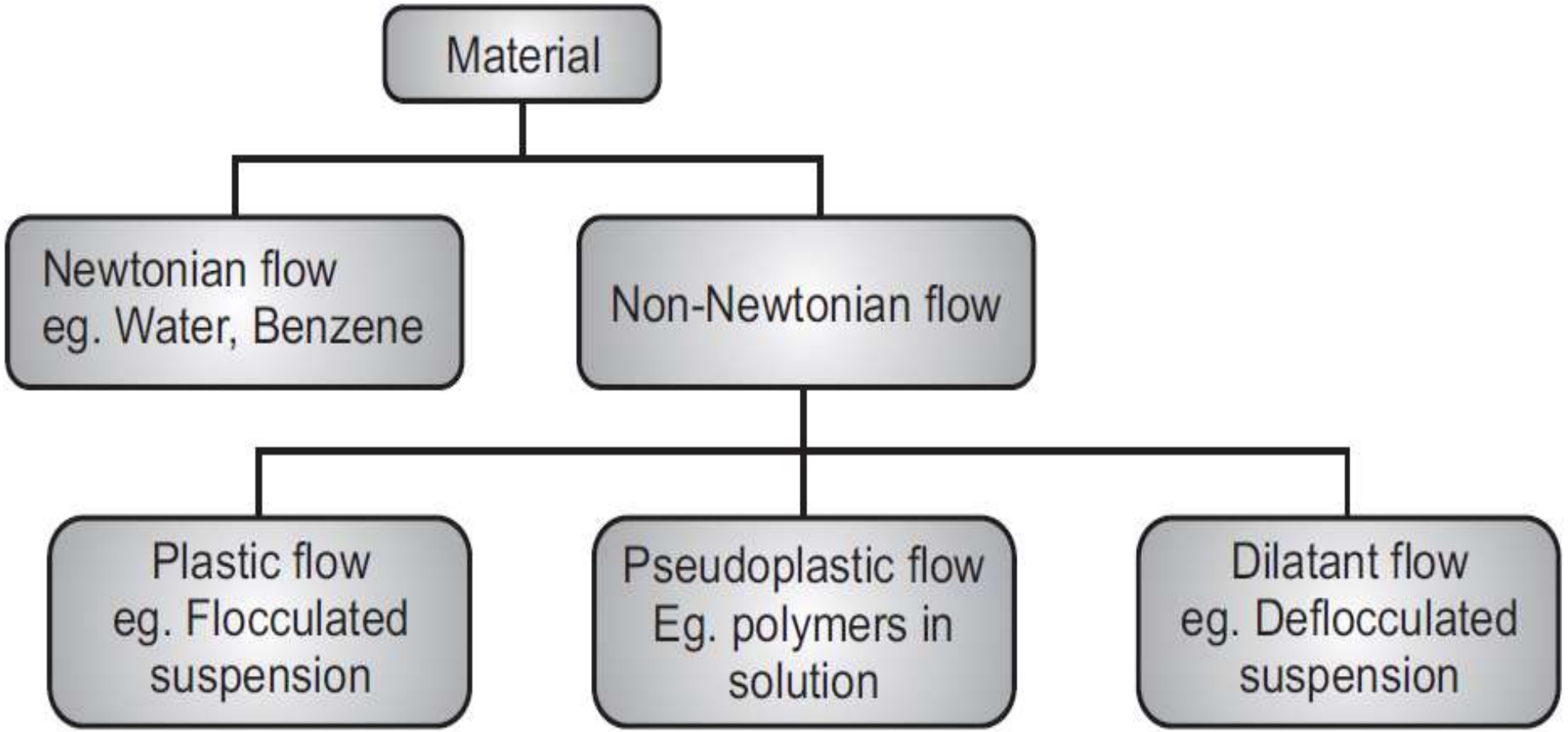
   Where,

   A is a constant which depends on the molecular weight and molar volume.

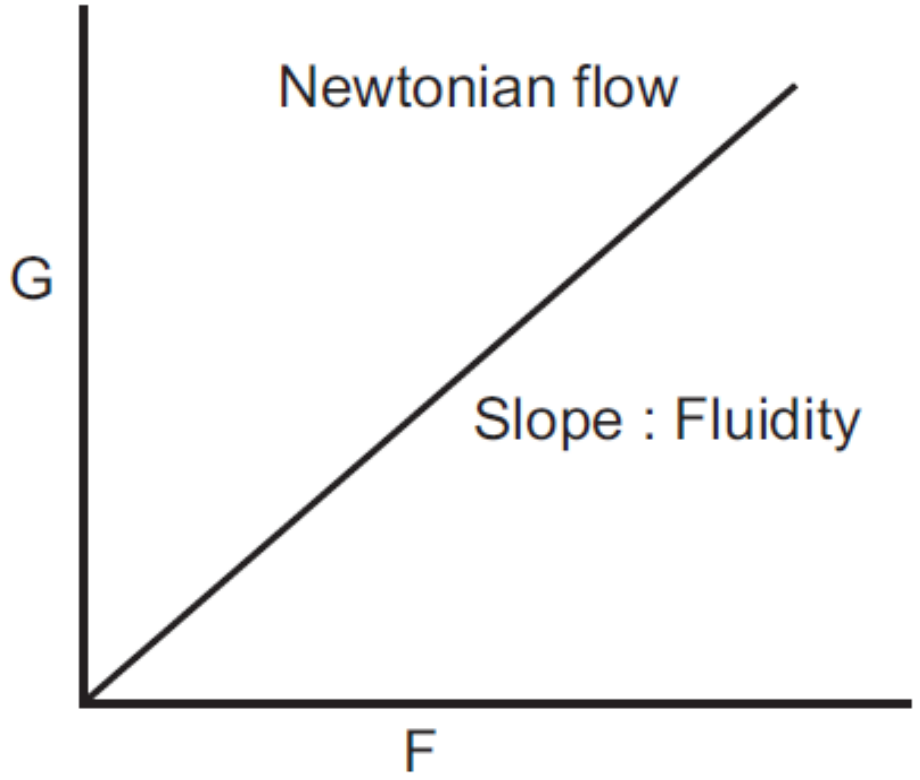
   E_v is an 'activation energy' required to initiate the flow between the molecules.

   For example, several mineral oils lose about 10 % of their viscosity if the temperature is only increased by 1 K.
3. **Shear rate:** The viscosity of most materials depends on the shear rate, i.e. the load.
4. **Pressure:** If great pressure is exerted on a material, its viscosity may increase as particles are organised in a more tight structure (resulting in more interaction possibilities).
5. Other influencing factors are the pH value, magnetic and electric field strength.

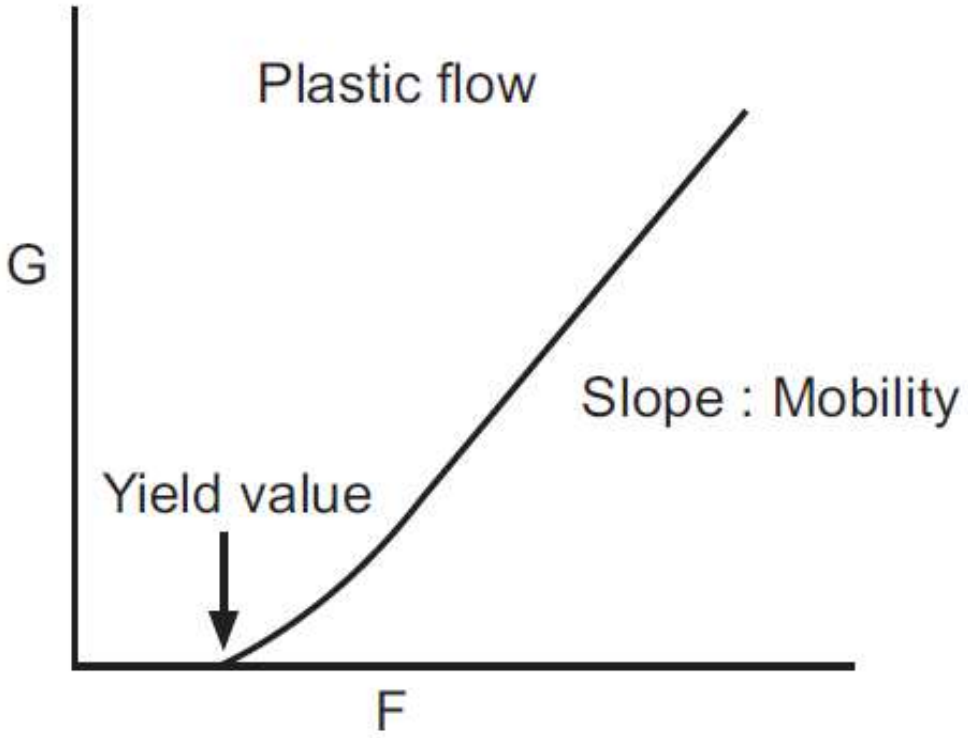
TYPES OF FLOW



Newtonian Flow

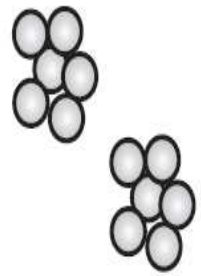


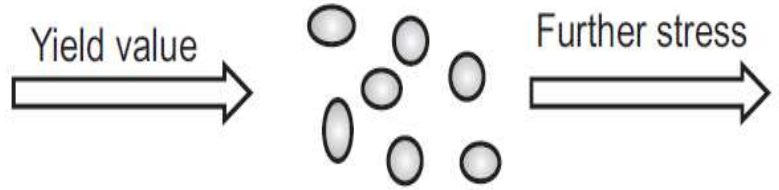
Plastic Flow



Flocculated suspension show plastic flow.

Bingham bodies





- Floccules at rest
- Particles aggregation
- Strong inter-particle contacts

- Particles individual
- Contacts: nil

$$U = \frac{F - f}{G}$$

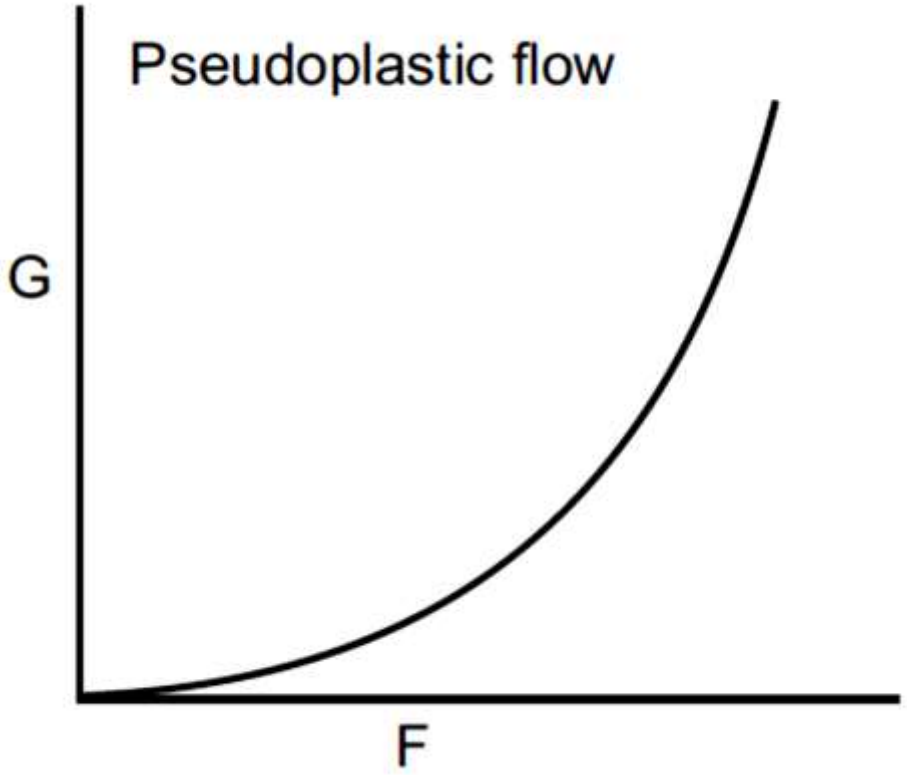
Where,

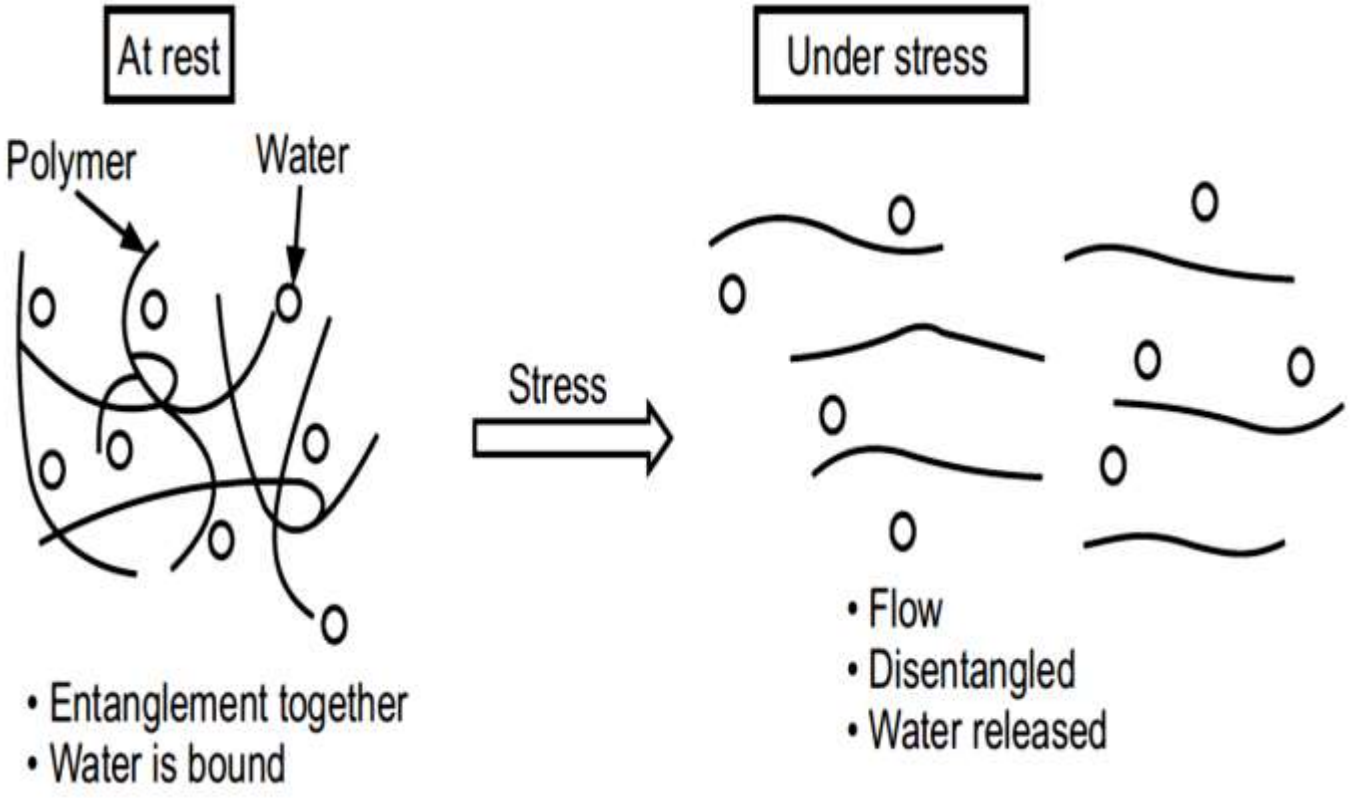
F : Shearing stress,

f : Yield value,

G : Rate of shear.

Pseudoplastic Flow





$$F^N = \eta G$$

where, N is a number given to the exponent.

In case;

N = 1, It is Newtonian flow.

N > 1, It is Pseudoplastic flow.

Taking log of both sides, equation (2.11) can be written as

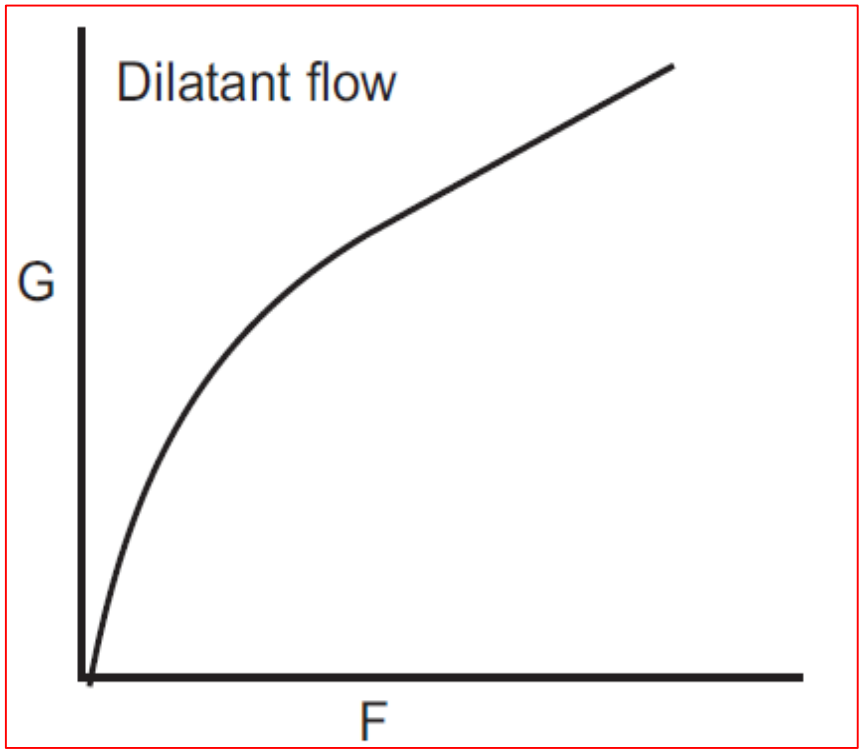
$$N \log F = \log \eta + \log G$$

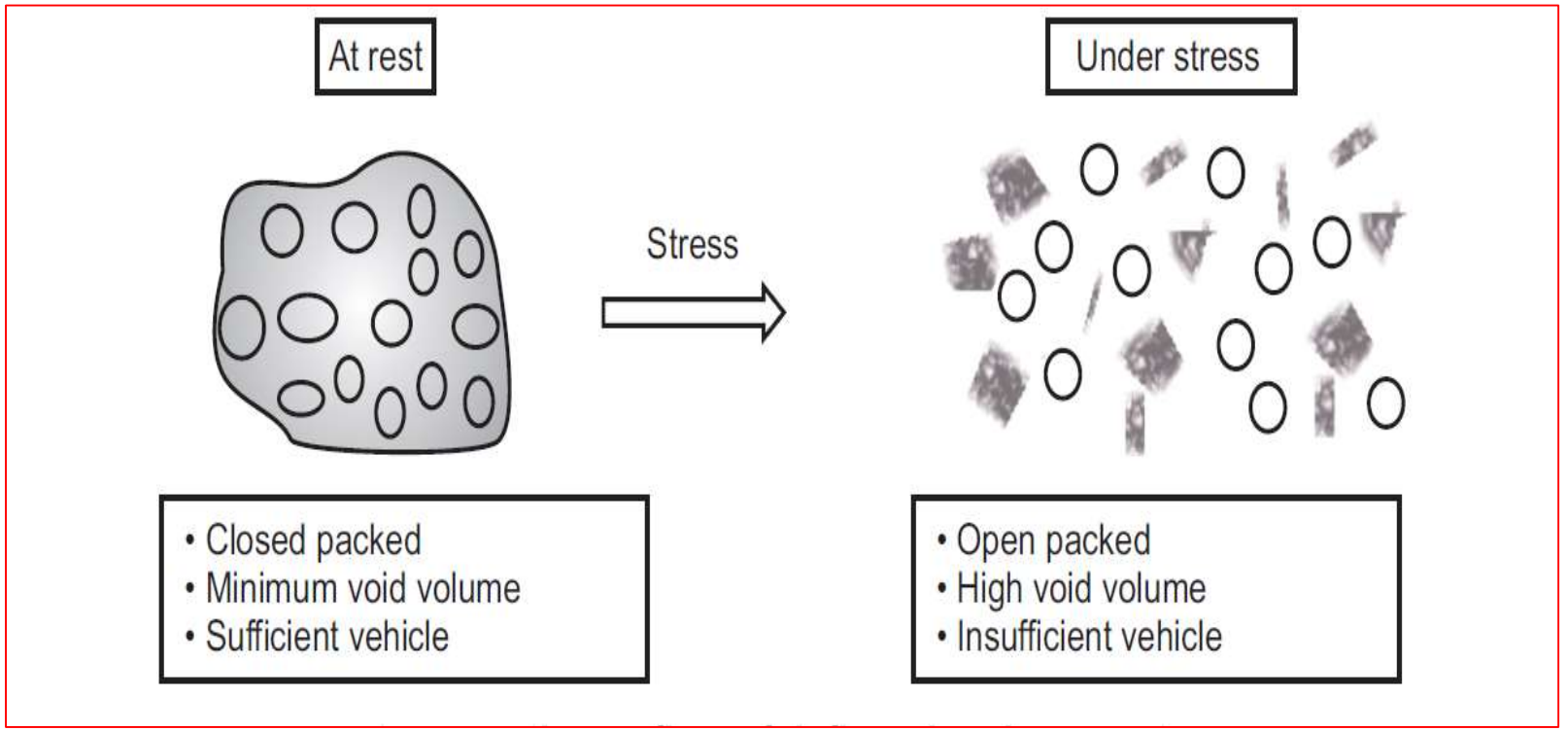
On rearrangement, we get

$$\log G = N \log F - \log \eta$$

Equation (2.13) represents a straight line.

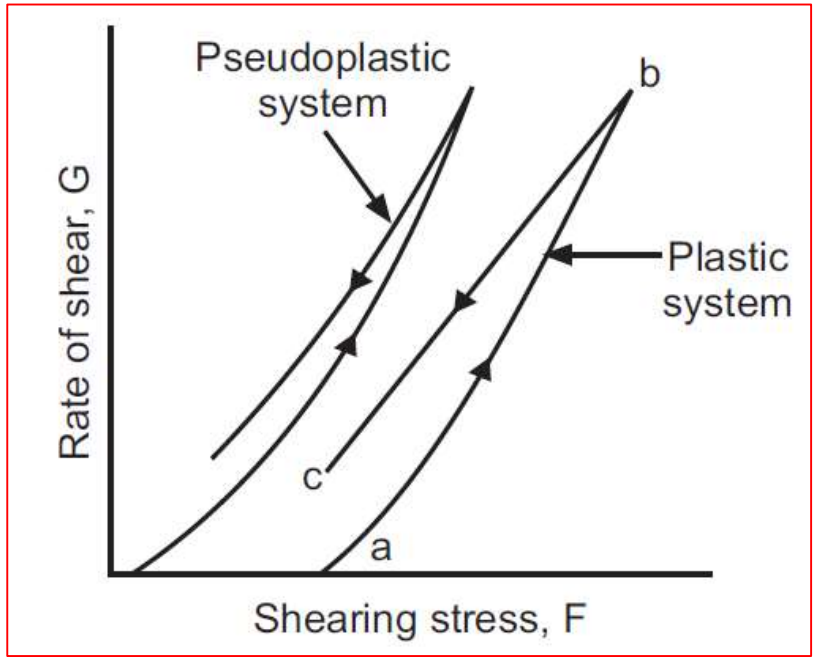
Dilatant Flow

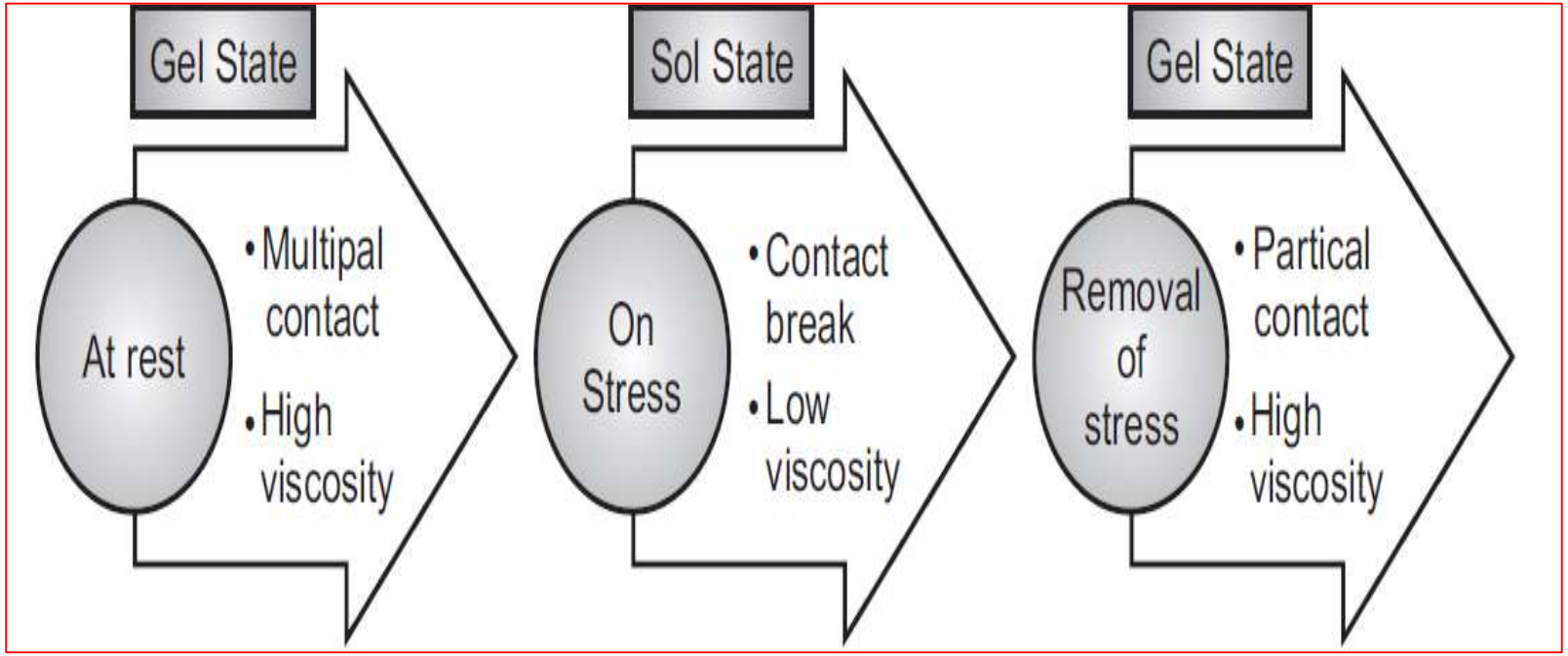




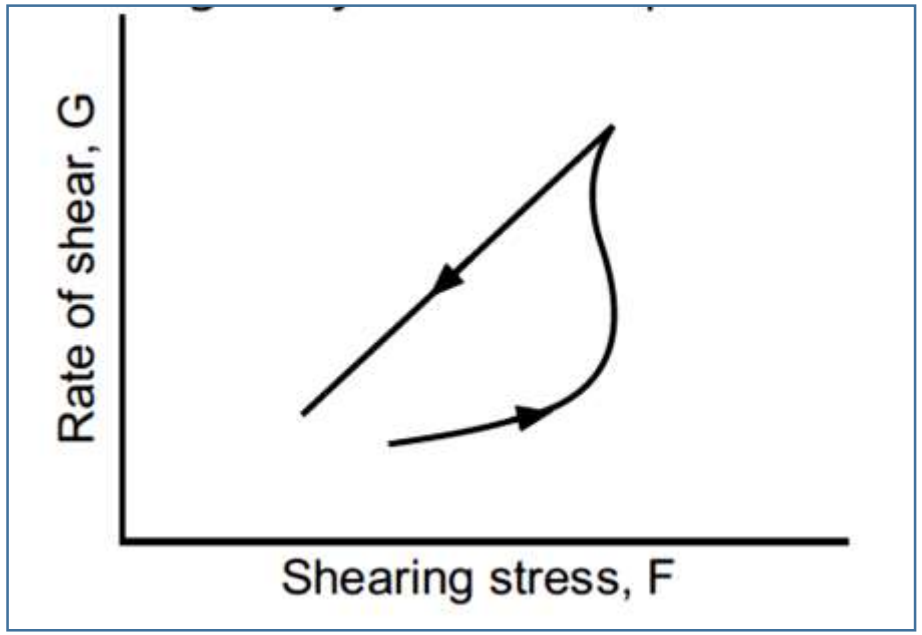
THIXOTROPY

Thixotropy is defined as **'an isothermal and comparatively slow recovery, on standing of material, of a consistency lost through shearing'.**



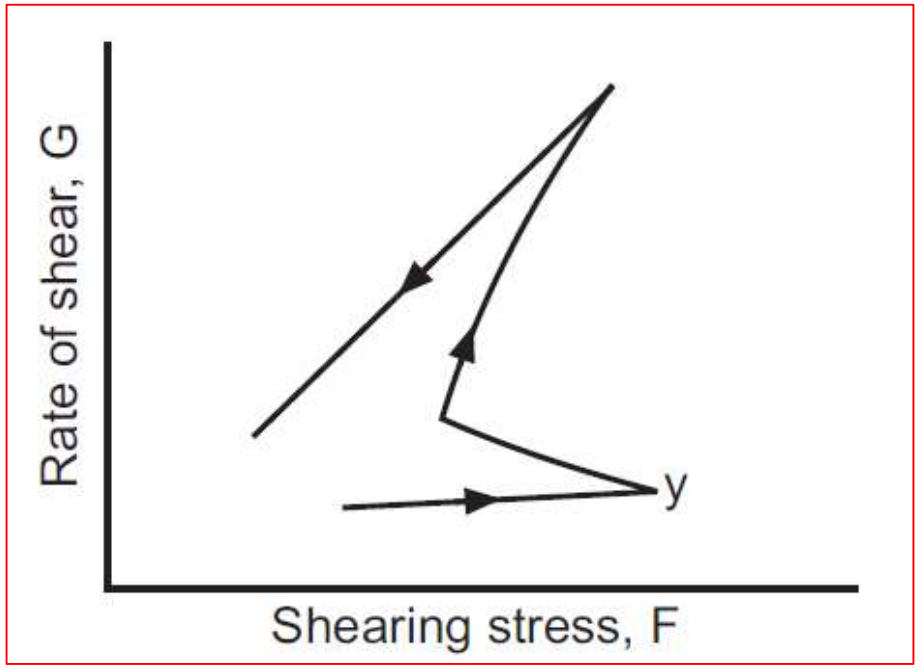


Bulges



Concentrated aqueous magma (gel) of Bentonite (10-15% w/w)

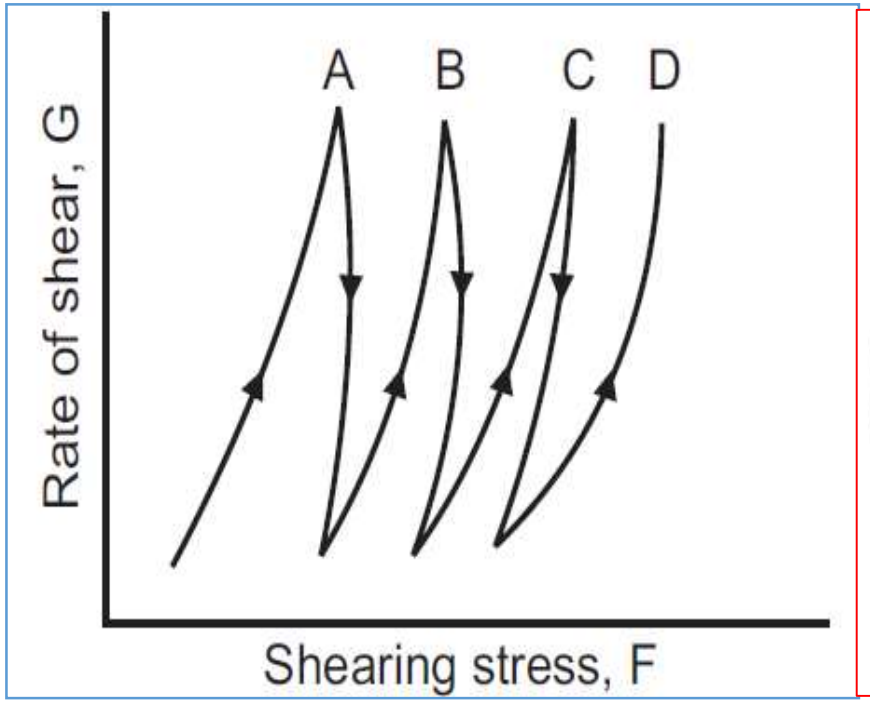
Spurs

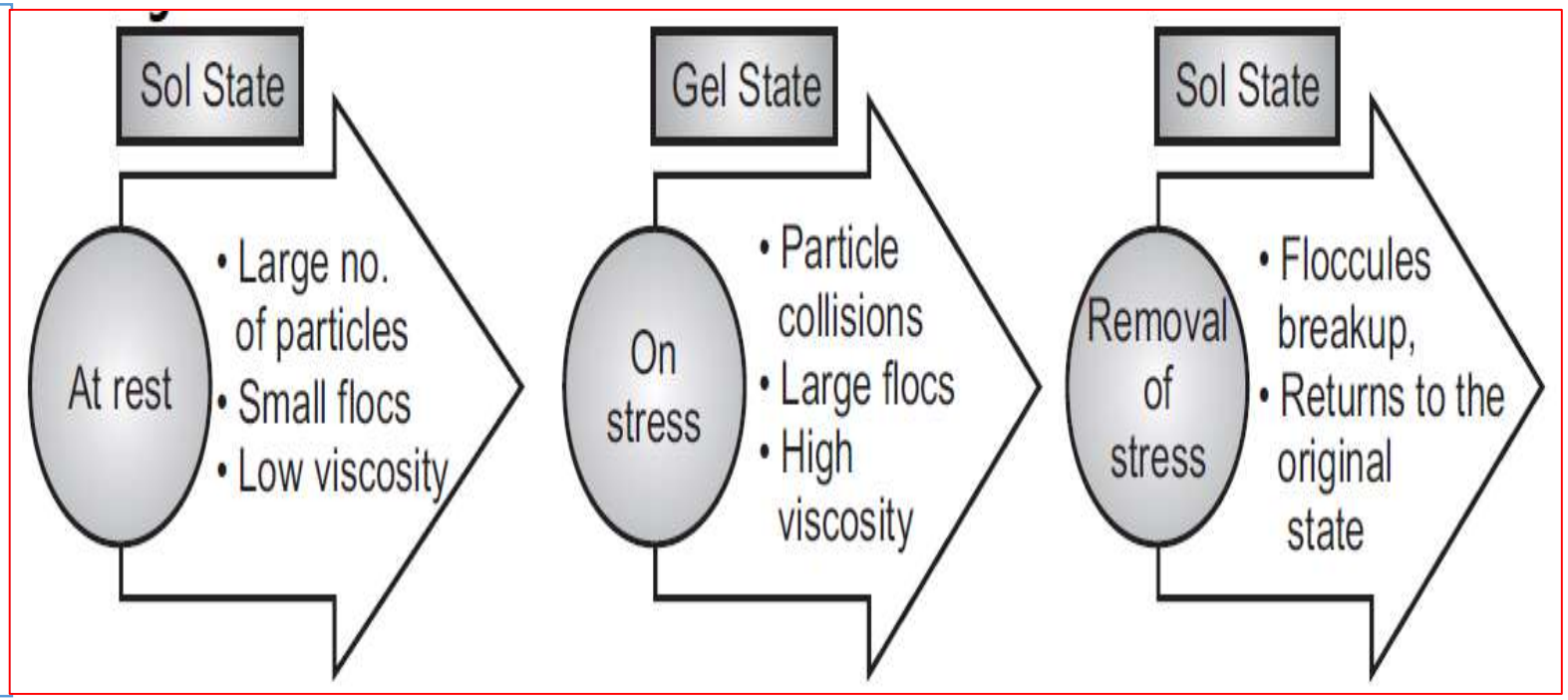


Procaine penicillin gel shows

NEGATIVE THIXOTROPY

Magnesia magma





Negative Thixotropy and Dilatant Flow:

1. Dilatant systems are deflocculated and the volume of solids is high (more than 50%),
2. Negative thixotropy is seen in a flocculated system containing low solid content (1 to 10%).

Applications:

Thixotropy is a desirable property particularly in emulsions, suspensions and creams.

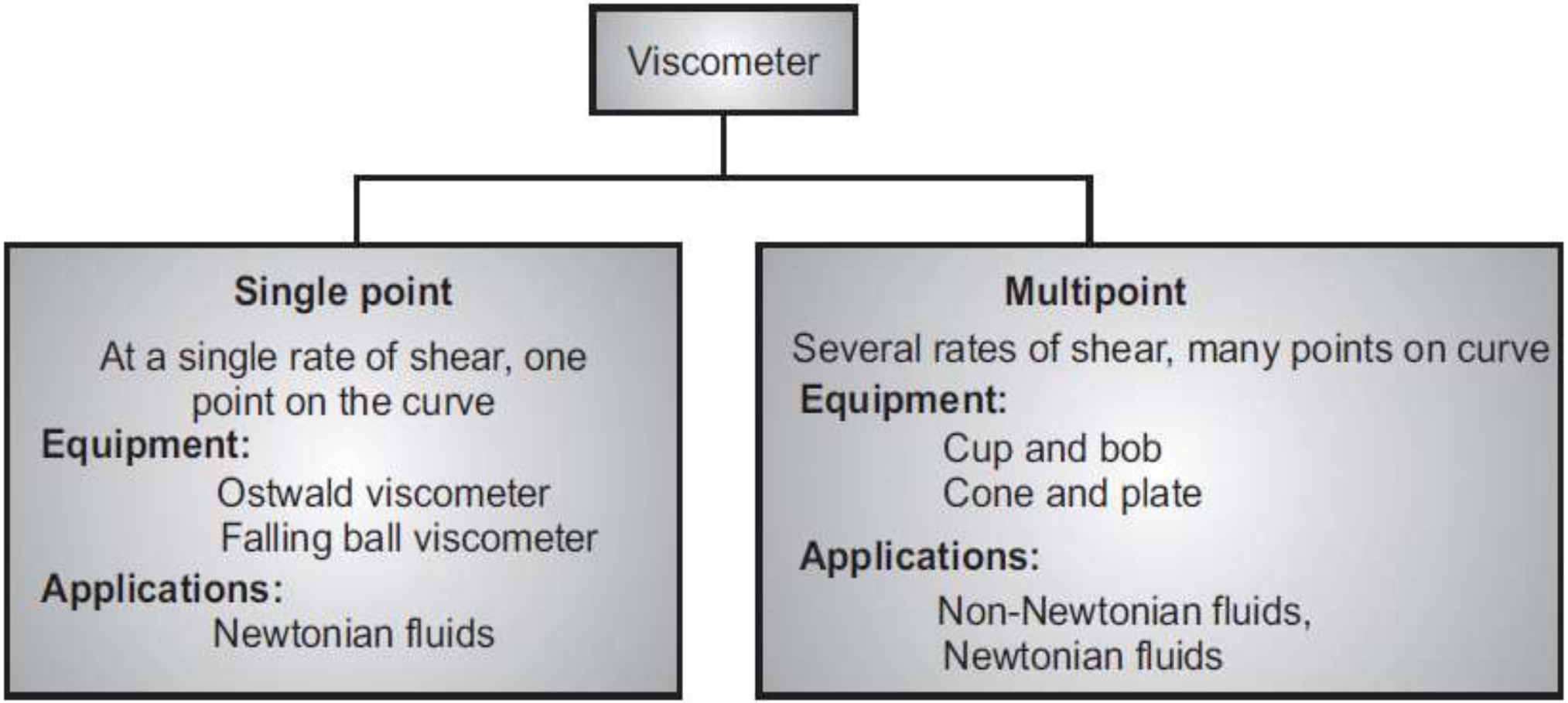
1. **Stability of suspension:** The greater the thixotropy, the higher is the physical stability of the suspension. During storage, a suspension should have high consistency (gel) in the container, so that the suspended particles do not settle rapidly.

   On moderate shaking, the suspension should become fluid (sol), so that the contents can be poured easily from the container. Thus, the principles of thixotropy are useful in dispensing and administration of a dose. At rest, the suspension regains its original consistency. These gel-sol-gel transformations improve the physical stability of dosage forms.

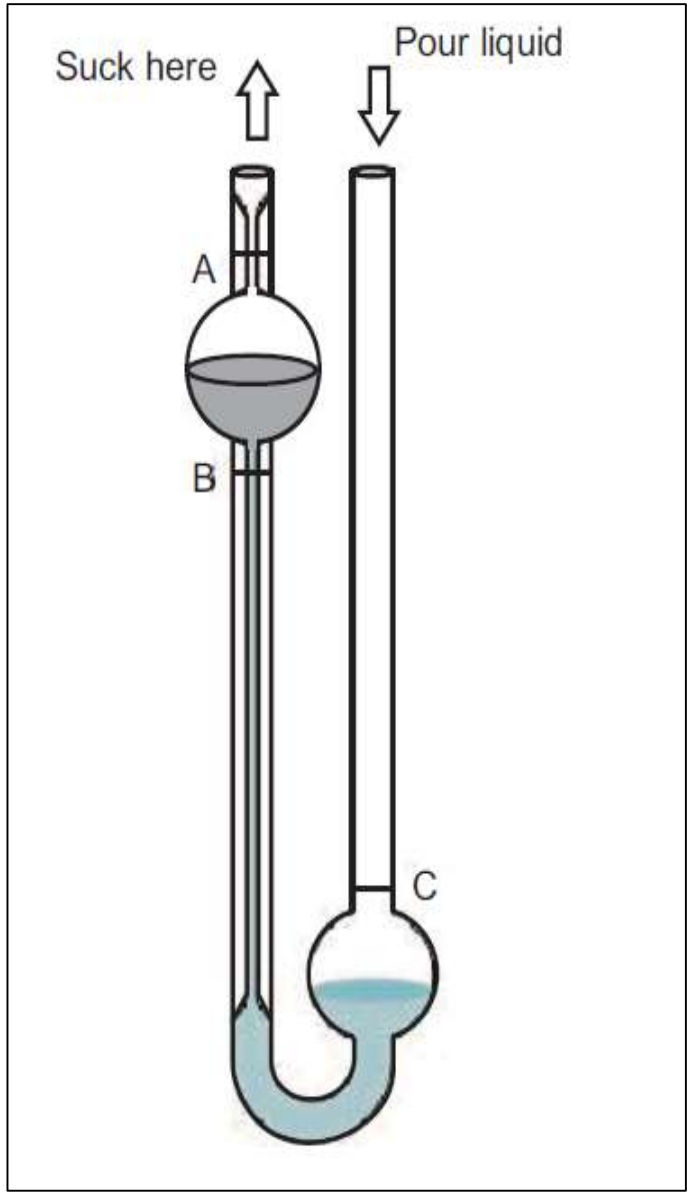
2. **Sustained release of drug:** The degree of thixotropy is related to the specific surface of penicillin used.

   Parenteral suspension containing 40 to 70% of procaine penicillin G in water has higher inherent thixotropy. While injecting the preparation, the structure of the suspended particles breaks down so that the product can pass through the hypodermic needle. After injection, the original structure of gel will be rebuilt. This leads to depot of the procaine penicillin G at the site of injection in the muscle, from, which it is slowly released so as to provide sustained release of drug in the body.

MEASUREMENT OF VISCOSITY



Capillary Viscometer (Ostwald Method)



Pioseulle's Equation for Viscosity

$$\eta = \frac{\pi r^2 P t}{8 l V} \longrightarrow \eta = K P t \quad \textbf{But,} \quad (P = \rho g).$$

$$\eta = K \rho t$$

Viscosity for unknown liquid; $\eta_1 = K \rho_1 t_1$

Viscosity for Water; $\eta_w = K \rho_w t_w$

The expression for relative viscosity $\left(\frac{\eta_1}{\eta_w}\right)$ can be derived as;

$$\frac{\eta_1}{\eta_w} = \frac{K \rho_1 t_1}{K \rho_w t_w} = \frac{\rho_1 t_1}{\rho_w t_w}$$

OR

$$\eta_1 = \frac{\rho_1 t_1}{\rho_w t_w} \eta_w$$

Where,

η_1 : Viscosity of unknown liquid,

η_w : Viscosity of water.

Rolling / Falling-Ball Viscometer

Rolling-ball viscometer: Angles between 10° and 80°.

Falling-ball viscometer: Angle is 80° or greater.

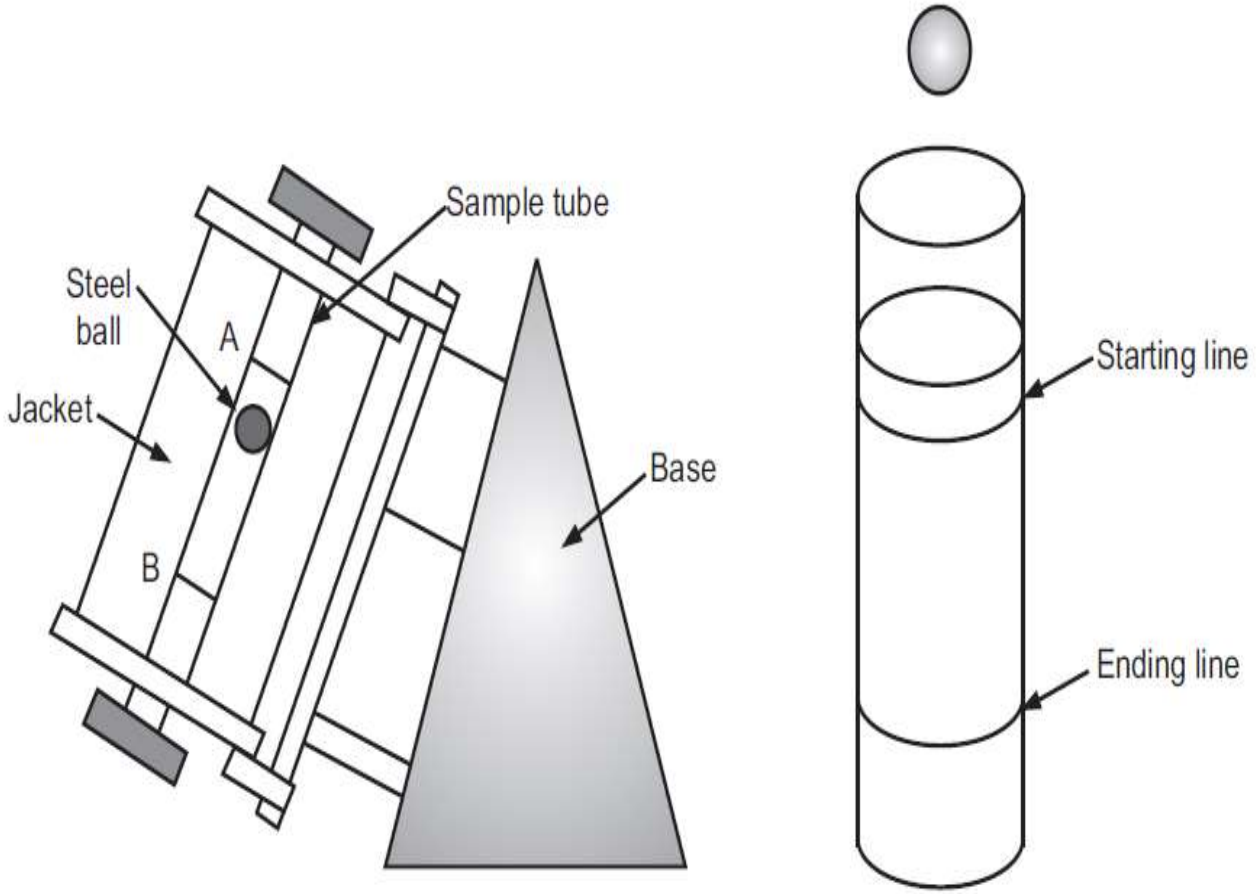


Fig. 2.17: Rolling / Falling-Ball viscometer

The viscosity of Newtonian liquid is calculated from:

$$\eta = t(\rho_b - \rho_f) B$$

Where,

η : The viscosity of liquid

t : The time travel in second

ρ_b : A density of ball

ρ_f : A density of liquid

B : A constant for ball.

Cup and Bob Viscometers

Classification:

According to rotation of cup and bob viscometers are classified as;

(i) The Couette type of viscometer:

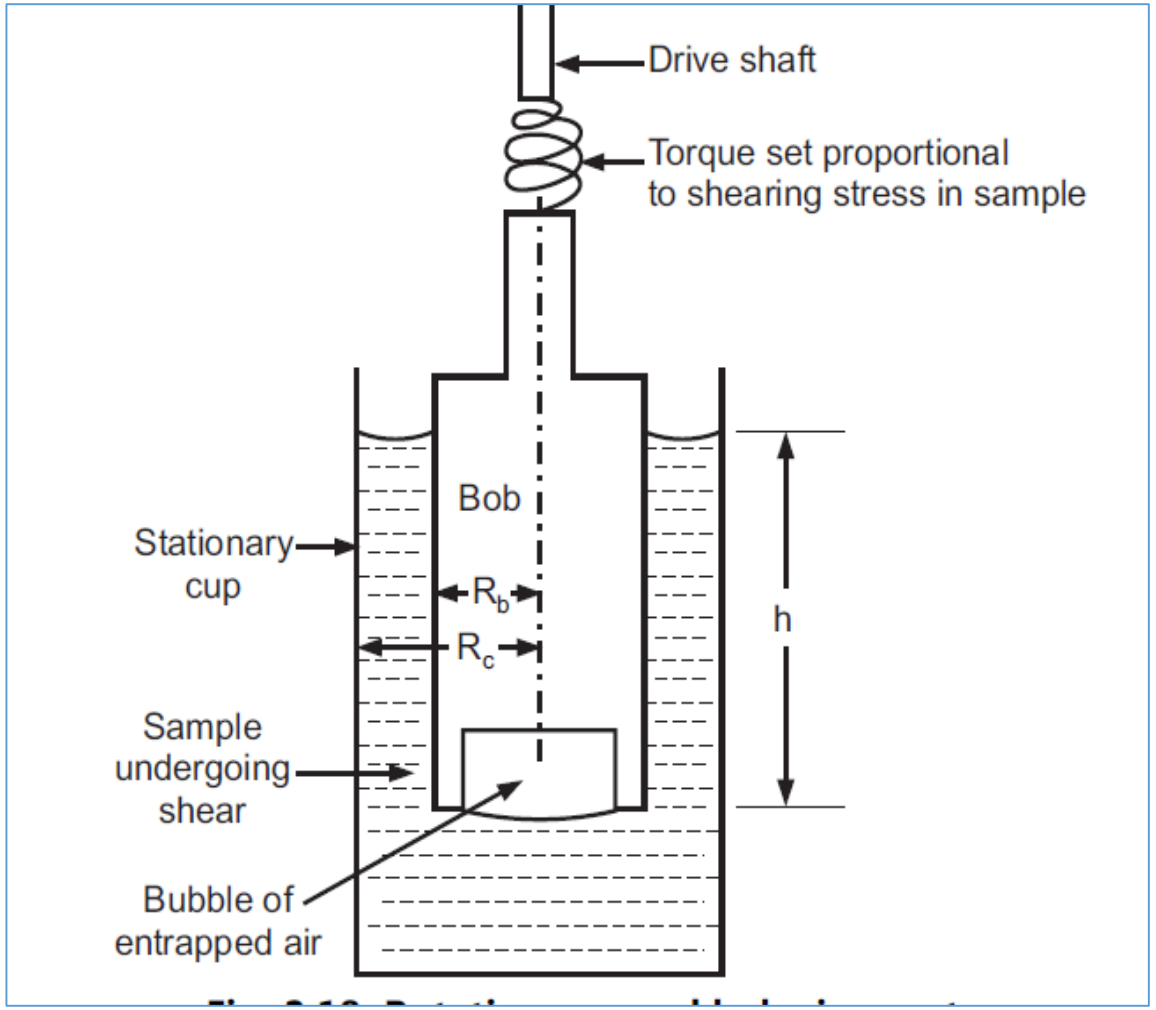
The cup is rotating and bob is stationary.

Example: MacMichael viscometer.

(ii) The Searle type of viscometer:

The cup is stationary and bob is rotating.

Example: Stormer instrument



Plug flow:

Mathematical Treatment:

For a rotational viscometer, shearing stress and shearing rate relationship can be expressed;

$$\Omega = \frac{1}{\eta}\frac{T}{4\pi h}\left[\frac{1}{R_b^2} - \frac{1}{R_c^2}\right] \quad \ldots (2.23)$$

Where,

Ω : The angular velocity in radians sec^{-1} produced by T,

T : The torque in dynes cm,

h : The depth to which the bob is immersed in the liquid,

R_b : The radius of the bob,

R_c : The radius of the cup.

It is frequently more convenient to combine all the constants (h, R_b and R_c) in equation (2.23), with the result that:

$$\eta = K_v\frac{T}{\Omega} \quad \ldots (2.24)$$

In which, K_v is a constant for the instrument.

With the modified Stormer viscometer, Ω is a function of v, the rpm is generated by the weight w, in grams, that is proportional to T. Equation (2.24) may then be written as:

$$\eta = K_v\frac{w}{v} \quad \ldots (2.25)$$

K_v may be obtained by analyzing material of known viscosity in poise.

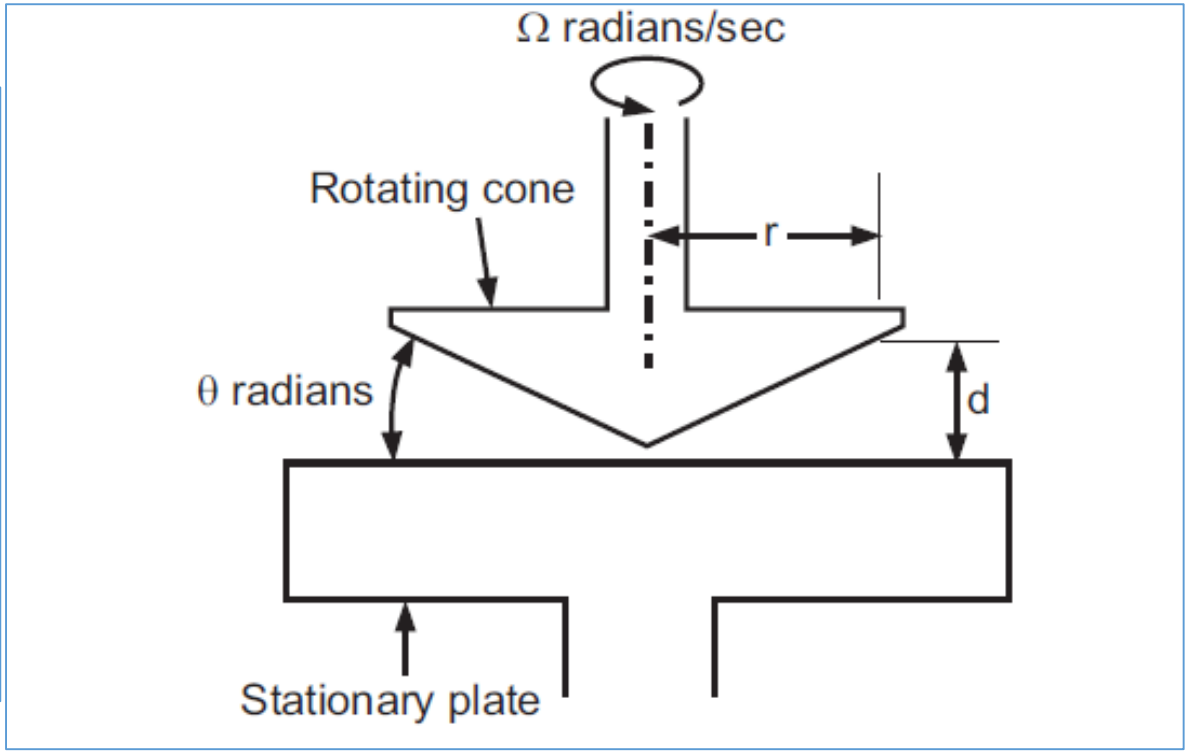
The equation for **plastic viscosity** when employing the Stormer viscometer is

$$U = K_v\frac{w - w_f}{v} \quad \ldots (2.26)$$

Cone and Plate Viscometer

Advantages:

1. The rate of shear is constant throughout the entire sample being sheared. Plug flow is not observed.
2. The sample required is small, 0.1 to 0.2 ml.
3. Cleaning and filling is easy.
4. Less time is required for temperature equilibration.



Mathematical Treatment:

For small values of θ in radians, the viscosity is determined;

$$\eta = \frac{3\theta}{2\pi R_b^2}\left(\frac{T}{\Omega}\right)$$

Where,

η : The viscosity.

Ω : The angular velocity in radians sec^{-1} produced by T,

T : The torque in dynes cm.

R_b : The radius of the cone,

θ : The angle formed by cone and plate.

It is frequently more convenient to combine all the constants (θ and R_b) in equation (2.28), with the result that:

$$\eta = C\frac{T}{\Omega} \quad \ldots (2.29)$$

Where, C is constant for instrument and it is obtained by analyzing material of known viscosity in poise.

Plastic Viscosity:

The plastic viscosity 'U' is estimated by equation

$$U = C_f . \frac{T - T_f}{v} \quad \ldots (2.31)$$

and yield value

$$f = C_f \times T_f \quad \ldots (2.32)$$

In which T_f is torque at the shearing stress an C_f is an instrumental constant.

VISCOELASTICITY

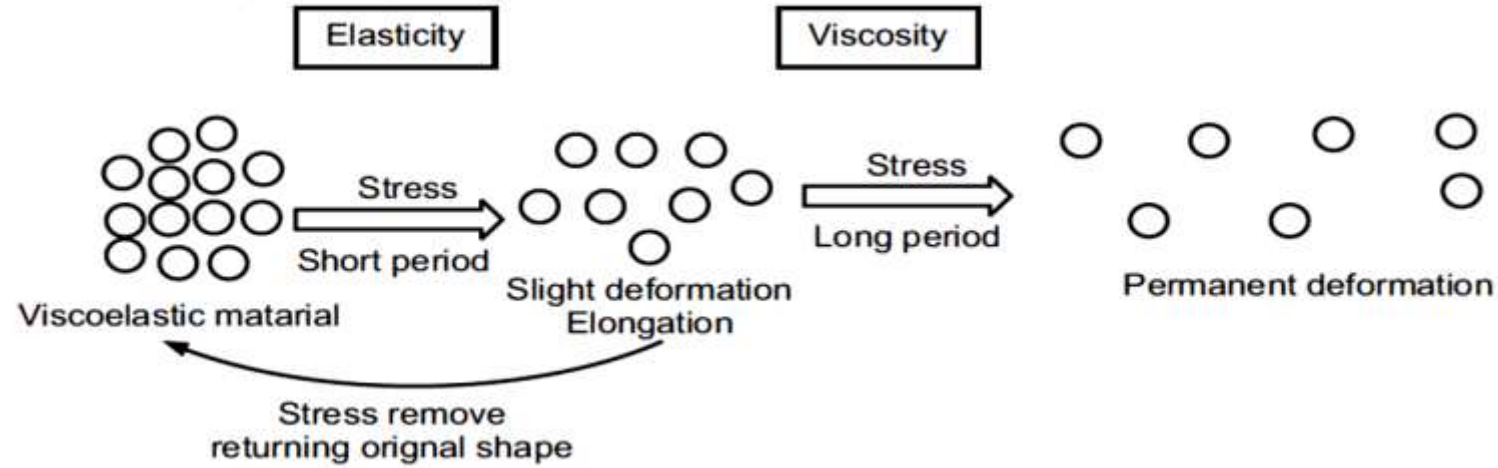
Viscoelastic measurements are based on the mechanical properties of materials that exhibit;

1. Viscous properties of liquids and
2. Elastic properties of solids.

Some example of viscoelastic substance;

1. In pharmacy: Creams, lotions, ointments, suppositories, suspensions, colloidal dispersing, emulsifying, and suspending agents.
2. Biologic materials: Blood, sputum and cervical fluid.

Here, rotational viscometer is not useful for determination of viscoelastic properties of substance, because rotational viscometers and similar flow instruments yield large deformations and may produce false results.



A semisolid is considered to demonstrate both solid and liquid characterises. The flow of a Newtonian fluid is expressed by using equation (2.3),

$$\eta = \frac{F}{G}$$

Relating shear stress F and shear rate G.

A solid material, on the other hand, is not characterized by flow but rather by elasticity, and its behaviour is expressed by the equation for a spring (derived from Hooke's law of general physics):

$$E = \frac{F}{y} \qquad \ldots (2.33)$$

Where,

E is the elasticity (dyne cm^{-2}),

F is the stress,

y is the strain.

Deformation of Solids

Plastic and elastic deformation	https://www.youtube.com/watch?v=YKpvYF0hVDE
Stress and Strain	https://www.youtube.com/watch?v=AnwrItEvqh4 https://www.youtube.com/watch?v=x-UiYHyAUaM
Heckel Equation	https://www.youtube.com/watch?v=6e6rTXM6uIo
Modulus Of Elasticity	https://www.youtube.com/watch?v=My-kVdbOfME

BP 403 T. PHYSICAL PHARMACEUTICS-II (Theory)

Unit-IV
Micromeritics

Subject Teacher:
Dr. Prashant Khade

Syllabus ...

(A) Introduction and Pharmaceutical Importance, Particle Size and Distribution, Particle Shape, Particle Volume, Particle Number, Surface Area, Methods for determining Particle Size, Particle Volume Measurement.

(B) Specific Surface, Method for Determining Surface Area.

(C) Derived Properties of Powder: Porosity, Packing Arrangement, Densities, Bulkiness, Flow properties of Powder, Angle of repose, Factors Affecting Flow of Powder.

Micromeritics is defined as 'the science and technology of small particles'.

Particle size (µm)	Approximate sieve size	Example
0.5-10	--	Suspension and fine emulsions.
10-50	--	Flocculated suspension and coarse emulsion.
50-100	325-140	Fine powder.
150-1000	100-18	Coarse powder.
1000-3360	18-6	Average granule size.

Unit

The unit of particle size used most frequently in micromeritics is the micrometer, µm, also called the micron, µ,

$$1\ \mu m = 10^{-6}\ m$$

$$1\ m\mu \text{ or } nm = 10^{-9}\ m$$

One must not confuse µm with mµ (millimicron). The millimicron now is most commonly referred to as the nanometer (nm).

PARTICLE SIZE

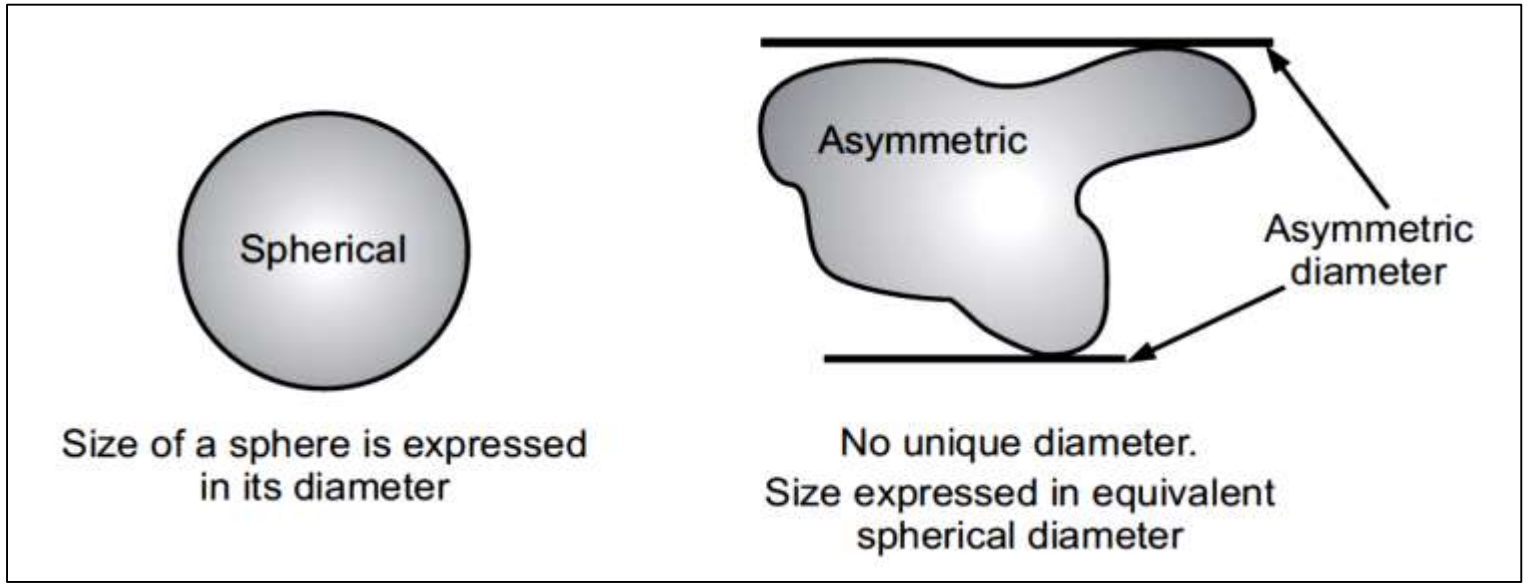
Pharmaceutical Importance

1. Drug release and dissolution:
2. Absorption and drug action:
3. Stability:
4. Dose uniformity:

In collection of particles there is uneven distribution of particles size means more than one size of particles i.e. **polydisperse sample** and same size of particles in collection of particles is termed as **mono-size or monodisperse.**

Also shapes of particles in collection are **non-uniform.** Some particle is **spherical in shape** and some are **asymmetric.**

The size of a sphere is readily expressed in terms of its diameter.



Equivalent Spherical Diameter for Asymmetric Particles

1. **Surface diameter, (d_s)** is the diameter of a sphere having the same surface area as that of the asymmetric particle.
2. **Volume diameter, (d_v)** is the diameter of a sphere having the same volume as that of the asymmetric particle.
3. **Projected diameter, (d_p)** is the diameter of a sphere having the same area as of the asymmetric particle when viewed under a microscope.
4. **Stokes diameter, (d_{st})** which describes an equivalent sphere undergoing sedimentation at the same rate as the asymmetric particle. Stokes' diameter is determined from sedimentation studies on the suspended particles.

Average Particle Size

Equation for Average Particle Size:

Edmundson has derived a general equation for the average particle size, whether it is an arithmetic, geometric, or harmonic mean diameter.

$$d_{mean} = \sqrt[p]{\frac{\sum nd^{P+f}}{\sum nd^{f}}}$$

In equation (4.1),

n : The number of particles in a size range,

d : The equivalent diameters,

p : An index related to the size of an individual particle, since d raised to the power p = 1, p = 2 or p = 3 is an expression of the particle length, surface or volume, respectively.

The value of the index p also decides whether;

(a) arithmetic mean: p is positive

(b) geometric mean: p is zero or

(c) harmonic mean: p is negative.

f : The frequency index, has values of 0, 1, 2 or 3, then the size frequency distribution is expressed in terms of the total number, length, surface or volume of the particles, respectively.

1. Length number mean (d_{in}):

When, p = 1 and f = 0

In this case, equation (4.1) is expressed as follows:

$$d_{ln} = \frac{\sum nd}{\sum n}$$

This mean is satisfactory if size range is narrow and distribution is normal. These conditions are rarely found in pharmaceutical powders.

2. Surface-number mean (d_{sn}):

When, p = 2 and f = 0

In this case, equation (4.1) is expressed as follows:

$$d_{sn} = \sqrt{\frac{\sum nd^2}{\sum n}}$$

It refers to particles having average surface area.

3. Volume-numbers mean (d_{vn}):

When, p = 3 and f = 0

In this case, equation (4.1) is expressed as follows:

$$d_{vn} = \sqrt[3]{\frac{\sum nd^3}{\sum n}}$$

Refers to particle having average weight and is related inversely to N, the number of particles per gram of material.

4. Surface-Length or Length-Weight Mean (d_{vs}):

When, p = 1 and f = 1

In this case, equation (4.1) is expressed as follows:

$$d_{sl} = \frac{\sum nd^2}{\sum nd}$$

This is not practical significant.

5. Volume-Surface or Surface-Weight Mean (d_{vs})

When, p = 1 and f = 2

In this case, equation (4.1) is expressed as follows

$$d_{vs} = \frac{\sum nd^3}{\sum nd^2}$$

Important pharmaceutically, because inversely related to the specific surface area Sw.

6. Weight-moment or volume-weighted mean (d_{wm}):

When, p = 1 and f= 3

In this case, equation (4.1) is expressed as follows

$$d_{wm} = \frac{\sum nd^4}{\sum nd^3}$$

This diameter has limited pharmaceutical significances.

PARTICLE SIZE DETERMINATION

Particle size of drugs in pharmaceutical products can affect:
1. The release of drugs from dosage forms that are administered orally, parenterally, rectally and topically.
2. The physical stability and pharmacologic response of suspensions, emulsions and tablets.
3. Flow properties and mixing of granules and powders in tablet and capsule manufacture.

Methods to estimate particle size are:

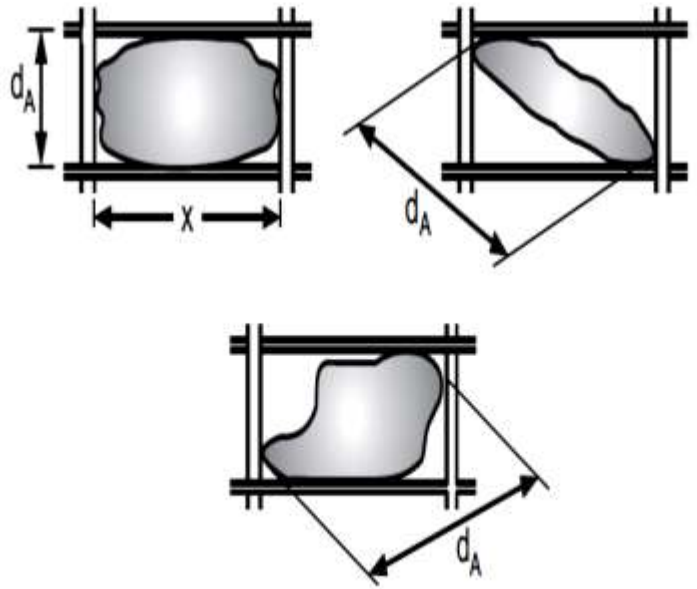
1. Sieve method
2. Optical microscopy
3. Sedimentation method
4. Coulter counter method

Sieve Method

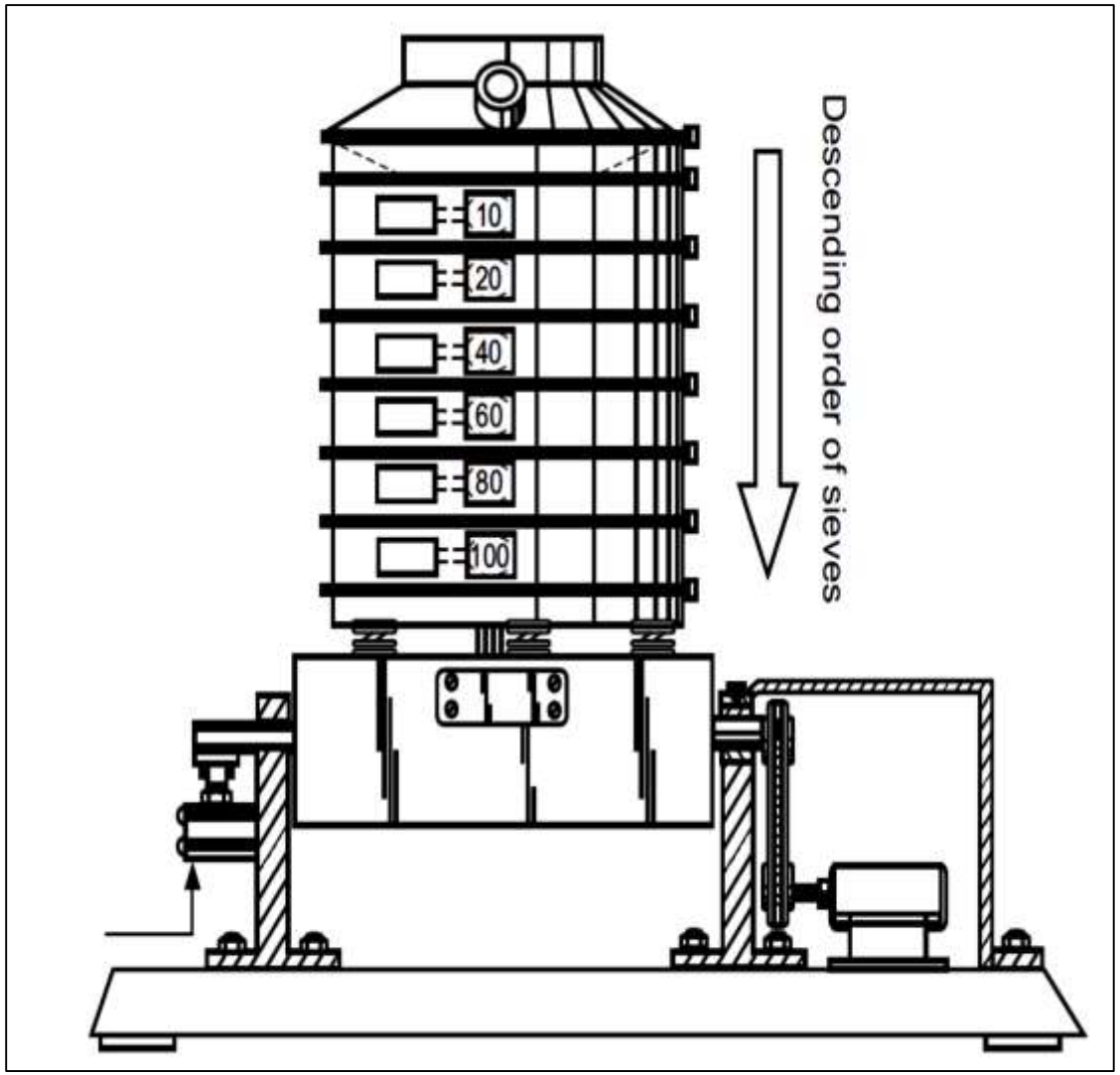
Range of Analysis:
45 μm- 1000 μm,

Equivalent Diameter:

Sieve diameter, d_{sieve}, is defined as 'the width of the minimum square aperture through which the particle will pass' and shown diagrammatically in Fig. 4.2 for different-shaped particles.



Sieve number	Aperture size micrometer	Sieve number	Aperture size micrometer
10	1700	44	325
12	1400	60	250
16	1000	85	35
22	710	100	36
25	600	120	34
30	500	150	36
36	425	170	35



Advantages of Sieve Method:

1. It is inexpensive, simple and rapid with reproducible result.

Disadvantages of Sieve Method:

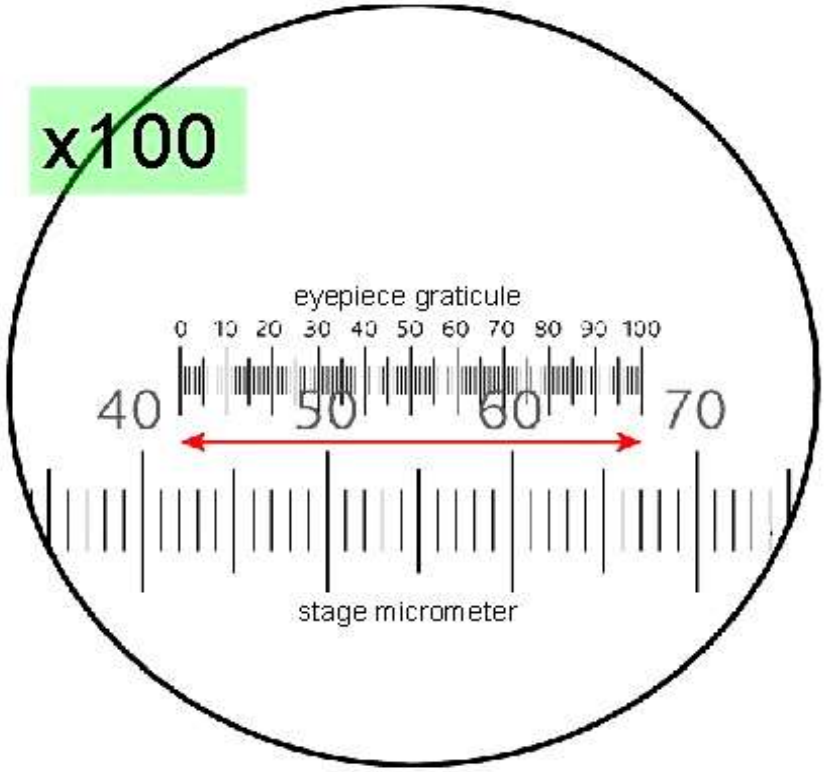
1. Lower limit of the particle size is 45 µm.
2. If powder is not dry, apertures become clogged with particles, leading to improper sieving.
3. During shaking, attrition (particles colliding with each other) occurs causing size reduction of particles. This leads to errors in estimation.

Optical Microscopy

Range of Analysis: 0.2 µm to 100 µm.

Equivalent Diameter:

In this method, the size is expressed as projected diameter (dp) which describes the diameter of a sphere having the same area as the asymmetric particle when observed under a microscope



Stage micrometer
67- 42 = 25 'stage units'

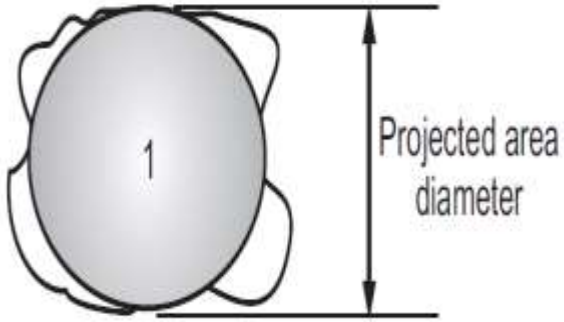
We know each 'stage unit'
= 10µm

So in total we have
25 x 10µm = 250µm

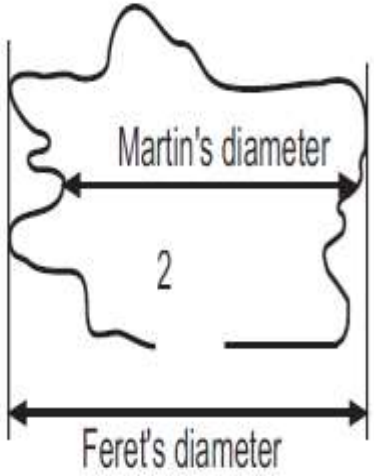
250µm = 100 'eyepiece' units

So each eyepiece unit
= 250/100 = 2.5µm

The micrometer can now be removed as the eyepiece graticule is now calibrated.



(a) Projected area diameter



(b) Martin's and Feret's diameter

Other Diameters:

1. **Projected area diameter:** Diameter of circle having same area as the projected area of the particle resting in stable position. [Fig. 4.4 (a)]
2. **Feret's diameter:** The mean value of the distance between pairs of parallel tangents to the projected outline of the particle. This can be considered as the boundary separating equal particle areas. [Fig. 4.4 (b)]
3. **Martin's diameter:** The mean chord length of the projected outline of particle. [Fig. 4.4 (b)]

Advantages of Optical Microscopy:

1. Easy and simple.
2. Contamination and aggregation of particles can be detected.
3. Small quantity of sample required.

Disadvantages of Optical Microscopy:

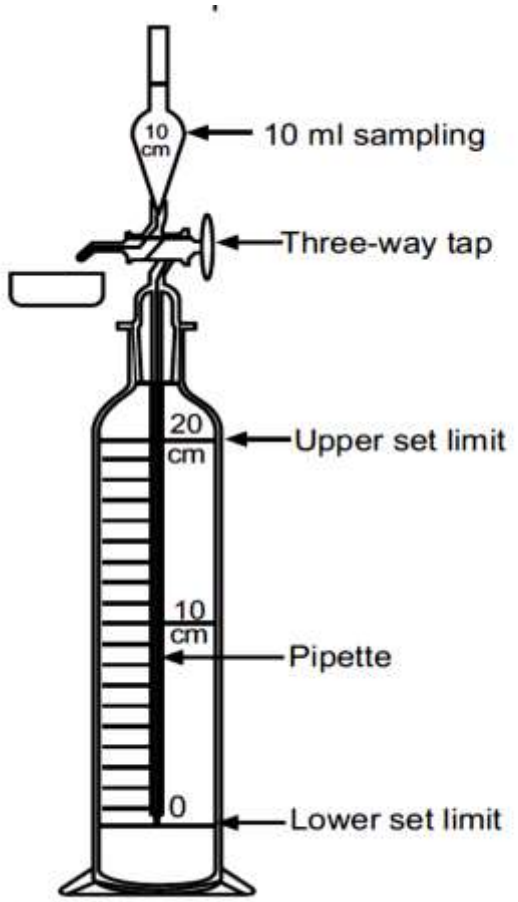
1. This method is time consumable and tedious.
2. Result is not reproducible.

Sedimentation Methods

Range of Analysis: 1 µm to 200 µm.

Equivalent Diameters:

In this method, size is expressed as Stoke's diameter, dst, which describe the diameter of an equivalent sphere having the same rate of sedimentation as that of the asymmetric particle.



ı. 4.5: Diagram of Andreasen pipette

$$v = \frac{h}{t} = \frac{d_{st}^2 (\rho_s - \rho_o)\, g}{18\, \eta} \qquad \ldots (4.8)$$

Where,

v : Rate of sedimentation,

h : Distance of fall in time, t (Height of liquid above the lower end of the pipette at the time of sampling),

η : Viscosity of the medium,

ρ_s : Density of the particles,

ρ_0 : Density of the dispersion medium,

g : Acceleration due to gravity,

d_{st} : Stokes diameter of particle.

The equation (4.8) is rearranged to get the Stokes' diameter, d_{st} of particles.

$$d_{st} = \sqrt{\frac{18\, \eta h}{(\rho_s - \rho_o)\, g\, t}} \qquad \ldots (4.9)$$

Limitation for Stokes' equation: Flow should be laminar:

Type of flow is determined by Reynolds number, R_e, which is defined as follows:

$$R_e = \frac{v\, d\, \rho_o}{\eta} \qquad \ldots (4.10)$$

Where all symbols have the same meaning as in equation (4.8).

In case,

R_e = or < 0.2, flow is laminar, Stokes' equation is applicable, but

R_e > 0.2, flow is turbulences, Stokes' equation is not applicable

Therefore, combining equations (4.8) and (4.10), we get

$$v = \frac{d_{st}^2\, (\rho_s - \rho_o)\, g}{18\, \eta} = \frac{R_e\, \eta}{d_{st}\, \rho_o} \qquad \ldots (4.11)$$

$$d_{st}^3 = \frac{18\, \eta^2\, R_e}{(\rho_s - \rho_o)\, g\, \rho_o} \qquad \ldots (4.12)$$

Equation (4.12) allows calculating maximum particle diameter when R_e > 0.2.

Advantages of Sedimentation:

1. Easy and simple.
2. Applicable in determination of stability of suspension and emulsion.

Disadvantages of Sedimentation:

1. One of the limitations of gravitational sedimentation is that below a diameter of approximately

5 μm, particle settling becomes prolonged because of diffusion and Brownian motion of particle.

2. Time consuming.

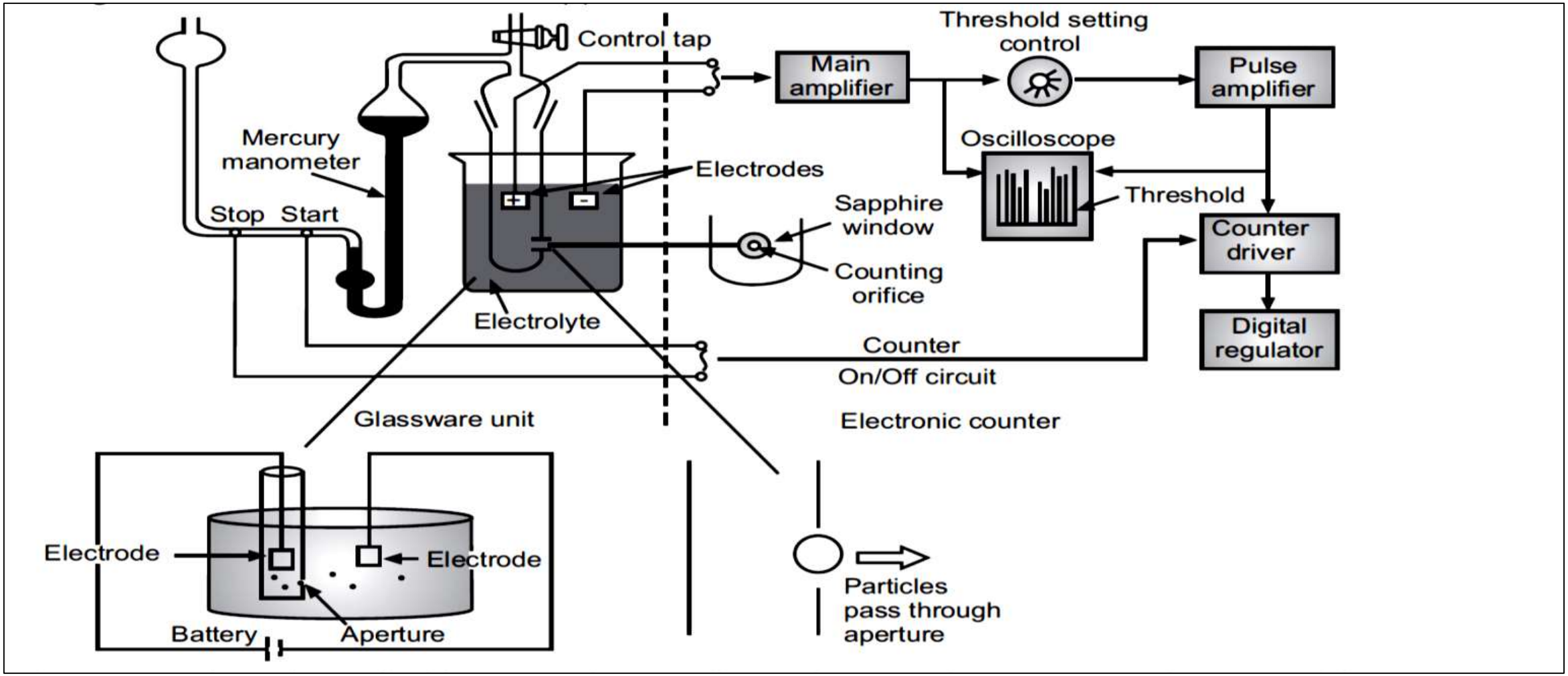
3. Large sample is required.

4. In case of turbulences flow, incorrect result.

Particle Volume Measurement (Coulter Counter Method)

Range of analysis: 0.5 µm to 500 µm

Equivalent diameters:
Size is expressed as volume diameter, d_v, which describe the diameter of the sphere having the same volume as that of the asymmetric particles.



Advantages of Coutler Counter Method:

1. 4000 particle per second can be counted.
2. Highly accurate method.
3. Short period required to determination of size.
4. It can be useful for determination of particle contamination in parenteral solution.

Disadvantages of Coutler Counter Method:

1. Unsuitable for water soluble drug, if hydrophilic drug disperse in non-polar solvent, it may produce adequate conductance.
2. It is expensive method.

PARTICLE NUMBER AND WEIGHT

Assuming particles are spherical in shape following relation may be obtained.

$$\text{Volume of a single particle} = \frac{1}{6}\pi d_{vn}^3 \quad \ldots (4.13)$$

$$\text{Mass of single particle} = \text{Volume} \times \text{Density}$$

$$\text{Mass of single particle} = \frac{1}{6}\pi d_{vn}^3 \rho \quad \ldots (4.14)$$

The following relation may be obtained.

$$\frac{\text{Weight of single particle}}{\text{1 particle}} = \frac{\text{Weight of powder (1 g)}}{\text{Number of particles, N}}$$

$$\frac{\frac{1}{6}\pi d_{vn}^3 \rho}{1} = \frac{1\ g}{N}$$

$$N = \frac{6}{\pi d_{vn}^3 \rho} \quad \ldots (4.15)$$

Powder Weight:

$$\text{Mass of powder} = \text{Number of particles} \times \text{Volume} \times \text{Density}$$

$$\text{Mass of powder} = N\frac{1}{6}\pi d_{vn}^3 \rho \quad \ldots (4.16)$$

The shape of the particle and density are independent of particle size. Considering the constant, π and ρ in equation (4.16), the relationship can be written as;

$$\text{Mass of powder} \propto N\ d_{vn}^3 \quad \ldots (4.17)$$

PARTICLE SIZE DISTRIBUTION

01. Number of Particles Versus Particle Size

Size range (μm)	Mean size range D (μm)	Number of particles (n)
0.50 - 1.00	0.75	2
1.00 - 1.50	1.25	10
1.50 - 2.00	1.75	22
2.00 - 2.50	2.25	54
2.50 - 3.00	2.75	17
3.00 - 3.50	3.25	8
3.50 - 4.00	3.75	5
		$\Sigma n = 118$

- 68% of the population lie ± 1σ from the mean,
- 95.5% lie within the mean ± 2σ and
- 99.7% lie within the mean ± 3σ.

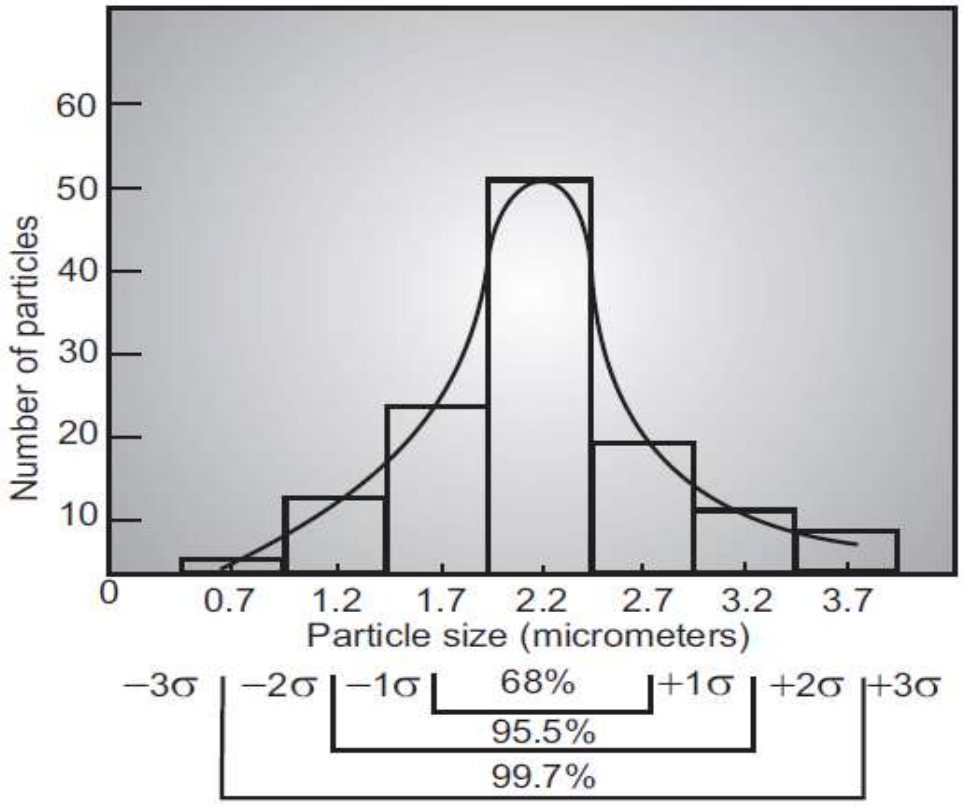
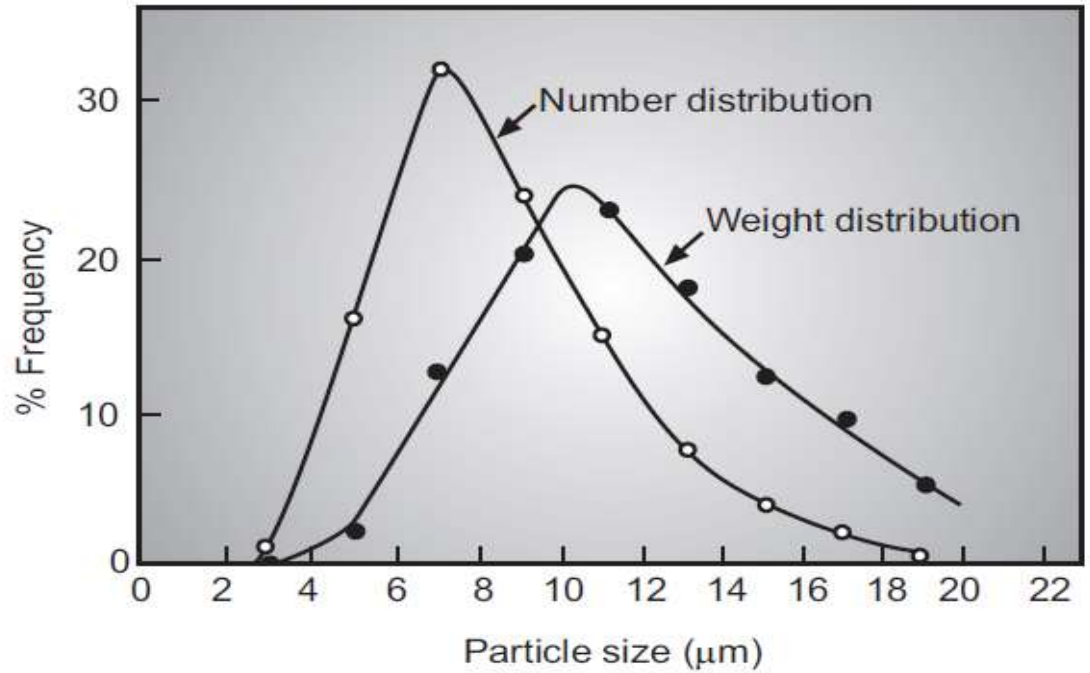
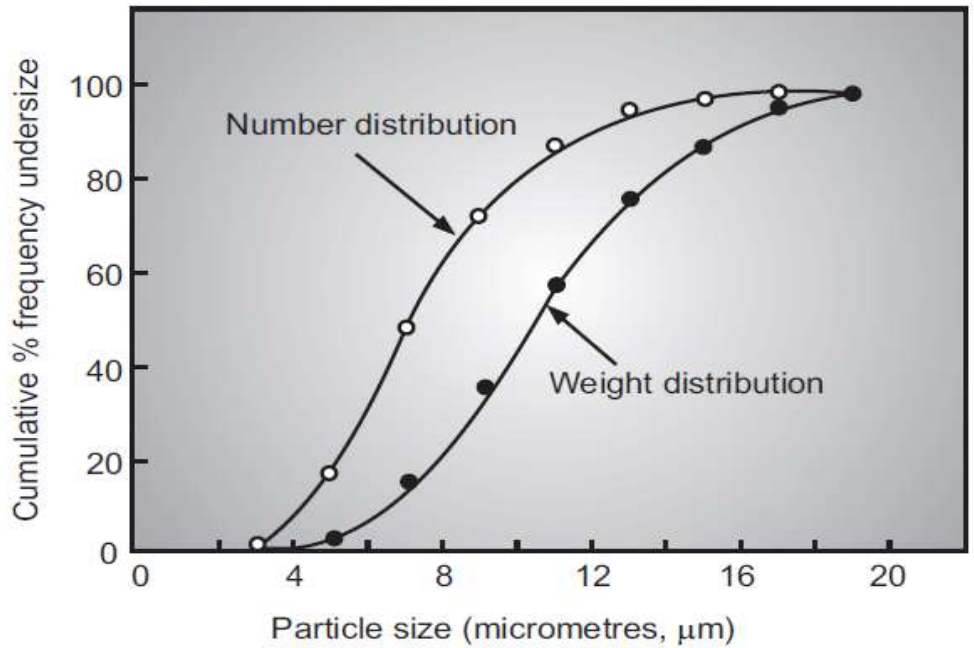


Fig. 4.7: Normal distribution curve

Size range (μm)	Mean size range, D (μm)	Log of mean size range, D (μm)	Number of particles, (n)	% n	Cumulative % frequency undersize (n)	% Weight (nd^3)	Cumulative % frequency undersize (Weight)
2 - 4	3	0.477	2	1	1	0.03	0.03
4 - 6	5	0.698	32	16	17	2.31	2.34
6 - 8	7	0.845	64	32	49	12.65	14.99
8 - 10	9	0.954	48	24	73	2.16	35.15
10 - 12	11	1.041	30	15	88	23.01	58.16
12 - 14	13	1.113	14	7	95	17.72	75.88
14 - 16	15	1.176	6	3	98	11.67	87.55
16 - 18	17	1.230	3	1.5	99.5	8.49	96.04
18 - 20	19	1.278	1	0.5	100	3.95	99.99
			Σn = 200				





PARTICLE SHAPE

	Surface area	Volume
Sphere	πd_s^2	$\frac{\pi d_s^3}{6}$
Asymmetric particle	$\alpha_s d_p^2$	$\alpha_v d_p^3$

Where, d_s and d_p are diameters of sphere and asymmetric particle respectively, α_s is surface area factor and α_v volume factor.

Solving for α_s and α_v by equating the appropriate properties (surface area and volume) provides:

$$\alpha_s = \frac{\pi d_s^2}{d_p^2} \quad \text{and} \quad \alpha_v = \frac{\pi d_s^3}{6 d_p^3} \qquad \ldots (4.18)$$

When $d_s = d_p$

$$\alpha_s = \pi = 3.124$$

and

$$\alpha_v = \frac{\pi}{6} = \frac{3.124}{6} = 0.524 \qquad \ldots (4.19)$$

Shape factor:

It is expressed as 'the ratio of surface and volume factor'.

$$\text{Shape factor} = \frac{\alpha_s}{\alpha_v} = \frac{3.124}{0.524} = 6 \qquad \ldots (4.20)$$

If shape factors is equal to or less than 6; particle is spherical in shape, is and **If shape factor is more than 6**; particle is asymmetric in shape.

PARTICLE SURFACE AREA – METHOD

Pharmaceutical Importance:

1. Particle size is inversely proportional to surface area.

2. The surface area of a particle plays an important role in flow and packaging properties of a powder.

3. Also dissolution and solubility of drug depend on the particle surface area. In this case, increase in surface area, increases the solubility of drugs.

Example:

(a) Bephenium hydroxynaphthoaie (an anthelmintic) is administered as a suspension. The I.P. prescribes the specific surface area limit of not less than7000 cm2/g. As the specific surface is reduced, the activity of the drug also falls because dissolution rate decreases.

(b) Griseofulvin, an antifungal antibiotic, should have the surface area of not less than 13000 to 17000 cm2/g. If it is less, the absorption of the drug will fall.

Specific Surface Area:

Specific surface is defined as 'the surface area per unit weight (Sw) or unit volume (Sv) ofthe material'.

Determination of S_v:

$$S_v = \frac{\text{Surface area of particles}}{\text{Volume of particles}} = \frac{\text{No. of particles} \times \text{Surface area of each particle}}{\text{No. of particles} \times \text{Volume of each particle}} \quad \ldots (4.21)$$

Substitute values from Table 4.5.

$$\therefore \quad S_v = \frac{n\,\alpha_s\,d^2}{n\,\alpha_v\,d^3} = \frac{\alpha_s}{\alpha_v\,d} \quad \ldots (4.22)$$

According to equation (4.20), $\frac{\alpha_s}{\alpha_v} = 6$, therefore equation (4.22) can be written as,

$$S_v = \frac{6}{d} \quad \ldots (4.23)$$

Determination of S_w:

$$S_w = \frac{\text{Surface area of particles}}{\text{Weight of particles}} = \frac{\text{No. of particles} \times \text{Surface area of each particle}}{\text{No. of particles} \times \text{Weight of each particle}}$$

$$= \frac{n\,\alpha_s\,d^2}{\text{No. of particles} \times \text{Density} \times \text{Volume}} \quad \ldots (4.24)$$

Substitute values from Table 4.5.

$$\therefore \quad S_w = \frac{n\,\alpha_s\,d_{vs}^2}{n\,\alpha_v\,d_{vs}^3\,\rho} = \frac{\alpha_s}{\alpha_v\,d_{vs}\,\rho} \quad \ldots (4.25)$$

According to equation (4.20), $\alpha_s / \alpha_v = 6$, therefore equation (4.25) can be written as,

$$\therefore \quad S_w = \frac{6}{d_{vs}\,\rho} \quad \ldots (4.26)$$

Equations (4.23) and (4.26) accurately determine the surface area of nonporous particle. But these equations have certain limitations. This equation is not applicable for porous particles, because surface area of porous particle is greater than nonporous particle, when projected diameter is same.

Air Adsorption Method

$$\frac{P}{y(P_o - P)} = \frac{1}{y_m b} + \frac{b - P}{y_m b} \cdot \frac{P}{P_o} \quad \ldots (4.27)$$

Where,

P : Partial vapour pressure of adsorbate gas in equilibrium (kPa),

P_0 : Saturated pressure of the adsorbate gas at – 196°C (kPa),

y : Volume of gas adsorbed at equilibrium (m*l*),

y_m : The mass of gas that 1 gram of solid adsorbent can take up when monolayer is completed,

b : Constant, proportional to heat of adsorption and latent heat of condensation of subsequent layers.

The specific surface area, S, is determined from y_m, the volume of gas adsorbed in a monolayer on the sample.

$$S = \frac{y_m \times N \times a}{m \times 22400} \quad \ldots (4.28)$$

Where,

S : Specific surface area (m^2/g),

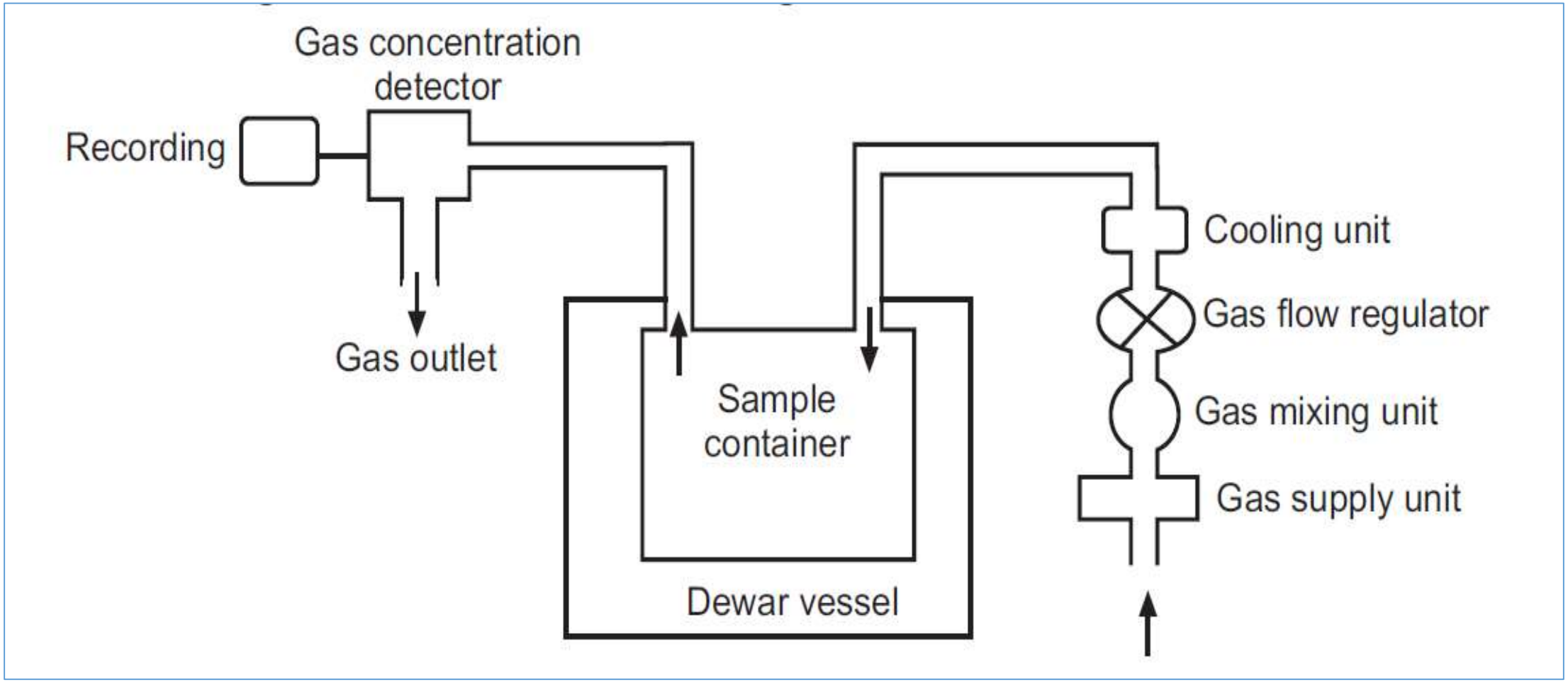
N : Avogadro constant,

a : Effective cross-sectional area of one adsorbate molecule (m^2),

m : Mass of the test powder (g),

Specific surface area is generally expressed in units of m^2/g.

Either of the methods described below can be used to measure the gas adsorption.


Gas concentration
detector
Recording
Gas outlet
Sample
container
Dewar vessel
Cooling unit
Gas flow regulator
Gas mixing unit
Gas supply unit

Air Permeability Method:

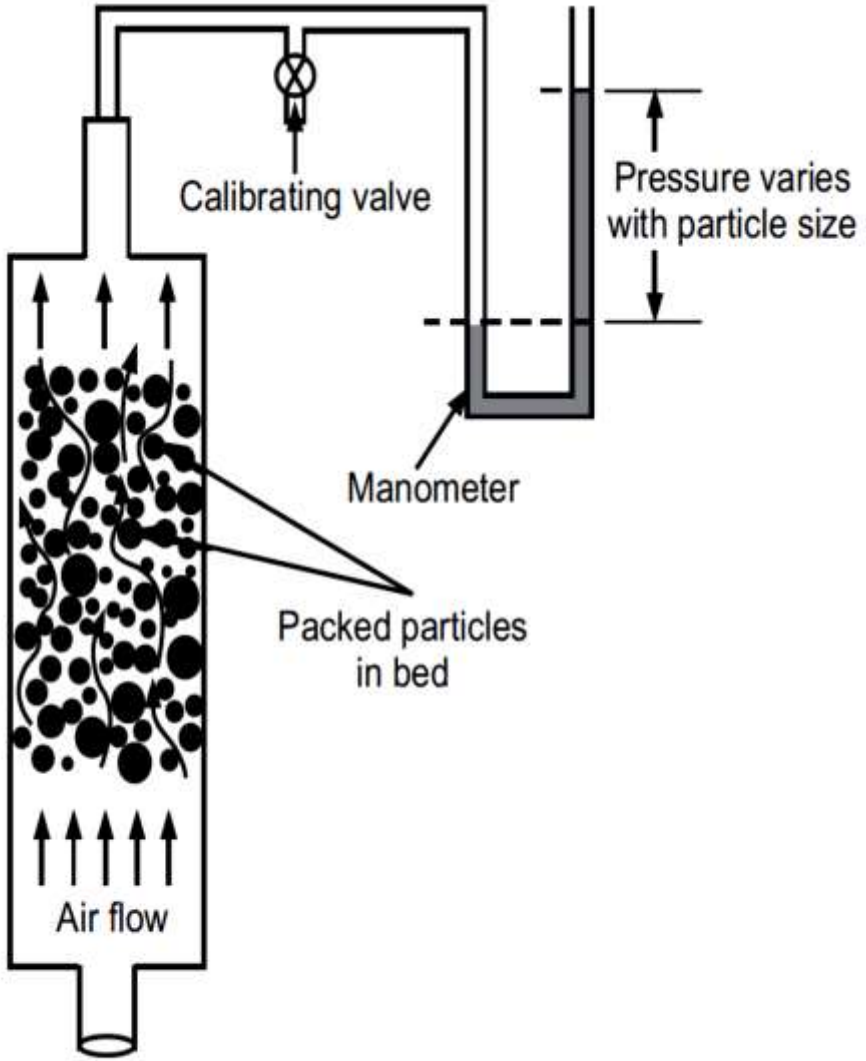


Fig. 4.14: Fisher subsieve size instrument

The Kozeny-Carman equation is used to estimate the surface area by this method.

$$S_w^2 = \frac{A}{\eta V}\,\frac{\Delta P t}{K l}\,\frac{\varepsilon}{(1-\varepsilon)^2} \qquad \ldots (4.29)$$

Where

A : Cross-sectional area of the bed (pack), cm^2

ΔP : Pressure difference of the plug,

t : Time of flow (seconds),

l : Length of the sample holder, (cm)

ε : Porosity of the powder,

S_w : Surface area per gram of the powder (cm/g),

η : Viscosity of the air (poise),

K : Constant (5.0±0.5) that accounts the irregular capillaries,

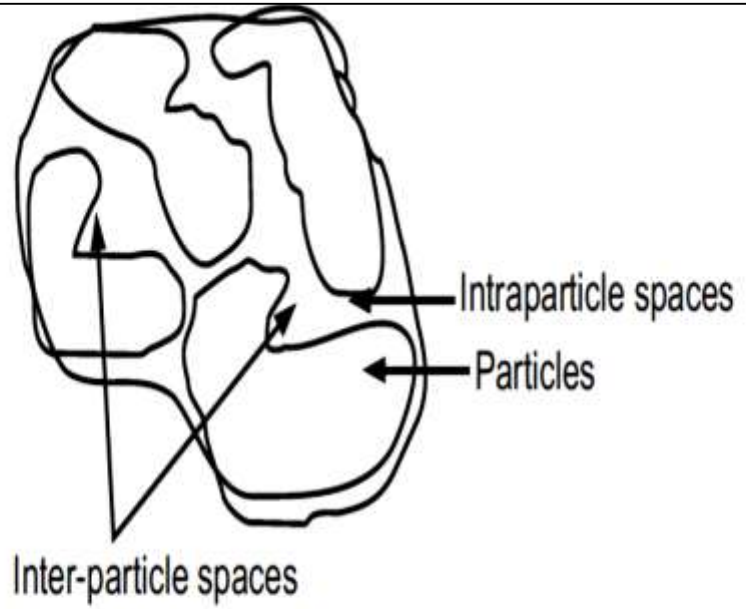
V : Volume of air flowing through the bed (cm^3).

DERIVED PROPERTIES OF POWDERS

Derived properties of powder enlist follow:

1. Porosity
2. Packing arrangement
3. Density
    a) Bulk density
    b) Tapped density
    c) True density
    d) Granule density
4. Bulkiness
5. Flow properties
6. Compaction

1. Porosity (Voids)



Bulk volume, V_b = Particles volume + Intraparticles spaces volume + Inter-particle spaces

True volume, V_p = Particles volume

Void volume, ε = Intraparticles spaces volume + Inter-particle spaces

Bulk volume, Vb:

V_b = Particles volume + Intraparticles space + Interparticles space

True volume, V_p

True volume, Vp = Volume of particles itself (means excluding volume of pores and capillary).

Void volume, v:

The volume of the spaces, known as the void volume, v, is given by the equation,

v = Vb – Vp

The porosity or voids ε of the powder is defined as 'the ratio of the void volume to the bulk volume of the packing'.

$$\therefore \quad \varepsilon = \frac{V_b - V_p}{V_b} = 1 - \frac{V_p}{V_b} \qquad \ldots (4.31)$$

Porosity is frequently expressed in per cent,

$$\varepsilon = \frac{V_b - V_p}{V_b} \times 100 \qquad \ldots (4.32)$$

Applications:

1. Dissolution: The surface area of porous particles is high. Dissolution rate of drug depend on the surface area. As surface area means porosity increases dissolution rate increases.

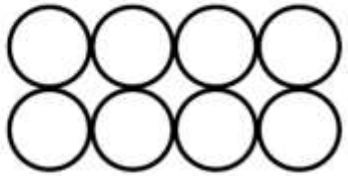
2. Disintegration rate: Drug is classified as porous and non-porous. Rate of disintegration of porous drug is high as compared to non-porous drug.

3. Adsorption: Charcoal is highly porous solid. Because of its porosity, it is used in poison treatment. It shows maximum adsorption of poison in GIT.

4. Diffusion: As porosity increases rate of diffusion also increases.

ε = 26 %

Particles closest packing



ε = 48 %

Particles loose packing

Packing Arrangements

1. **Real powders: neither spherical in shape nor uniform in size.**
   - It is to be expected that the particles of ordinary powders may have any arrangement intermediate between closest and loose.
   - Porosities are in between 30 and 50%.
2. **If the particles are of greatly different sizes:**
   - The smaller particles sift between the larger particles means filling gap between particles. In preparation of tablet, granules are compressed with fine powder minimizes the voids spaces between particles.
   - Porosities are below 26%.
3. **In powders containing flocculates or aggregates:**
   - The formation of bridges and arches in the packing.
   - The porosity may be above the theoretic maximum of 48%.

Density of Powder

Density is universally defined as 'weight per unit volume'.

Bulk Density

$$\text{Bulk density, } \rho = \frac{\text{Mass of powder}}{\text{Bulk volume}}$$

Procedure:

1. Pass a quantity of powder sufficient to complete the test through a **sieve** with apertures greater than or equal to 1.0 mm, 2. Into a dry graduated cylinder of 250 ml (readable to 2 ml), gently introduce, without compacting, approximately 100 g of the test sample (m) weighed with 0.1% accuracy.
3. Carefully level the powder without compacting, if necessary, and read the unsettled bulk volume (Vb) to the nearest graduated unit.
4. Calculate the bulk density in (g/ml) using the formula m/Vb.
5. Generally, replicated determinations are desirable for the determination of this property.

Applications:

1. Bulk density helps in selection of proper size of a container, packing material and mixing apparatus.
2. According to bulk density, size of capsule determines lower bulk density, therefore big size capsule is required.
3. Bulk density is used to check uniformity of drug.

Tapped Density

$$\text{Tapped density, } \rho = \frac{\text{Mass of powder}}{\text{Tapped volume}}$$

Tap volume, V_t = Particles Volume + Inter-Particles Volume

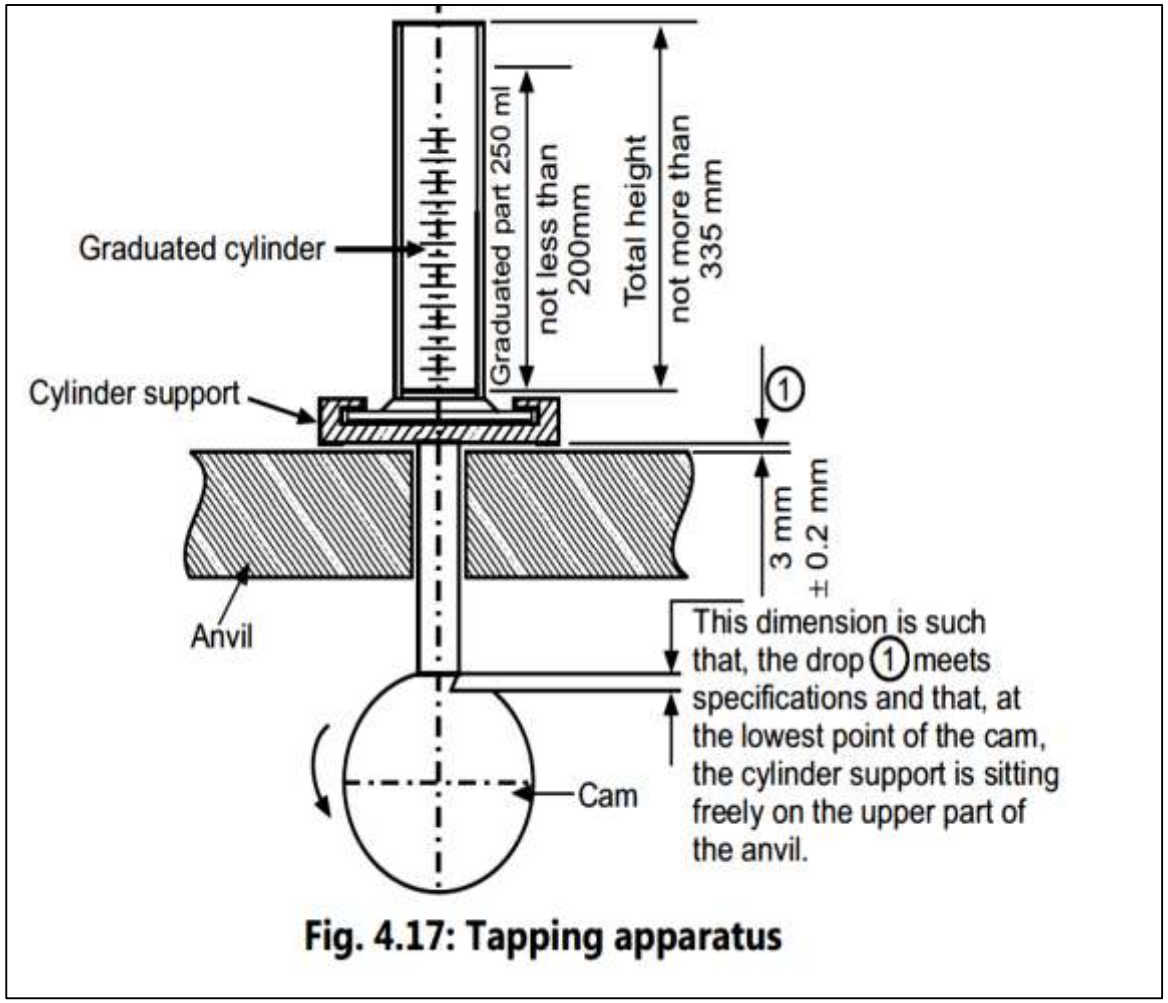


Fig. 4.17: Tapping apparatus

Apparatus:

(a) 250 ml graduated cylinder (readable to 2 ml) with a mass of 220 ± 44 g; and a settling apparatus capable of producing, in 1 minute, either nominally 250 ± 15 taps from a height of 3 ± 0.2 mm, or nominally 300 ± 15 taps from a height of 14 ± 2 mm.

The support for the graduated cylinder, with its holder, has a mass of450 ± 10 g.

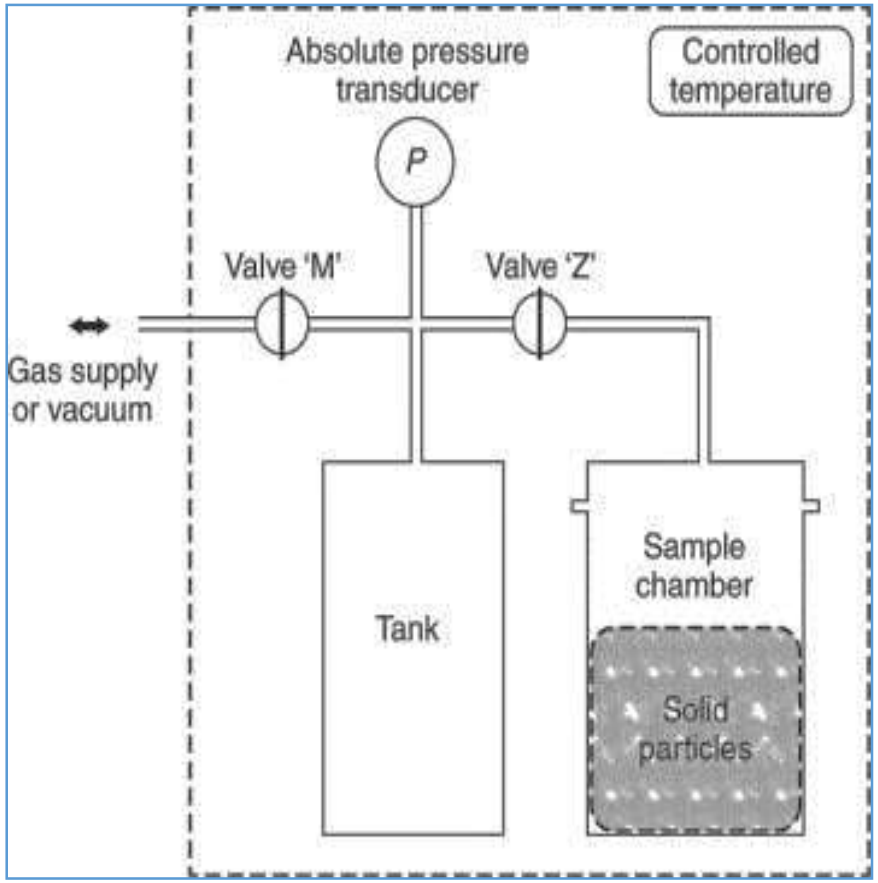
Procedure:

1. Proceed as described above for the determination of the bulk volume (Vb).
2. Secure the cylinder in the holder.
3. Carry out 10, 500 and 1250 taps on the same powder sample and read the corresponding volumes V10, V500 and V1250 to the nearest graduated unit. If the difference between V500 and V1250 is less than or equal to 2 ml, V1250 is the tapped volume.
4. If the difference between V500 and V1250 exceeds 2 ml, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than or equal to 2 ml.
5. Fewer taps may be appropriate for some powders, when validated.
6. Calculate the tapped density (g/ml) using the formula m/Vt in which Vt is the final tapped volume.
7. If it is not possible to use a 100 g test sample, use a reduced amount and a suitable 100 ml graduated cylinder (readable to 1 ml) weighing 130 ± 16 g and mounted on a holder weighing 240 ± 12 g. The modified test conditions are specified in the expression of the results.

True Density

True density, Lp, is the density of the actual solid material.
True volume, Vp = Particle volume itself. (Excluded volume of pores and intra-particles space)

Helium densitometer



1. **V1 = Volume of empty apparatus (Bulk volume of powder)**
2. A weighed amount of powder (M) is then introduced into the sample tube.
3. Adsorbed gases are removed from the powder by an out-gassing procedure.
4. Helium is again introduced.
5. The pressure is read on a mercury manometer, and by application of the gas laws, the volume of helium surrounding the particles and penetrating into the small cracks and pores is calculated.
V2 = Volume occupied by helium in pores and intra-particle space between particles (volume of voids)
6. The difference between the volume of helium filling the empty apparatus and the volume of helium in the presence of the powder sample yields the volume occupied by the powder.
Vp = V1 – V2
7. Knowing the weight of the powder, one is then able to calculate the true density.

2. Liquid Displacement Method:

This method is used to determine the true volume of non-porous solid.

Obtained density by this method is lower than the helium displacement method, because liquid such as water and ethanol cannot occupy the pores of particles.

Select the liquid in which powder is insoluble and heavy.

Method: Pycnometer or specific gravity bottle may be used:

1. Weigh the empty pycnometer, W_1
2. Then fill solid in pycnometer and weigh the pycnometer, W_2
3. $\therefore$ Weight of solid, $W_3 = W_2 - W_1$
4. Then weight of pycnometer with solid and filled with liquid, W_4
5. Weight of liquid displaced by solid = $W_4 - W_2$
6. True density is the weight of the powder divided by the weight of the liquid it displaces,

$$V_t = \frac{W_2 - W_1}{W_4 - W_2} \quad \ldots (4.33)$$

For example, if the weight of a sample of glass beads is 5.0 grams and the weight of water required to fill a pycnometer is 50.0 grams, then the total weight would be 55.0 grams. When the beads are immersed in the water and the weight is 53.0 grams or a displacement is of 2.0 grams of water, the density is 5.0 /2.0 = 2.5.

Granular Density (ρ_g)

The volume of the particles together with their intra-particle spaces then gives the granule volume, and from knowledge of the powder weight, the granular density is obtained.

$$\rho_g = \frac{\text{Granule weight}}{\text{Granule volume}}$$

Intra-particles porosity of granules:

It is determined by using values of granular volume and true volume.

$$\varepsilon_{\text{intra-particle}} = \frac{V_g - V_p}{V_g} = 1 - \frac{V_p}{V_g}$$

Therefore,

$$\varepsilon_{\text{intra-particle}} = 1 - \frac{W/\rho_p}{W/\rho_g} = 1 - \frac{\rho_g}{\rho_p}$$

Bulkiness

Bulk volume of powder is the reciprocal of bulk density, often called as bulkiness or bulk.

It is an important consideration in the packaging of powders. The bulk density of calcium carbonate may vary from 0.1 to 1.3, and the lightest or bulkiest type would require a container about 13 times larger than that needed for the heaviest variety.

Bulkiness increases with a decrease in particle size. In a mixture of materials of different sizes, however, the smaller particles sift between the larger ones and tend to reduce the bulkiness.

Flow Properties

1 . Angle of Repose

$$\theta = \tan^{-1}\frac{h}{r}$$

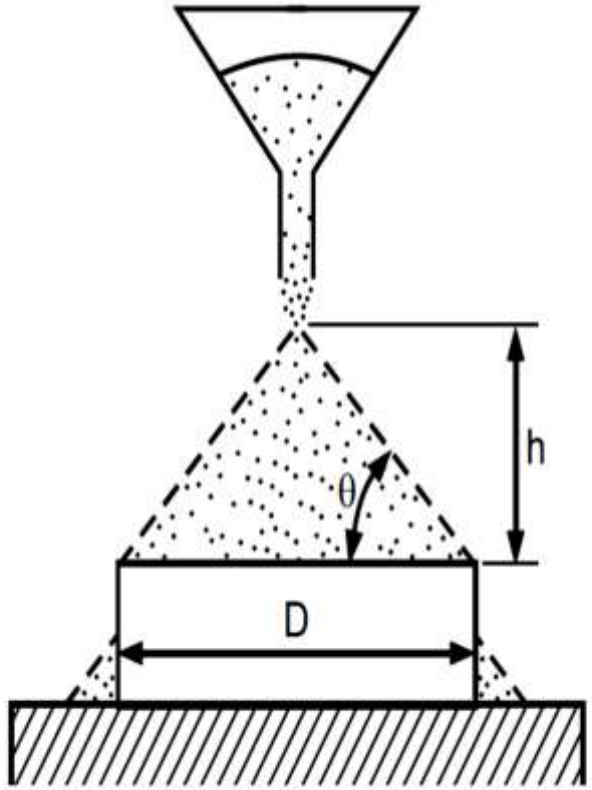


Fig. 4.18: Determination of angle of repose

Flow property	Angle of repose (°)
Excellent	25-30
Good	31-35
Fair: Aid not needed	36-40
Passable: may hang-up	41-45
Poor: must agitate and vibrate	46-55
Very poor	56-65
Very, very poor	More than 66

Compressibility Index

$$\text{Compressibility index} = \frac{V_b - V_t}{V_t} \times 100$$

V_b = Bulk volume

V_t = Tapped volume

Hausner Ratio:

$$\text{Hausner Ratio} = \frac{V_b}{V_t}$$

Compressibility Index (%)	Flow Character	Hausner Ratio
10	Excellent	1.00 – 1.11
11-15	Good	1.12 – 1.18
16-20	Fair	1.19 – 1.25
21-25	Passable	1.26 – 1.34
26-31	Poor	1.35 – 1.45
32-37	Very poor	1.46 – 1.59
>38	Very, very poor	> 1.60

Factors Affecting and Improvement of Flowability

1. Alteration of particle size and size distribution:

2. Alteration of particle shape or texture:

3. Alteration of surface forces:

4. Moisture content:

5. Use vibration-assisted hopper:

6. Use force feeder

BP 403 T. PHYSICAL PHARMACEUTICS-II (Theory)

Unit-V
Drug Stability

Subject Teacher:
Dr. Prashant Khade

UNIT-V **10 Hours**

Drug stability: Reaction kinetics: zero, pseudo-zero, first & second order(complex reaction:reversible, parallel and side reactions), units of basic rate constants, determination of reaction order. Physical and chemical factors influencing the chemical degradation of pharmaceutical product: temperature, solvent, ionic strength, dielectric constant, specific & general acid base catalysis,Simple numerical problems.Stabilization of medicinal agents against common reactions like hydrolysis & oxidation.Accelerated stability testing in expiration dating of pharmaceutical dosage forms.Photolytic degradation and its prevention

'The branch of physical chemistry which deals with the rate of reactions' is called chemical kinetics.

The study of chemical kinetics includes:

1. The rate of the reactions and rate laws.
2. The factors as temperature, pressure, concentration and catalyst, which influences the rate of a reaction.
3. The mechanism or the sequence of steps by which a reaction occurs.

Applications of Chemical Kinetics

1. Drug stability
2. Dissolution
3. Drug release
4. Pharmacokinetics
5. Drug action

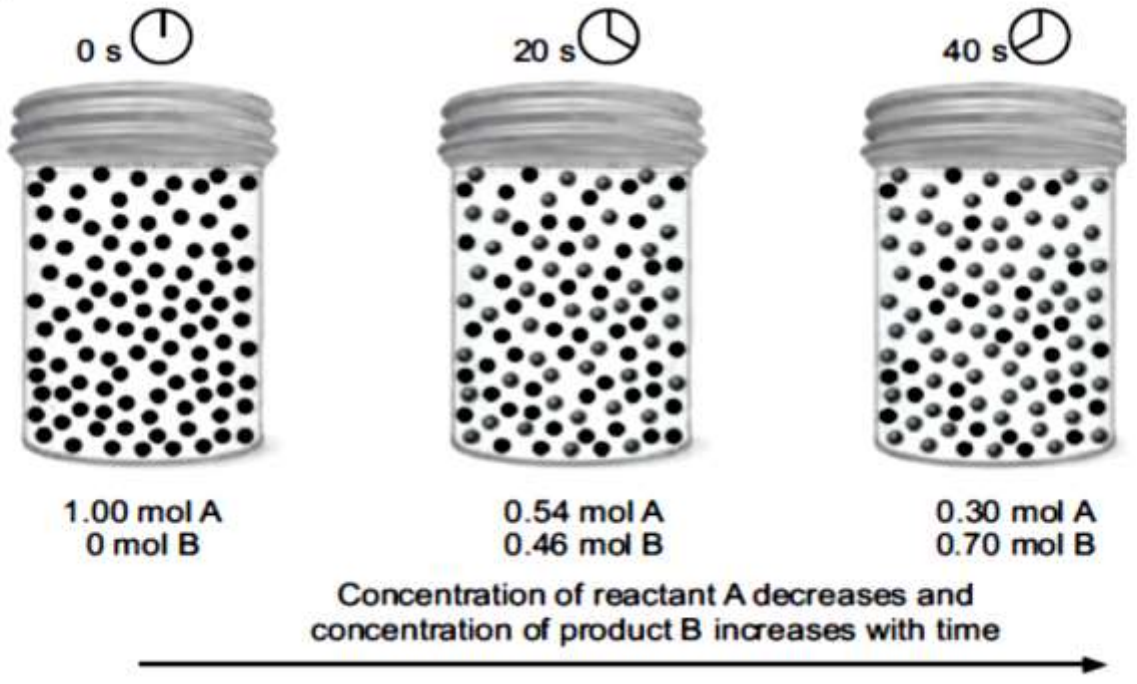
REACTION RATES

Rate of reaction is defined as, 'the change in the concentration of a reactant or a product with time (M/s)'.

Reactant → Products

A → B

The concentration of reactant 'A' decreases and concentration of product 'B' increases with time. [Fig. 3.1]



From given reaction:

$$\text{Rate of reaction} = \text{Rate of disappearance of A.}$$

$$= \text{Rate of appearance of B.}$$

Now, consider rate of disappearance of reactant A;

$$\text{Rate} = \frac{\text{Change in number of moles of A}}{\text{Change in time}}$$

$$\text{Rate} = -\frac{\Delta[A]}{\Delta t} = \frac{[A]_2 - [A]_1}{t_2 - t_1}$$

Where,

$[A]_1$: Concentration of reactant at time t_1.

$[A]_2$: Concentration of reactant at time t_2.

– ve sign indicates concentration of reactant A decreases.

Now, consider rate of appearance of product B;

$$\therefore \quad \text{Rate} = \frac{\text{Change in number of moles of B}}{\text{Change in time}}$$

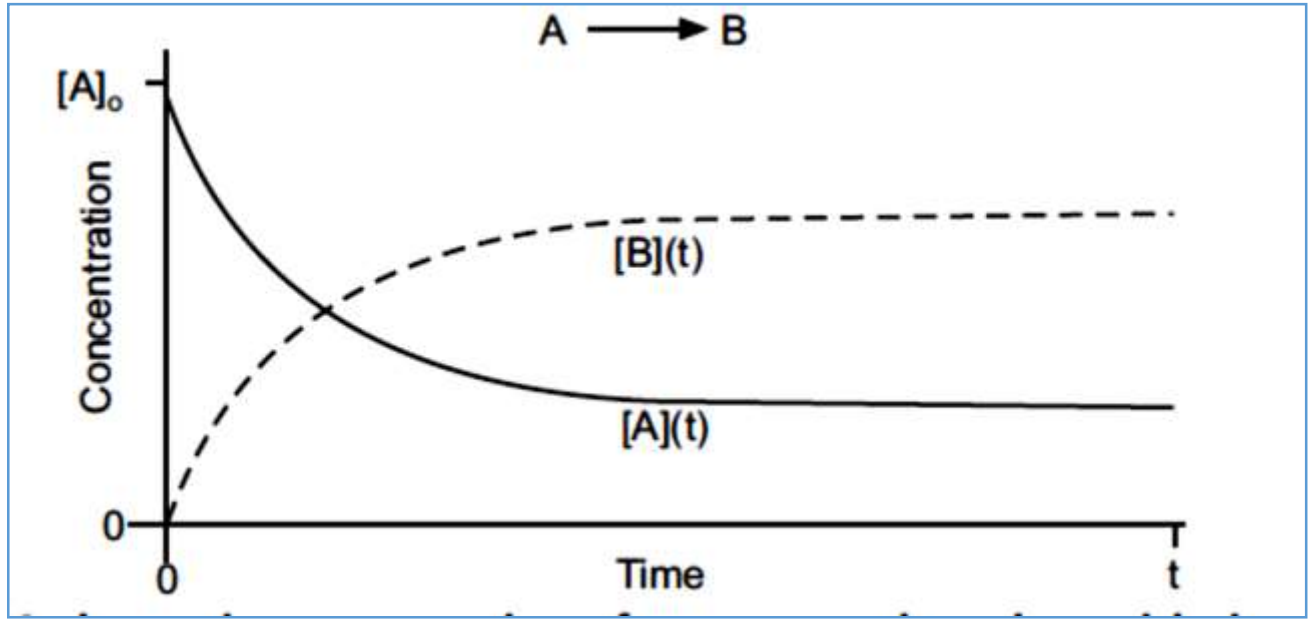
$$\text{Rate} = \frac{\Delta[B]}{\Delta t} = \frac{[B]_2 - [B]_1}{t_2 - t_1}$$

Where,

$[B]_1$: Concentration of product at time t_1

$[B]_2$: Concentration of product at time t_2

+ ve sign indicates concentration of product B increases.



Reaction rate has the units if concentration divided by time.

mole/litre sec or mol $l^{-1} s^{-1}$

mole/litre min or mol $l^{-1} min^{-1}$

mole/litre hour or mol $l^{-1} h^{-1}$

RATE LAWS

The rate of a reaction is directly proportional to the reactant concentrations, each concentration being raised to some power.

Let us consider a simple reaction;

$$\text{Reactant} \rightarrow \text{Products}$$

$$_nA \rightarrow B$$

$$\text{rate} \propto [A]^n$$

$$\text{rate} = k\,[A]^n$$

For a reaction; $_mA + _nB \rightarrow$ Products

The reaction rate with respect to A or B is determined by varying the concentration of one reactant, keeping that of the other constant. Thus, the rate of reaction may be expressed as

$$\text{Rate} = k\,[A]^m\,[B]^n$$

An expression which shows how the reaction rate is related to concentrations is called the **rate law or rate equation**.

The power (exponent) of concentration n or m in the rate law is usually a small whole number integer (1, 2, 3) or fractional. The proportionality constant k is called the rate constant for the reaction.

ORDER OF A REACTION

The order of a reaction is defined as 'the sum of the powers of concentrations in the rate law'.

Let us consider the example of a reaction which has the rate law

$$\text{Rate} = k [A]^m [B]^n$$

The order of such a reaction = m + n.

The order of a reaction can also be defined with respect to a single reactant. Thus, the reaction order with respect to A is m and with respect to B it is n.

The overall order of reaction (m + n) may range from 1 to 3 and can be fractional.

Classification of Reaction According to the Order:

Zero Order:	$m + n = 0$
First order:	$m + n = 1$
Second order:	$m + n = 2$
Third order:	$m + n = 3$

In case of zero order reaction: A reactant whose concentration does not affect the reaction rate is not included in the rate law. In effect, the concentration of such a reactant has the power 0. Thus, $[A]^{\circ} = 1$.

A zero order reaction is one whose rate is independent of concentration.

For example, the rate law for the reaction

$$NO_2 + CO \rightarrow NO + CO_2 \qquad \text{at } 200^{\circ}\text{ C is}$$

$$\text{Rate} = k[NO_2]^2$$

Here the rate does not depend on [CO], so this is not included in the rate law and the power of [CO] is understood to be zero. The reaction is **zero order** with respect to CO. The reaction is second order with respect to $[NO_2]$. The overall reaction order is 2 + 0 = 2.

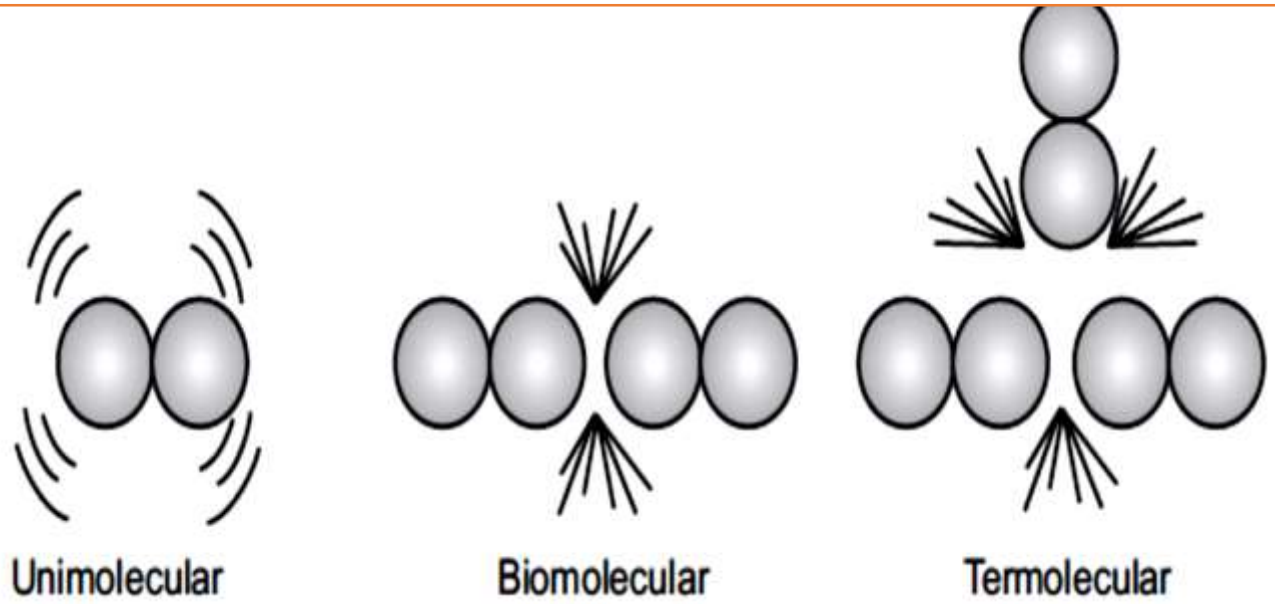
MOLECULARITY OF REACTION

Chemical reactions are classified into two types:

1. **Elementary Reactions:** Simple reaction which occurs in single step.
2. **Complex reactions:** Reactions which occurs in two or more steps.

Molecularity of Elementary Reaction

Molecularity of an Elementary Reaction is defined as 'total number of reactant molecules involved in a reaction'.

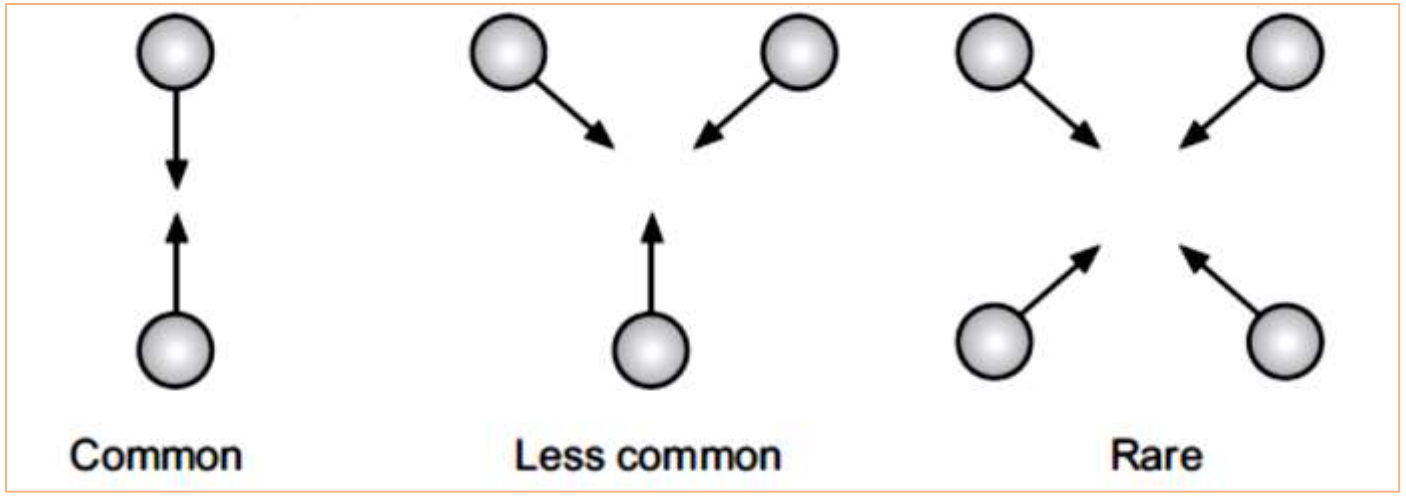


1. **Unimolecular (Molecularity = 1)**
   Example: $Br_2 \rightarrow 2\ Br.$
2. **Bimolecular (Molecularity = 2)**
   Example: $2H_2O_2 \rightarrow 2H_2O + O_2$
3. **Termolecular (Molecularity = 3)**
   Example: $2NO + O_2 \rightarrow 2NO_2$

Why High Molecularity Reactions are Rare?

The rarity of reactions with high molecularity can be explained on the basis of the **kinetic molecular theory.**

1) According to this theory, the rate of a chemical reaction is proportional to the number of collisions taking place between the reacting molecules.
2) The chances of simultaneous collision of reacting molecules will go on decreasing with increase in number of molecules.
3) Thus, the possibility of three molecules colliding together is much less than in case of bimolecular collision.
4) For a reaction of molecularity 4, the four molecules must come close and collide with one another at the same time. The possibility of their doing so is much less than even in the case of termolecular reaction.



Molecularity of a Complex Reaction

The decomposition reaction of N_2O_5

i.e., $2N_2O_5 \rightarrow 4NO_2 + O_2$

is an example of a complex reaction. It occurs by the following steps:

Step 1: $2N_2O_5 \rightarrow 2NO_2 + 2NO_3$ (slow)

Step 2: $NO_2 + NO_3 \rightarrow NO + NO_2 + O_2$ (slow)

Step 3: $NO + NO_3 \rightarrow 2NO_2$ (fast)

Molecularity versus Order of Reaction

	Order of Reaction	Molecularity of Reaction
1.	It is the sum of powers of the concentration terms in the rate law expression.	It is number of reacting species undergoing simultaneous collision in the elementary or simple reaction.
2.	It is an experimentally determined value.	It is a theoretical concept.
3.	It can have fractional value.	It is always a whole number.
4.	It can assume zero value.	It cannot have zero value.
5.	Order of a reaction can change with the conditions such as pressure, temperature, concentration.	Molecularity is invariant for a chemical equation.

INTEGRATED RATE LAW

1. Zero order reaction
2. Pseudo order reaction
3. First order reaction
4. Second order reaction
4. Third order reaction

1. Zero order reaction

Therefore, zero order reaction is defined as 'the reaction whose rate is independent of the reactant concentration and remains constant throughout the course of the reaction'.

Consider a zero order reaction,

$$A \rightarrow \text{Products}$$

The rate law of the reaction is given by

$$\text{Rate} = \frac{d[A]}{dt} = k[A]^0 = k \qquad \ldots (3.5)$$

On rearrangement it becomes:

$$d[A] = -k.dt \qquad \ldots (3.6)$$

On integration of the equation between limits $[A] = [A]_0$ at $t = 0$ and $[A] = [A]_t$ at $t = t$

we get

$$\int_{[A]_0}^{[A]_t} d[A] = -k \int_{t}^{0} dt \qquad \ldots (3.7)$$

Hence,

$$[A]_t - [A]_0 = -kt \qquad \ldots (3.8)$$

$$[A]_t = -kt + [A]_0 \qquad \ldots (3.9)$$

$$k = \frac{[A]_0 - [A]_t}{t} \qquad \ldots (3.10)$$

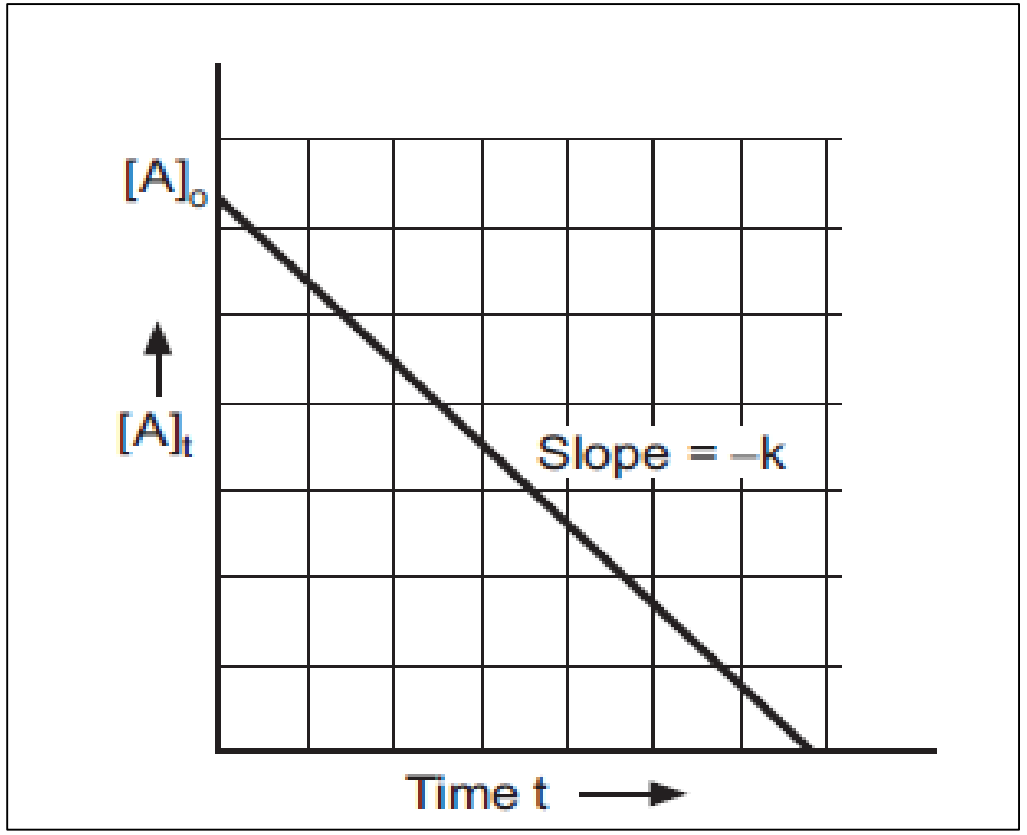
Unit of K of Zero Order Reactions

$$k = \frac{[A]_t - [A]_0}{t} = \frac{\Delta[A]}{t} = \frac{\text{mol}}{\text{lit}} \times \frac{1}{\text{Time}}$$

Thus, the unit of k is: **mol lit^{-1} time $^{-1}$.**

Graphical Representation

$$[A]_t = -kt + [A]_0$$
$$\downarrow \quad \downarrow \quad \downarrow$$
$$y = mx + c$$



Half Life of Zero Order Reaction

Half life is defined as 'the time required for concentration of reactant to reduce to half of its initial concentration'. Half life is represented as $\mathbf{t_{1/2}}$.

The integrated rate law for zero order reactions is given by equation (3.9).

$$[A]_t = -kt + [A]_0$$

Where;

$[A]_t$: The concentration of A that remains unreacted at time t.

$[A]_0$ is initial concentration at t = 0.

As per the definition; $\frac{[A]_0}{2}$ at $t = t_{1/2}$

Hence we can write; $[A]_t = \frac{[A]_0}{2}$ at $t = t_{1/2}$.

With this condition, equation (3.9) become,

$$\frac{[A]_0}{2} = -kt_{1/2} + [A]_0$$

$$kt_{1/2} = [A]_0 - \frac{[A]_0}{2} = \frac{[A]_0}{2}$$

$$t_{1/2} = \frac{[A]_0}{2k} \quad \ldots (3.11)$$

The unit for half life of reaction is time scale, i.e. sec/conc, min/conc, etc.

Shelf Life of Zero Order Reaction

Shelf Life is defined as 'the time required for the concentration of the reactant to reduce to 90% of its initial concentration'.

Shelf life is represented as $\mathbf{t_{90}}$ and has the unit time/conc. The shelf life equation can be derived as follows:

As per the definition; $\frac{90\ [A]_0}{100}$ or $0.9\ [A]_0$ at $t = t_{90}$

Hence, we can write; $[A]_t = 0.9\ [A]_0$ at $t = t_{90}$

With this condition equation (3.9) become,

$$0.9\ [A]_0 = -k\ t_{90} + [A]_0$$

$$k\ t_{90} = [A]_0 - 0.9\ [A]_0$$

$$t_{90} = \frac{[A]_0 - 0.9\ [A]_0}{k} = \frac{0.1\ [A]_0}{k} \qquad \ldots (3.12)$$

Equation (3.12) uses to determine shelf life of zero order reaction.

2 Pseudo Order Reaction

A reaction in which one of the reactants is present in a large excess shows an order different from the actual order. The experimental order which is not the actual one is referred to as the pseudo order. Since for elementary reactions molecularity and order are identical, pseudo–order reactions may also be called pseudo molecular reactions. Let us consider a reaction

$$A + B \rightarrow \text{Products}$$

In which, the reactant **B is present in a large excess**. Since it is an elementary reaction, its rate law can be written as

$$\text{Rate} = k\,[A][B]$$

As B is present in large excess, its concentration remains practically constant in the course of reaction. Thus, the rate law can be written as

$$\text{Rate} = k\,[A]$$

Where the new rate constant $k' = k\,[B]$. Thus, the actual order of the reaction is second–order but in practice it will be first–order. Therefore, the reaction is said to have a **Pseudo–First Order.**

Example of Pseudo order reaction:

Hydrolysis of Sucrose: Sucrose upon hydrolysis in the presence of a dilute mineral acid gives glucose and fructose.

$$\underset{\text{Sucrose (excess)}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}$$

If a large excess of water is present, $[H_2O]$ is practically constant and the rate law may be written

$$\text{Rate} = k\,[C_{12}H_{22}O_{11}]\,[H_2O]$$

$$= k'\,[C_{12}H_{22}O_{11}]$$

The reaction though of second order is experimentally found to be of first order. Thus, it is a pseudo–first–order reaction.

3. First Order Reaction

Consider a simple general first order reaction

$$A \rightarrow \text{Products}$$

The differential rate law for this first order reaction is

$$-\frac{d[A]}{dt} = k[A] \qquad \ldots (3.13)$$

Where

[A] : The concentration of the reactant A that remains unreacted at time t.

$\frac{-d[A]}{dt}$: The rate of reaction measured at time t at which A is convened to the products.

By rearranging and integrating the equation (3.13) between the limits $[A] = [A]_0$ at $t = 0$ and $[A] = [A]_t$ at $t = t$, we write,

$$\int_{[A]_0}^{[A]_t} \frac{d[A]}{[A]} = -k \int_0^t dt \qquad \ldots (3.14)$$

Where,

$[A]_0$: The initial concentration of A at $t = 0$

$[A]_t$: The concentration of A that remains unreacted at $t = t$.

On performing the integration, we get;

$$\ln [A]_t - \ln [A]_0 = -kt \quad \text{or} \quad \ln \frac{[A]_t}{[A]_0} = -kt \qquad \ldots (3.15)$$

Hence,
$$kt = \ln \frac{[A]_0}{[A]_t} \qquad \ldots (3.16)$$

$\therefore$
$$k = \frac{1}{t} \ln \frac{[A]_0}{[A]_t} \qquad \ldots (3.17)$$

Converting into $\log_{10}$ the integrated rate law becomes

$$k = \frac{2.303}{t} \log_{10} \frac{[A]_0}{[A]_t} \qquad \ldots (3.18)$$

If we let

'a' mol lit^{-1} (that is M) as the initial concentration of A at t = 0

'x' as the concentration of A that decreases during time t from the beginning of the reaction. Also x means concentration of product at time t.

Then the concentration of A that remains unreacted at time t is given by;

$$[A]_t = [A]_0 - x = a - x$$

Where, $[A]_0$ = a mol lit^{-1}.

With these values the equation (3.18) can be written as:

$$k = \frac{2.303}{t} \log_{10} \left(\frac{a}{a - x} \right) \qquad \ldots (3.19)$$

Units of k

The integrated rate law for the first order reaction is given by equation (3.18).

$$k = \frac{2.303}{t} \log_{10} \frac{[A]_0}{[A]_t}$$

Because, $\log_{10} \frac{[A]_0}{[A]_t}$ is a unitless quantity. The unit of k will be given by reciprocal of time.

For example, t is expressed in s and k will have the unit s^{-1}.

Graphical Representation

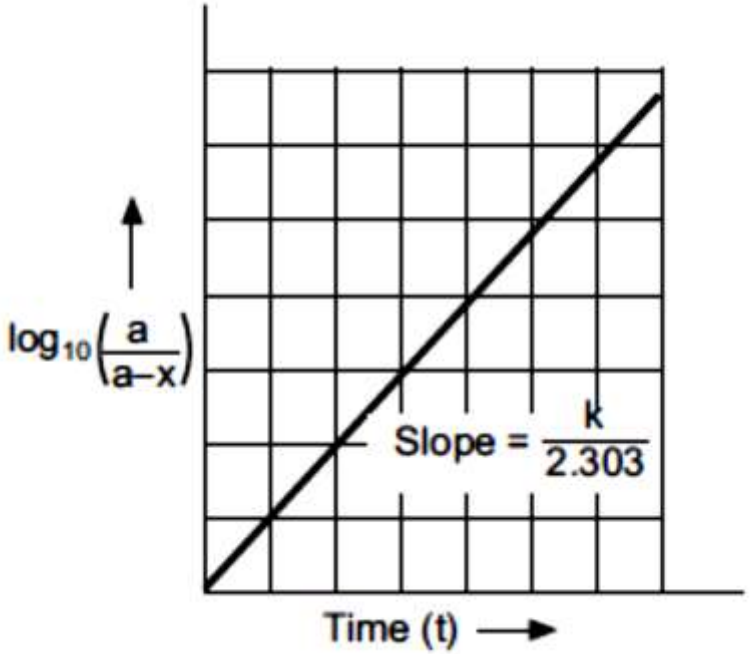
3. The equation (3.19) on rearrangement gives

$$k = \frac{2.303}{t} \log_{10} \left(\frac{a}{a - x} \right)$$

Rearranging, $\log_{10} \left(\frac{a}{a - x} \right) = \frac{kt}{2.303} t + 0$

$\downarrow \quad \downarrow \quad \downarrow$

$y = mx + c$



Half Life of First Order Reactions ($t_{1/2}$)

The half life of a reaction is defined as, 'the time needed for the reactant concentration to fall to one half of its initial value'.

Where,

As per the definition; $\frac{[A]_0}{2}$ at $t = t_{1/2}$

Hence we can write; $[A]_t = \frac{[A]_0}{2}$ at $t = t_{1/2}$.

For the first order reaction the integrated rate law is given by the equation (3.18).

$$k = \frac{2.303}{t} \log_{10} \frac{[A]_0}{[A]_t}$$

$$k = \frac{2.303}{t_{1/2}} \log_{10} \frac{[A]_0}{[A]_0/2}$$

$$t_{1/2} = \frac{2.303}{k} \log_{10} 2 = \frac{2.303}{k} \times 0.301$$

$$\therefore \quad t_{1/2} = \frac{0.693}{k} \qquad \ldots (3.23)$$

The equation (3.23) implies that the half life of a first order reaction is constant and is independent of the reactant concentration.

Shelf Life of First Order Reaction

As per the discussion in section 3.6.1.5.

The shelf life equation can be derived as follows:

As per the definition; $\frac{90\ [A]_0}{100}$ or $0.9\ [A]_0$ at $t = t_{90}$

Hence we can write; $[A]_t = 0.9\ [A]_0$ at $t = t_{90}$

For the first order reaction, the integrated rate law is given by the equation (3.18).

$$k = \frac{2.303}{t} \log_{10} \frac{[A]_0}{[A]_t}$$

$$k = \frac{2.303}{t_{90}} \log_{10} \frac{[A]_0}{0.9\ [A]_0} = \frac{2.303}{k} \log_{10} \left(\frac{1}{0.9}\right) = \frac{2.303}{k} \log_{10} (1.11)$$

$$t_{90} = \frac{0.105}{k} \qquad \ldots (3.24)$$

The equation (3.24) implies the shelf life of a first order reaction.

Second Order Reaction

Consider the following reaction:

$$A + B \longrightarrow \text{Products}$$

As per the definition, the rate equation for second order is,

$$-\frac{dA}{dt} = -\frac{dB}{dt} = k_2 [A] [B] \quad \ldots (3.26)$$

Let us consider,

'a' and 'b' be the initial concentrations of A and B, respectively,

'x' be the concentration of each species reacting in t.

Substituting these terms in equation (3.26)

$$\frac{dx}{dt} = k_2 (a - x) (b - x) \quad \ldots (3.27)$$

Now, consider a case where a = b (both A and B have the same concentration).

Then the above equation changes to equation (3.28)

$$\frac{dx}{dt} = k_2 (a - x)^2 \quad \ldots (3.28)$$

Integrate equation (3.28) employing the conditions x = 0 at t = 0 and x = x at t = t.

$$\int_0^x \frac{dx}{(a - x)^2} = k_2 \int_0^t dt \quad \ldots (3.29)$$

On performing the integration, we get;

$$\frac{1}{(a - x)} - \frac{1}{(a - 0)} = k_2 (t - 0) \quad \ldots (3.30)$$

$$\frac{a - a + x}{a(a - x)} = k_2 t \quad \ldots (3.31)$$

$$k_2 = \frac{1}{at} \cdot \frac{x}{(a - x)} \quad \ldots (3.32)$$

Equation (3.32) is the integral equation for second order reaction, where a = b.

Unit of k_2 moles/litre, then k_2 has units litre/mol. time (min).

Graphical Representation

The integrated rate law given by equation (3.30) is,

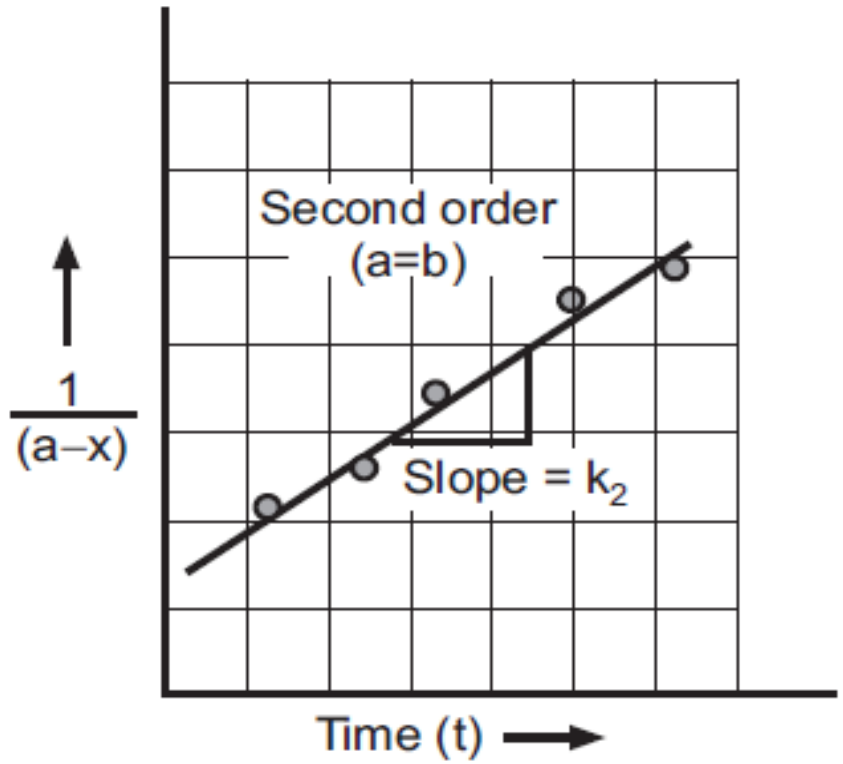
$$\frac{1}{(a-x)} - \frac{1}{(a-0)} = k_2 (t-0)$$

On rearrangement, equation (3.30) can be written as,

$$\frac{1}{(a-x)} = k_2 t + \frac{1}{a}$$

$$\downarrow \quad \downarrow \quad \downarrow$$

$$y = mx + c$$



Half Life of Second Order Reactions ($t_{1/2}$)

For the second order reaction, the integrated rate law is given by the equation (3.30).

$$k_2 t = \frac{1}{(a - x)} - \frac{1}{a}$$

$$\therefore \quad k_2 t = \frac{1}{[A]_t} - \frac{1}{[A]_0} \qquad \ldots (3.34)$$

At $t_{1/2}$;

$$k_2\, t_{1/2} = \frac{1}{1/2\,[A]_0} - \frac{1}{[A]_0} = \frac{2}{[A]_0} - \frac{1}{[A]_0} \qquad \ldots (3.35)$$

$$t_{1/2} = \frac{1}{k_2\,[A]_0} \qquad \ldots (3.36)$$

Shelf Life of Second Order Reaction

For the second order reaction, the integrated rate law is given by the equation (3.30)

$$k_2 t = \frac{1}{(a - x)} - \frac{1}{a}$$

$$\therefore \quad k_2 t = \frac{1}{[A]_t} - \frac{1}{[A]_0}$$

At t_{90};

$$k_2 t_{90} = \frac{1}{0.9\,[A]_0} - \frac{1}{[A]_0} = \frac{[A]_0 - 0.9\,[A]_0}{0.9\,[A]_0 \cdot [A]_0} = \frac{0.1\,[A]_0}{0.9\,[A]_0^2} \qquad \ldots (3.37)$$

$$t_{90} = \frac{0.11}{[A]_0\, k_2} \qquad \ldots (3.38)$$

	Zero order	First order	Second order
Rate Law	$-\frac{d[A]}{dt} = k$	$-\frac{d[A]}{dt} = k[A]$	$-\frac{d[A]}{dt} = k[A]^2$
Integrated Rate Law	$k = \frac{[A]_0 - [A]_t}{t}$	$k = \frac{2.303}{t} \log_{10} \frac{[A]_0}{[A]_t}$	$k_2 t = \frac{1}{[A]_t} - \frac{1}{[A]_0}$
Units of k	mol lit^{-1} $time^{-1}$	s^{-1}	$conc^{-1}$ $time^{-1}$
Half life	$t_{1/2} = \frac{[A]_0}{2k}$	$t_{1/2} = \frac{0.693}{k}$	$t_{1/2} = \frac{1}{k_2 [A]_0}$
Shelf life	$t_{90} = \frac{0.1\ [A]_0}{k}$	$t_{90} = \frac{0.105}{k}$	$t_{90} = \frac{0.11}{[A]_0 k_2}$

DETERMINATION OF ORDER OF REACTION

1. Using Integrated Rate Equations

Example: Decomposition of H_2O_2 in aqueous solution. The decomposition of H_2O_2 in the presence of platinum as catalyst.

$$H_2O_2 \xrightarrow{Pt} H_2O + O$$

A solution of H_2O_2 when titrated against $KMnO_4$ solution at different time intervals gave the following results:

Time (minutes)	**0**	**10**	**20**
Volume of $KMnO_4$ used for 10 ml H_2SO_4	23.8 ml	14.7 ml	9.1 ml

Show that the decomposition of H_2O_2 is a first order reaction.

Solution: The integrated rate equation for first order reaction is

$$k = \frac{2.303}{t} \log\left(\frac{a}{a-x}\right)$$

Since volume of $KMnO_4$ used in the titration is measure of concentration of H_2O_2 in solution,

$\therefore$ $a = 23.8$ ml

$(a - x) = 14.7$ when $t = 10$ min.

$(a - x) = 9.1$ when $t = 20$ min.

$$k = \frac{2.303}{10} \log \left(\frac{23.8}{14.7}\right)$$

$$= 0.2303 (\log 23.8 - \log 14.7)$$

$$= 0.2303 (1.3766 - 1.1673) = 0.04820$$

and

$$k = \frac{2.303}{20} \log \left(\frac{23.8}{9.1}\right)$$

$$= 0.10165 (\log 23.8 - \log 9.1)$$

$$= 0.10165 (1.3766 - 0.9595) = 0.04810$$

Since the value of k is almost constant, the decomposition of H_2O_2 is a **first order reaction.**

2. Graphical Method

3. Using Half–Life Period

1. Two separate experiments: different initial concentrations of a reactant.
2. The progress of the reaction in each case is recorded by analysis.
3. When the initial concentration is reduced to one-half, the time is noted.
4. Let the **initial concentrations** in the two experiments be **$[A_1]$ and $[A_2]$,**
5. while **times for completion of half change** are **t_1 and t_2** respectively

We know that, half–life period for a first order reaction is independent of the initial concentration, [A]. We also know:

$$\text{half-life} \propto \frac{1}{[A]} \text{ for } 2^{nd} \text{ order reaction}$$

$$\text{half-life} \propto \frac{1}{[A]^2} \text{ for } 3^{rd} \text{ order reaction}$$

$$\text{half-life} \propto \frac{1}{[A]^{n-1}} \text{ for } n^{th} \text{ order reaction}$$

Substituting values of initial concentrations and half–life periods from the two experiments, we have

$$t_1 \propto \frac{1}{[A_1]^{n-1}}$$

$$t_2 \propto \frac{1}{[A_2]^{n-1}} \quad \ldots (3.39)$$

and

$$\frac{t_2}{t_1} = \left[\frac{A_1}{A_2}\right]^{n-1} \quad \ldots (3.40)$$

$$(n-1) \log \left[\frac{A_2}{A_1}\right] = \log \left[\frac{t_1}{t_2}\right] \quad \ldots (3.41)$$

Solving for n, the order of reaction

$$n = 1 + \frac{\log \left[\frac{t_1}{t_2}\right]}{\log \left[\frac{A_2}{A_1}\right]} \quad \ldots (3.42)$$

4. The Differential Method

This method was suggested by Van't Hoff and, therefore, it is also called **Van't Hoff's differential method.** According to it, the rate of a reaction of the n^{th} order is proportional to the n^{th} power of concentration.

$$-\frac{dC}{dt} = k C^n \quad \ldots (3.43)$$

Where C = concentration at any instant. In two experiments performed with different initial concentrations, we can write

$$-\frac{dC_1}{dt} = k C_1^n \quad \ldots (3.44)$$

$$-\frac{dC_2}{dt} = k C_2^n \quad \ldots (3.45)$$

Taking logs of the equation (3.44) and (3.45)

$$\log\left(-\frac{dC_1}{dt}\right) = \log k + n \log C_1 \qquad \ldots (3.46)$$

$$\log\left(-\frac{dC_2}{dt}\right) = \log k + n \log C_2 \qquad \ldots (3.47)$$

On substracting equation (3.47) from (3.46), we get

$$n = \frac{\log\left(-\frac{dC_1}{dt}\right) - \left(-\frac{dC_2}{dt}\right)}{\log C_1 - \log C_2} \qquad \ldots (3.48)$$

To find n, [– dc/dt] in the two experiments is determined by plotting concentrations against time (t). The slope, [– dc/dt] at a given time interval is measured by drawing tangents. Using the values of slopes [– dc_1/dt] and [– dc_2/dt] in the equation (3.48), n can be calculated.

5 Ostwald's Isolation Method

This method is employed in determining the order of complicated reactions by 'isolating' one of the reactants so far as its influence on the rate of reaction is **concerned.** Suppose the reaction under consideration is:

$$A + B + C \longrightarrow \text{Products}$$

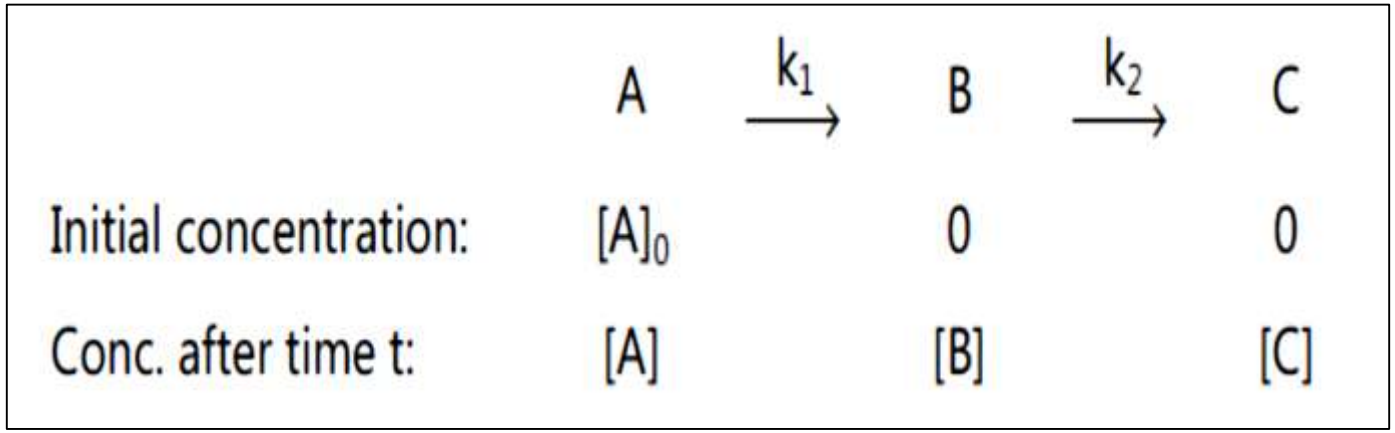
The order of the reaction with respect to A, B and C is determined. For the determination of the order of reaction with respect to A, B and C, they are taken in a large excess so that their concentrations are not affected during the reaction. The order of the reaction is then determined by using any of the methods described earlier. Likewise, the order of the reaction with respect to B and C is determined. If n_A, n_B and n_C are the orders of the reaction with respect to A, B and C respectively, the order of the reaction n is given by the expression.

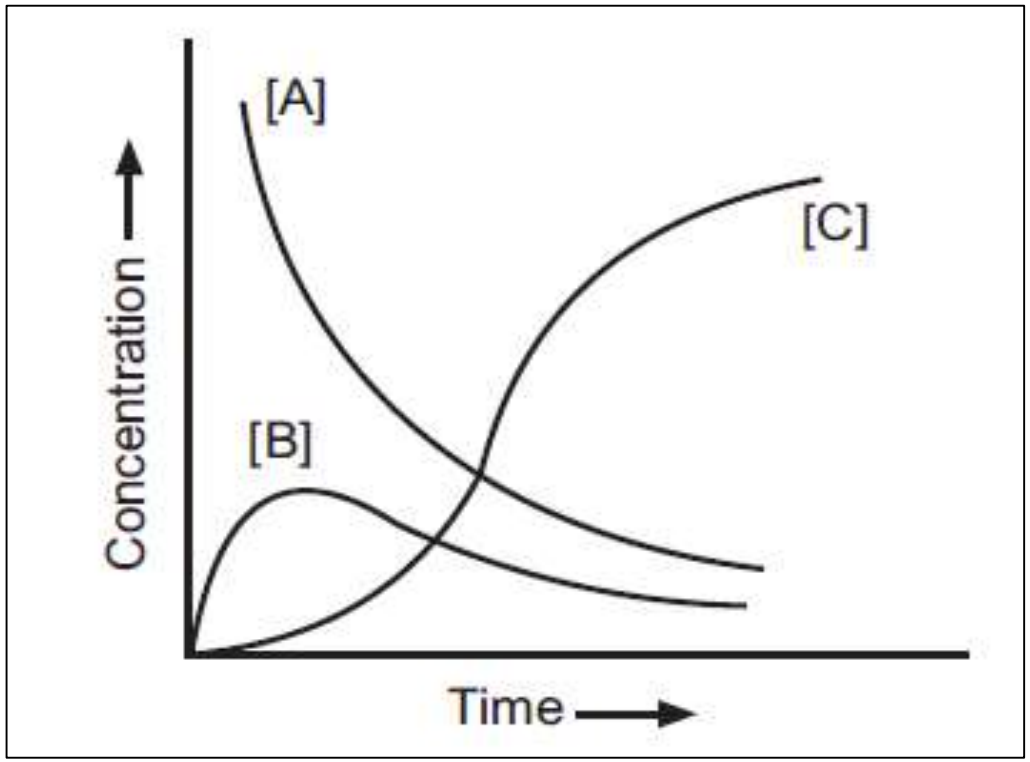
$$n = n_A + n_B + n_C \qquad \ldots (3.49)$$

SIMULTANEOUS REACTIONS/COMPLEX REACTIONS

1. Consecutive reactions

1. The reactions in which the final product is formed through one or more intermediate steps are called **consecutive reactions**.
2. These are also known as **sequential reactions**.





Examples of First Order Consecutive Reactions:

(a) Decomposition of dimethyl ether in gaseous phase

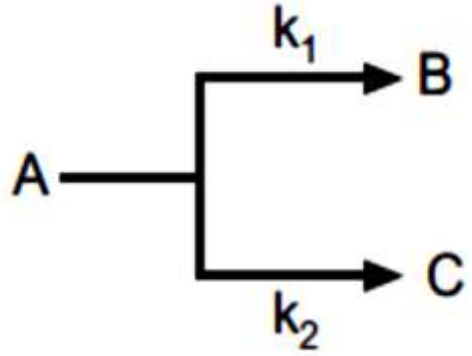
$$CH_3COCH_3 \xrightarrow{k_1} CH_4 + HCHO \xrightarrow{k_2} H_2 + CO$$

(b) Decomposition of ethylene oxide

$$\underset{\diagdown O \diagup}{CH_2 — CH_2} \xrightarrow{k_1} (CH_3CHO) \xrightarrow{k_2} CH_4 + CO$$

2. Parallel or Side Reactions

The reaction in which the maximum yield of the products is obtained is called the **main or major reaction** while the other reaction (or reactions) is called **side or parallel reactions.**

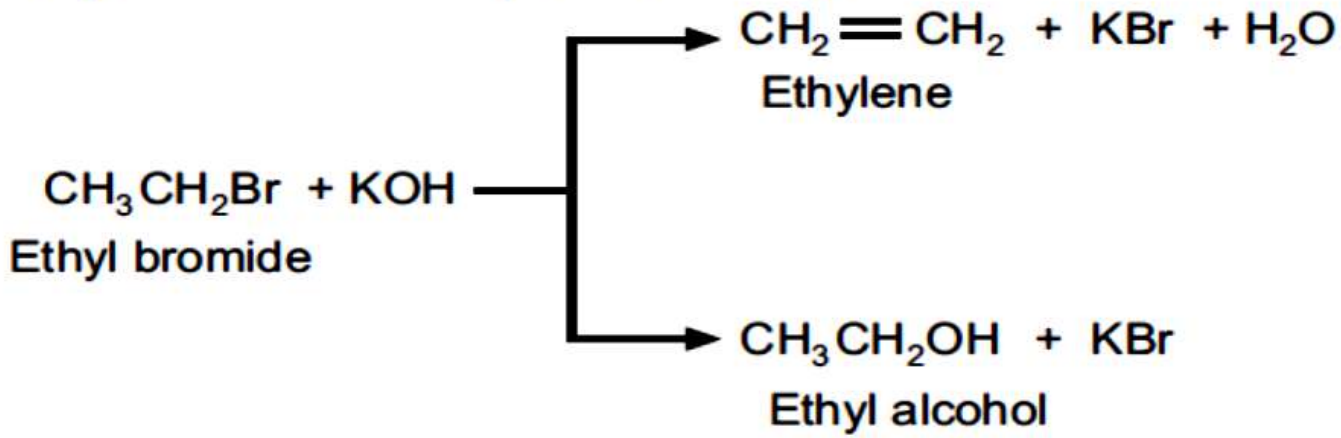


In the above reaction, the reactant A gives two products B and C separately in two different reactions with rate constants k_1 and k_2 respectively.

If $\mathbf{k_1 > k_2}$ the reaction A ⟶ B will be the **major reaction** and A ⟶ C will be the **side or parallel reaction**.

Examples of Parallel or Side Reactions

(a) Reaction of ethyl bromide with potassium hydroxide



3 Reversible or Opposing Reactions

$$A \underset{k_b}{\overset{k_f}{\rightleftharpoons}} B$$

Initial concentration	$[A]_0$	0
Conc. after time t	[A]	[B]

where k_f and k_b are the rate constants of the forward and backward reaction respectively.

The overall rate of reaction is given by:

Rate of Reaction = Rate of Forward Reaction – Rate of Backward Reaction

Examples of Opposing Reactions:

(a) Dissociation of hydrogen iodides

$$2HI \underset{k_b}{\overset{k_f}{\rightleftharpoons}} H_2 + I_2$$

(b) Isomerisation of cyclopropane into propene

$$\underset{\text{cyclopropane}}{\overset{\Delta}{}} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \underset{\text{Propene}}{CH_3 - CH = CH_2}$$

TYPES OF DEGRADATION PATHWAY

1. Physical Degradation

A. Crystallization of Amorphous Drugs:

1. Formulate poorly water–soluble drugs in their **amorphous state**.

2. This is because, the **solubility of amorphous materials** is generally higher than that of the same substances in their crystalline state

However, amorphous substances tend to change to their more thermodynamically **stable crystalline state with time.**

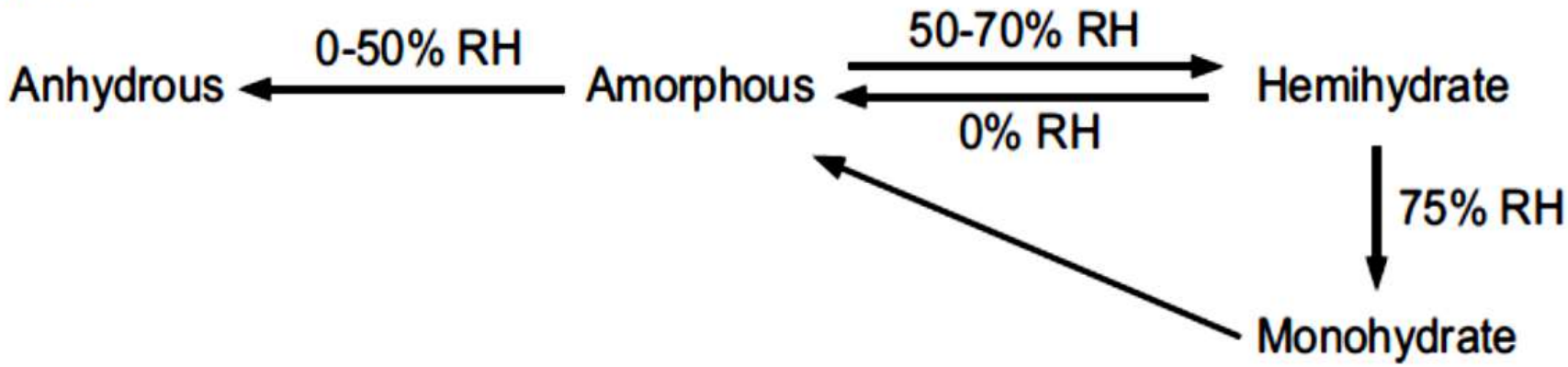
Therefore, crystallization of amorphous drug substances may occur during long–term storage and may lead to drastic changes in the **release characteristics of the drug and, hence, changes in its clinical and toxicological** behavior.

Changes in crystal habit during storage have been reported for many drug substances.

Some examples are discussed below.

(a) Amorphous nifedipine, coprecipitated with polyvinylpyrrolidone, undergoes partial crystallization during storage under high–humidity conditions. This change from a largely amorphous state to a partially crystalline state resulted in altered dissolution and solubility behaviour.

(b) Oxyphenbutazone, which can exist in an amorphous state and three different crystalline states (anhydrous, monohydrate and hemihydrate), exhibits crystallization and polymorphic transitions during storage depending on humidity, as illustrated in Fig. 3.19.



B. Polymorphism:

Polymorphs are different crystalline forms of the same drug.

Because, these forms have different free energy or chemical potentials, depending on temperature conditions, transitions between polymorphs occur.

Polymorphic transitions during storage may alter critical properties of drugs because the solubility and dissolution rate of drug substances generally vary with changes in their crystalline form.

From a storage perspective, temperature and humidity affect polymorphic transitions.

Transitions between anhydrous and hydrated forms have been reported for many drug substances such as **raclopride, theophylline, nitrofurantoin, sulfaguanidine and phenobarbital.**
Again, significant differences in solubility can exist between the anhydrous and hydrated forms of the same drug.

C. Formation and Growth of Crystals:

Drug substances and excipients in solid dosage forms, such as tablets and granules, may **recrystallize or sublime** onto the surface of the dosage form during storage. So–called **whisker crystallization** was observed in tablets of **ethenzamide and caffeine anhydride**.

Crystallization of ethenzamide and caffeine anhydride tablets was enhanced at **higher and lower humidity**, respectively.

Aspirin tablets exhibited whisker crystallization of salicylic acid, a degradation product.
This was found to change the tablet strength and to be dependent on tablet pore size.

D. Sublimation:

Nitroglycerin, which is a liquid with a significant vapour pressure, sublingual tablets exhibit significant variations in **drug content during storage** owing to inter-tablet migration through the vapour phase

This transfer was inhibited by adding water–soluble, **non–volatile fixing agents such as polyethylene glycol.**

E. Moisture Adsorption:

Moisture adsorption during storage can also affect the physical stability of pharmaceuticals, leading to changes in such properties as appearance and dissolution rate.

For example, the adsorption of moisture by aspirin crystals was enhanced by adding hydrophilic excipients.

F. Loss of Volatile Constituents:

Medicinal agents such as **iodine, camphor, menthol, ethyl alcohol, anesthetic ether, chloroform** have a tendency to evaporate from the product during storage.

Similarly, **nitroglycerine tablets** may lose its potency owing to volatilisation of the medicament.

The preventive measures include keeping the product in well–closed containers, and storing it in cool place.

G. Loss of Water:

Loss of vehicle (water) from the product leads to decrease in weight, rises in concentration of drug and increases potency.

Efflorescent substances, such as borax, caffeine and quinidine sulphate, have a natural tendency to lose water.

Products such as emulsions and semisolids exhibit cracking.

Loss of water depends on temperature and humidity.

Preventive measures include preserving the product in a well–closed container and storing it in a cool place.

Chemical Degradation and Preventive Measures

Hydrolysis:

(a) Ester and amide groups drugs: They are reacting with one molecule of water and undergo hydrolysis. Ester groups break faster than amide groups.

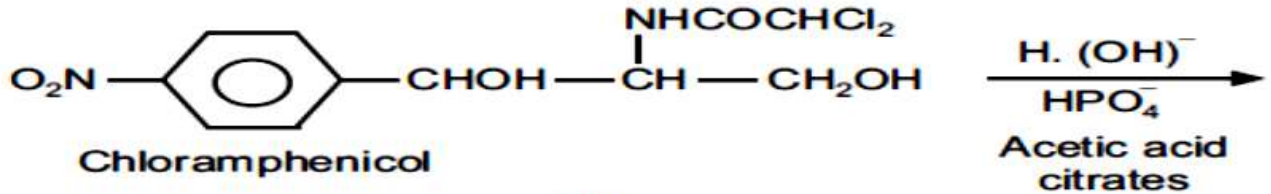
Drugs that contain ester groups, such as aspirin,

Aspirin — Salicylic acid — Acetic acid

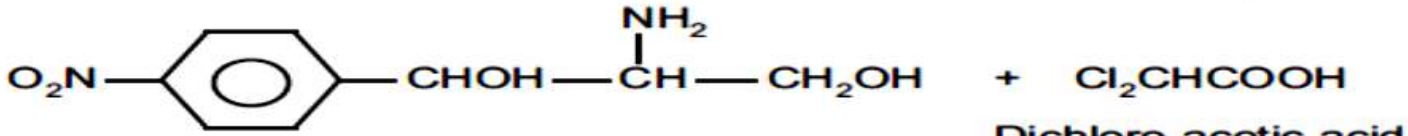
Aspirin undergo hydrolysis reaction and formation of salicylic acid and acetic acid but this decamped product, salicylic acid shows gastric irritation.

Drugs that contain amide groups, such as Chloramphenicol,

Amides undergo hydrolysis, though at a slower rate than esters. Chloramphenicol belongs to the category of amides. Its decomposition is influenced by acids, bases, phosphate ions, acidic and citrate buffers.



Chloramphenicol



Dichloro acetic acid

Protection against Hydrolysis:

Hydrolysis reactions are known to occur in presence of moisture, catalytic species H^+ and $(OH)^-$. Protective measures should aim at eliminating the influence of these factors on the drug.

Removal of water: As the presence of water is responsible for hydrolysis, it is better to avoid its contact with the drug in the preparation.

Example:

(a) Storing the drug in dry form. When desired, reconstitute the product. Example is streptomycin dry powder for injection.
(b) Using water–immiscible vehicle for the dispersion of drug. Example is aspirin in silicone fluid.

Buffers: Drug may be stabilized by the use of buffers. The pH of the solution should be adjusted so that the drug will have maximum stability and therapeutic activity.

Example: Pilocarpine is highly active in alkaline pH, because it exists in the unionized (free base) form. But at alkaline pH, it is highly irritating to the eye and also decomposes rapidly. Therefore, to prevent hydrolysis, acidic pH has to be selected. Select a buffer with low buffer capacity so that when administered in the eye, the pH gradually rises and releases the free base for drug action. For this reason, boric acid buffer of a pH 5.0 with low buffer capacity is selected.

Complexation: Drug may be stabilized by the complexation of drug with complexing agent.

Example: Hydrolysis of benzocaine in aqueous solution can be inhibited by the addition of caffeine which forms a complex. As a result of complexation, the attack of catalytic species on benzocaine may be reduced. Now, the rate of hydrolysis depends on the amount of free uncomplexed benzocaine present in solution. As the amount of caffeine increases, more and more amount of benzocaine will be complexed. This leads to decreased hydrolysis. Thus, the shelf life of the product can be prolonged. Other drugs which may be stabilized by complexation are procaine, tetracaine etc.

Suppression of solubility: When the solubility of a drug decreases, the concentration of drug in solution phase will be decreased. Hence, the rate of hydrolysis is reduced.

Example:

(a) Citrates, dextrose, sorbitol and gluconates, when combined with drugs, the solubility of drugs will be suppressed, probably because of decreased hydration of drug molecules.

(b) **Salts:** The degradation of penicillin can be prevented by using poorly soluble salt of procaine penicillin in the dosage form.

(c) **Derivatives:** Poorly water soluble derivatives such as esters (higher fatty acids) of drugs can be used to reduce the tendency of hydrolysis. Examples are erythromycin propionate, erythromycin stearate, chloramphenicol palmitate etc.

Oxidation:

Oxidation involves the removal of electrons from a molecule. The reaction between the compounds and molecular oxygen is called **autooxidation.** In fats and oils, autooxidation of unsaturated fatty acids proceeds in the presence of atmospheric oxygen, light and traces of heavy metals or organic peroxides. For example, the rate of oxidation of ascorbic acid is increased by a factor of 10^5, when copper ions are present in the concentration of 0.002 M.

The general principles that govern an oxidation reaction may be listed as follows:

(a) The presence of atomospheric oxygen (also air) promotes the rate of oxidation.

(b) Since oxidation frequently involves free radicals, chain reactions occur. Light provides the necessary energy to initiate the oxidation process.

(c) The presence of trace metals also accelerates the rate of oxidation.

(d) Organic peroxides promote the chain initiation and propagate the oxidation reaction.

(e) Drugs are either weak acids or bases. Therefore, these may be available as ionic forms or neutral molecules. Oxidation reaction between ionic species proceeds faster than with neutral molecules (to a large extent it is solubility related phenomenon).

(f) Oxidation reactions are catalysed by H^+ and OH^- ions. Hydroxyl ions catalyse oxidation faster than hydrogen ions. Alkaline solutions are known to react with atmospheric oxygen and form oxides.

Influence of Trace Metal:

When solutions are free from traces of copper, ascorbic acid is not oxidized by molecular oxygen to a measurable extent, except in alkaline solutions. However, even traces of copper lead to the rapid oxidation of ascorbic acid. When CO and KCN are added to the above reaction mixture, they form complexes with metal ions, and therefore, oxidation of ascorbic acid is inhibited. These reactions demonstrate the influence of cupric ion on the oxidation of ascorbic acid.

$$\text{Ascorbate ion in solution} \xrightarrow[\text{Slow oxidation}]{Cu^{2+}} \text{Semi quinone} \xrightarrow[\text{Rapid oxidation}]{O_2} \text{Dehydro ascorbic acid}$$

Influence of air on oxidation:

The rate of decomposition decreases when higher concentration of ascorbic acid is used. It is presumed that a part of the ascorbic acid reacts with oxygen and thus depletes free oxygen. When air is bubbled through the reaction mixture, the rate of oxidation is enhanced. When dissolved oxygen is maintained at saturation level, the reaction rate remains constant. Therefore, oxygen is responsible for the autooxidation reactions.

Protection against Oxidation:

Oxidation reactions are known to occur in presence of oxygen, trace metals, H^+ and $(OH)^-$ ions. Protective measures should aim at eliminating the influence of these factors on the drug.

Antioxidants: Antioxidants mean this excipient inhibit the oxidation reaction.

Oil soluble antioxidant: These agents act by breaking the free radical chain reactions at the step of chain propagation.

Tocopherols are the naturally occurring antioxidants. Other examples are butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate etc. These are widely used in foods, cosmetics and drugs.

Water–soluble antioxidants: They act by preferentially undergoing oxidation instead of the drug itself.

Example is ascorbic acid, cysteine, acetylcysteine, thioglycolic acid etc.

Chelating agents: Addition of a chelating agent to a product will be useful when traces of heavy metals catalyse the oxidation. Substances such as EDTA (ethylerrediamine tetraacetic acid) citric acid and tartaric acid, form complexes with heavy metals. Thus, metal ions are not available to catalyse the oxidation. For example, addition of EDTA to the buffer system prevents the degradation of drugs such as prednisolone and ascorbic acid.

Vehicles: Usually water is used as a solvent for most products. The replacement of water by other solvents is often employed as a means of stabilizing drugs. But, other solvents when used in combination with water, they have catalyzing effect on oxidation.

Therefore careful consideration should be given while attempting to use solvents for stabilizing drugs.

Micellar solubilisation: Surfactants such as polysorbate 80 enhance the rate of oxidation of ascorbic acid at low concentration, but protect above its critical micelle concentration (cmc), presumably by entrapping the drugs in the spherical micelles. Sometimes, spherical micelles offer a site for surface adsorption of catalytic ions and enhance rate of reaction.

Buffers: Buffer system imparts stability when oxidation is catalysed by H^+ or $(OH)^-$ ions. Choose a buffer with appropriate pH to maintain maximum stability of the product.

Prevent the exposure to light: Light is responsible for oxidation. The preparation is protected from light by employing amber coloured bottles or using appropriate packaging material.

Oxygen free environment: Oxygen enhances oxidative degradation. Therefore, air is replaced with inert gases such as nitrogen or carbon dioxide.

Low temperature storage: Since high temperature enhances the rate of reaction, the product is stored in a cool place.

3. Isomerism:

Some drugs often have same structural formula, but possess different stereochemical configurations. Interconversion of one stereochemical form into another leads to inactive or less active drugs.

[I] Optical Isomerisation:

In solutions, the optically active form (+ve or –ve form) of a drug gets converted into its enantiomorph (+ve and –ve form). This process continues until the two forms are equal in concentration. The optical activity at equilibrium will be zero, i.e., optically inactive.

A few examples are:

(–) Adrenaline (greater biological activity) ⟶ (±) Adrenaline (+ and – is 50 : 50) less potent

(–) Hyocyamine (more intense mydriatic) —Heat or alkali / light⟶ (±) Hyocyamine (less potent)

Preventive measures: The product is protected from light and heat. Optimum pH has to be maintained for maximum activity.

[II] Geometric Isomerisation:

In this case, the compounds exist as trans and cis isomers, based on their relative spatial configuration of groups around a double bond (s). These changes may bring about corresponding changes in its biological activity.

For example:

Vitamin A palmitate (all trans) ⟶ 6-mono-cis derivative + 2, 6-di-cis derivative

More active ⟶ Less active

4. Absorption of carbon dioxide:

Solutions absorb carbon dioxide from the atmosphere. For example:

$$\text{Sodium hexobarbitone (IV injection)} \xrightarrow[\text{hydrolysis}]{\text{salt}} \text{Solution (Basic pH)} \xrightarrow[CO_2]{\text{absorb}} \text{Hexobarbitone precipitate (Acidic pH)}$$

Preventive measures: The product is stored in well–filled and well–closed containers. Manufacturers supply the product as a dry sterile powder. The instruction should be — to dissolve the drug before use in CO_2 free sterile water for injection.

5. Decarboxylation:

These types of reactions are normally observed when a parenteral solution contains sodium carbonate. During autoclaving (for sterilisation), the carboxylic acid groups will be knocked off.

Examples: Sodium p–aminosalicylic acid (PAS, anti–TB drug) Procaine hydrochloride (local anesthetic)

$$\text{Procain (clear solution)} \xrightarrow{\text{hydrolysis}} \text{p-amino benzoic acid} \xrightarrow{-CO_2} \text{Aniline liquid} \xrightarrow{\text{light}} \text{Dark coloured liquid}$$

Preventive measures: Carbon dioxide gas is passed into the solution for one minute. The container is sealed so as to be air–tight prior to autoclaving.

Stability Testing

Definitions:

1. **Stability of a pharmaceutical product** may be defined as 'the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, toxicological, protective and informational specifications'.
2. In other words, it is the extent to which a product retains, within the specified limits, throughout its period of storage and use, the same properties and characteristics possessed at the time of its packaging.

Reasons to Study Stability of Pharmaceutical Product

1. The stability of the active ingredient(s); interaction between active ingredients and excipients.
2. The effect of light, heat and moisture conditions encountered during shipment, storage and handling.
3. In addition, degradation reactions like oxidation, reduction, hydrolysis or racemization, which can play vital role in stability of a pharmaceutical product.
4. Stability depends on such conditions like concentration of reactants, pH, radiation, catalysts etc.
5. A pharmaceutical product may undergo change in appearance, consistency, content uniformity, clarity (solution), moisture contents, particle size and shape, pH, package integrity thereby affecting its stability.
6. Such physical changes may be because of impact, vibration, abrasion, and temperature fluctuations such as freezing, thawing or shearing etc.
7. Stability of a pharmaceutical product can also be affected because of microbiological changes like growth of microorganisms in non-sterile products and changes in preservative efficacy.

Climatic zone	Climate/ Definition	Major countries/ Regions	MAT*/ Mean Annual Partial Water Vapour Pressure	Long-term testing conditions
(I)	Temperate	United Kingdom, Northern Europe, Russia, United States	≤15°C ≤11 hPa	21°C/45% RH
(II)	Subtropical and Mediterranean	Japan, Southern Europe	> 15-22°C />11-18 hPa	25°C/60% RH
(III)	Hot and Dry	Iraq, India	>22°C/≤15 hPa	30°C/35% RH
(IV) (a)	Hot and humid	Iran, Egypt	>22°C/>15-27 hPa	30°C/65% RH
(IV) (b)	Hot and very humid	Brazil, Singapore	>22°C/>27 hPa	30°C/75% RH

* MAT - Mean annual temperature measured in open air.

This guideline was published by **WHO (Word Health Organization)** for Stability testing of active pharmaceutical ingredients and finished pharmaceutical products for **Climatic zone III and IV.**

Also, **The ICH (International Conference of Harmonization) Q 1 A** guideline for Stability testing of active pharmaceutical ingredients and finished pharmaceutical products for **Climatic zone I and II.**

Points in Stability Testing

(a) **Selection of batches:** Least three primary batches of the drug should normally be provided. The batches should be manufactured to a minimum of pilot scale by the same synthesis route as production batches.

(b) **Container Closure System:** The stability studies should be conducted on the drug substance packaged in a container closure system.

(c) **Testing Frequency:** For long–term studies, frequency of testing should be sufficient to establish the stability profile of the API.

(i) **For long term studies:** The frequency of testing at the long term storage condition should normally be every 3 months over the first year (e.g. 0, 3, 6, 9 and 12 months), every 6 months over the second year.

(ii) **For accelerated storage condition:** A minimum of three time points, including the initial and final time points (e.g., 0, 3 and 6 months) from a 6 month study is recommended.

(iii) **For intermediate term studies:** When testing at the intermediate storage condition is called for as a result of significant change at the accelerated storage condition, a minimum of four time points, including the initial and final time points (e.g., 0, 6, 9, 12 months), from a 12 month study is recommended.

(d) Storage Conditions

(A) General Case:

Study	Storage condition	Minimum time period covered by data at submission
Long-term[a]	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH or 30°C ± 2°C/75% RH ± 5% RH	12 months or 6 months
Intermediate[b]	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

(B) Storage in a Refrigerator:

Study	Storage condition	Minimum time period covered by data at submission
Long-term	5°C ± 3°C	12 months
Accelerated[a]	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH or 30°C ± 2°C/75% RH ± 5% RH	6 months

(C) For Storage in a Freezer:

Study	Storage condition	Minimum time period covered by data at submission
Long-term	– 20°C ± 5°C	12 months

(D) For Storage below –20°C:

APIs intended for storage below –20°C should be treated on a case–by–case basis.

General Anatomy of Urinary System

By

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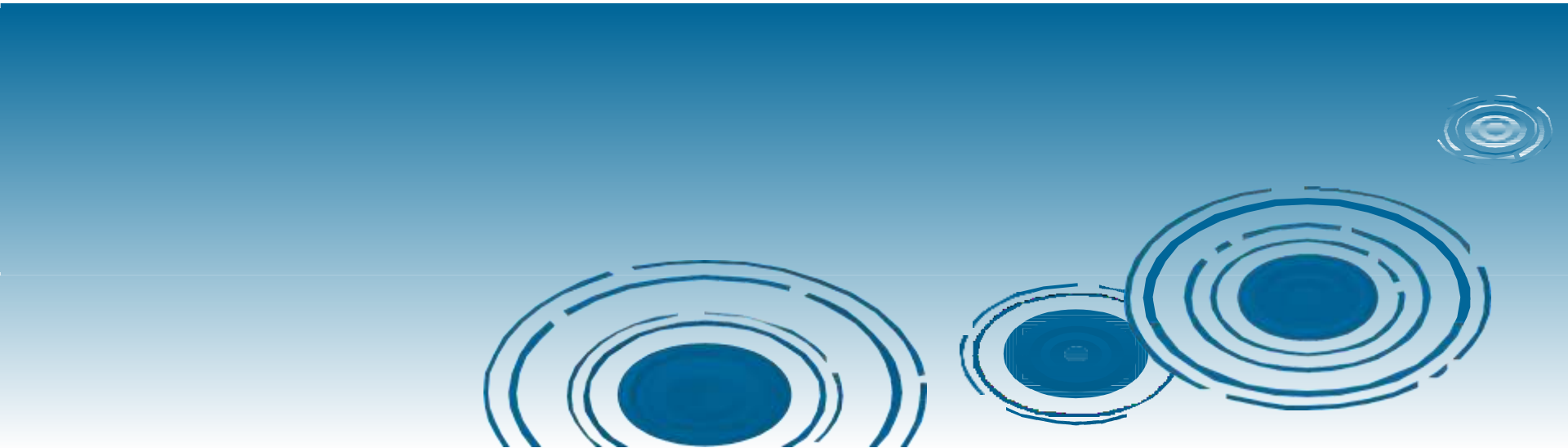
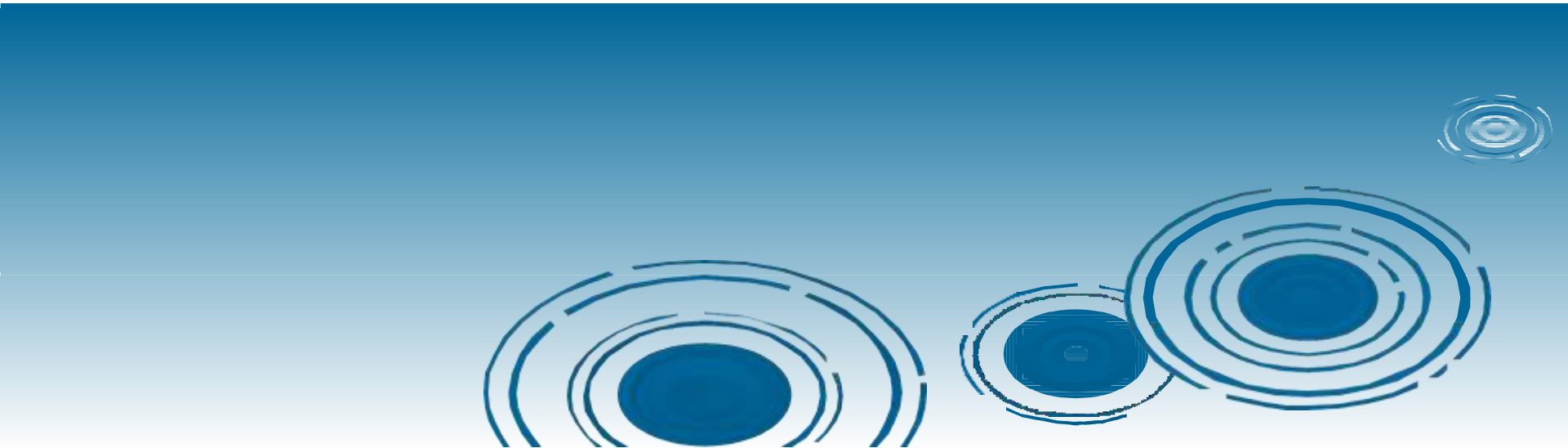
Introduction

- The **urinary system**, also known as the **renal system**
- The urinary system refers to the structures that produce and conduct urine to the point of excretion.



URINARY SYSTEM ORGANS

- Kidneys (2)
- Ureters (2)
- Urinary bladder
- Urethra



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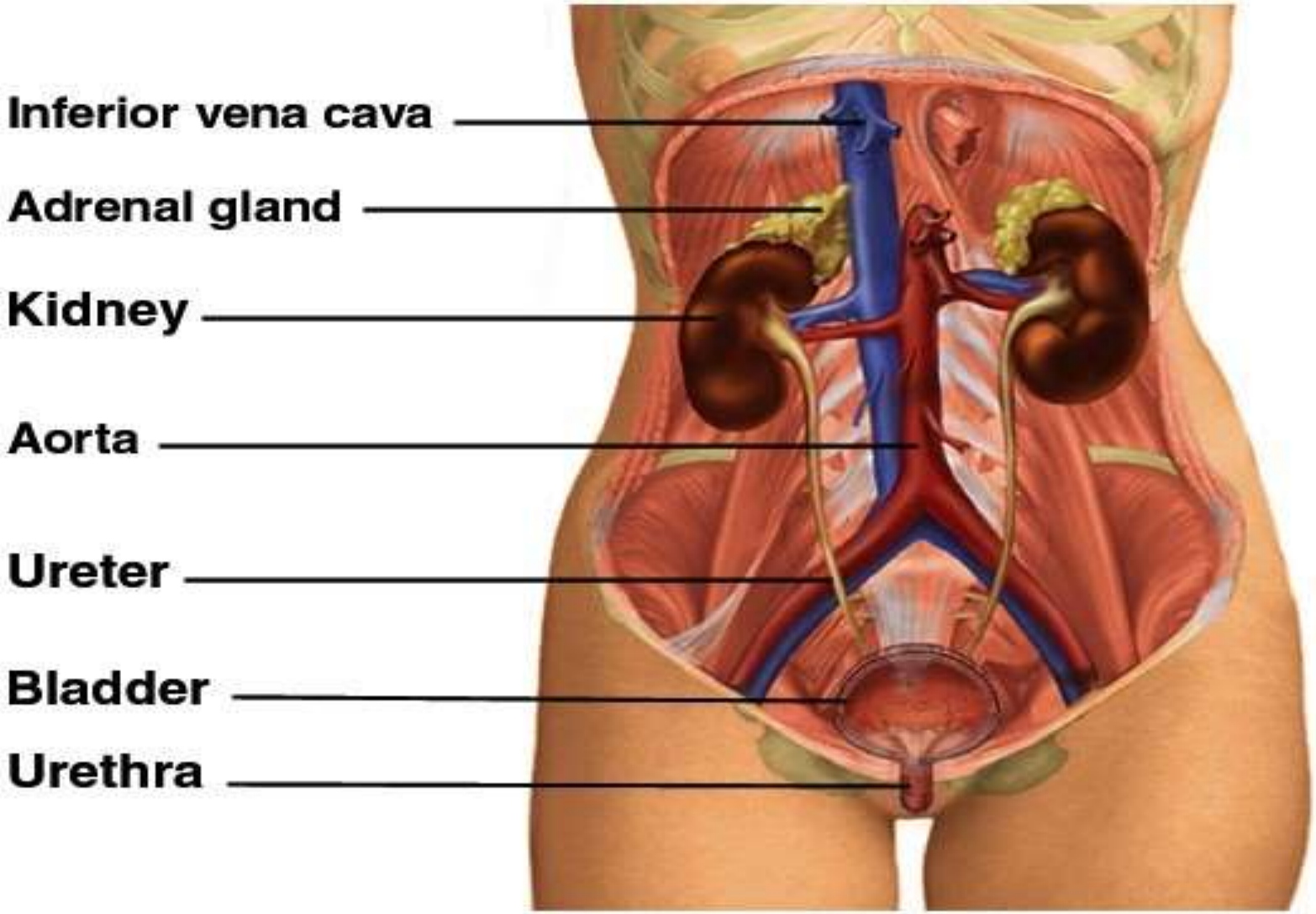
Urinary System

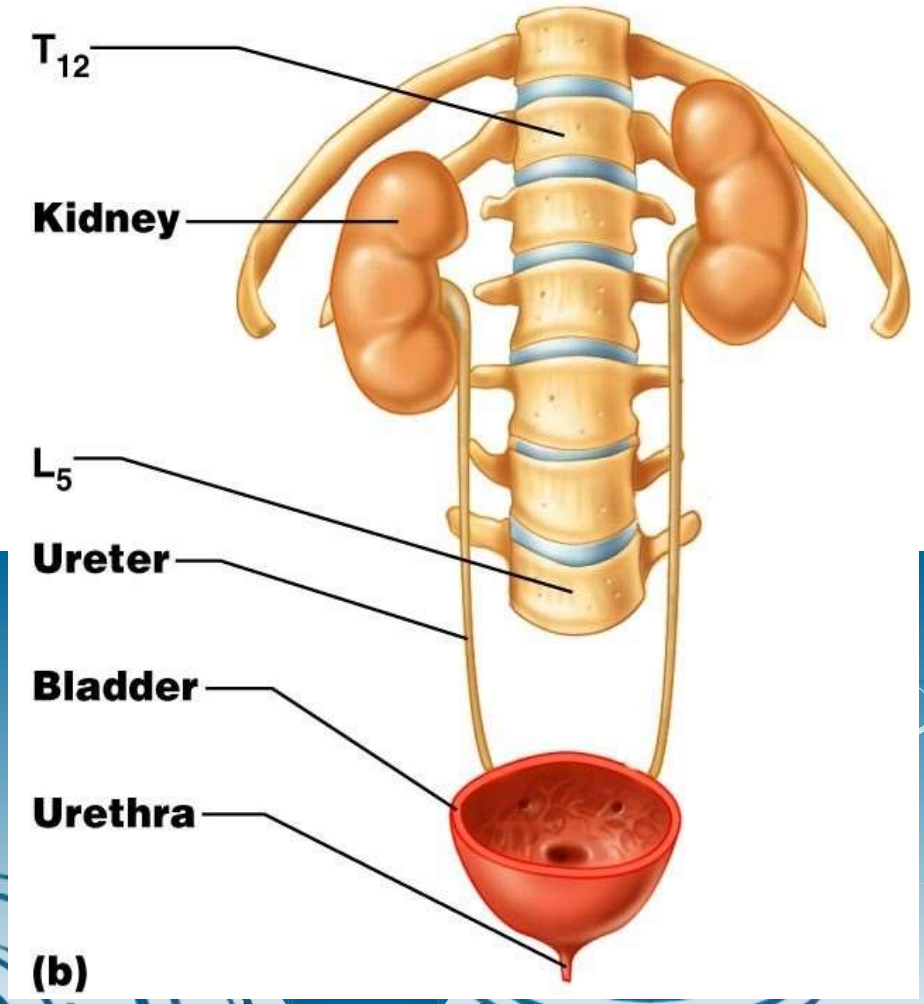


Kidney

- The human body normally has two paired kidneys, one on the left and one on the right.
- The functional unit of the kidney is nephron.
- Urine is formed by nephrons

Location and External Anatomy of Kidneys

- Located **retroperitoneally**
- Lateral to T_{12}–L_3 vertebrae
- Average kidney
  - 12 cm tall, 6 cm wide, 3 cm thick



Protected by three connective tissue layers

- Renal fascia
  - Attaches to abdominal wall
- Adipose capsule
  - Fat cushioning kidney
- Renal capsule
  - Fibrous sac
  - Protects from trauma and infection

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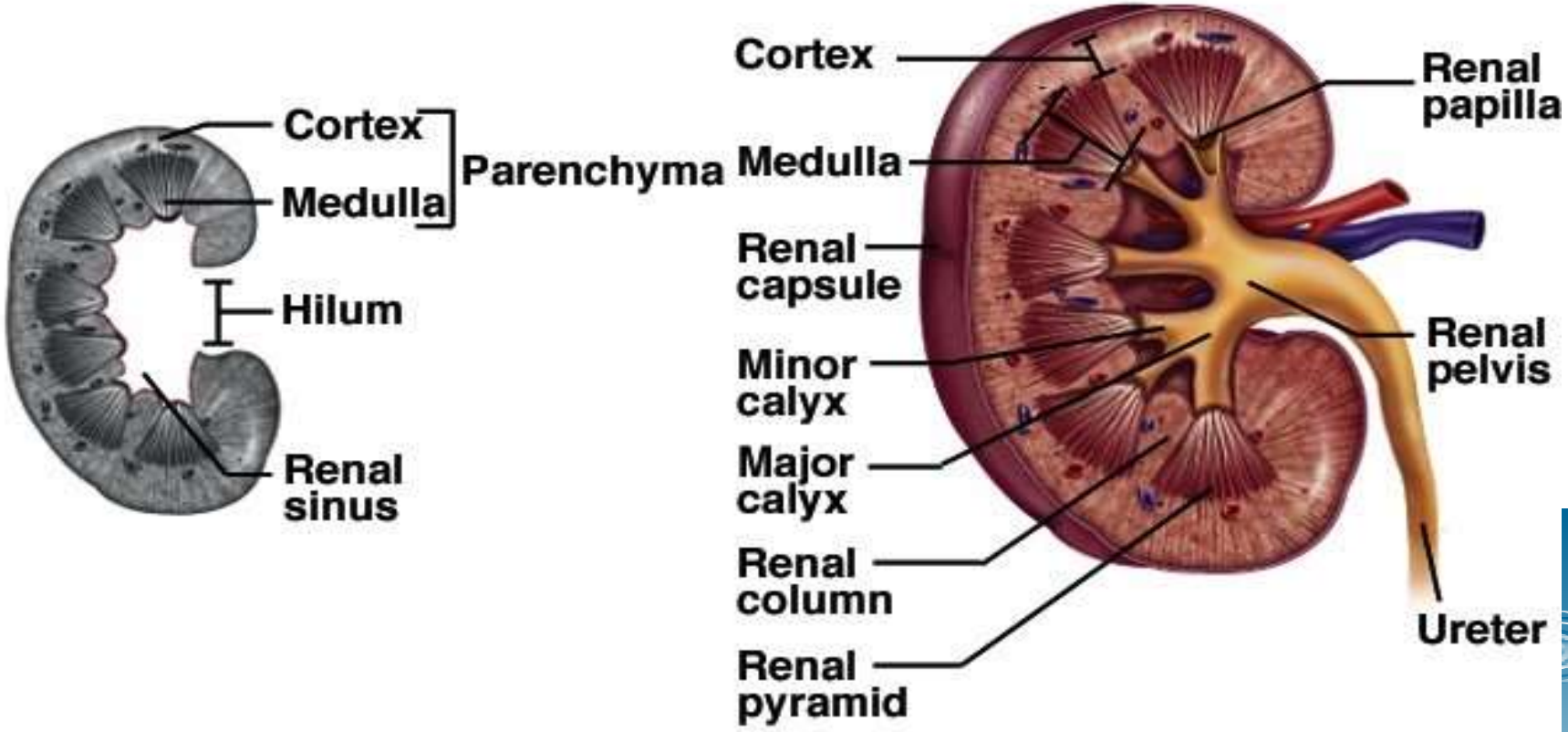
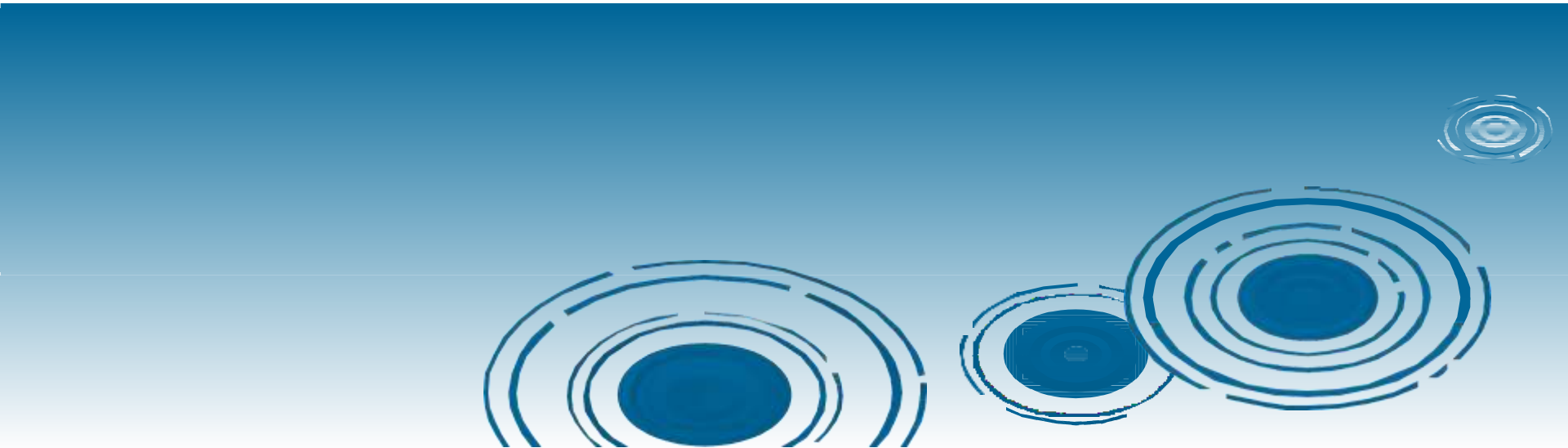
Anatomy of Kidney



KIDNEY

Gross anatomy

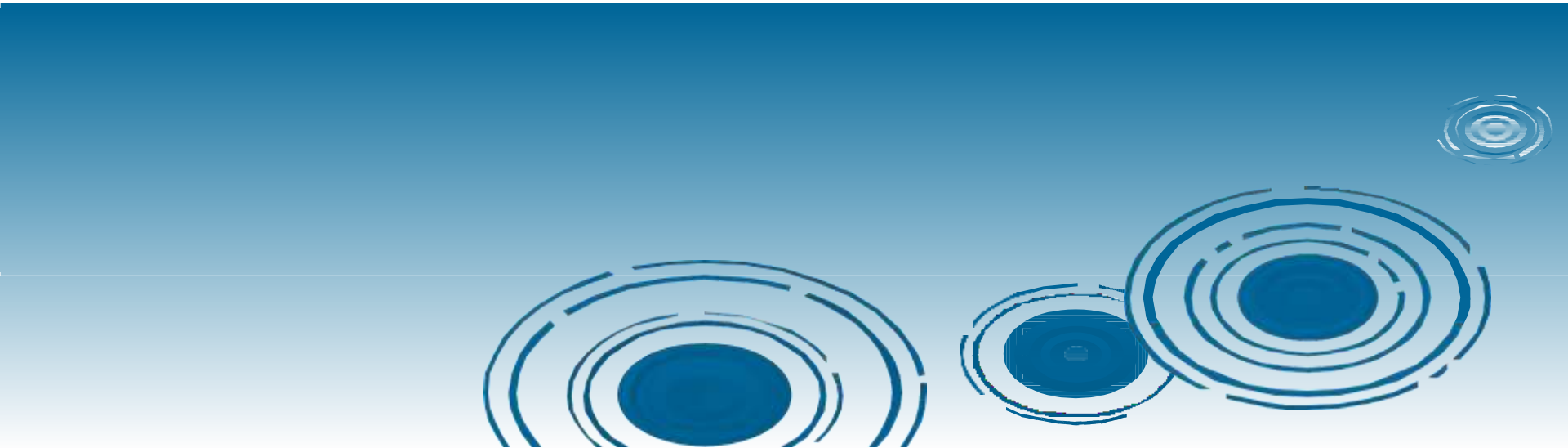
- Renal parenchyma
- Renal sinus

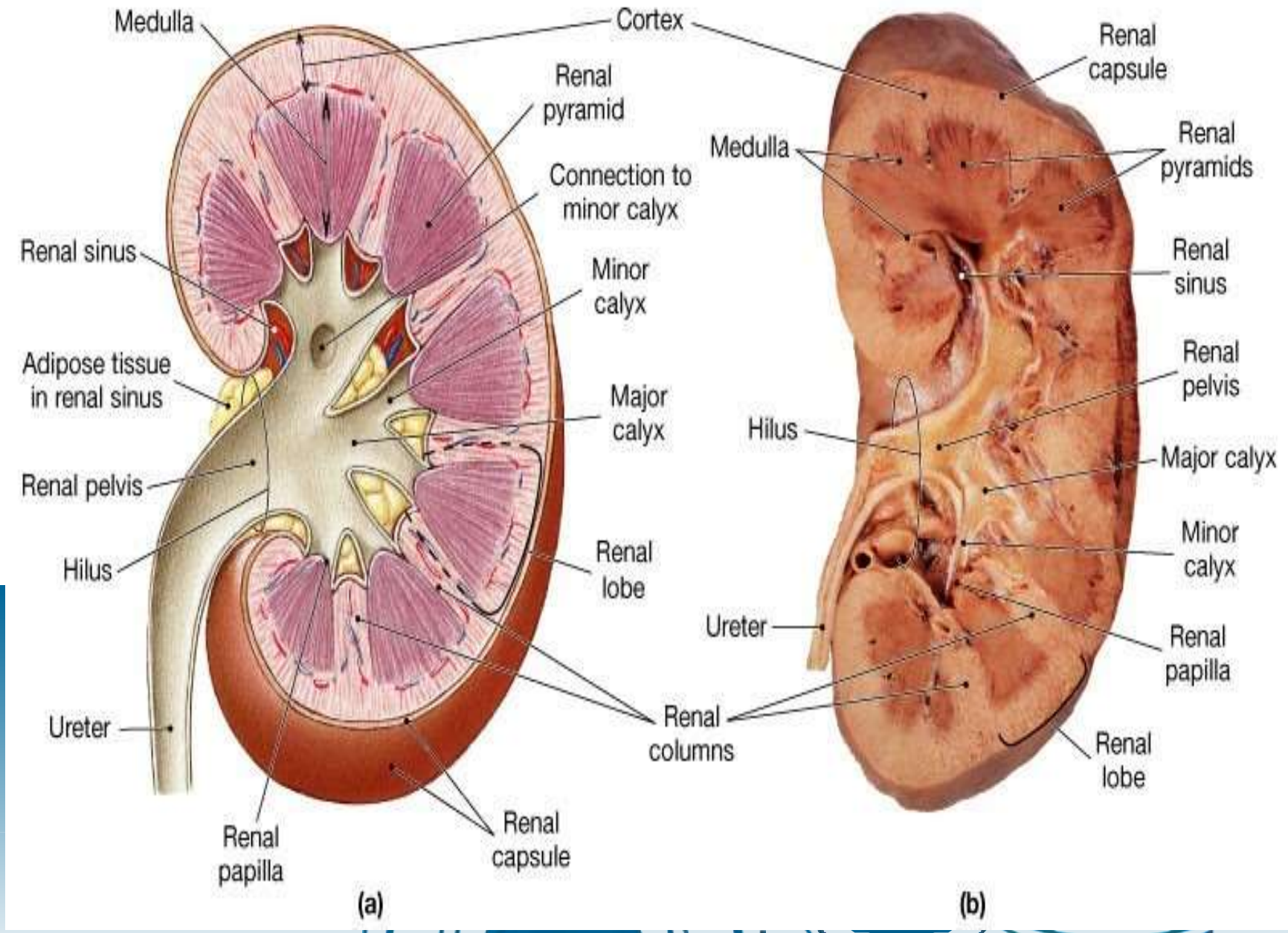


KIDNEY ANATOMY

Renal parenchyma

- Two zones
  - Outer cortex
  - Inner medulla




Medulla
Cortex
Renal pyramid
Connection to minor calyx
Renal sinus
Minor calyx
Adipose tissue in renal sinus
Major calyx
Renal pelvis
Hilus
Renal lobe
Ureter
Renal columns
Renal papilla
Renal capsule
(a)
Renal capsule
Medulla
Renal pyramids
Renal sinus
Renal pelvis
Hilus
Major calyx
Minor calyx
Ureter
Renal papilla
Renal lobe
(b)

Anatomy of the kidneys

- Superficial outer cortex and inner medulla
  - The medulla consists of 6-18 renal pyramids
  - The cortex is composed of roughly 1.25 million nephrons

KIDNEY ANATOMY

Renal parenchyma

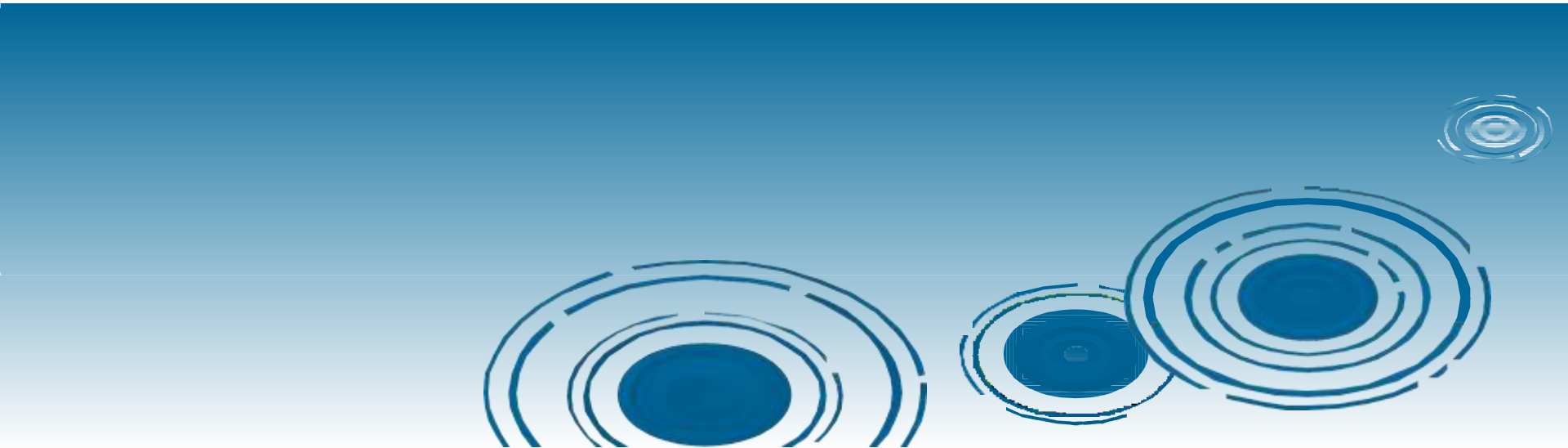
- Renal pyramids
  - Extensions of cortex (renal columns) divide medulla into 6 – 10 renal pyramids
  - Pyramid + overlying cortex = Lobe
  - Point of pyramid = Papilla
  - Papilla nested in cup (minor calyx)
  - 2 – 3 minor calices ▪ Major calyx
  - 2 – 3 major calices ▪ Renal pelvis
  - Renal pelvis ▪ Ureter

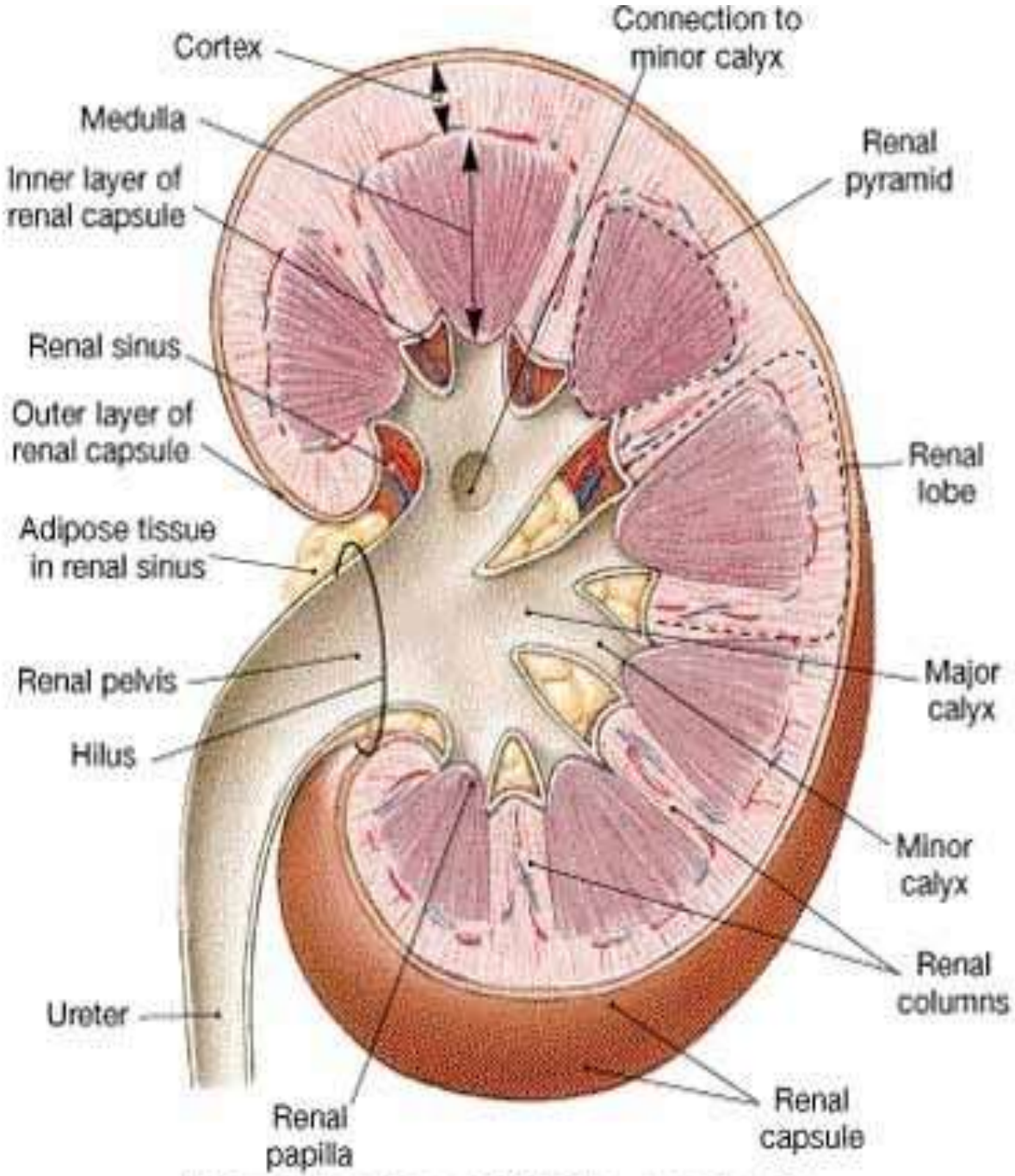
KIDNEY ANATOMY

Renal sinus

- Surrounded by renal parenchyma
- Contains blood & lymph vessels, nerves, urine-collecting structures
- **Hilus**
  - On concave surface
  - Vessels and nerves enter and exit

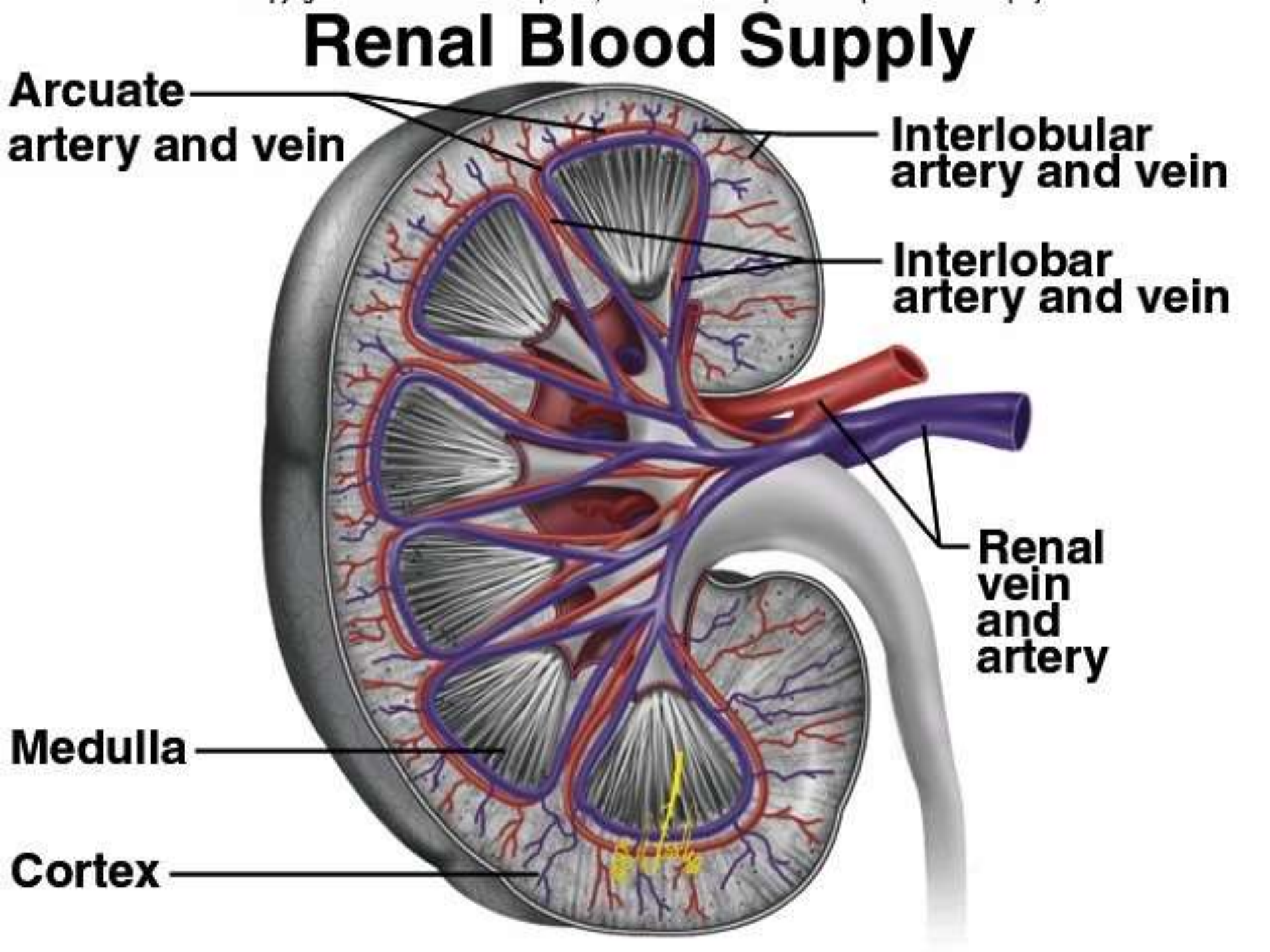
- Major and minor calyces along with the pelvis drain urine to the ureters





(a) Frontal section of left kidney, anterior view

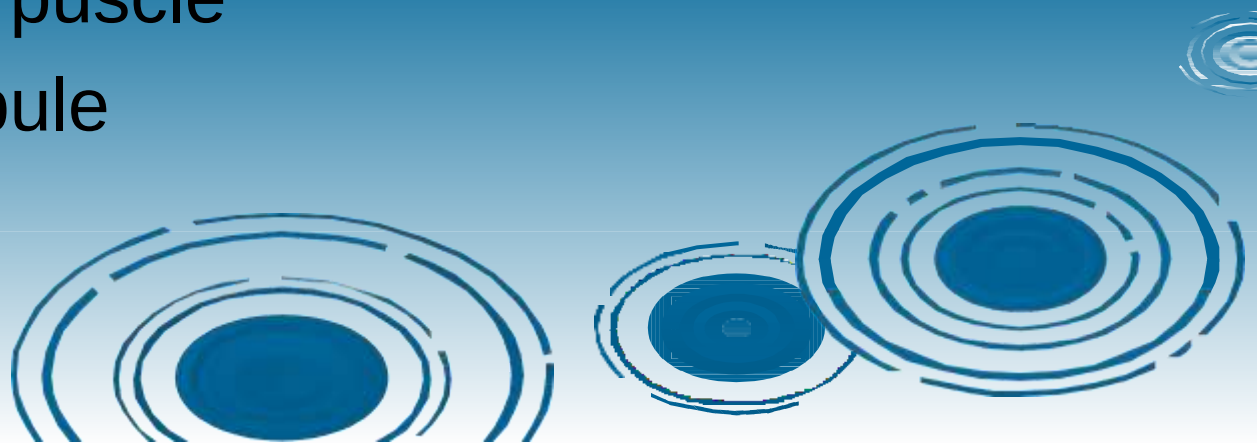
Renal Blood Supply



NEPHRONS

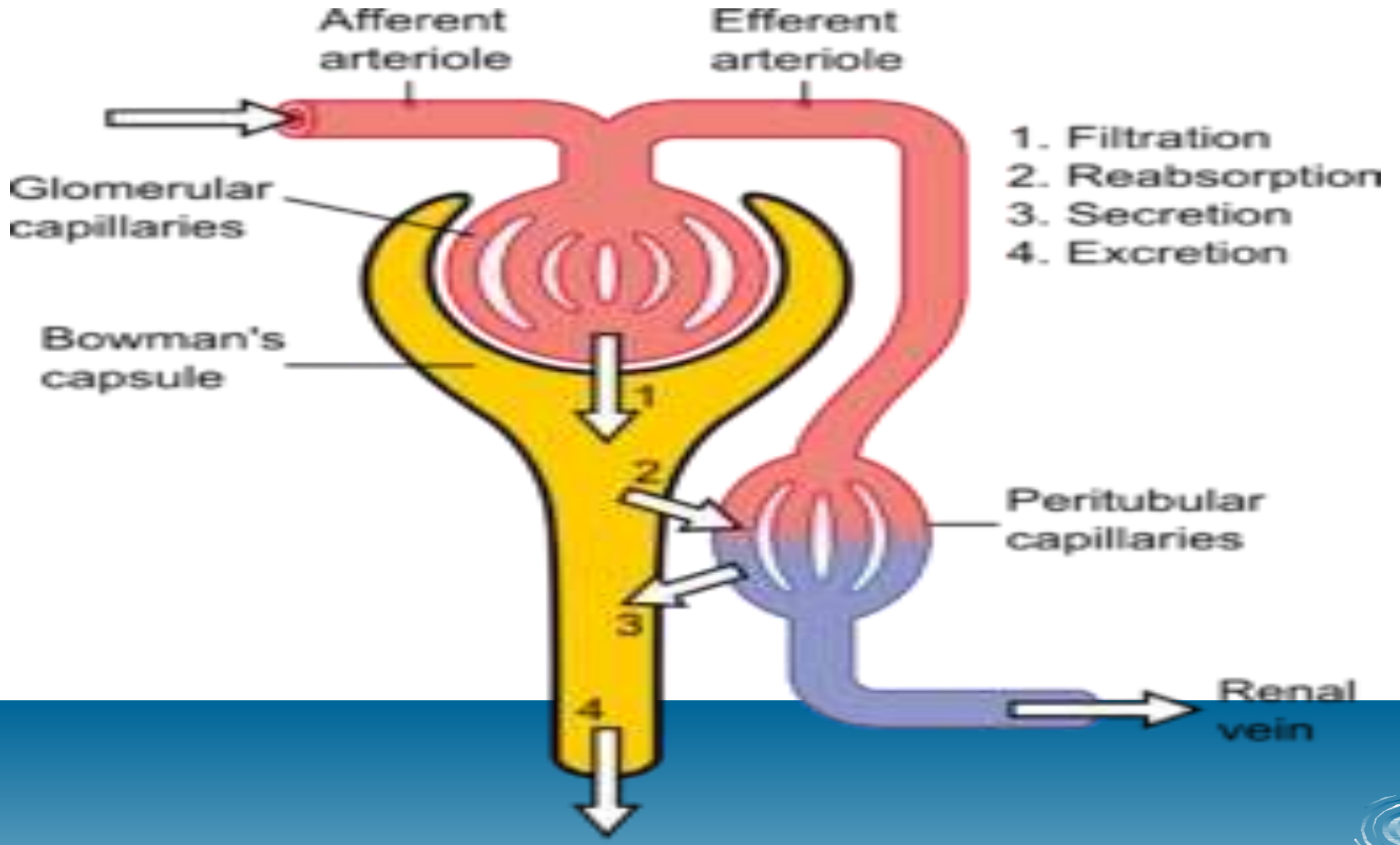
Nephrons

- Functional units of kidney
- ~1.2 million per kidney
- Three main parts
  - Blood vessels
  - Renal corpuscle
  - Renal tubule



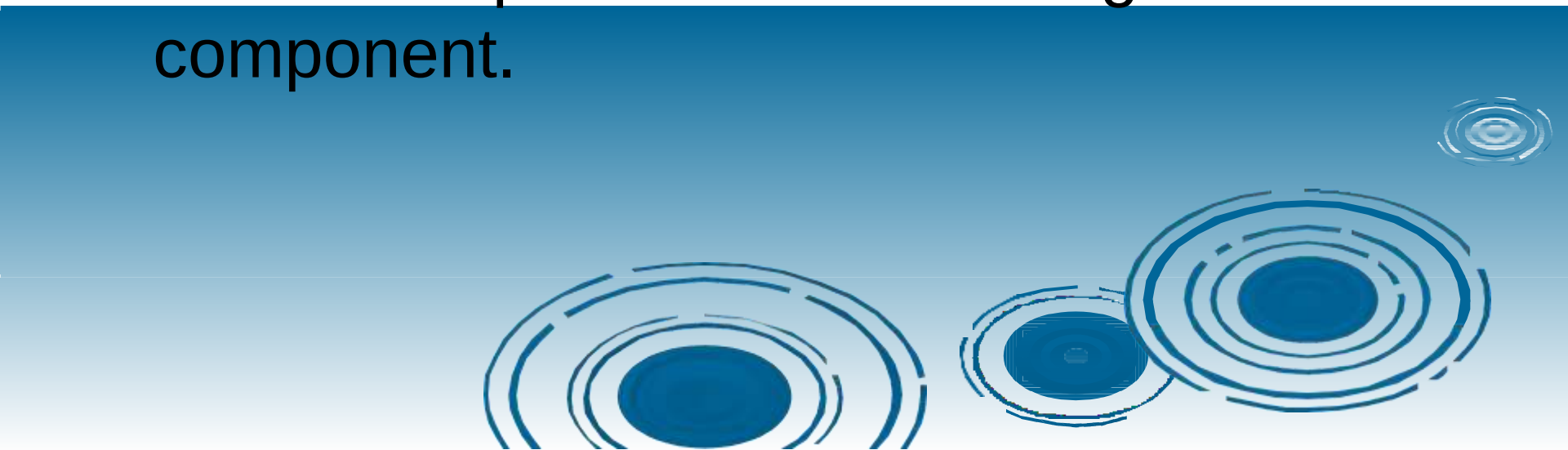
Blood vessels servicing kidney

- Glomerulus
  - Fenestrated capillaries
  - Capillary filtration in glomerulus initiates urine production
  - Filtrate lacks cells & proteins
  - Drained by efferent arteriole
  - ·Peritubular capillaries
  - · · · · Renal vein


Afferent arteriole
Efferent arteriole
1. Filtration
2. Reabsorption
3. Secretion
4. Excretion
Glomerular capillaries
Bowman's capsule
1
2
3
4
Peritubular capillaries
Renal vein
Urinary excretion
Excretion = Filtration – Reabsorption + Secretion

Renal corpuscle

- Composed of a glomerulus and the Bowman's capsule,
- The renal corpuscle is the beginning of the nephron.
- It is the nephron's initial filtering component.



Glomerulus

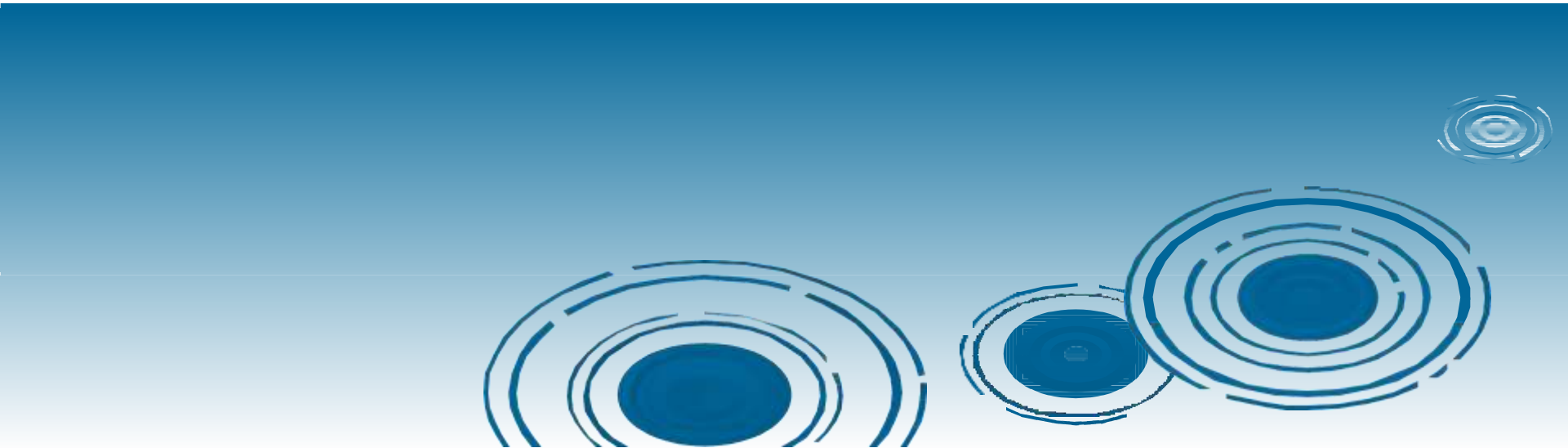
- The glomerulus is a capillary tuft that receives its blood supply from an afferent arteriole of the renal circulation.
- The glomerular blood pressure provides the driving force for water and solutes to be filtered out of the blood and into the space made byBowman's capsule

- The remainder of the blood passes into the efferent arteriole.
- The diameter of efferent arterioles is smaller than that of afferent arterioles, increasing the hydrostatic pressure in the glomerulus.

Bowman's capsule

- The Bowman's capsule, also called the glomerular capsule.
- surrounds the glomerulus.
- It is composed of a visceral inner layer formed by specialized cells called podocytes.
- Parietal outer layer composed of simple squamous epithelium.

- Fluids from blood in the glomerulus are filtered through the visceral layer of podocytes, resulting in the glomerular filtrate.

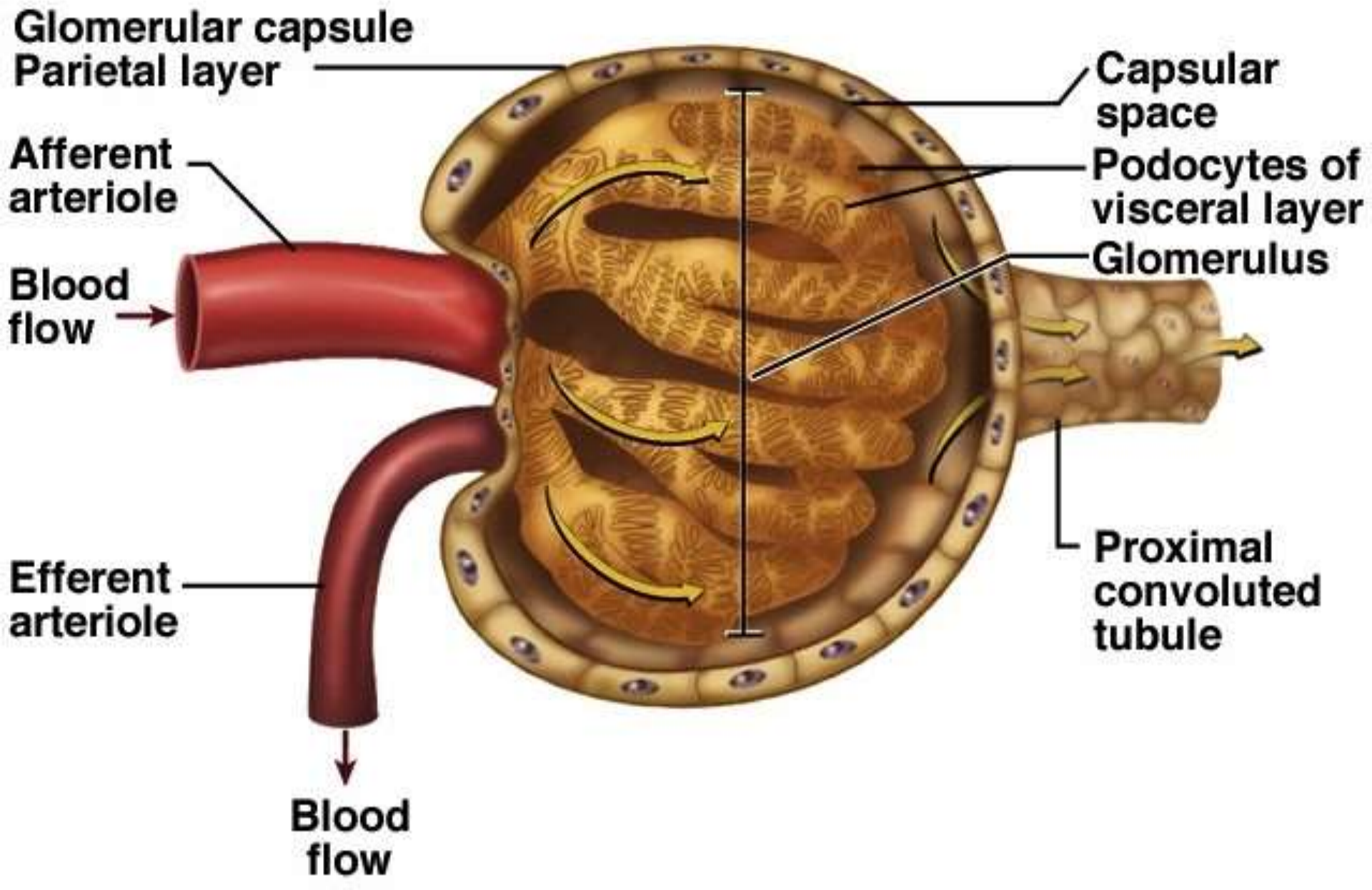


NOTE

Renal corpuscle

- Glomerulus plus capsule
- Glomerulus enclosed in two-layered glomerular capsule
  - “Bowman’s capsule”
- Fluid filters from glomerular capillaries
  - “Glomerular filtrate”
- Fluid collects in capsular space
- Fluid flows into renal tubule

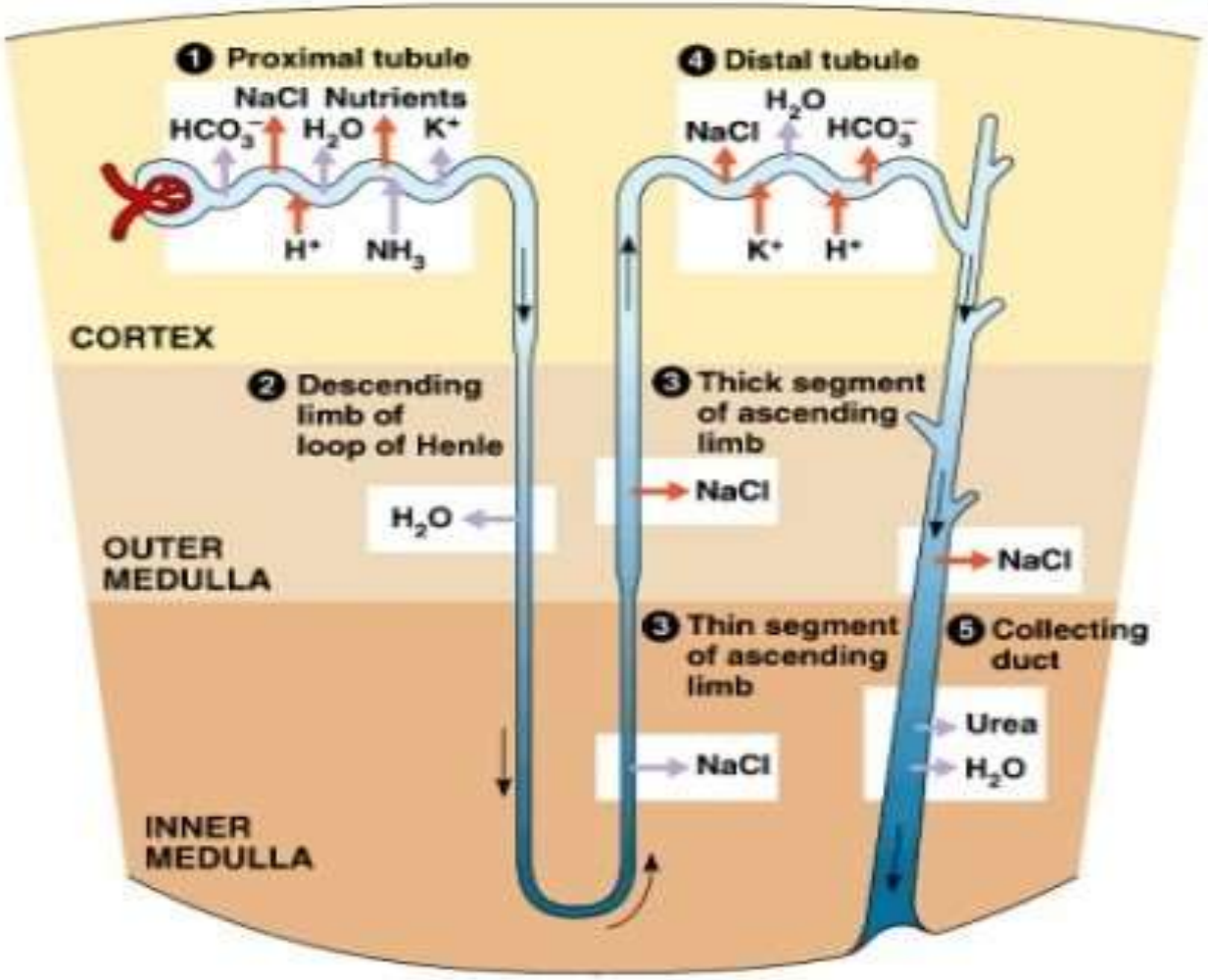
Renal Corpuscle



Renal tubule

- Leads from glomerular capsule
- Ends at tip of medullary pyramid
- ~3 cm long
- Four major regions
  - Proximal convoluted tubule
  - Nephron loop
  - Distal convoluted tubule
  - Collecting duct

Nephron-Tubular System



1. Proximal convoluted tubule
2. Descending loop of Henle
3. Ascending loop of Henle
4. Distal convoluted tubule
5. Collecting duct

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Proximal convoluted tubule (PCT)

- Arises from glomerular capsule
- Longest, most coiled region
- lies in cortex
- lined by simple cuboidal epithelium with brush borders which help to increase the area of absorption greatly.)
- Prominent microvilli
  - Function in absorption

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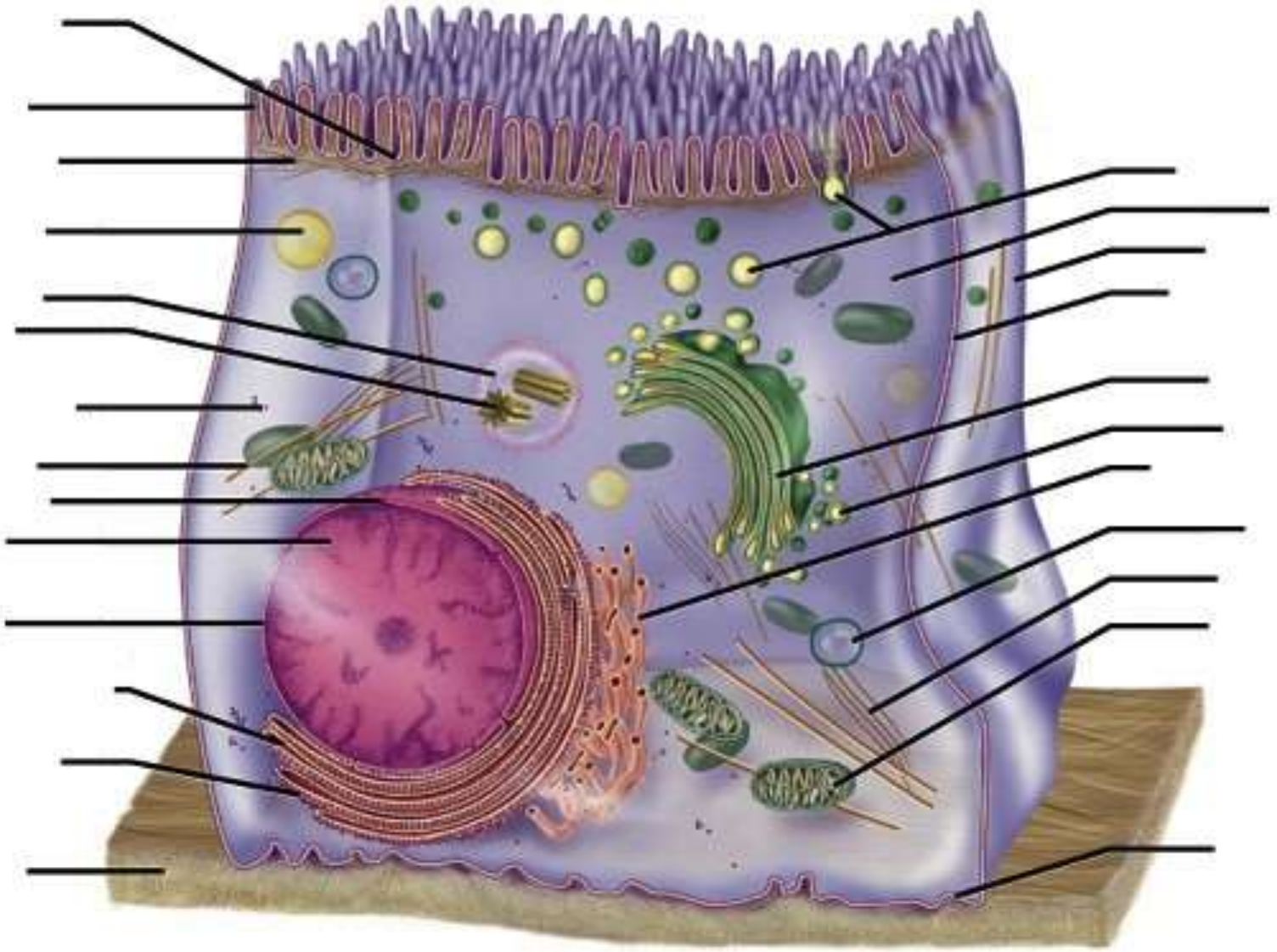
Cell Stucture



Nephron loop (“Loop of Henle”)

- “U” – shaped, distal to PCT
- lies in medulla

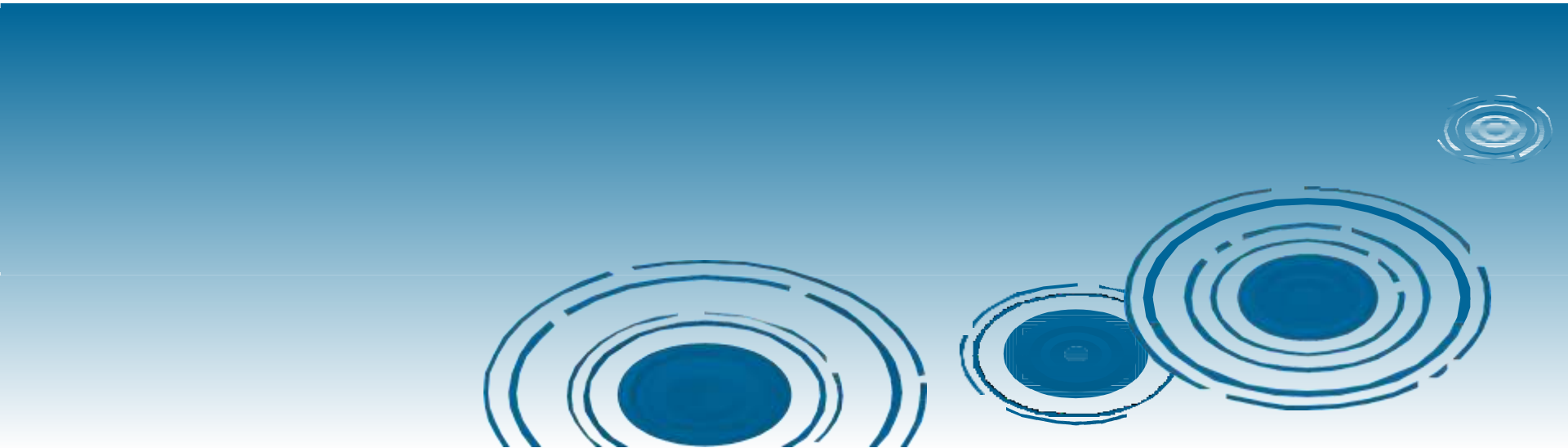
2 parts

- Descending limb of loop of Henle
- Ascending limb of loop of Henle

Ascending limb of loop of Henle

- The ascending limb of loop of Henle is divided into 2 segments:
- Lower end of ascending limb is very thin and is lined by simple squamous epithelium.
- The distal portion of ascending limb is thick and is lined by simple cuboidal epithelium.
- Thin ascending limb of loop of Henle
- Thick ascending limb of loop of Henle (enters cortex and becomes DCT-distal convoluted tubule.)

- Thick segments
  - Active transport of salts
  - High metabolism, many mitochondria
- Thin segments
  - Permeable to water
  - Low metabolism



Distal convoluted tubule (DCT)

- Coiled, distal to nephron loop
- Shorter than PCT
- Less coiled than PCT
- Very few microvilli
- Contacts afferent and efferent arterioles
- Contact with peritubular capillaries

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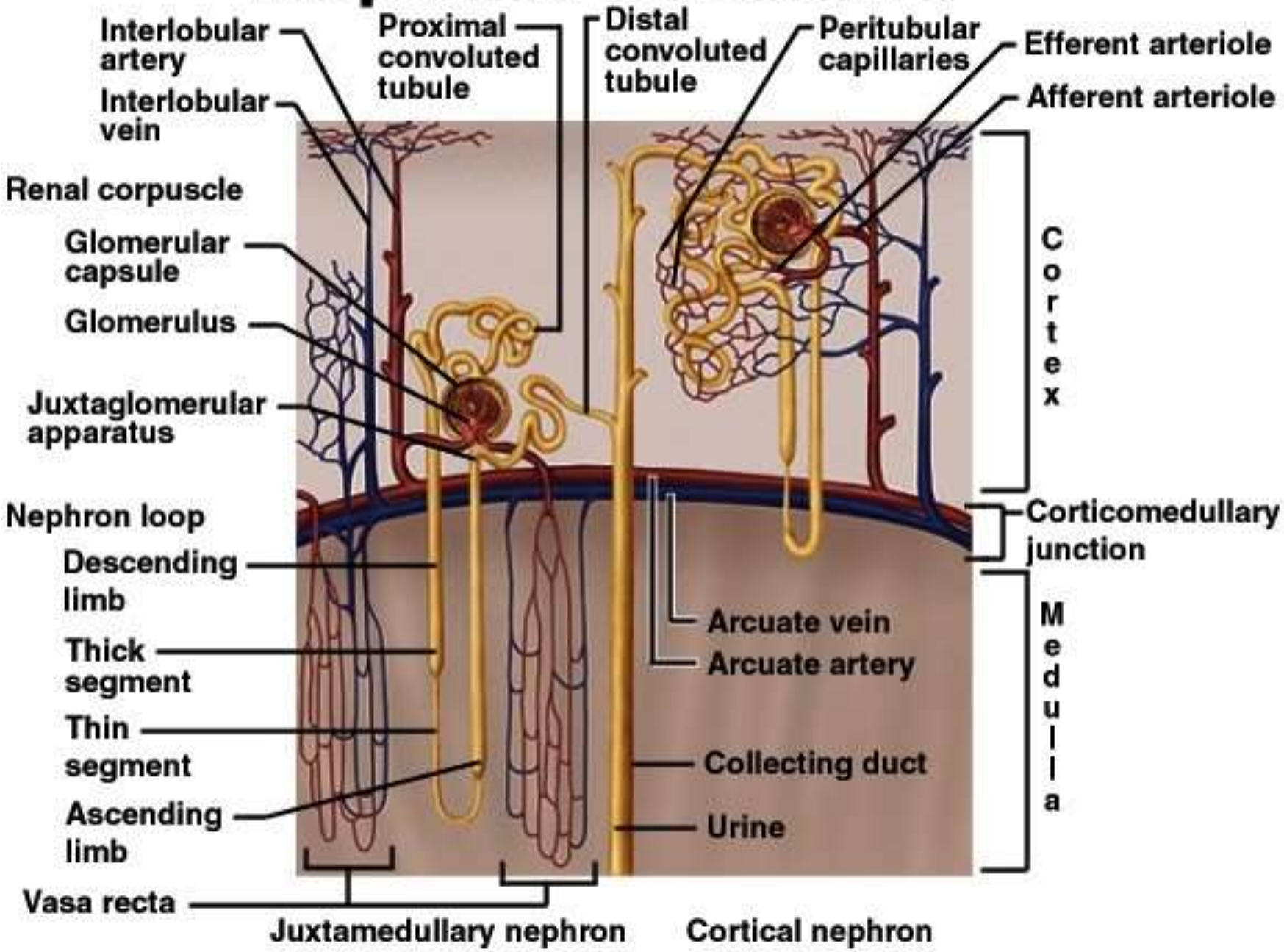
Nephron Structure



Collecting duct

- DCTs of several nephrons empty into a collecting duct
- Passes into medulla
- Several merge into papillary duct (~30 per papilla)
- Drain into minor calyx

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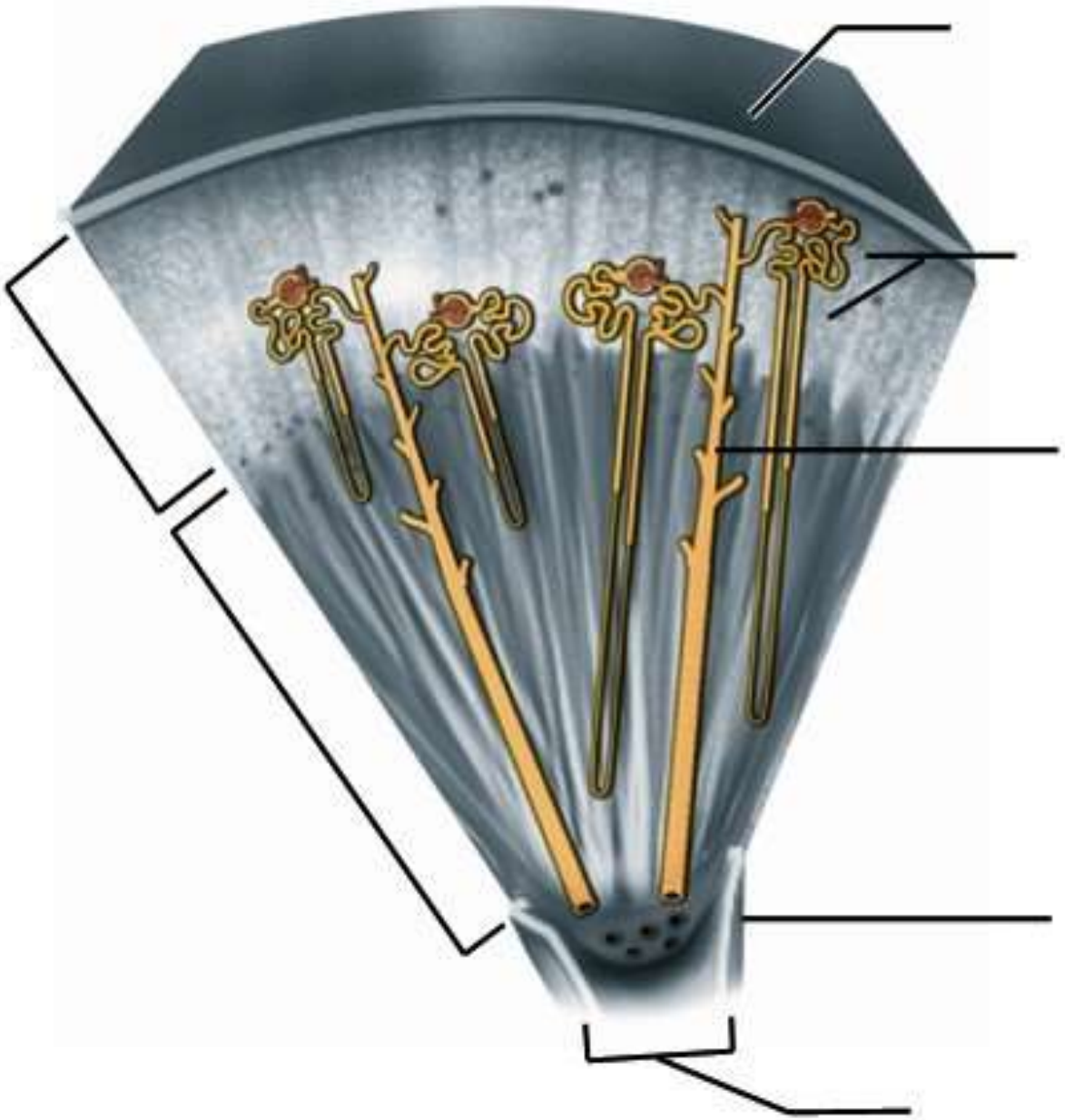
Kidney Lobe



CLASSES

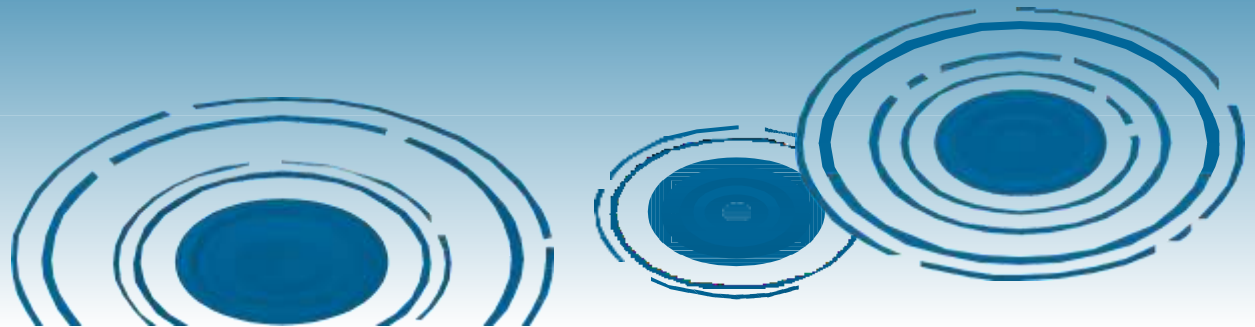
The two general classes of nephrons are

- Cortical nephrons
- Juxtamedullary nephrons

which are classified according to the

- length of their Loop of Henle
- location of their renal corpuscle.

- All nephrons have their renal corpuscles in the cortex.
- *Cortical* nephrons have their Loop of Henle in the renal medulla near its junction with the renal cortex,
- Loop of Henle of juxtamedullary nephrons is located deep in the renal medulla;



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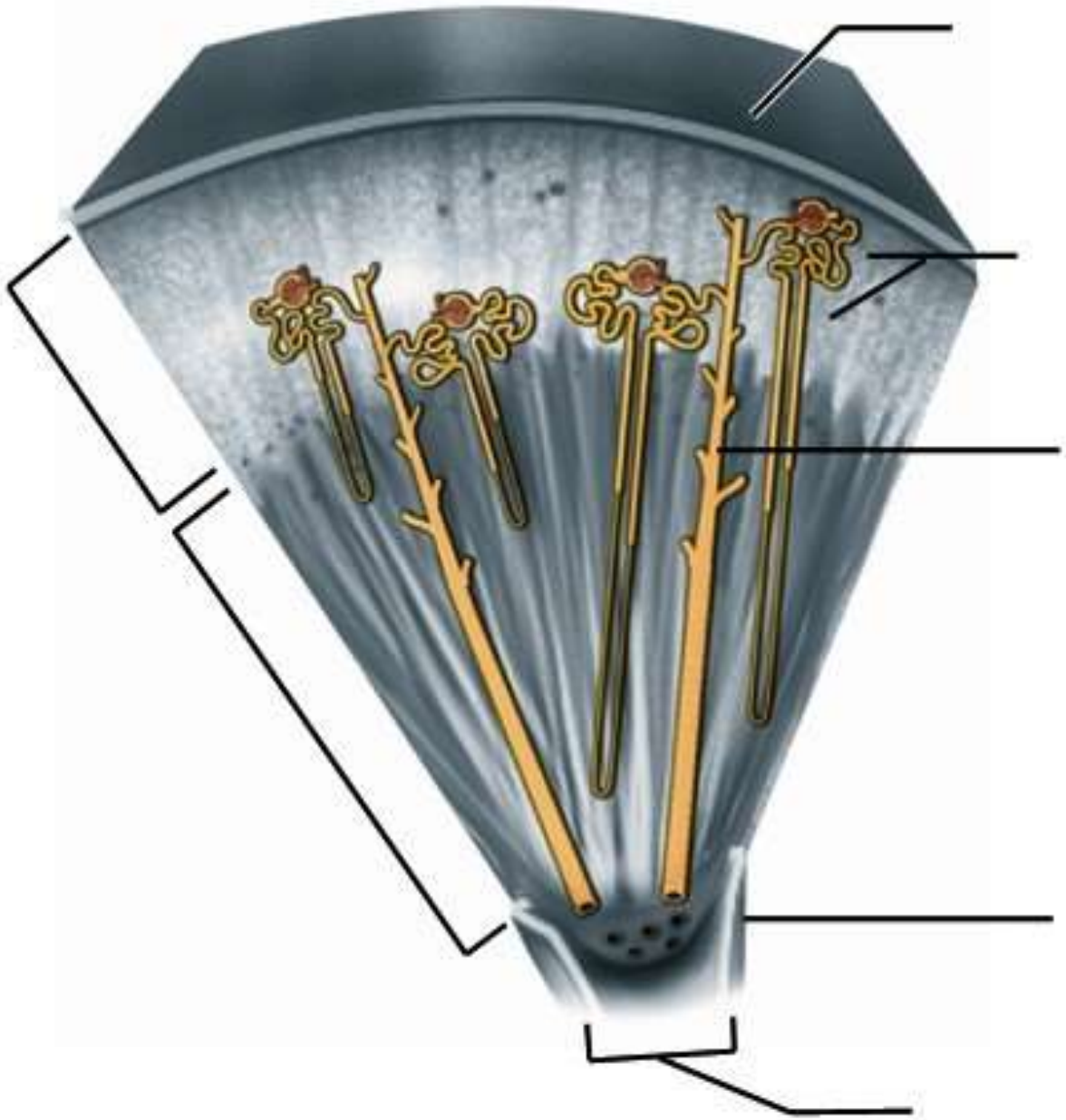
Kidney Lobe



URINE FORMATION

Overview

- Blood plasma ▪▪▪▪ Urine
- Four steps
  - Glomerular filtration
  - Tubular reabsorption
  - Tubular secretion
  - Water conservation

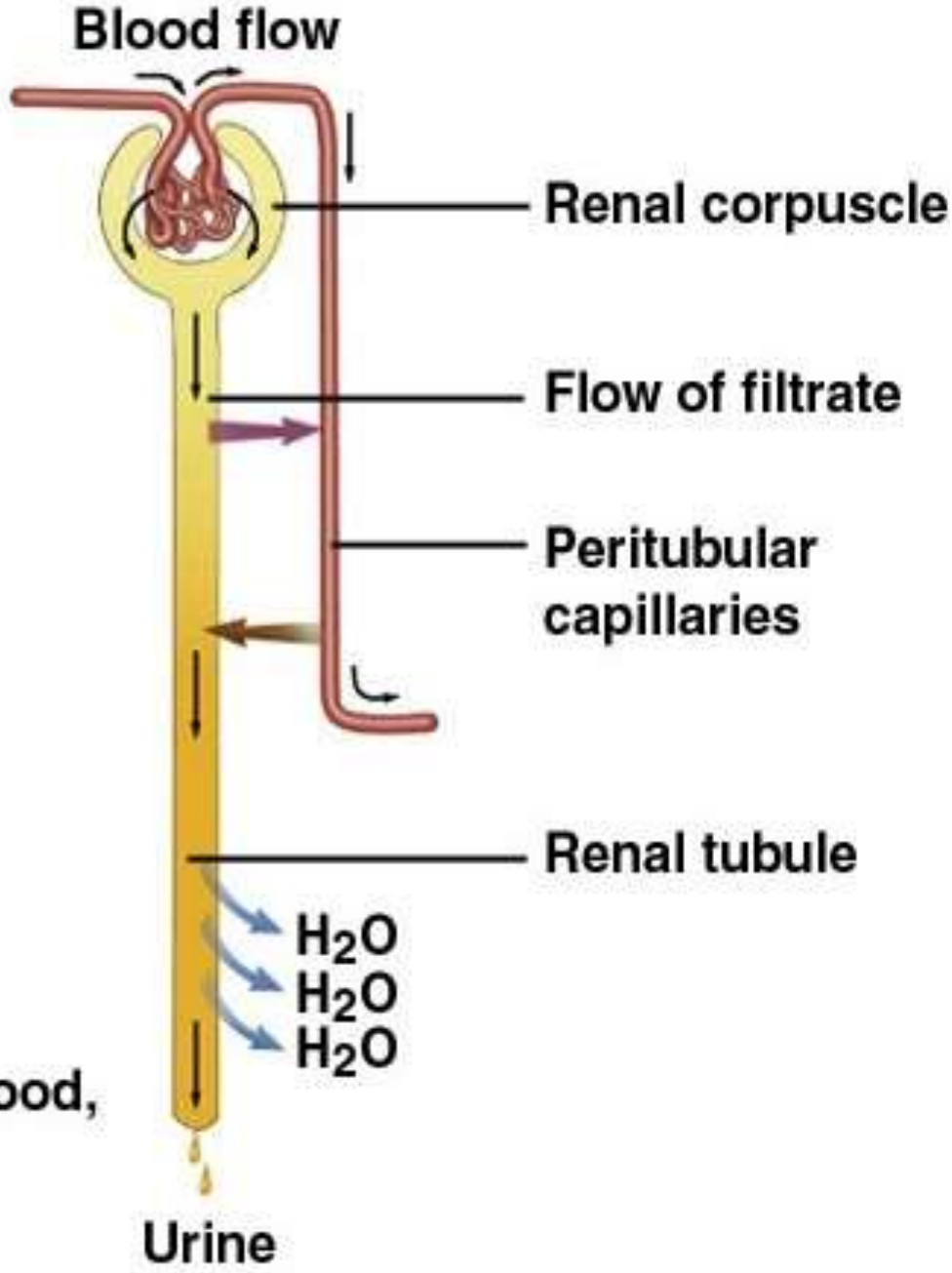
Urine Formation

① **Glomerular filtration**
Creates a plasmalike filtrate of the blood

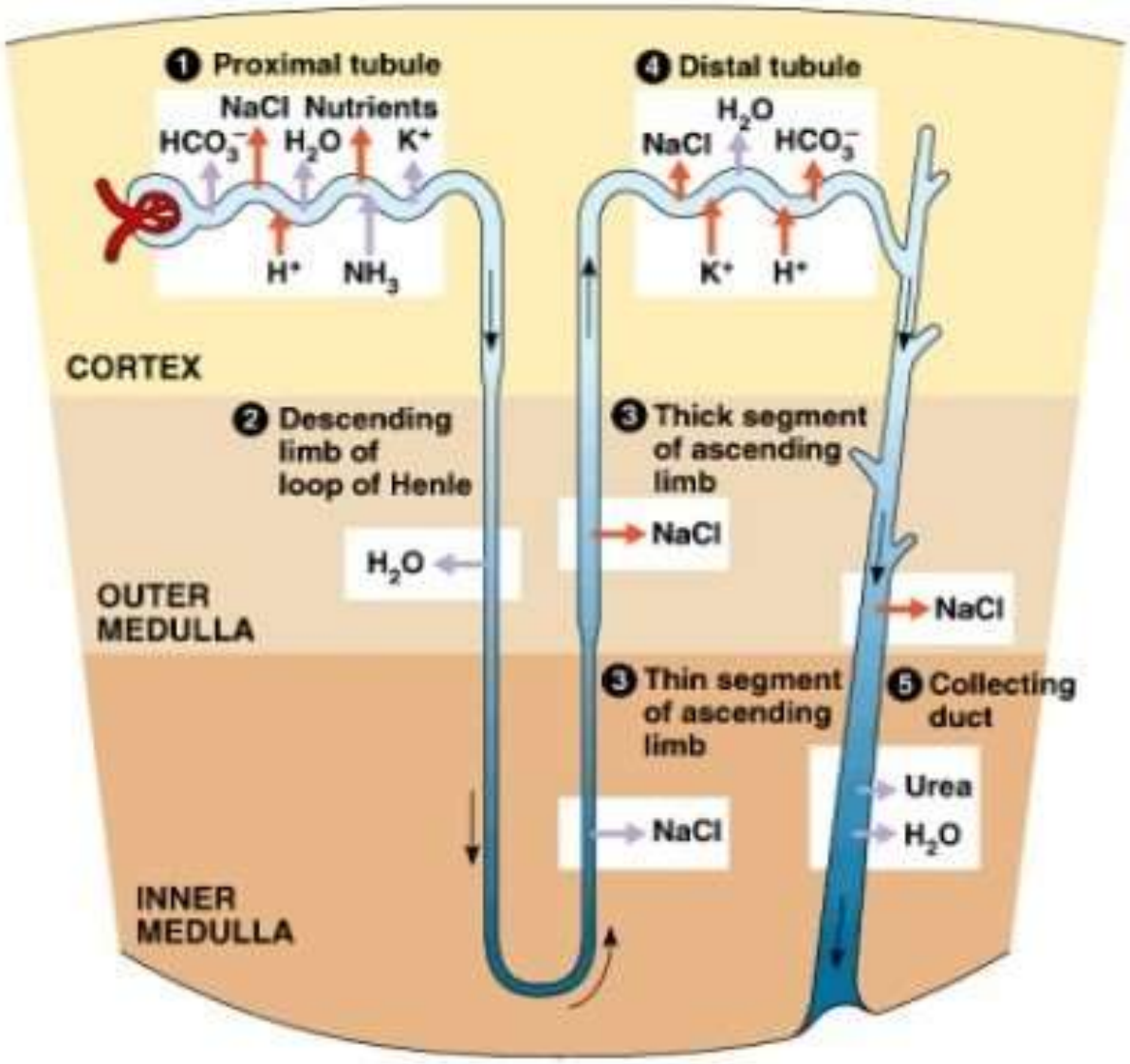
② **Tubular reabsorption**
Removes useful solutes from the filtrate, returns them to the blood

③ **Tubular secretion**
Removes additional wastes from the blood, adds them to the filtrate

④ **Water conservation**
Removes water from the urine and returns it to blood, concentrates wastes



Nephron-Tubular System



1. Proximal convoluted tubule
2. Descending loop of Henle
3. Ascending loop of Henle
4. Distal convoluted tubule
5. Collecting duct

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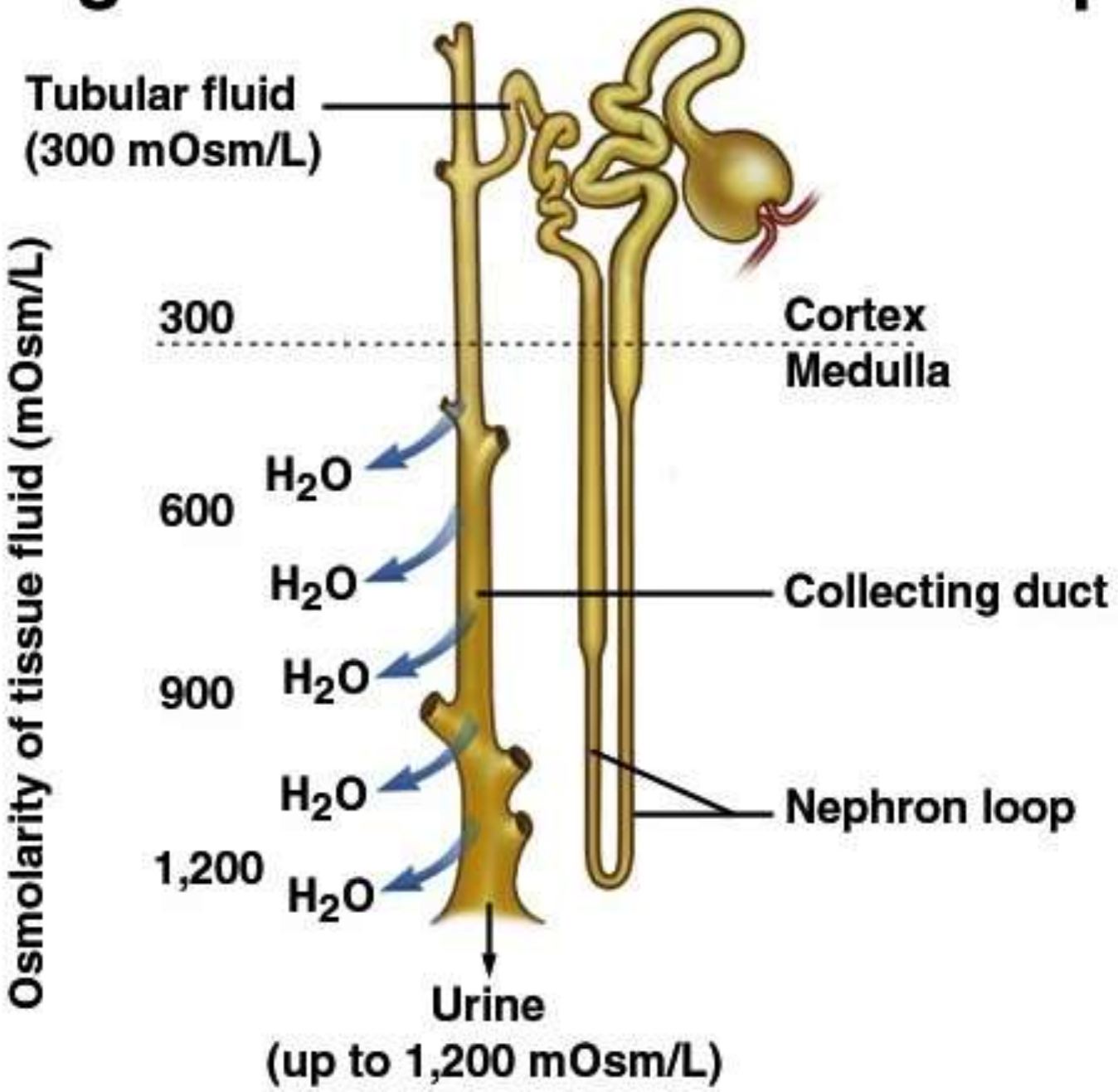
Collecting Duct and Water Reabsorption

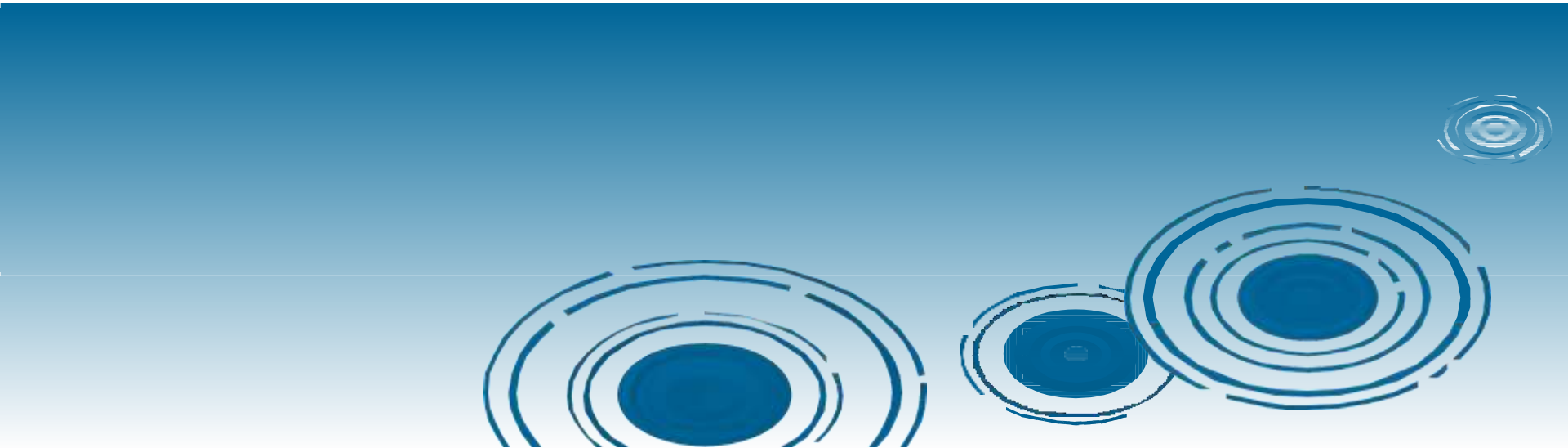


KIDNEY FUNCTIONS

- Regulate blood volume, pressure
- Regulate fluid osmolarity
- Secrete renin
- Secrete erythropoietin (EPO)
- Regulate P_{CO_2}, Acid-Base balance
- Synthesize calcitriol (Vitamin D)
- Detoxify free radicals, drugs
- Gluconeogenesis

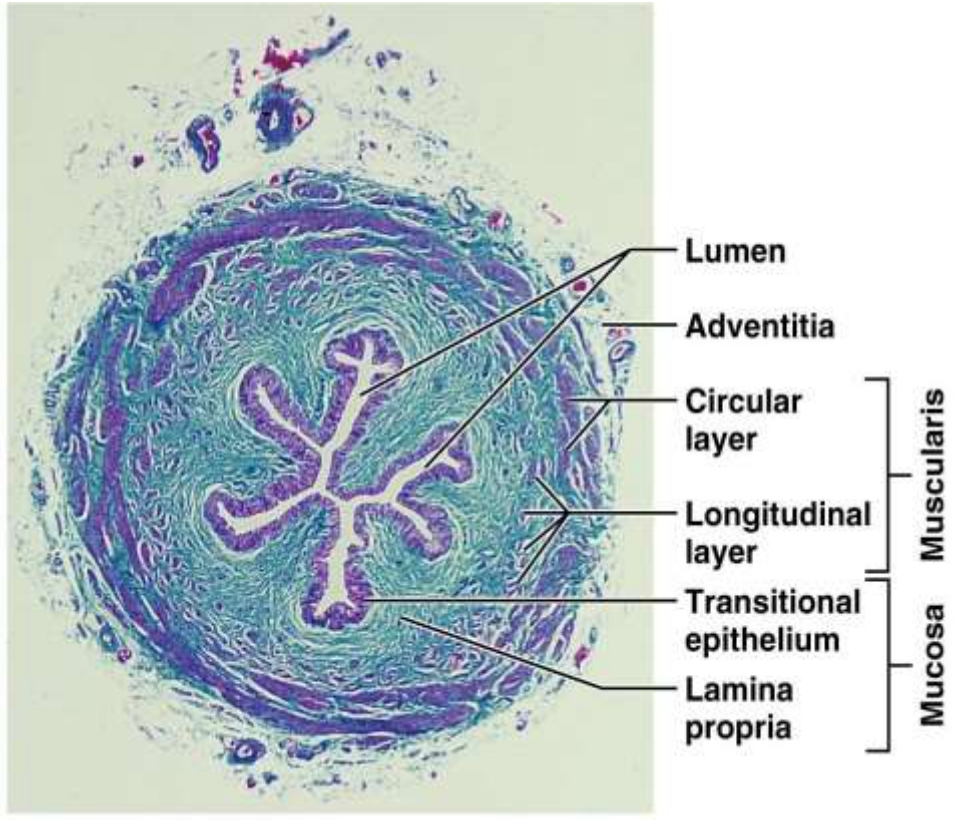
The Ureters

- Pair of muscular tubes
- Extend from renal pelvis to the bladder
- Oblique entry into bladder prevents backflow of urine

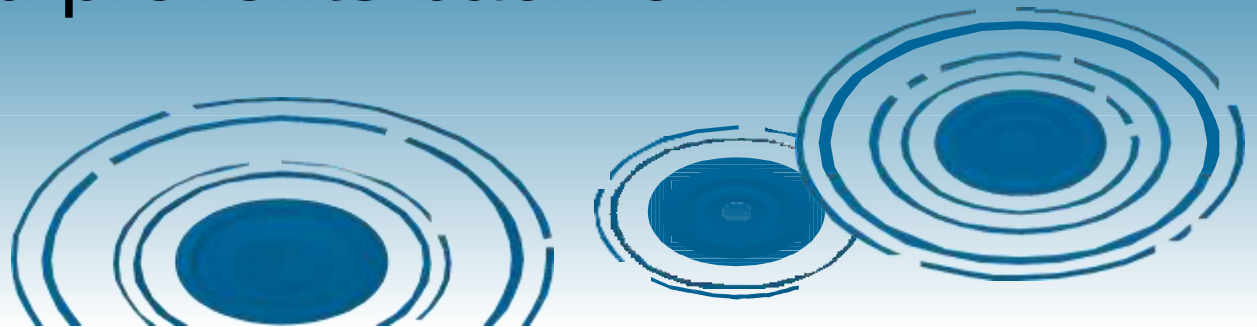


Histology of Ureter

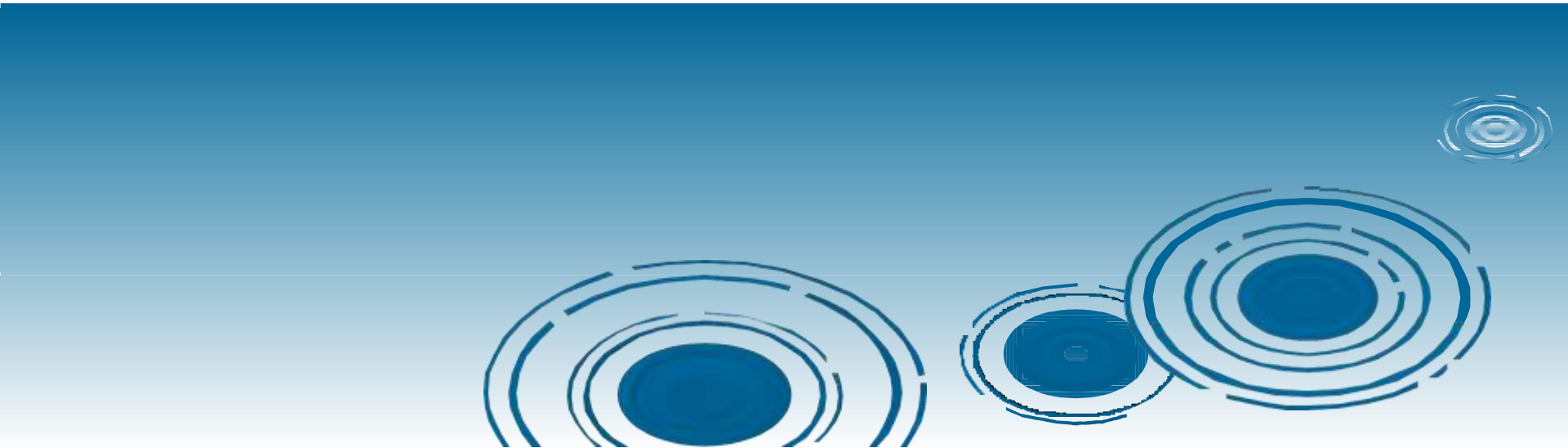
- Mucosa – **transitional epithelium**
- **Muscularis** – two layers
  - Inner longitudinal layer
  - Outer circular layer
- **Adventitia** – typical connective tissue



- Carry urine from kidneys to urinary bladder via peristalsis
  - Rhythmic contraction of smooth muscle
- Enter bladder from below
- Pressure from full bladder compresses ureters and prevents backflow



- Small diameter
- Easily obstructed or injured by kidney stones (renal calculi)



Urinary Bladder

- A collapsible muscular sac
- Stores and expels urine
  - Full bladder – spherical
    - Expands into the abdominal cavity
  - Empty bladder – lies entirely within the pelvis

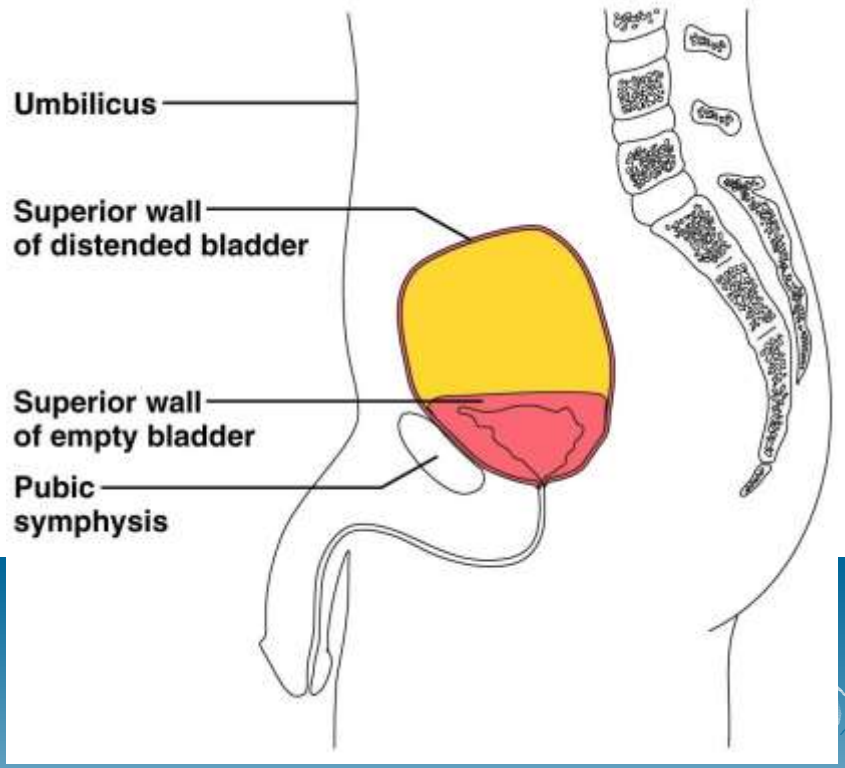
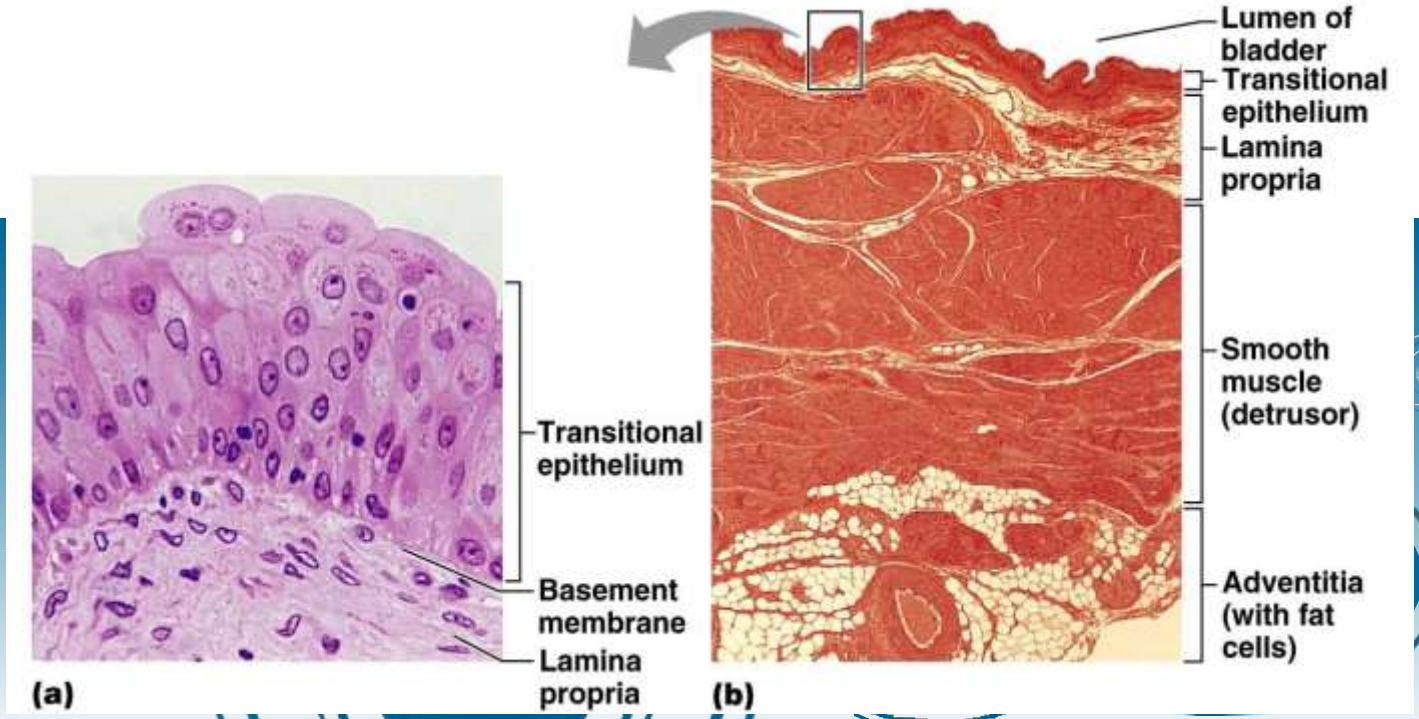


Figure 23.13

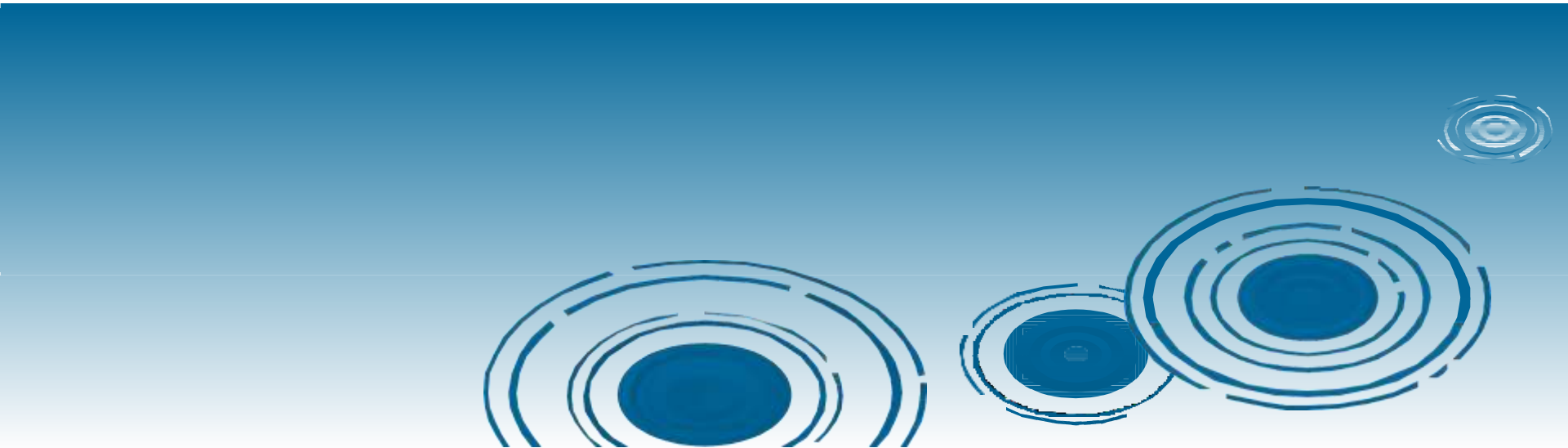
Urinary Bladder

- Wall of bladder
  - Mucosa - transitional epithelium
  - Muscular layer - detrusor muscle
  - Adventitia



Urinary bladder

- Wrinkles termed rugae
- Openings of ureters common site for bladder infection



Urethra

- Conveys urine from body
- Internal urethral sphincter
  - Retains urine in bladder
  - Smooth muscle, involuntary
- External urethral sphincter
  - Provides voluntary control over voiding of urine

Urethra in female

- 3 – 4 cm long in females
  - Bound by connective tissue to anterior wall of vagina
  - Urethral orifice exits body between vaginal orifice and clitoris

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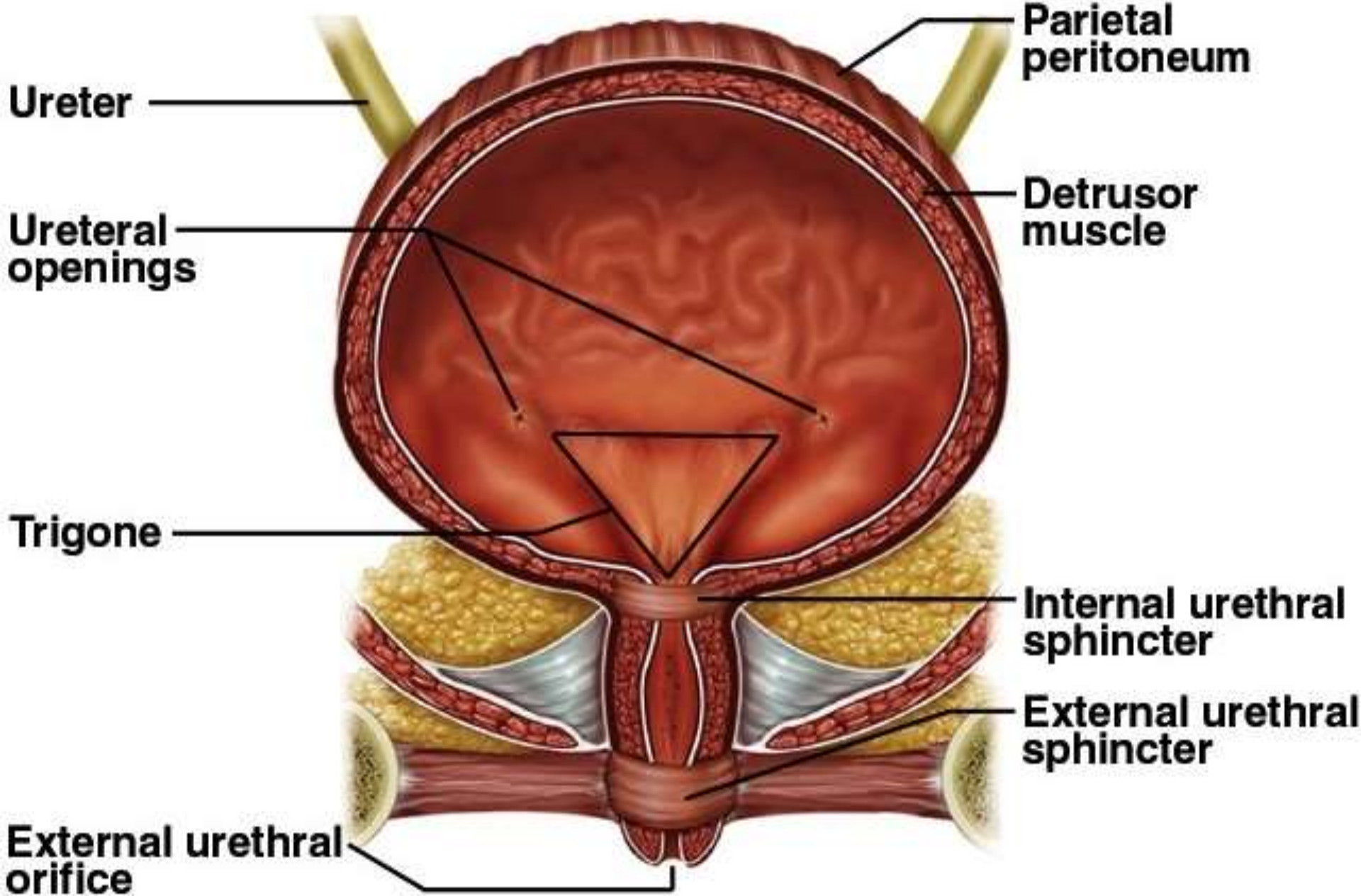
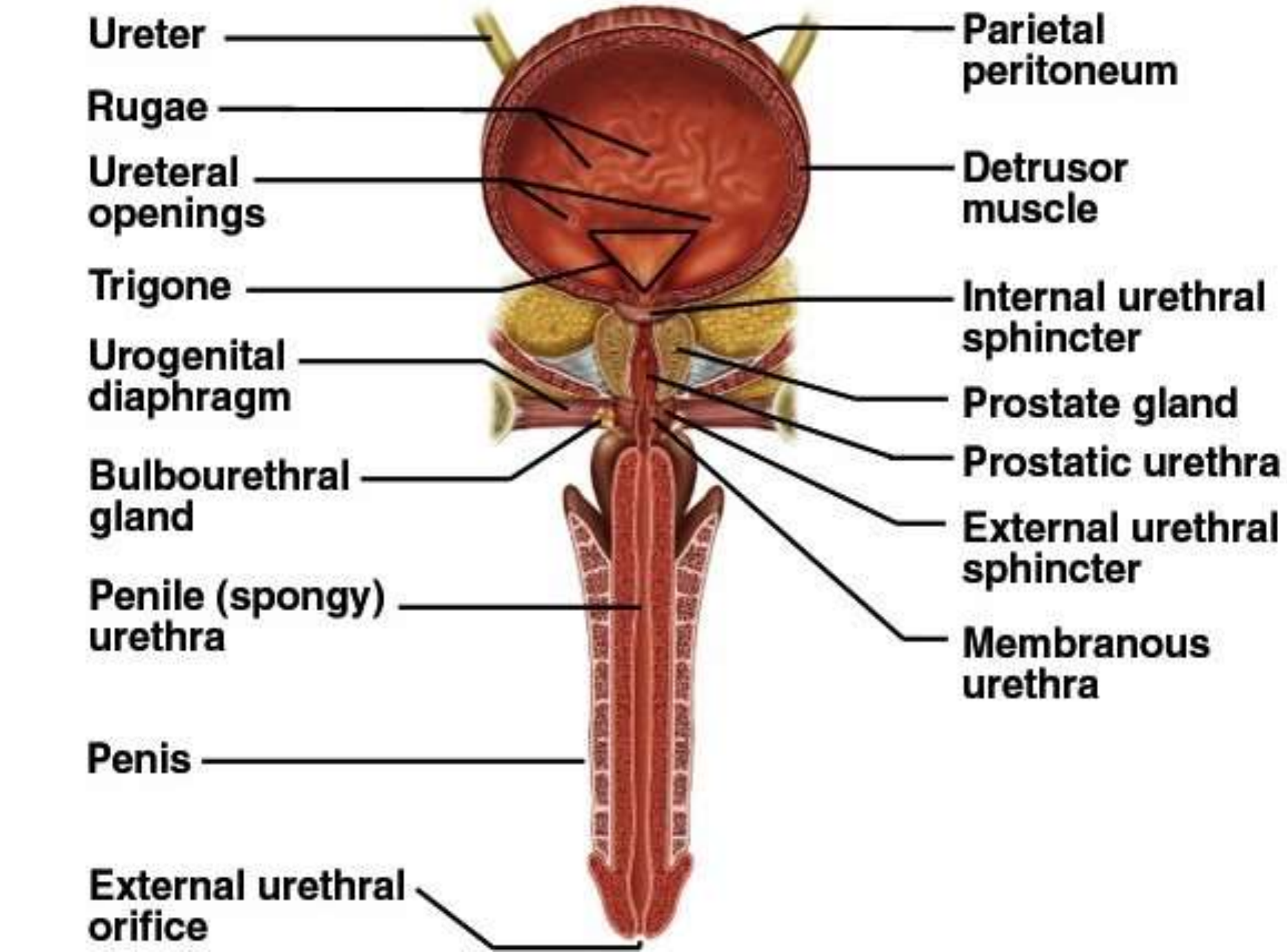
Urinary Bladder and Urethra, Female



Urethra in male

- ~18 cm long in males
  - Prostatic urethra
    - ~2.5 cm long, urinary bladder ▪ prostate
  - Membranous urethra
    - ~0.5 cm, passes through floor of pelvic cavity
  - Penile urethra
    - ~15 cm long, passes through penis

Urinary Bladder and Urethra, Male



URINE ELIMINATION

Urination (micturition)

- ~200 ml of urine held
- Distension initiates desire to void
- Internal sphincter relaxes involuntarily
  - Smooth muscle
- External sphincter voluntarily relaxes
  - Skeletal muscle
  - Poor control in infants
- Bladder muscle contracts
- Urine forces through urethra

Urinary System

- Kidneys – produce urine
- Ureters –transport urine to bladder
- Urinary bladder - stores urine
- Urethra transports urine to exterior

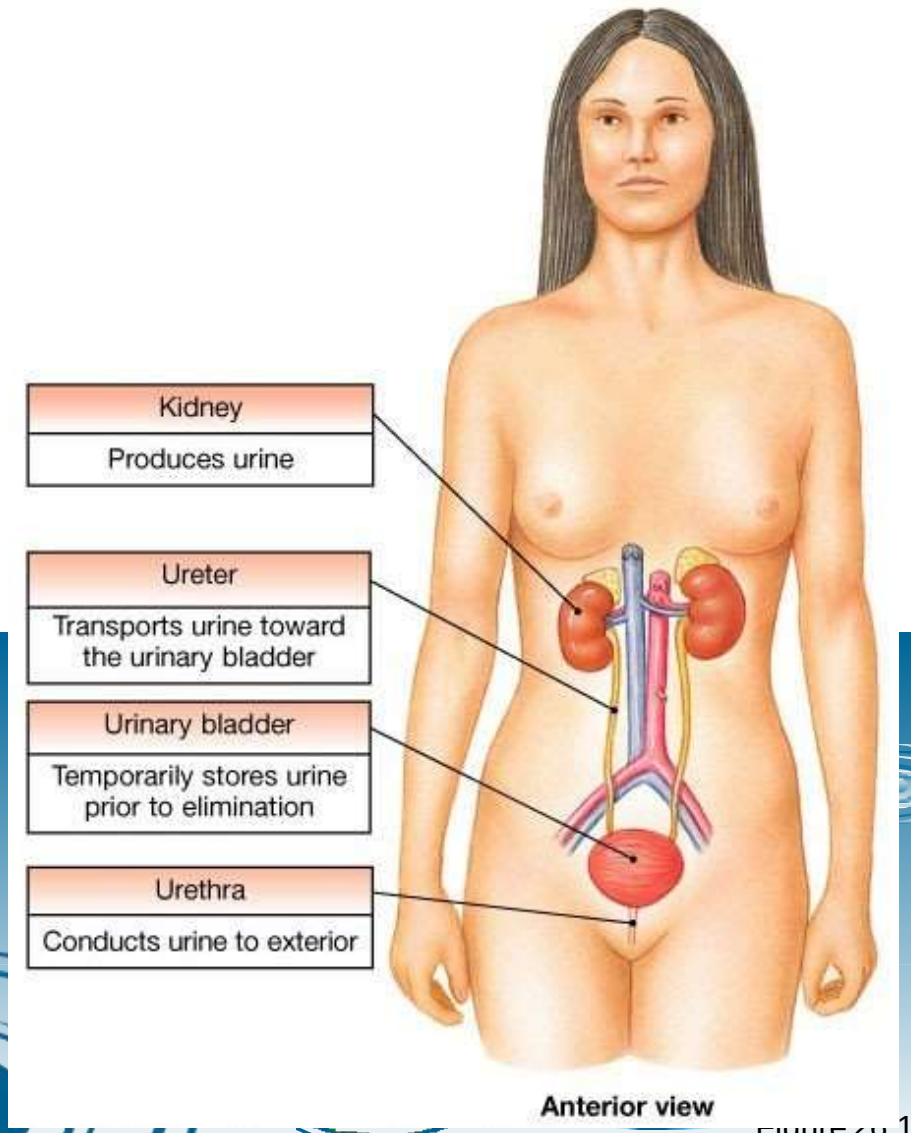
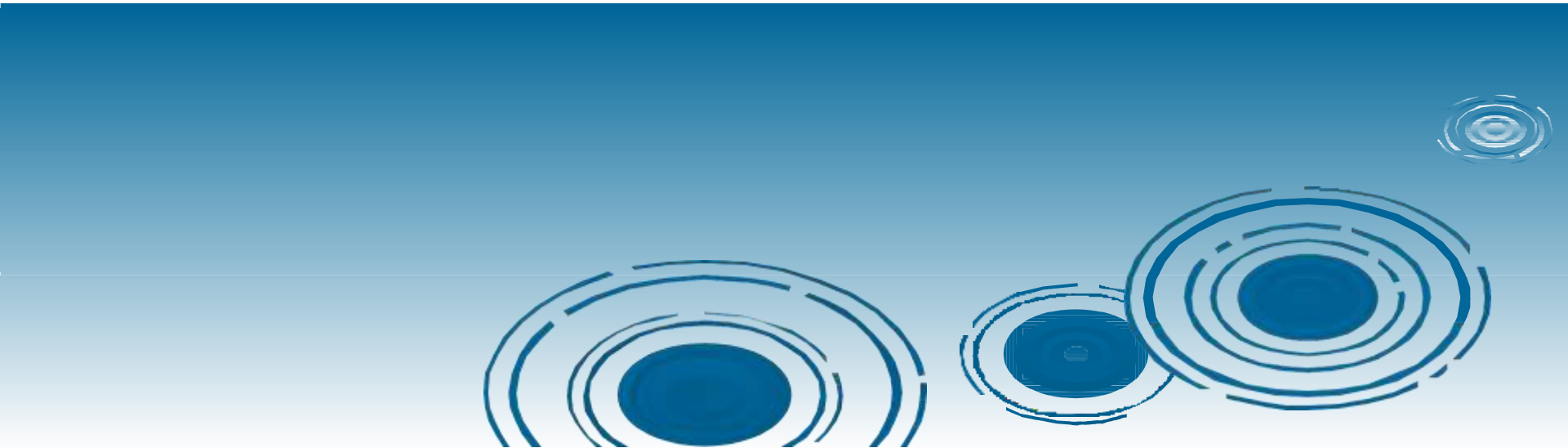


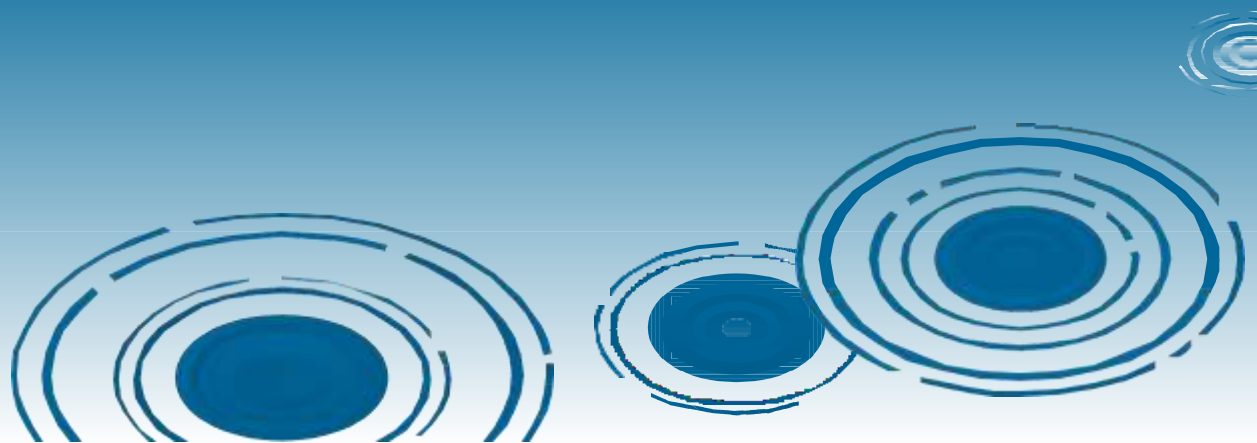
Figure 26.1

Functions of the urinary system

- Homeostatic regulation of blood plasma
  - Regulating blood volume and pressure
  - Regulating plasma ion concentrations
  - Stabilizing blood pH
  - Conserving nutrients



- Filter many liters of fluid from blood
- Excretion - The removal of organic waste products from body fluids
  - Urea
  - Uric acid
  - Creatinine
- Elimination - The discharge of waste products into the environment



Pune District Education Association's
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UV-VISIBLE SPECTROSCOPY INSTRUMENTATION

Presented by: Dr Vijaya U. Barge

Vice Principal & Professor

LEARNING OBJECTIVES:

After completing this sub-unit the students should be able:

- To learn principle measurement of radiation.
- To study about electronic transitions after absorption of radiation.
- To know the factors affecting spectral shift.
- To learn about theory of spectroscopy and Beer-Lambert law and its deviations.
- To study instrumentation used in UV-Visible spectroscopy.To study components of UV-Visible spectrophotometer.
- To learn about applications of UV-Visible spectroscopy.

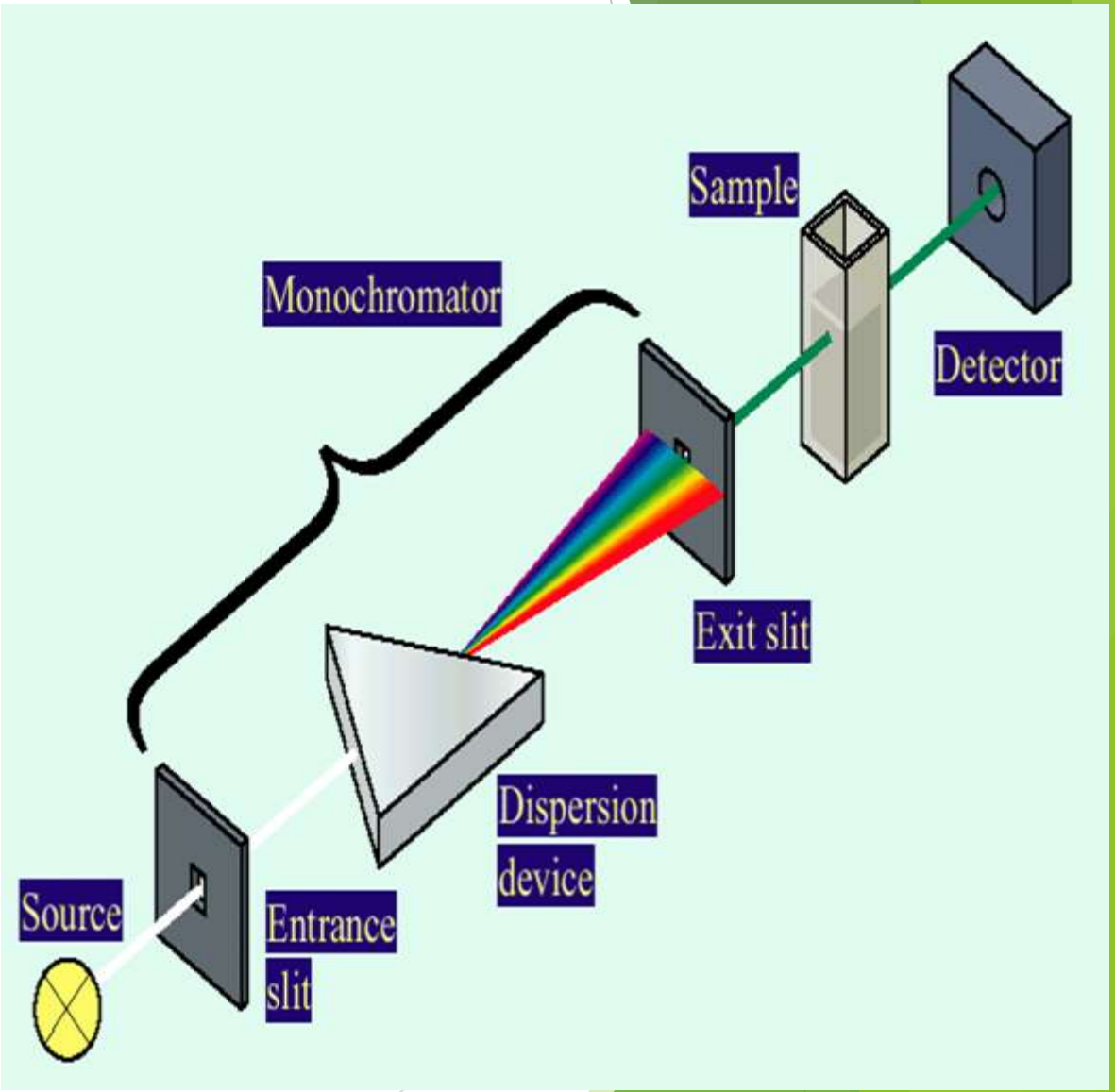
INTRODUCTION

- Spectroscopy is the branch of Science that deals with the study of interaction of electromagnetic radiation with matter.
- Spectrometer is an instrument design to measure the spectrum of a compound .
- Instrument used to measure the absorbance in UV (200 400nm) or Visible (400-800nm) region is called UV Visible Spectrophotometer.
- A Spectrophotometer records the degree of absorption by a sample at different wavelengths and the resulting plot of absorbance (A) versus wavelength (2) is known as a Spectrum.

INSTRUMENTATION

- COMPONENTS OF SPECTROPHOTOMETER

1) Source
2) Wavelength selectors or Dispersing devices
3) Sample compartment
4) Detector
5) Recorder



LIGHT SOURCE

Requirements:

- It should be stable.
- It should provide continuous radiation .
- It must be of the sufficient intensity for the transmitted energy to be detected at the end of the optical path.

TYPES OF LAMP :

1) HYDROGEN DISCHARGE LAMPS
2) DEUTERIUM LAMPS
3) TUNGSTEN LAMP
4) XENON DISCHARGE LAMP
5) MERCURY ARC LAMP

HYDROGEN DISCHARGE LAMP

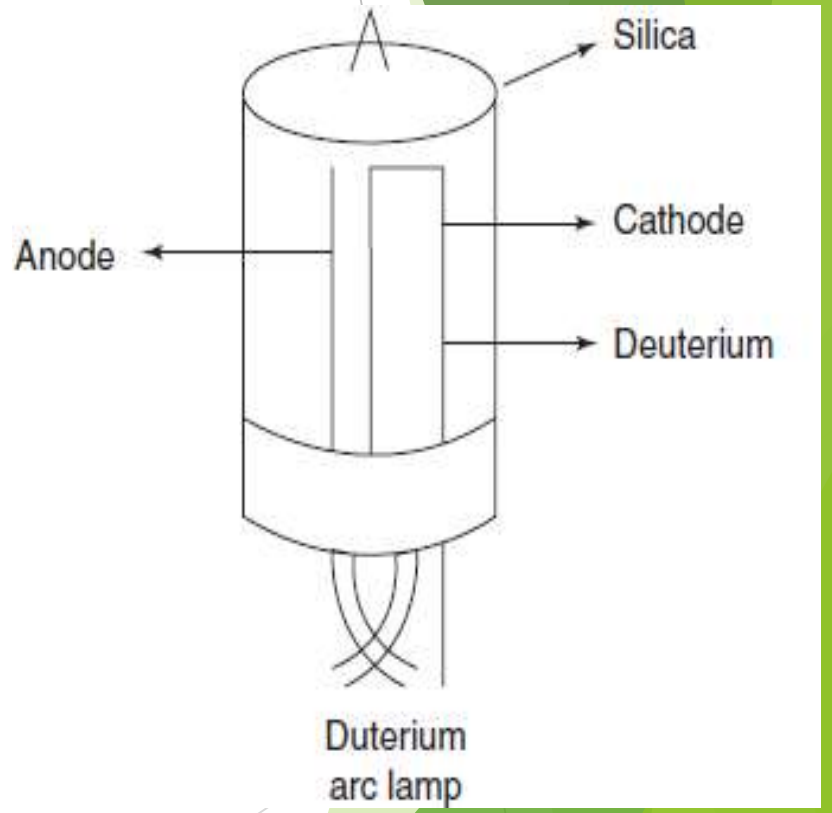
- In these lamps a pair of electrodes is enclosed in a glass tube filled with hydrogen gas under relatively high pressure.
- It is a continuous source.
- Covers a range 160-375nm.
- Stable, robust and widely used.

PROCESS

1) High voltage current is passed through the electrodes
2) Discharge of electrons occurs
3) Excitation of hydrogen molecules
4) Cause emission of UV radiation.

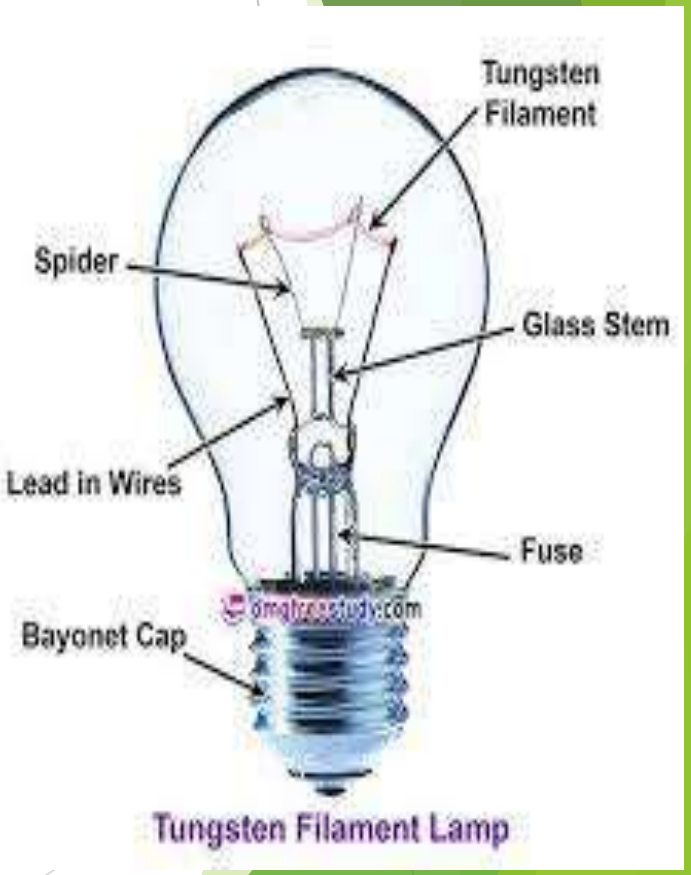
DEUTERIUM LAMPS

- Similar to hydrogen discharge lamp.
- Deuterium is filled in place of hydrogen.
- The intensity of radiation emitted is 3 to 5 times the intensity of a hydrogen lamp.
- More expensive than hydrogen lamp.
- Used when high intensity is required.



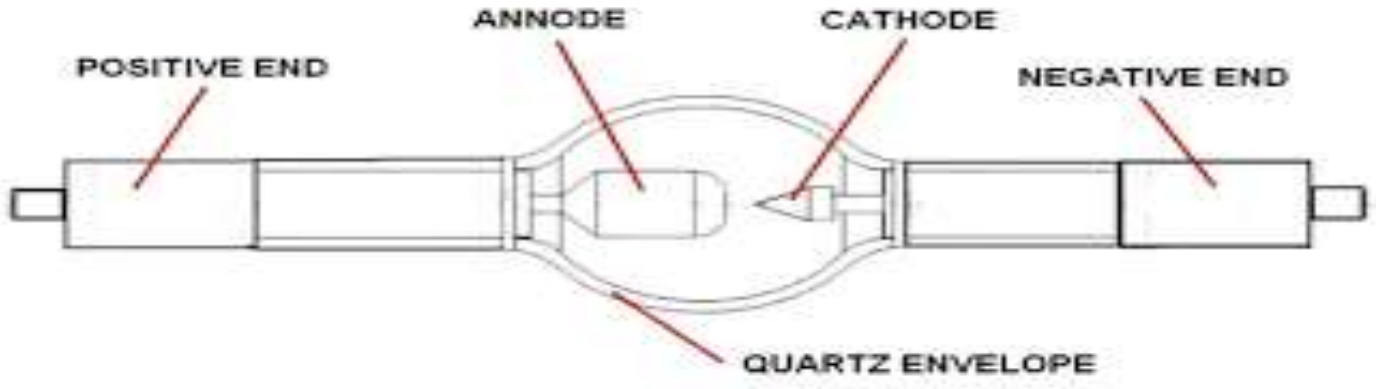
TUNGSTEN LAMP

- Similar in its functioning to an electric bulb.
- It provides a supply of radiation in the wavelength range of 320-2500nm.
- Continuous source of light.
- When tungsten filament is heated to incandescence by an electric current ,the light is produced.
- The glass bulb enclosing the filament contains a low pressure of inert gas, usually argon.
- Small amounts of halogen like iodine is added to improve the intensity (Tungsten-Iodine lamp).



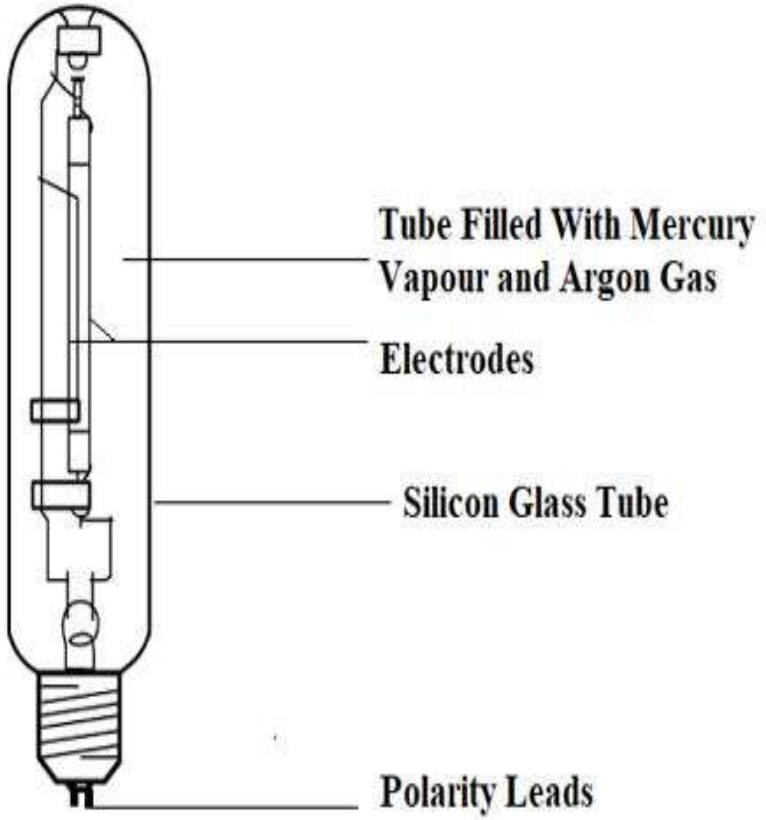
XENON DISCHARGE LAMP

- Xenon gas is stored in lamps at 10-30 atm pressure.
- It contain 2 tungsten electrodes that are separated by a distance of about 8mm.
- When current passes through xenon cause thermal excitation.
- It produces greater UV radiation than the hydrogen lamp.
- Continuous source.



MERCURY ARC LAMP

- In this mercury vapour is stored under high pressure and the excitation of mercury atoms is done by electric discharge.
- Mercury vapour at high pressure give intense lines on continuous background above 350nm.
- Low pressure mercury vapour gives an additional line at 254nm.



WAVELENGTH SELECTOR

- Converts polychromatic light to monochromatic light.
- Filters and Monochromators are used for this purposes.

FILTERS :

- It is frequently necessary to filter or remove wide bands of radiation from a signal.
- Filters isolate a wider band than the monochromators.

MONOCHROMATORS

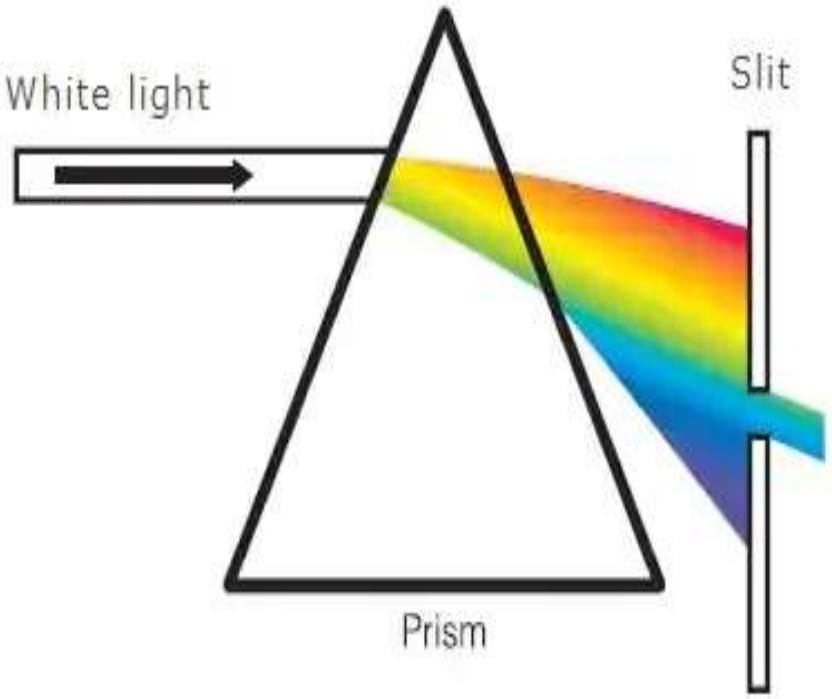
- It is used to disperse the heterochromic radiation into its component wavelength and to permit the isolation of desired portion of the spectrum .
- It consists of an entrance slit, an exit slit and a dispersing device either a prism or grating.
- Materials of construction should be selected withcare to suit the range in which it has to work.

For eg :

- Quartz for ultraviolet.
- Normal glass for visual range.
- Alkali halides for IR region.
- Gratings are cheaper than prism.

PRISM

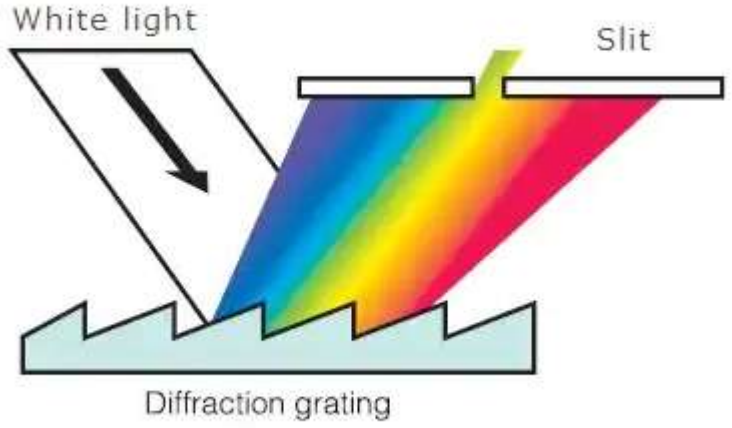
- Made up of glass , quartz or fused silica.
- Quartz or fused silica is the choice of material of UV spectrum.
- When white light is passed through the glass prism, dispersion of polychromatic light in rainbow occurs .
- Now by the rotation of the prism different wavelengths of the spectrum can be made to pass through in exit slit on the sample.
- The effective wavelength depends on the dispersive power of prism material and the optical angle of the prism.

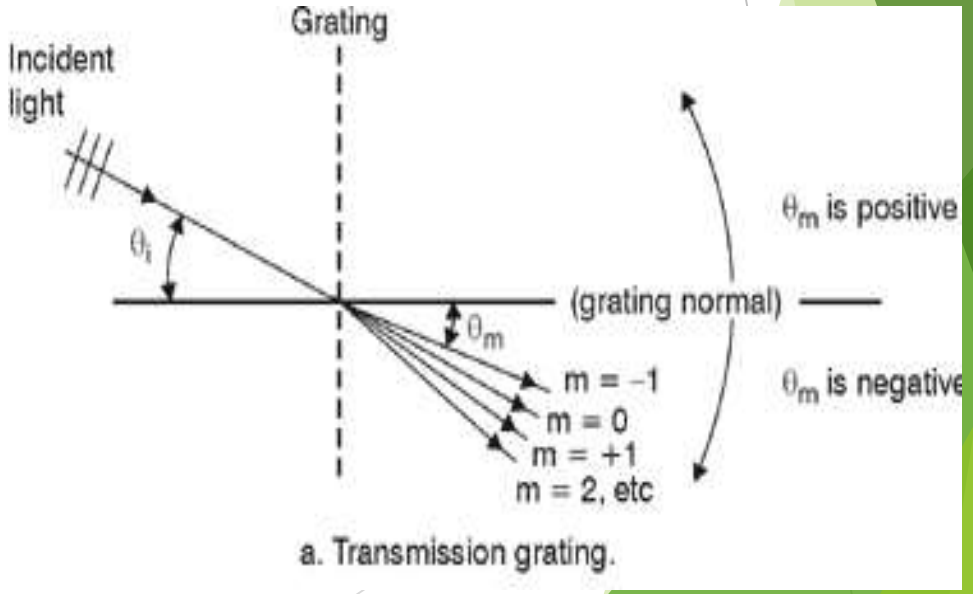


GRATING MONOCHROMATOR

- **Gratings are of 2 types**

1. Diffraction grating
2. Transmission grating





a. Transmission grating.

DIFFRACTION GRATING

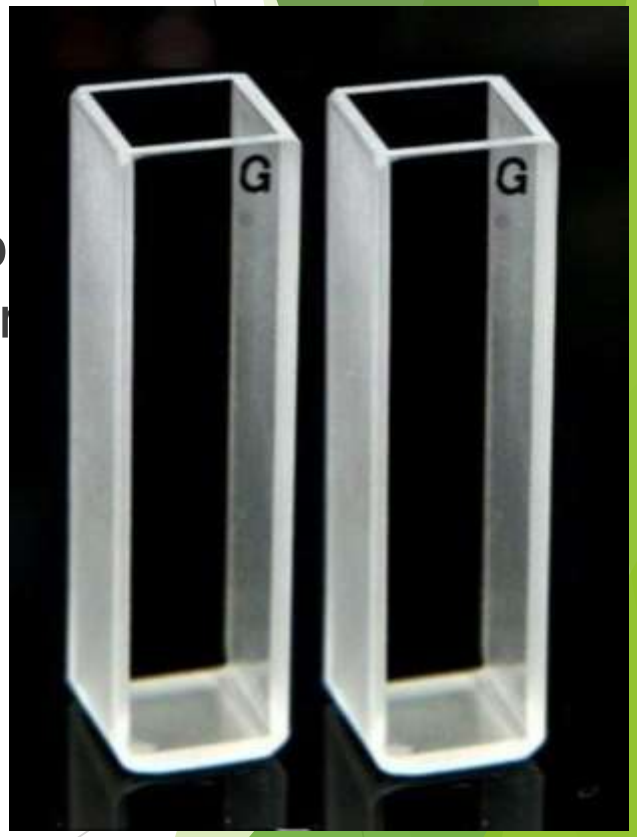
- More refined dispersion of light is obtained by means of diffraction gratings.
- These consists of large no.of parallel lines(grooves) about 15000-30000/inch is ruled on highly polished surface of aluminum.
- To make the surface reflective, a deposit of aluminum is made on the surface. In order to minimize to greater amounts of scattered radiation and appearance of unwanted radiation of other spectral orders, the gratings are blazed to concentrate the radiation into a single order.

TRANSMISSION GRATING

- It is similar to diffraction grating but refraction takes place instead of reflection.
- Refraction produces reinforcement.
- This occurs when radiation transmitted through grating reinforces with the partially refracted radiation.

SAMPLE HOLDER/ CUVETTES

- The cells or cuvettes are used for handling liquid samples.
- The cell may either be rectangular or cylindrical in nature.
- For study in UV region the cells are prepared from quartz o fused silica whereas colour corrected fused glass is used fo visible region.
- Cleaning is carried out washing with distilled water or with dilute alcohol, acetone.
- The surfaces of absorption cells must be kept scrupulously clean.
- No fingerprints or blotches should be present on cells.



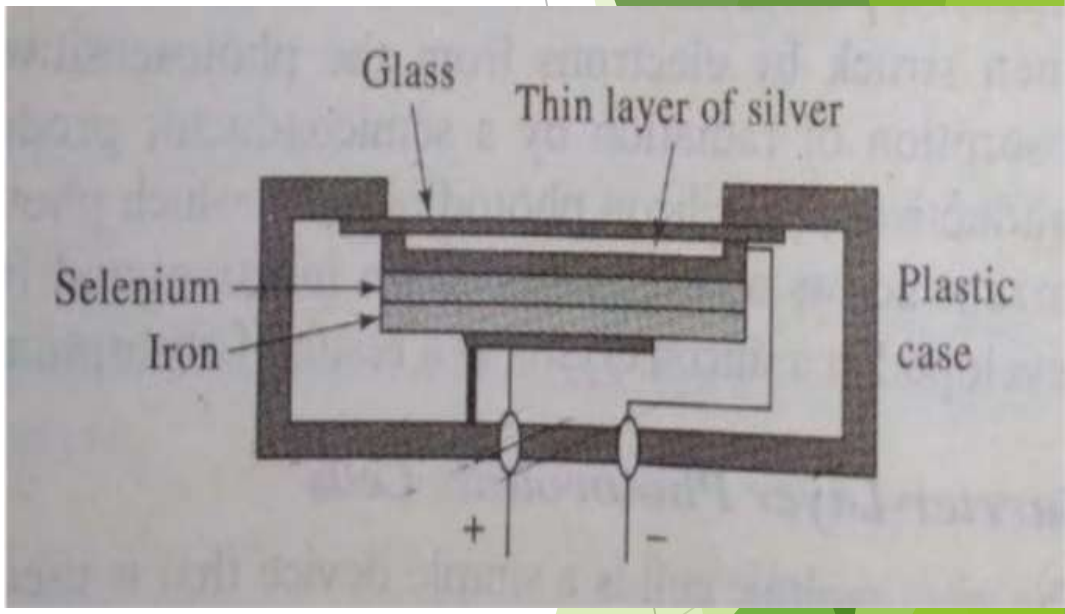
DETECTORS

- Device which converts light energy into electrical signals, that are displayed on readout devices.
- The transmitted radiation falls on the detector which determines the intensity of radiation absorbed by sample.
- The followed types of detectors are employed in instrumentation of absorption spectrophotometer.

1) Barrier layer cell/Photovoltaic cell.
2) Phototubes/Photo emissive tube.
3) Photomultiplier tube.

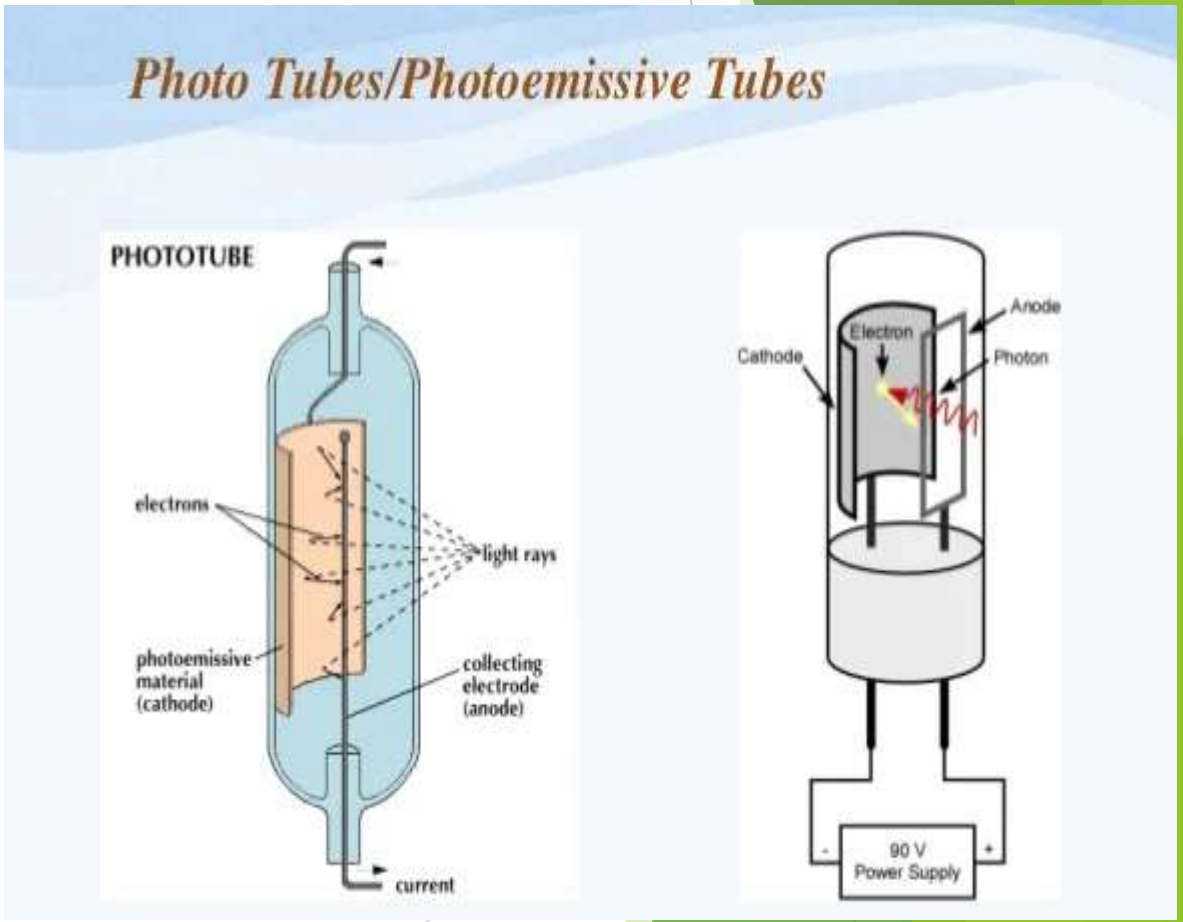
BARRIER LAYER CELL/ PHOTOVOLTAIC CELL

- The detector has a thin film metallic layer coated with silver or gold and act as another electrode.
- It also has a metal base plate which act as another electrode.
- These 2 layers are separated by a semiconductor layer of selenium.
- This creates a potential difference between two electrodes & causes the flow of current.
- When it is connected to galvanometer, a flow of current observed which is proportional to the intensity and wavelength of light falling on it.
- When light radiation falls on selenium layer, electrons become mobile and are taken up by transparent metal layer.



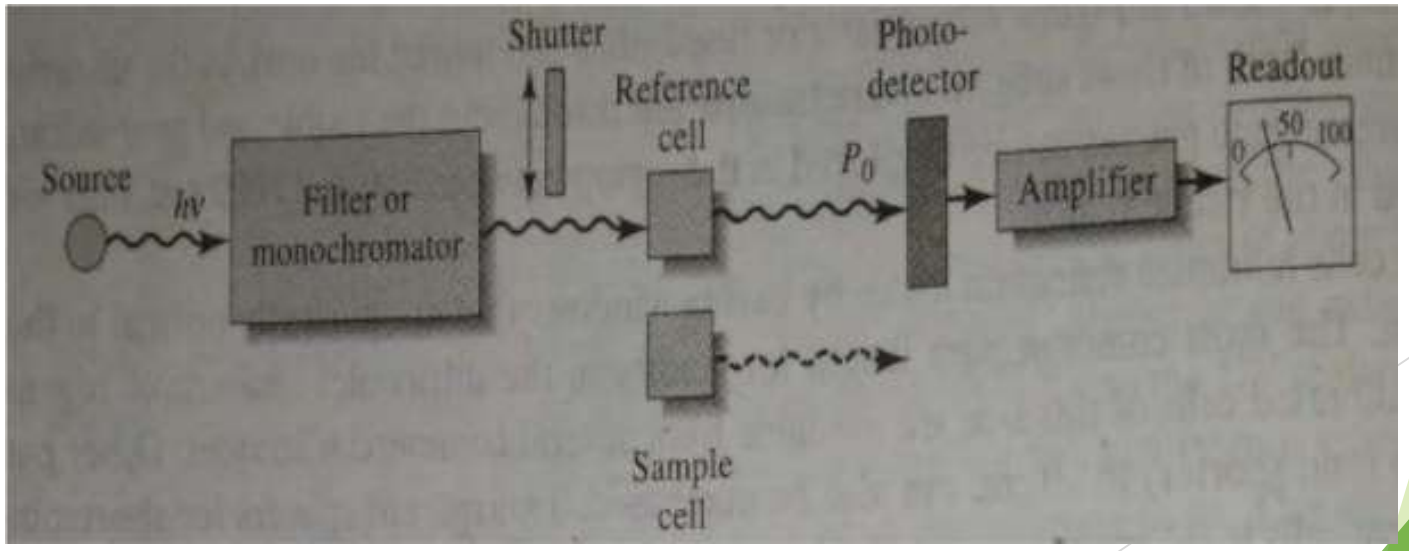
PHOTOTUBES/PHOTOEMISSIVE TUBES

- Consists of a evacuated glass tube with a photocathode and collector anode.
- The surface of photocathode is coated with a layer of elements like cesium, silver oxide or mixture of them.
- When radiant energy falls on photosensitive cathode, electrons are attracted to anode causing current to flow.
- More sensitive compared to barrier layer cell and therefore widely used.



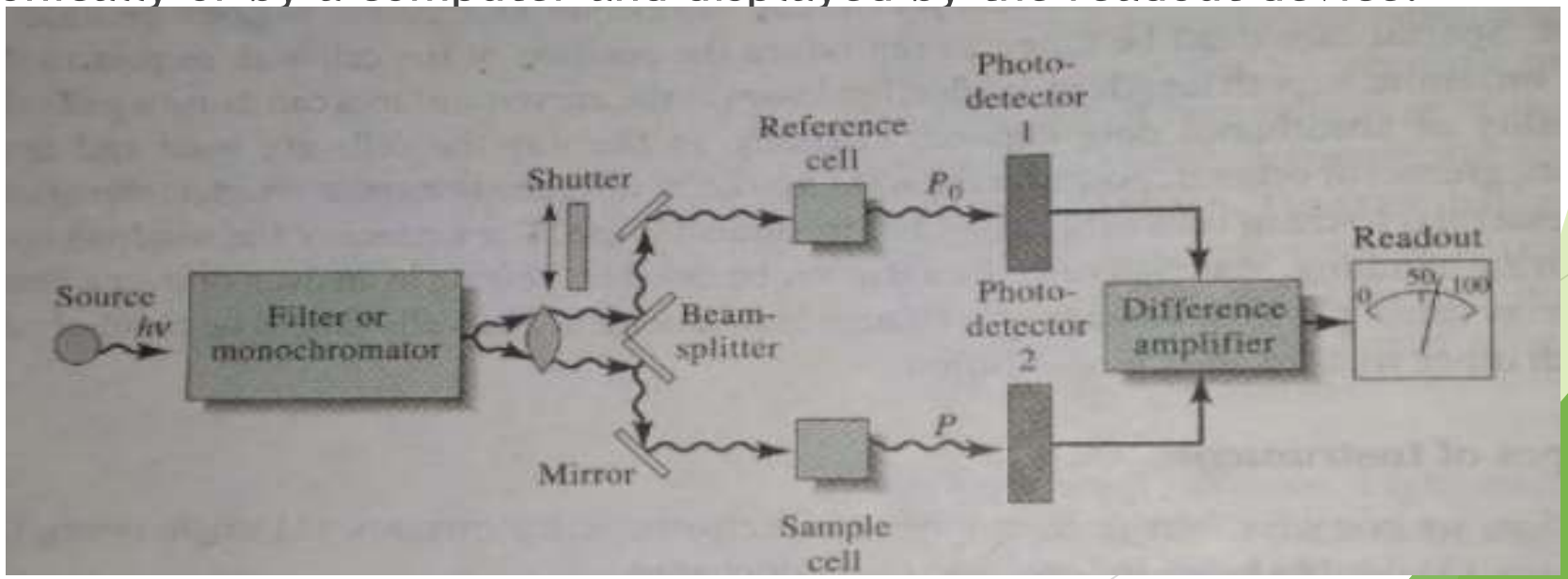
INSTRUMENT DESIGN
SINGLE-BEAM SPECTROPHOTOMETER

- A beam of radiation pass through a single cell, the reference cell is used to set the absorbance scale at zero for the wavelength to be studied.
- It is then replaced by sample cell to determine the absorbance of the sample at that wavelength.
- This was earliest design and is still use in both teaching and industrial labs.
- It requires a stabilized voltage supply to avoid errors resulting from changes in the beam intensity.



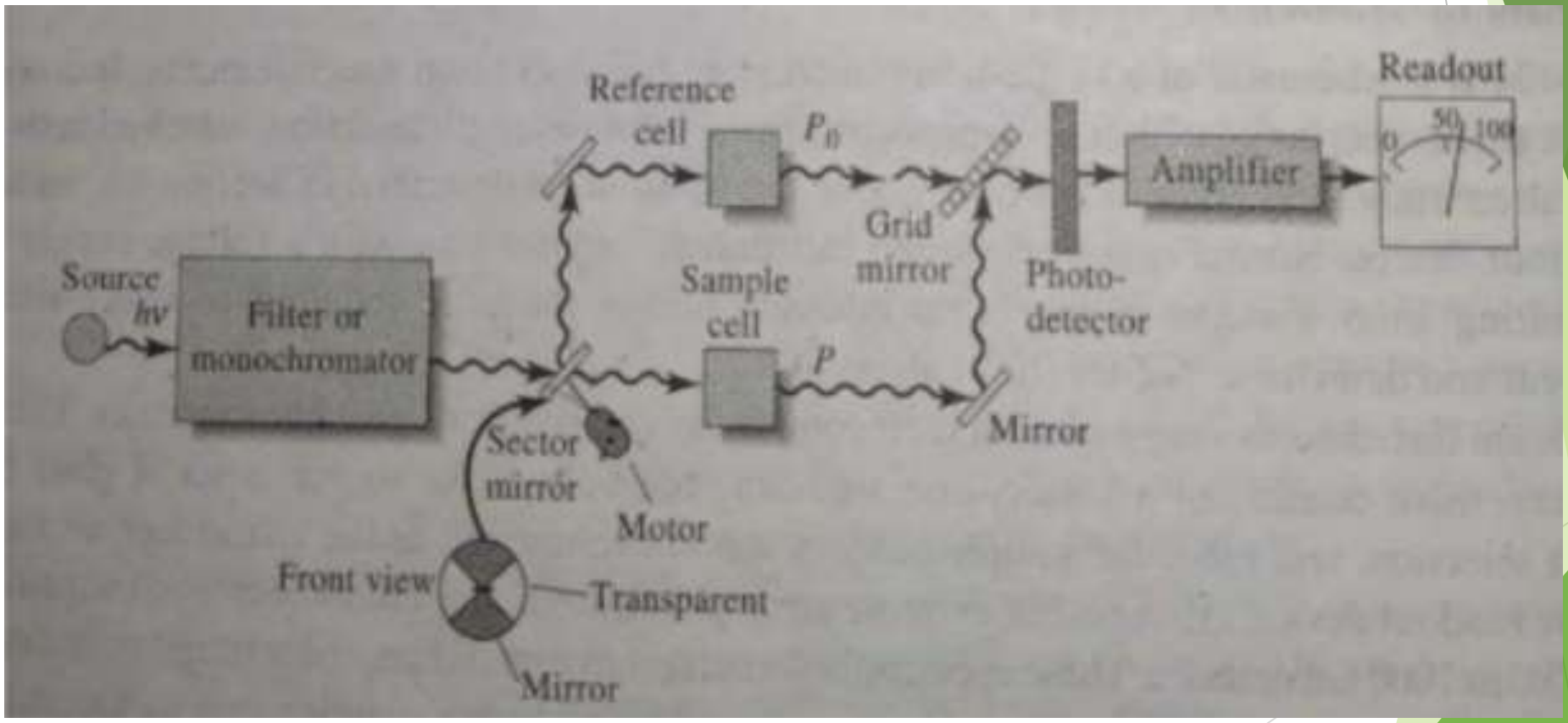
DOUBLE-BEAM SPECTROPHOTOMETER
DOUBLE-BEAM-IN-SPACE INSTRUMENT

- Two beams are formed in space by a V-shaped mirror called beam splitter.
- One beam passes through the reference solution to a photodetector, and the 2nd simultaneously traverses the sample to a 2nd matched detector.
- The 2 outputs are amplified, and their ratio or logarithm of their ratio is determined electronically or by a computer and displayed by the readout device.



DOUBLE-BEAM-IN-TIME INSTRUMENT

- The beams are separated in time by a rotating sector mirror that directs the entire beam from the monochromator first through the reference cell and then through the sample cell.
- The pulses of radiation are recombined by another sector mirror, which transmits one pulse to other to the transducer.
- The motor-driven sector mirror is made up of pie shape segments, half of which are mirrored and half of which are transparent.
- The mirrored sections are held in place by blackened metal frames that periodically interrupt the beam and prevent its reaching the transducer.
- The double-beam-in-time approach is generally preferred because of the difficulty in matching the 2 detectors needed for the double-beam-in-space design.


Reference cell
P_0
Readout
0
50
100
Amplifier
Grid mirror
Photo-detector
Source
hv
Filter or monochromator
Sample cell
P
Mirror
Sector mirror
Motor
Front view
Transparent
Mirror

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Thank
You

History of Microbiology

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CONTENTS OF PRESENTATION

- **Defination of Virus**
- **Properties of viruses**
- **Morphology of Virus Partical**
  - Size
  - Nucleic Acid / Genome
  - Capsid and Envelope
  - **Symmetry of Capsid**
  - **Structure**
  - Bacteriophages
  - Animal Viruses
  - **Multiplication and Cultivation of Viruses**
  - **Applications of Viruses**

Defination of Virus

A virus is an infectious agent made up of nucleic acid (DNA or RNA) wrapped in a protein coat called a capsid.

Viruses have no nucleus, no organelles, no cytoplasm or cell membrane—Non-cellular

This is why it does NOT belong to any kingdom

Properties of Viruses

- Obligate intracellular parasites of bacteria, protozoa, fungi, algae, plants, and animals.
- Ultramicroscopic size, ranging from 20 nm up to 450 nm (diameter).
- Not cellular in nature; structure is very compact and economical.
- Do not independently fulfill the characteristics of life.
- Inactive macromolecules outside the host cell and active only inside host cells.
- Basic structure consists of protein shell (capsid) surrounding nucleic acid core.
- Nucleic acid can be either DNA or RNA but not both.
- Nucleic acid can be double-stranded DNA, single-stranded DNA, single-stranded RNA, or double-stranded RNA.
- Molecules on virus surface impart high specificity for attachment to host cell.
- Multiply by taking control of host cell's genetic material and regulating the synthesis and assembly of new viruses.
- Lack enzymes for most metabolic processes.
- Lack machinery for synthesizing proteins.

❖Morphology of Virus Particle

❖Size

- As a group, viruses represent the smallest infectious agents their size places them in the realm of the ultramicroscopic.
- This term means that most of them are so minute (0.2 m) that an electron microscope is necessary to detect them
- More than 2,000 bacterial viruses could fit into an average bacterial cell, and more than 50 million polioviruses could be accommodated by an average human cell.
- Animal viruses range in size from the small parvoviruses around 20 nm [0.02 m] in diameter to poxviruses that are as large as small bacteria up to 450 nm [0.4 m] in length
- Some cylindrical viruses are relatively long (800 nm [0.8 m] in length) but so narrow in diameter (15 nm [0.015 m])
- Viruses are also referred to I terms of their genome size.Two of the largest known viruses,Pandoravirus and Mimivirus have a genome size of 1-2.5 Mbp.[Megabase pair;Mbp=106 base pairs of DNA]

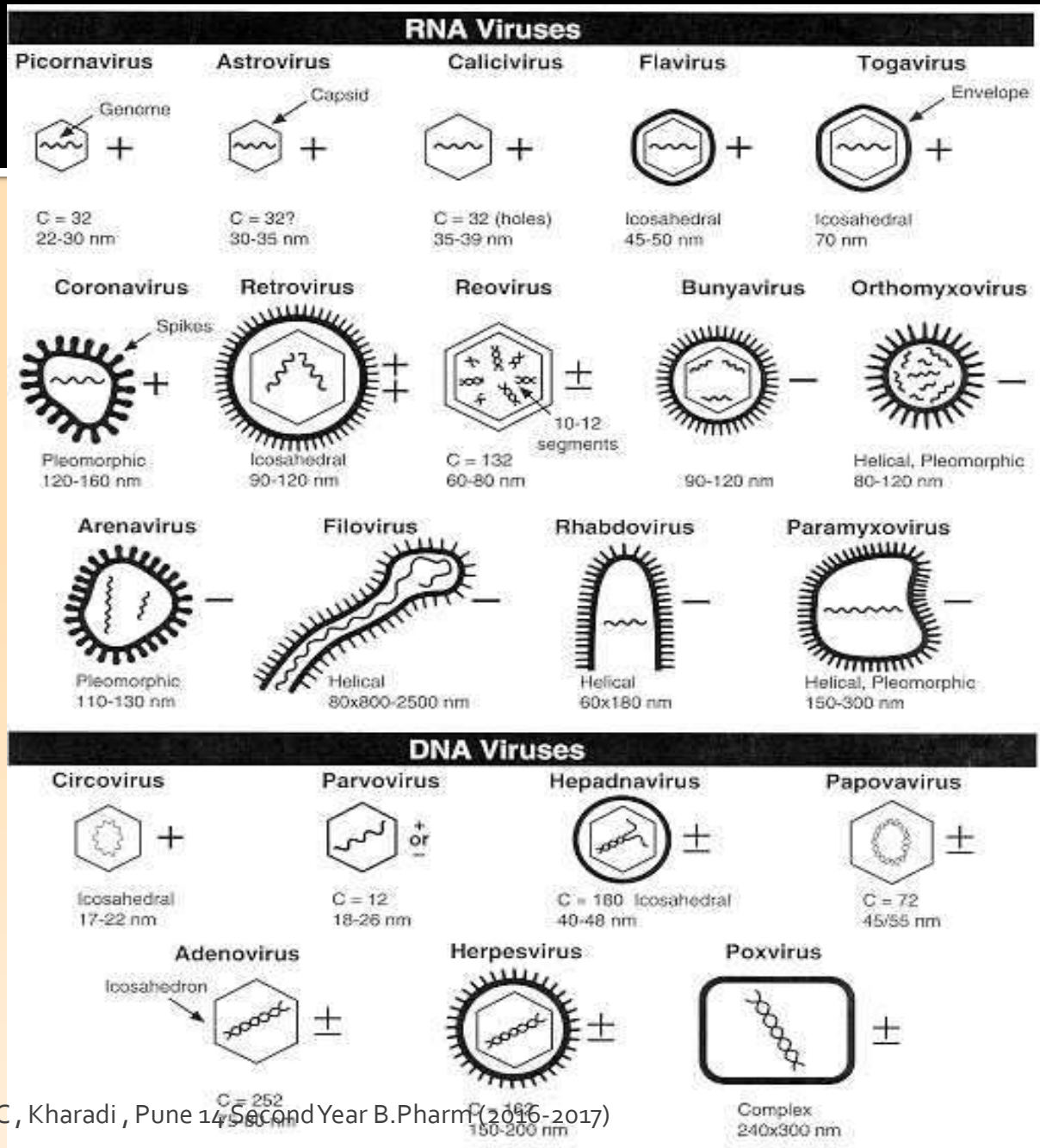
❖Nucleic Acid /Genome

- The sum total of the genetic information carried by an organism is known as its **genome [DNA or RNA]** It is either single-stranded [ss] or double stranded [ds]
- RNA can be present as the plus strand , referred to as the sense strand which is same as mRNA. Its complimentary form , the anti-sense RNA [minus RNA] that does not make a meaningful coding message , ,can also be the genome .
- In some viruses the nucleic acid is in several separate strands .

Schemes of 21 virus families infecting humans showing a number of distinctive criteria:

an envelope or (double-) capsid nucleic acid genome:
+, Sense strand;
-, antisense strand;
±, dsRNA or DNA;
0, circular DNA;

C, number of capsomers , where known;
nm, dimensions of capsid , or envelope when present;



❖The Capsid

- All viruses have a protein **capsid,*** nucleic acid in the central core.
- The assembly of nucleic acid within the capsid is called **nucleocapsid**

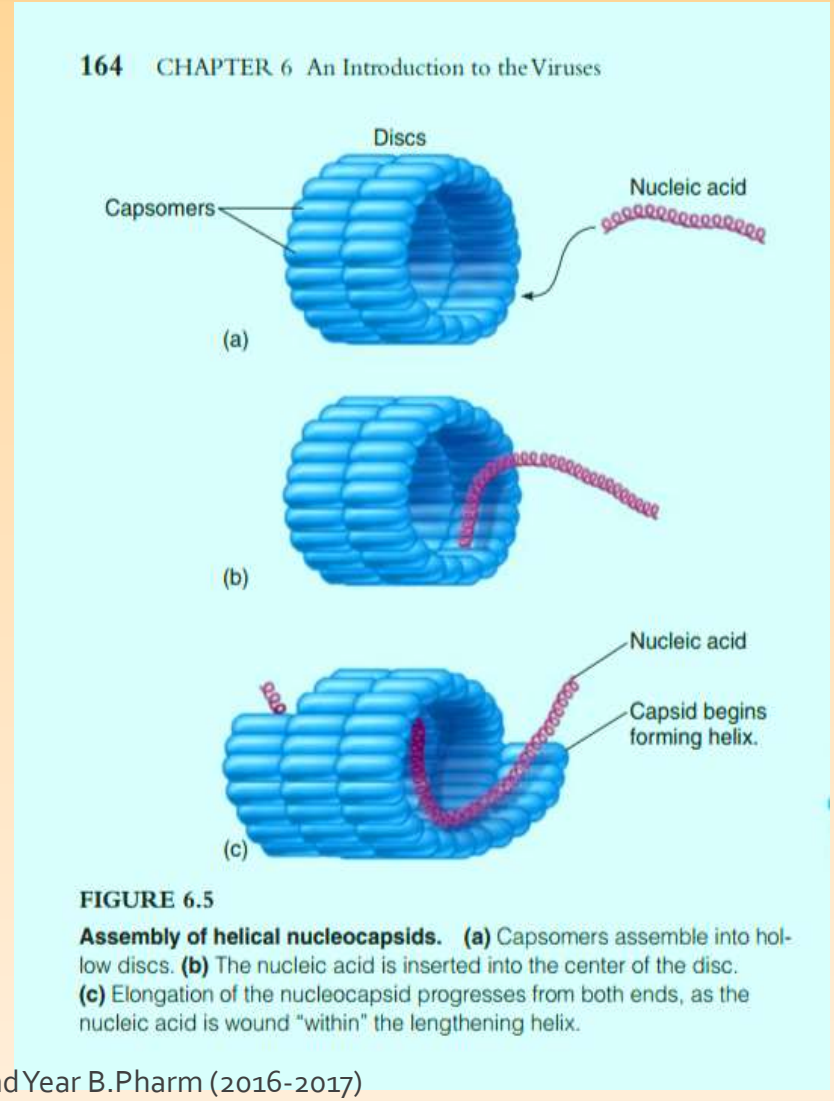
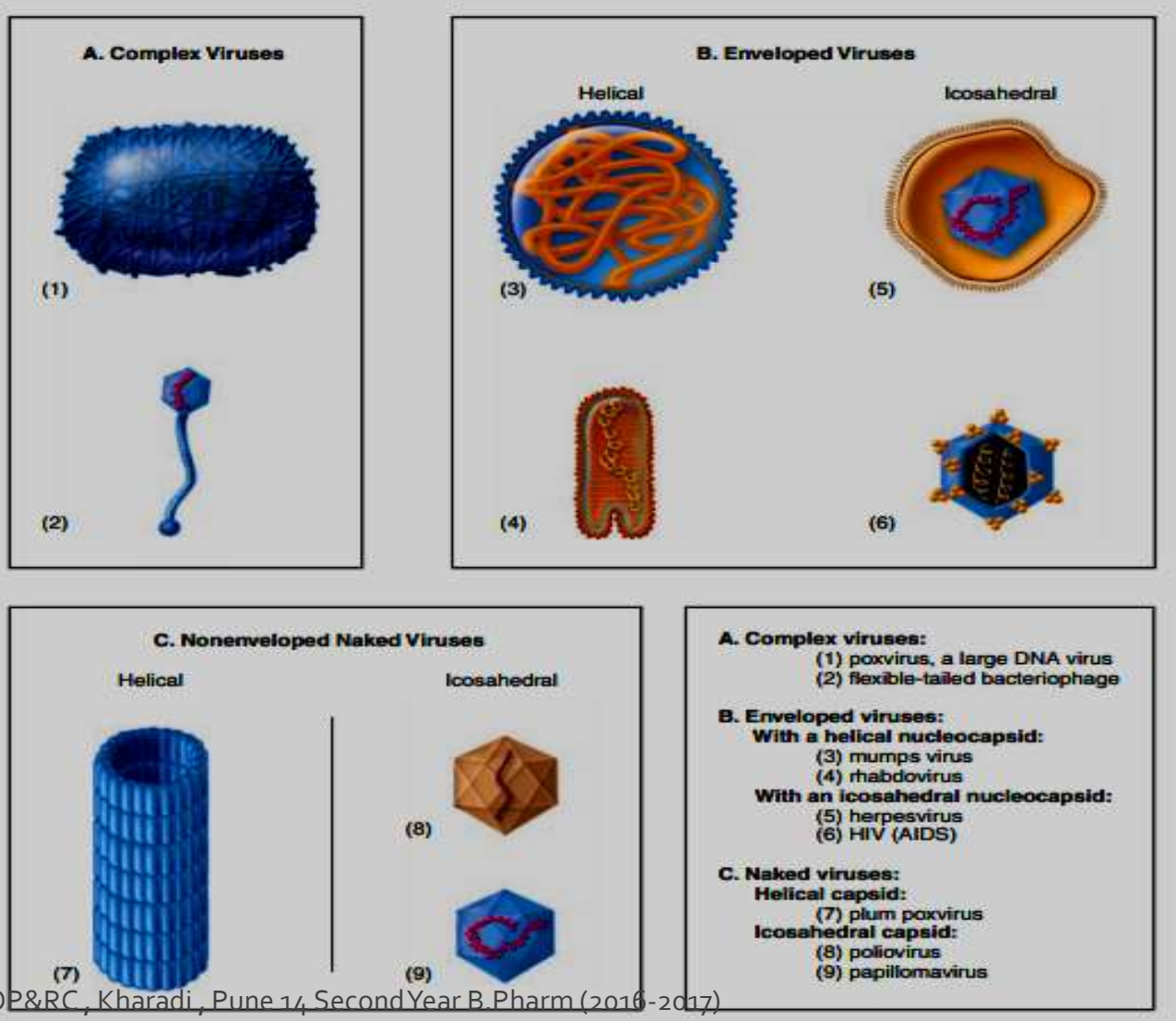


FIGURE 6.5

Assembly of helical nucleocapsids. **(a)** Capsomers assemble into hollow discs. **(b)** The nucleic acid is inserted into the center of the disc. **(c)** Elongation of the nucleocapsid progresses from both ends, as the nucleic acid is wound "within" the lengthening helix.

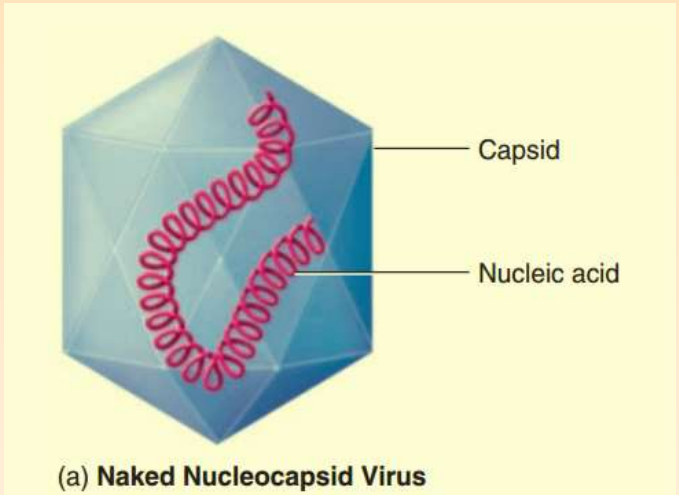
Symmetry of Capsid

- The different virus shapes are
  - cuboid
  - elongated
  - coiled
  - bullet shaped
  - filamentous

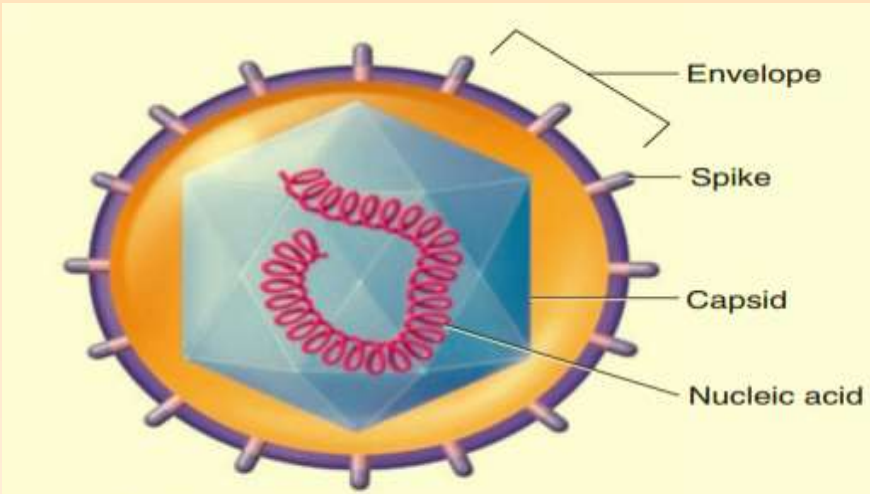


The Envelope

- Members of 13 of the 20 families of animal viruses possess an additional covering external to the capsid called an envelope, which is usually a modified piece of the host's cell membrane
- Envelop may or may not have spikes jutting from its surface
- *Viruses* that consist of only a nucleocapsid are considered naked viruses

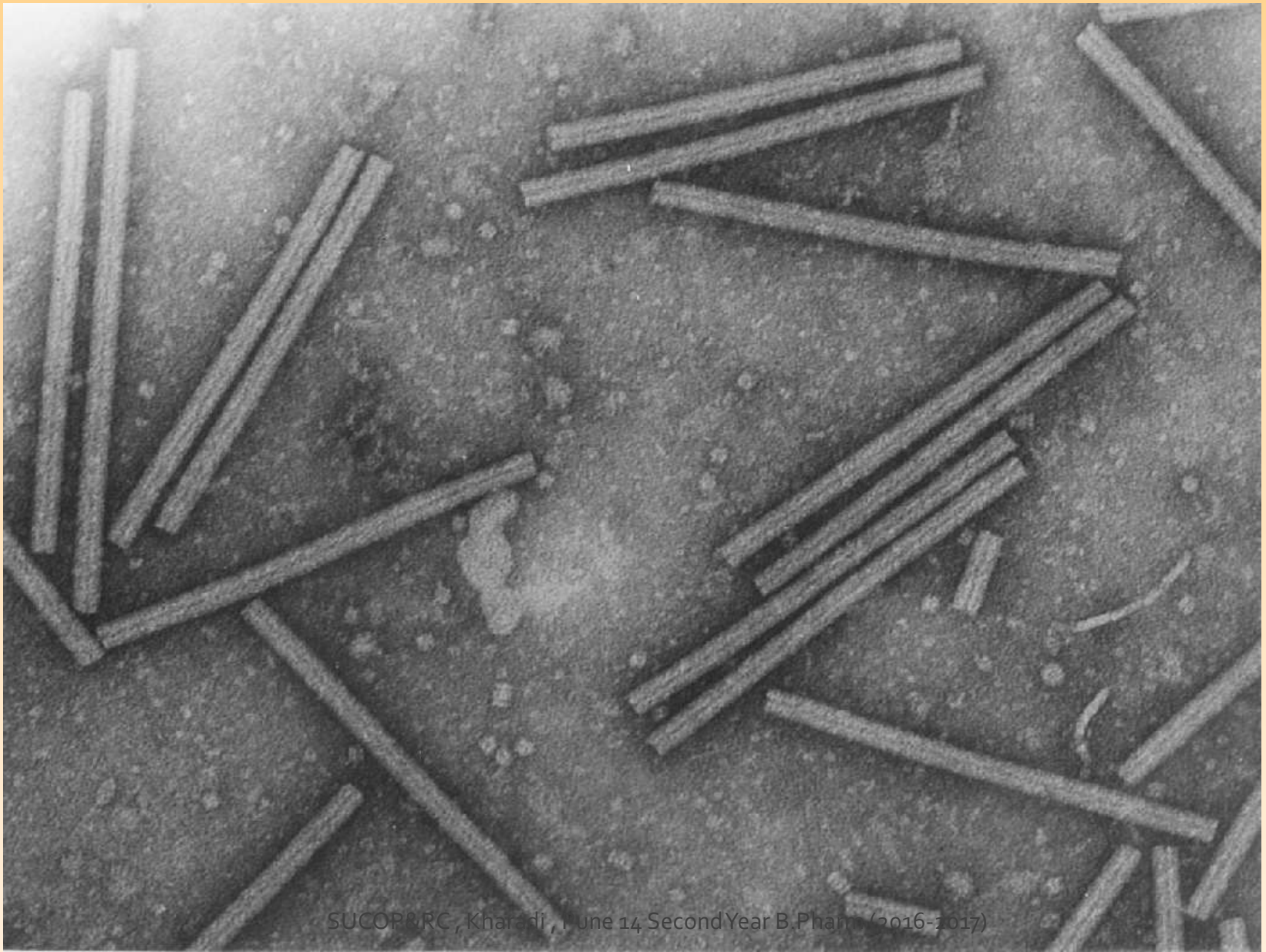


(a) **Naked Nucleocapsid Virus**

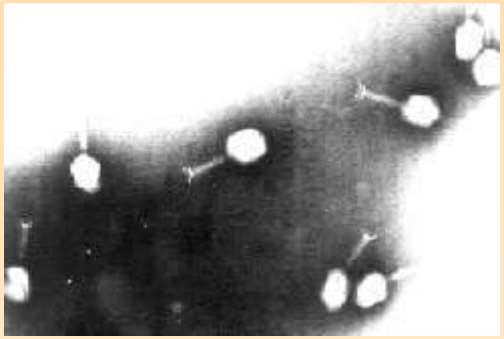


(b) **Enveloped Virus**

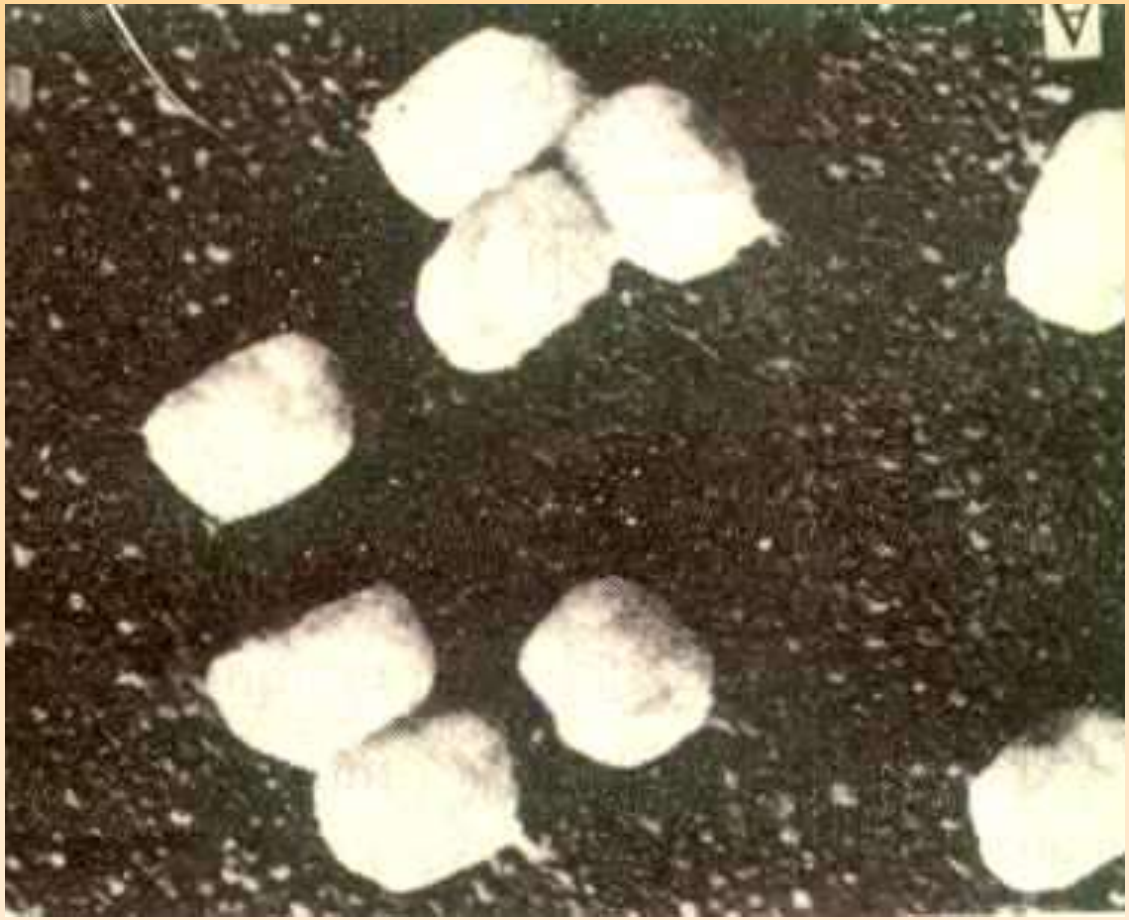
Shapes of Viruses :Rod-shaped



Tadpole-shaped

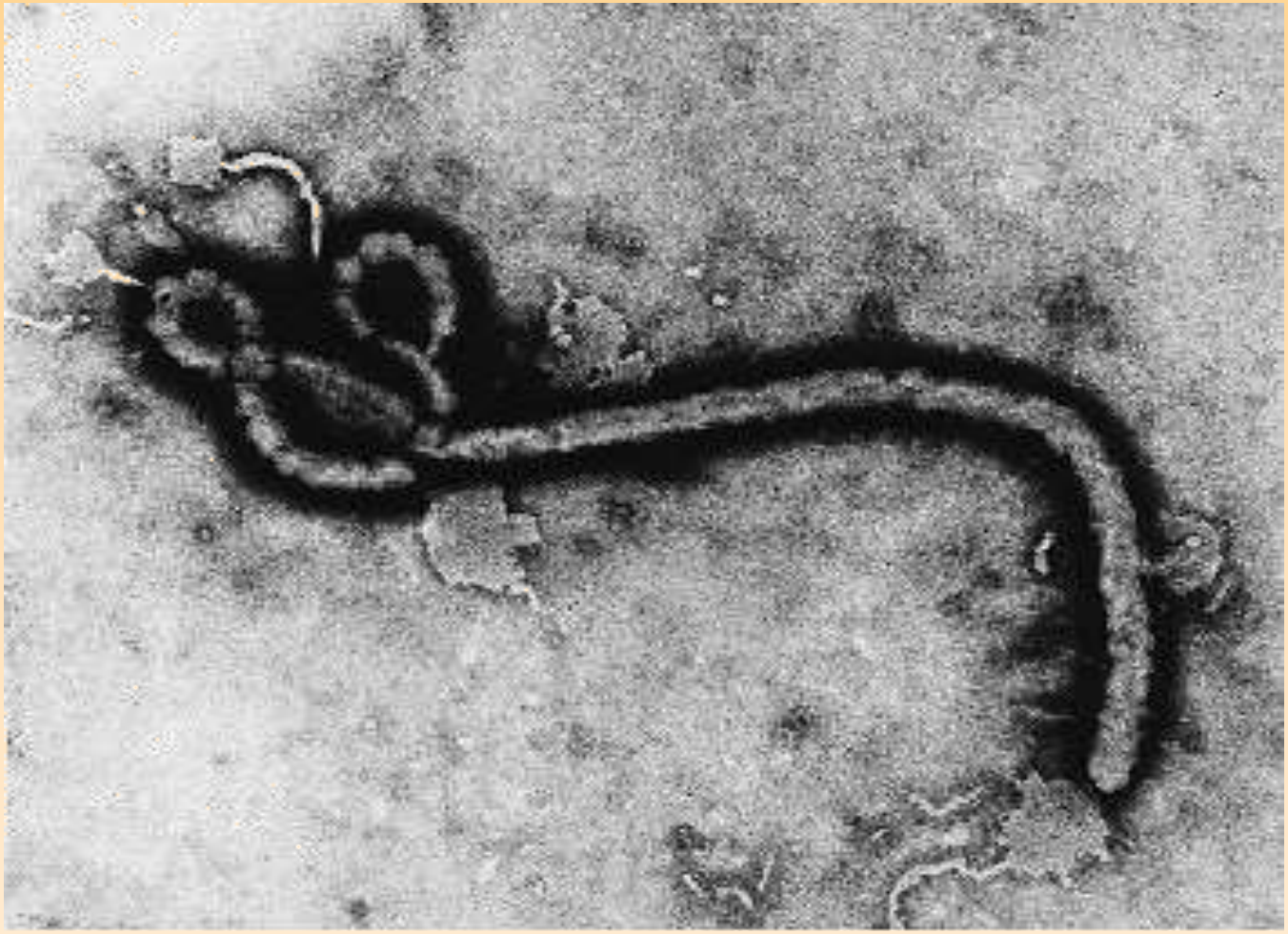


Shapes of Viruses :Brick-shaped



Shapes of Viruses: Spherical

Shapes of Viruses :Filament



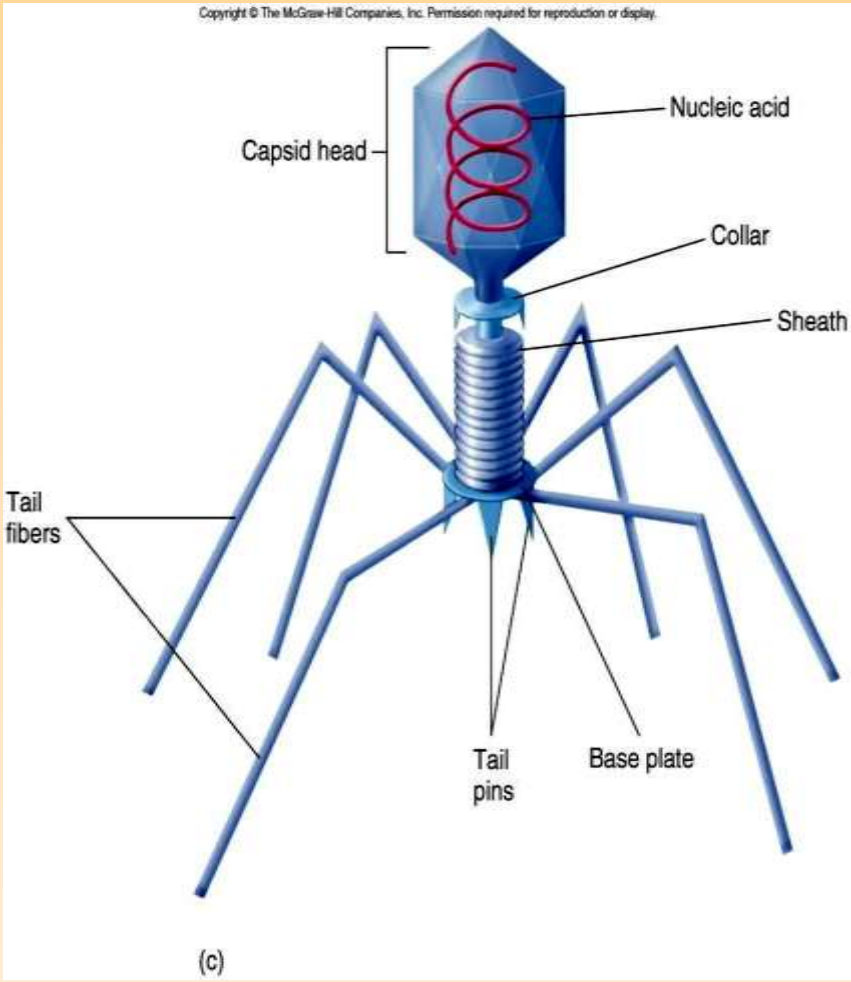
Shapes of Viruses :Bullet-shaped

❖Structure

❖ Structure of Bacteriophages

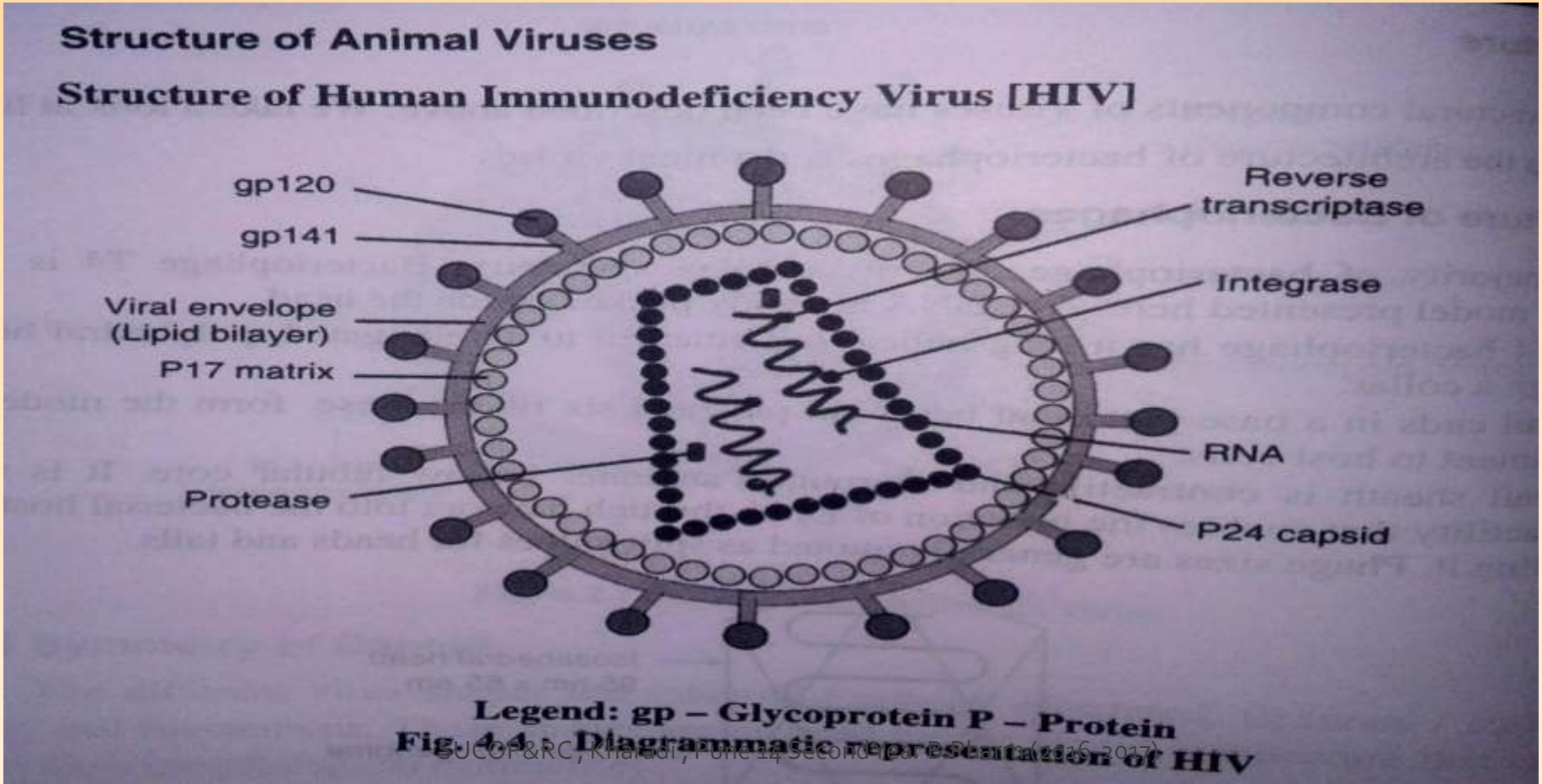
❖ [T4 Bacteriophage]

- A dsDNA is tightly packed inside head of T4 bacteriophage .
- The tail ends in a base plate that bears six fibers.
- The tail is contractile and surrounds an inner hollow tubular core . It enables The injection of DNA into host.



❖ Structure of Animal Viruses

[Human Immunodeficiency Virus(HIV)]



❖Human Immunodeficiency Virus [HIV]

- HIV belong to the genus Lentivirus of family Retroviridae . It is dsRNA virus having two identical plus strands .
- It contains three important enzymes Reverse transcriptase , Integrase and protease
- The envelope of HIV is made up of two layers of phospholipids .Spikes of giycoprotein are embedded in envelope layers
- The matrix protein p 17 lies between the envelope and core .The capsid protein is p 24.

❖Life cycle of Bacteriophages

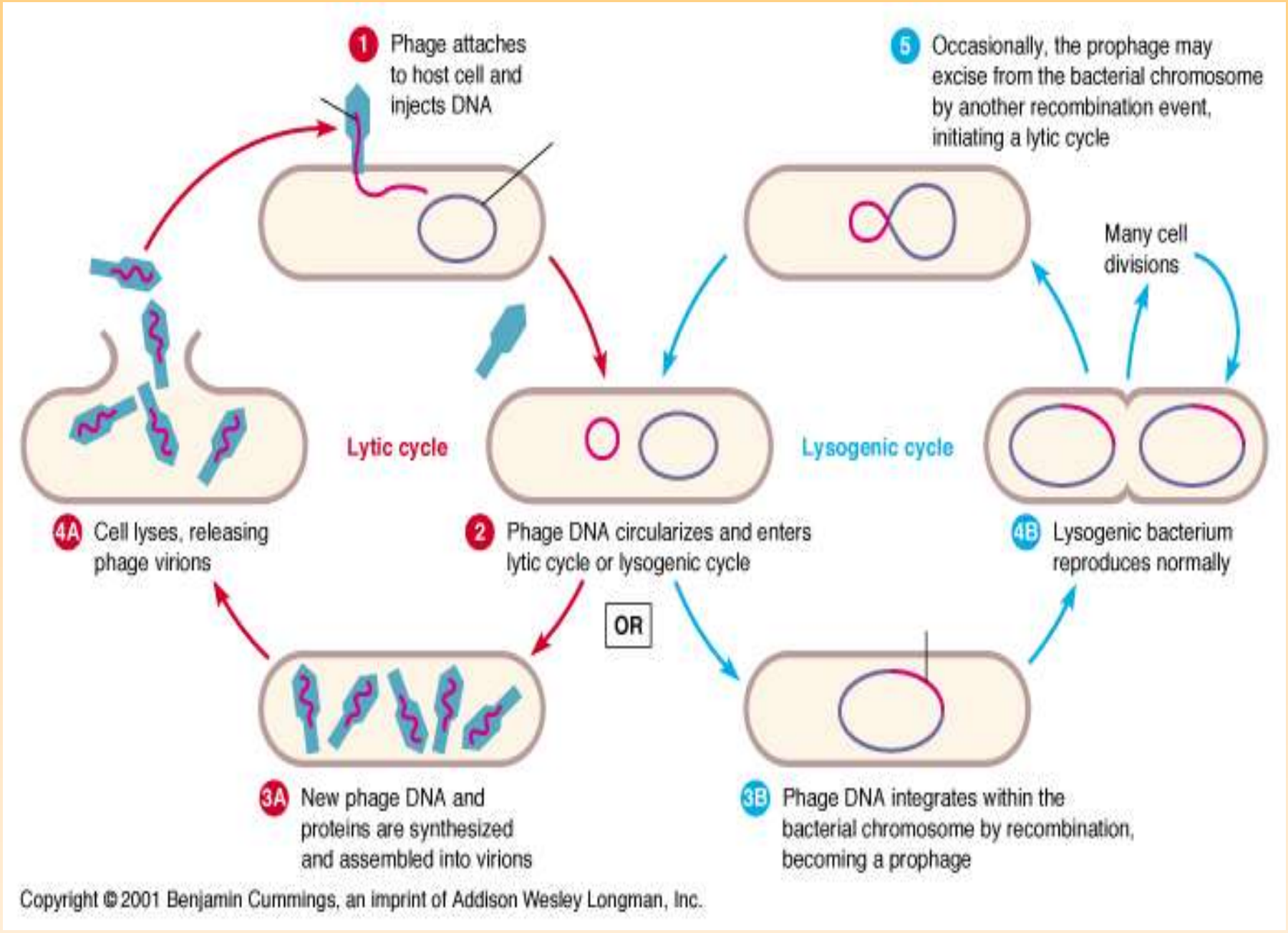
1. Attachment
2. Penetration
3. Biosynthesis
4. Maturation
5. Release

❖Multiplication of Viruses

Multiplication of bacteriophage

- *The Lysogenic Cycle*

- The **lysogenic cycle** replicates the phage genome without destroying the host
- The viral DNA molecule is incorporated into the host cell's chromosome
- This integrated viral DNA is known as a **prophage**
- Every time the host divides, it copies the phage DNA and passes the copies to daughter cells
- An environmental signal can trigger the virus genome to exit the bacterial chromosome and switch to the lytic mode
- Phages that use both the lytic and lysogenic cycles are called **temperate phages**


1 Phage attaches to host cell and injects DNA
5 Occasionally, the prophage may excise from the bacterial chromosome by another recombination event, initiating a lytic cycle
Many cell divisions
Lytic cycle
Lysogenic cycle
4A Cell lyses, releasing phage virions
2 Phage DNA circularizes and enters lytic cycle or lysogenic cycle
4B Lysogenic bacterium reproduces normally
OR
3A New phage DNA and proteins are synthesized and assembled into virions
3B Phage DNA integrates within the bacterial chromosome by recombination, becoming a prophage
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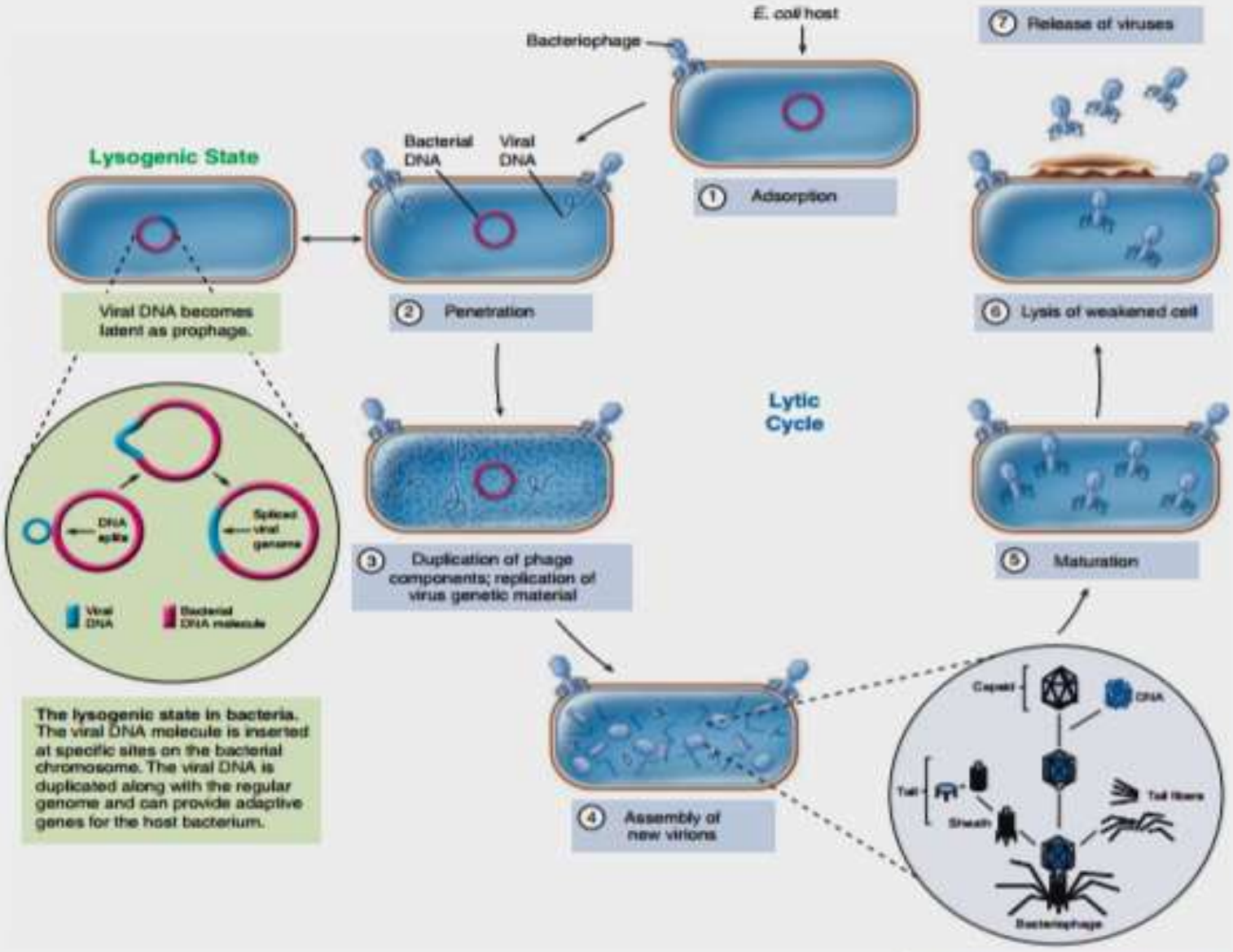
The Lytic Cycle

- The **lytic cycle** is a phage reproductive cycle that culminates in the death of the host cell
- The lytic cycle produces new phages and digests the host's cell wall, releasing the progeny viruses
- A phage that reproduces only by the lytic cycle is called a **virulent phage**
- Bacteria have defenses against phages, including **restriction enzymes** that recognize and cut up certain phage DNA

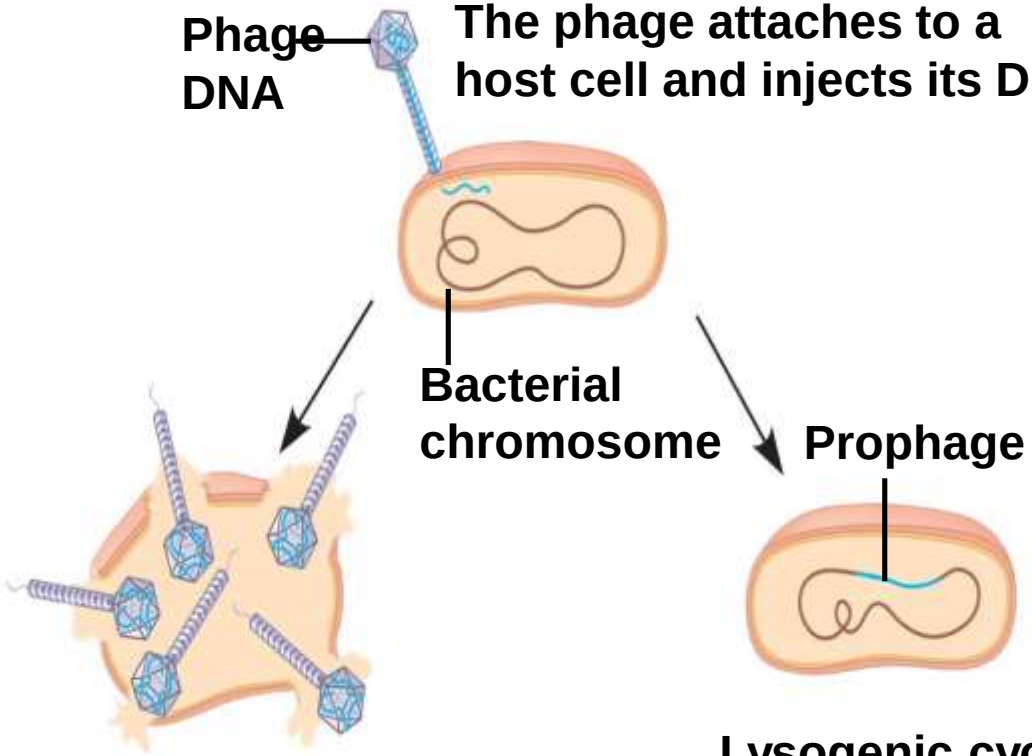


The lysogenic state in bacteria. The viral DNA molecule is inserted at specific sites on the bacterial chromosome. The viral DNA is duplicated along with the regular genome and can provide adaptive genes for the host bacterium.

Bacteriophage assembly line. First the capsomers are synthesized by the host cell. A strand of viral nucleic acid is inserted during capsid formation. In final assembly, the prefabricated components fit together into whole parts and finally into the finished viruses.

FIGURE 6.17

Events in the multiplication cycle of T-even bacteriophages. The lytic cycle (1–7) involves full completion of viral infection through lysis and release of virions. Occasionally the virus enters a reversible state of lysogeny (left) and is incorporated [illegible]



Lytic cycle

- Virulent or temperate phage
- Destruction of host DNA
- Production of new phages
- Lysis of host cell causes release of progeny phages

Lysogenic cycle

- Temperate phage only
- Genome integrates into bacterial chromosome as prophage, which (1) is replicated and passed on to daughter cells and (2) can be induced to leave the chromosome and initiate a lytic cycle

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❖Life cycle of Animal Viruses

1. Adsorption
2. Penetration
3. Uncoating
4. Synthesis
5. Assembly
6. Release

1. Adsorption

- Virus encounters susceptible host cells
- Adsorbs specifically to receptor sites on the cell membrane
- Because of the exact fit required, viruses have a limited **host range**

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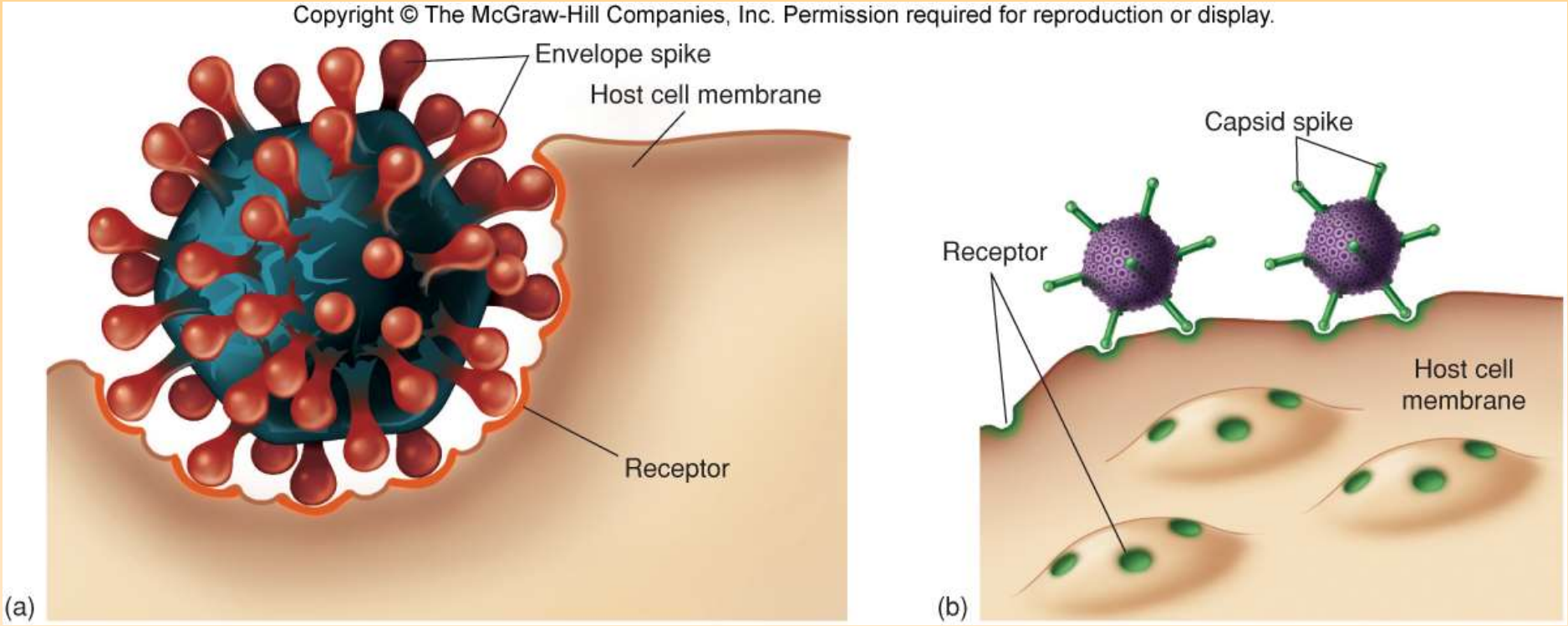

Figure 6.12

2. Penetration

- Flexible cell membrane of the host is penetrated by the whole virus or its nucleic acid
- Endocytosis: entire virus engulfed by the cell and enclosed in a vacuole or vesicle
- The viral envelope can also directly fuse with the host cell membrane

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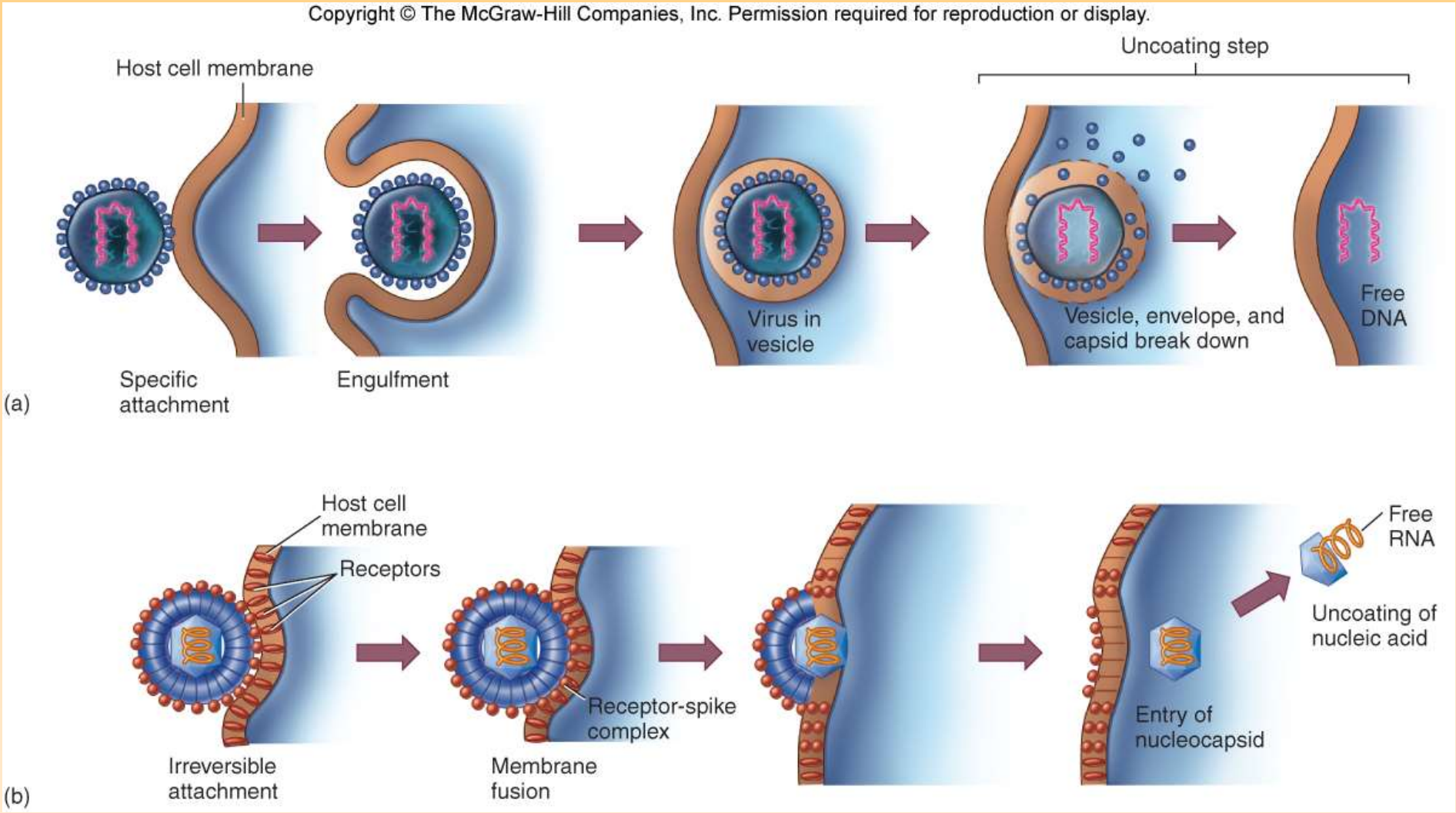

3. Uncoating

- Enzymes in the vacuole dissolve the envelope and capsid
- The virus is now **uncoated**

4. Synthesis

- Free viral nucleic acid exerts control over the host's synthetic and metabolic machinery
- DNA viruses- enter host cell's nucleus where they are replicated and assembled
  - DNA enters the nucleus and is transcribed into RNA
  - The RNA becomes a message for synthesizing viral proteins (translation)
  - New DNA is synthesized using host nucleotides
- RNA viruses- replicated and assembled in the cytoplasm

5. Assembly

- Mature virus particles are constructed from the growing pool of parts

6. Release

- Nonenveloped and complex viruses are released when the cell lyses or ruptures
- Enveloped viruses are liberated by **budding** or **exocytosis**
- Anywhere from 3,000 to 100,000 virions may be released, depending on the virus
- Entire length of cycle- anywhere from 8 to 36 hours

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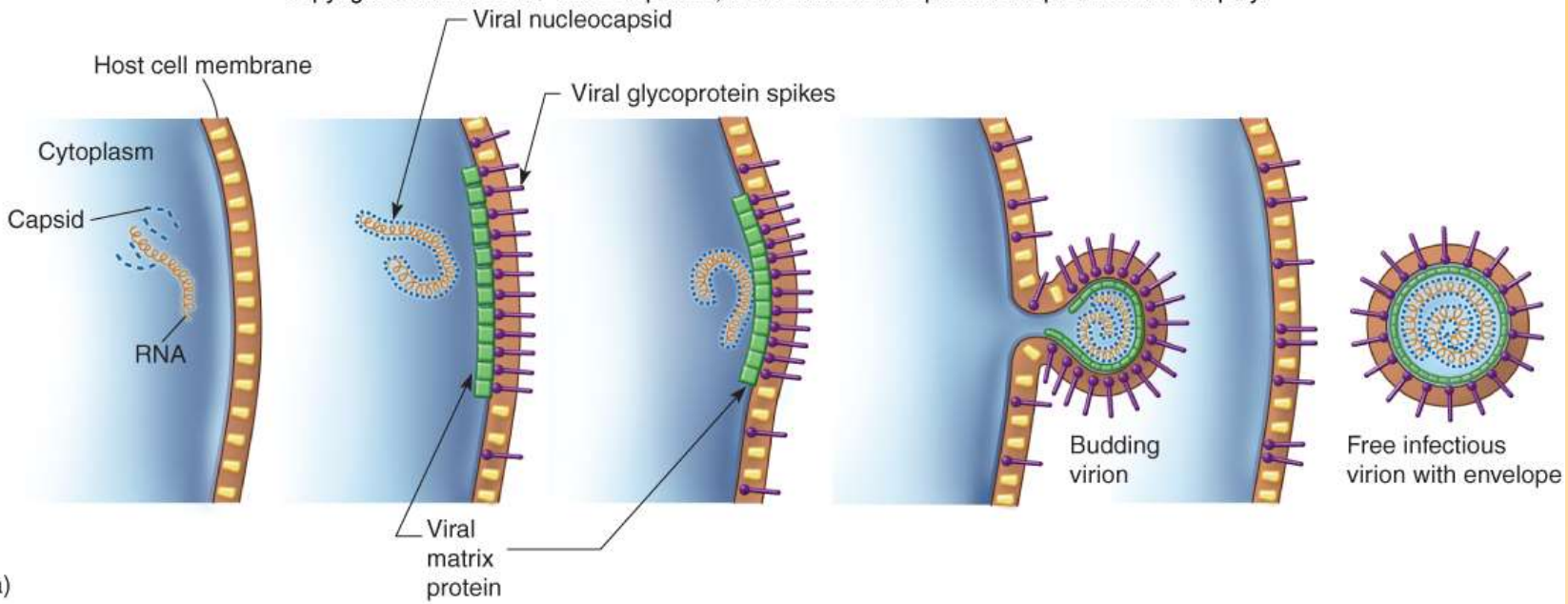

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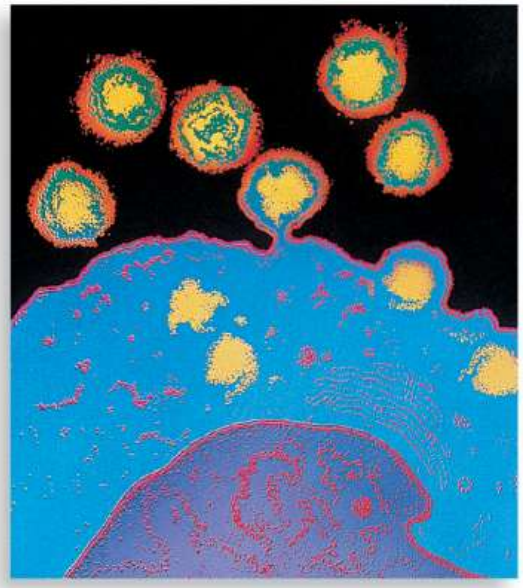

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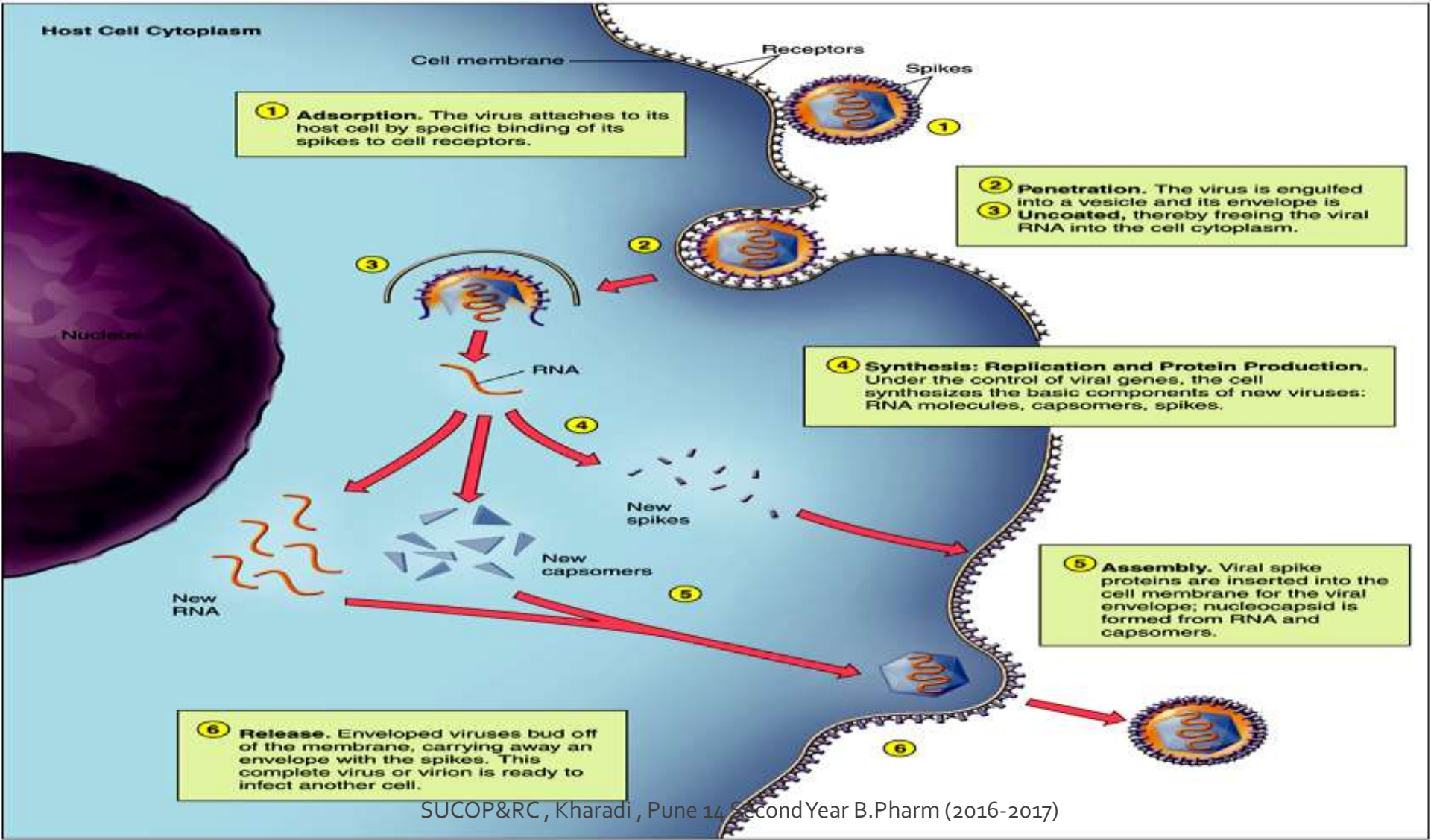
Multiplication of Animal Viruses



Techniques in Cultivating and Identifying Animal Viruses

- Primary purposes of viral cultivation
  - To isolate and identify viruses in clinical specimens
  - To prepare viruses for vaccines
  - To do detailed research on viral structure, multiplication cycles, genetics, and effects on host cells
- Using Live Animal Inoculation
  - Specially bred strains of white mice, rats, hamsters, guinea pigs, and rabbits
  - Occasionally invertebrates or nonhuman primates are used
  - Animal is exposed to the virus by injection
- Using Bird Embryos
  - Enclosed in an egg- nearly perfect conditions for viral propagation
  - Chicken, duck, and turkey are most common
  - Egg is injected through the shell using sterile techniques

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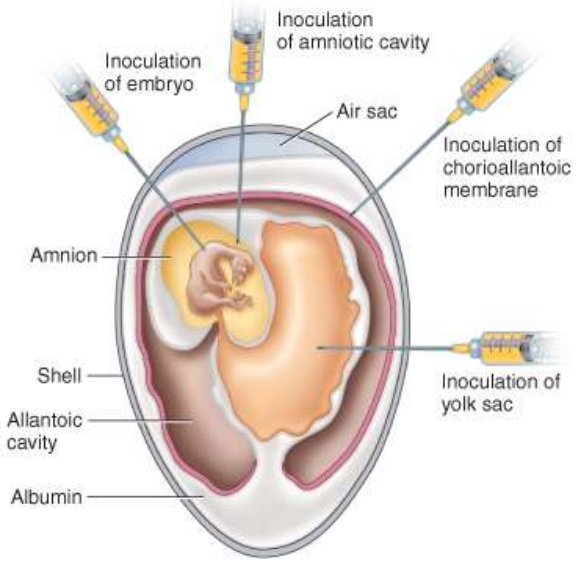

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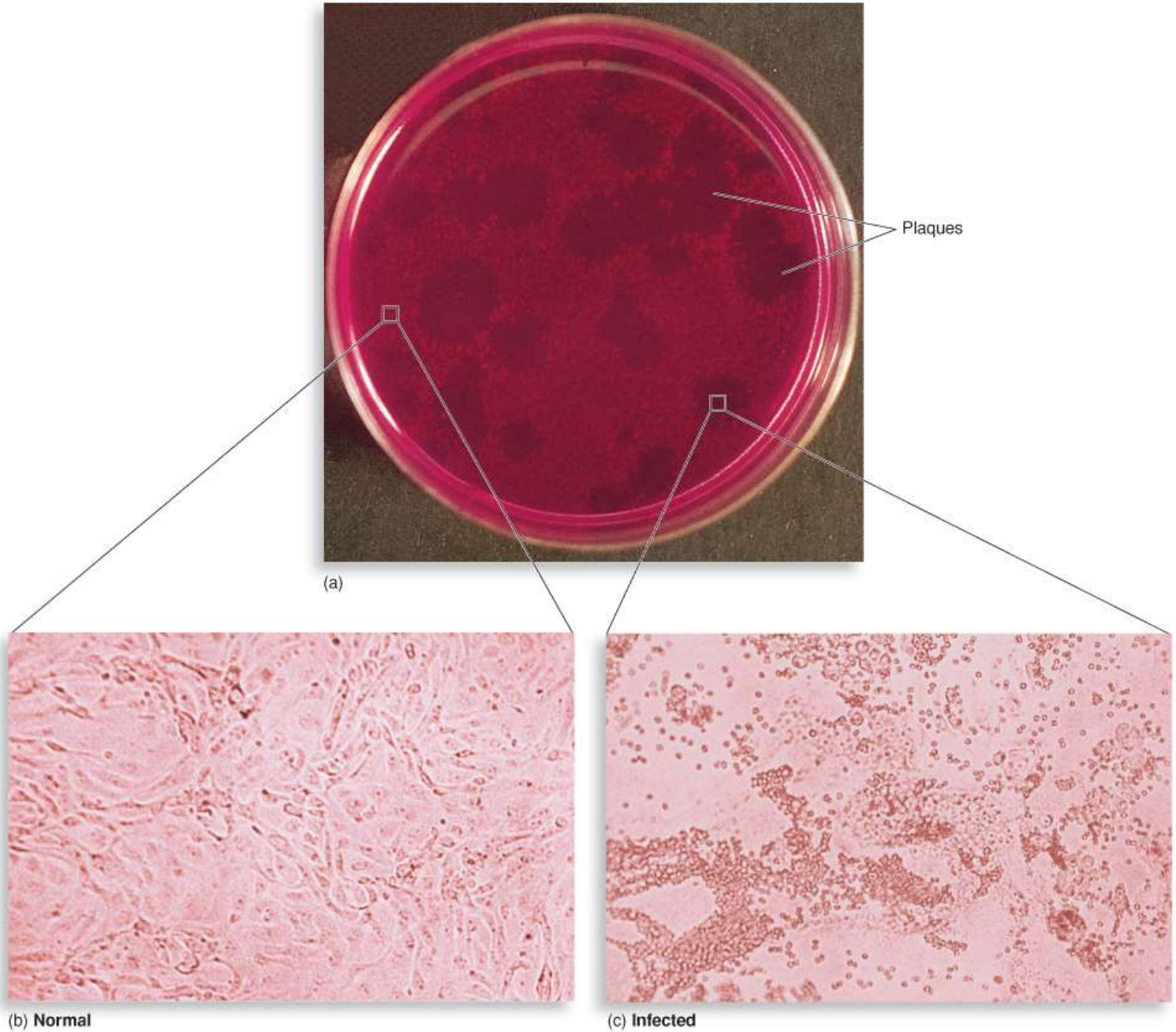

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Application of Viruses

- Animal viruses are used as vectors to introduce specific genes into animal cells this is known as gene therapy
- Phage therapy is a concept that entails the use of bacteriophage to kill bacteria . Phages are very specific , hence do not have the side effects of drug.
- In various pharmaceutical industries , phages are used to control unwanted bacteria in the production line .
- Phages have been used for the detection of pathogenic bacteria .
- Animal viruses are employed for the production of interferons .
- Animal viruses are useful in studies of cell transformation .
- Bacteriophage and animal viruses play a crucial role in the development of molecular cell biology study.

❖QUESTIONS

1. What are viruses ? How they are different from typical living cell ? How does the multiplication of human viruses takes place ? how are human viruses cultivated ?
2. Draw digram of T – even bacteriophages ?
3. Write short note on multiplication of human viruses ?
4. Write short note on lytic cycle ?
5. Write short note on lysogenic cycle ?
6. Explain in short different techniques used for cultivation of viruses ?
7. Draw the structure of bacteriophages ?
8. Write short note on HIV ?
9. Write note on cultivation of viruses ?
10. Write note on tumour viruses ?

Thank you

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X-Ray Crystallography

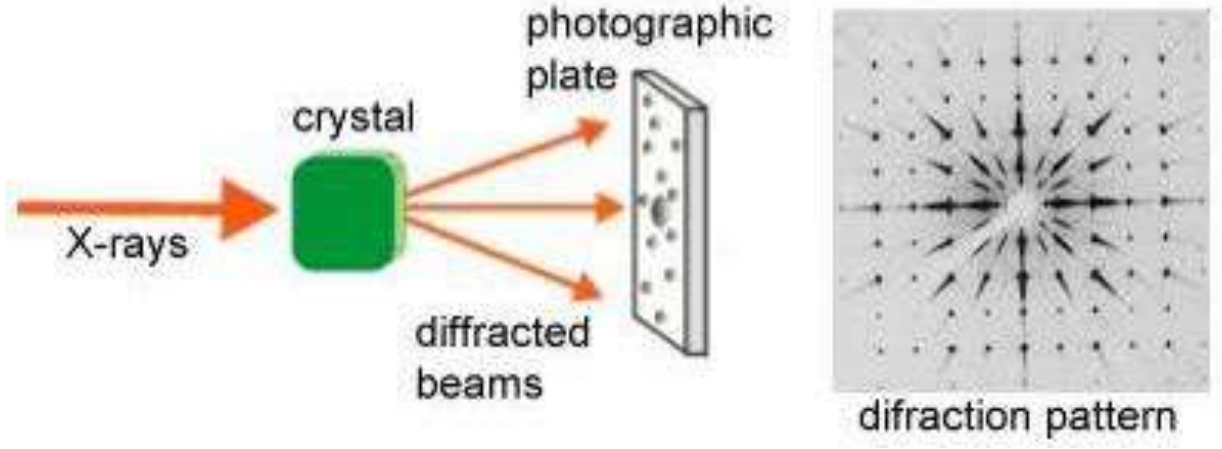
Presented by : Dr. Vijaya U. Barge
(Vice- Principal & Professor)

- Contents:

1. Introduction
2. Production of x ray
3. X-ray diffraction methods
4. Bragg‘s law

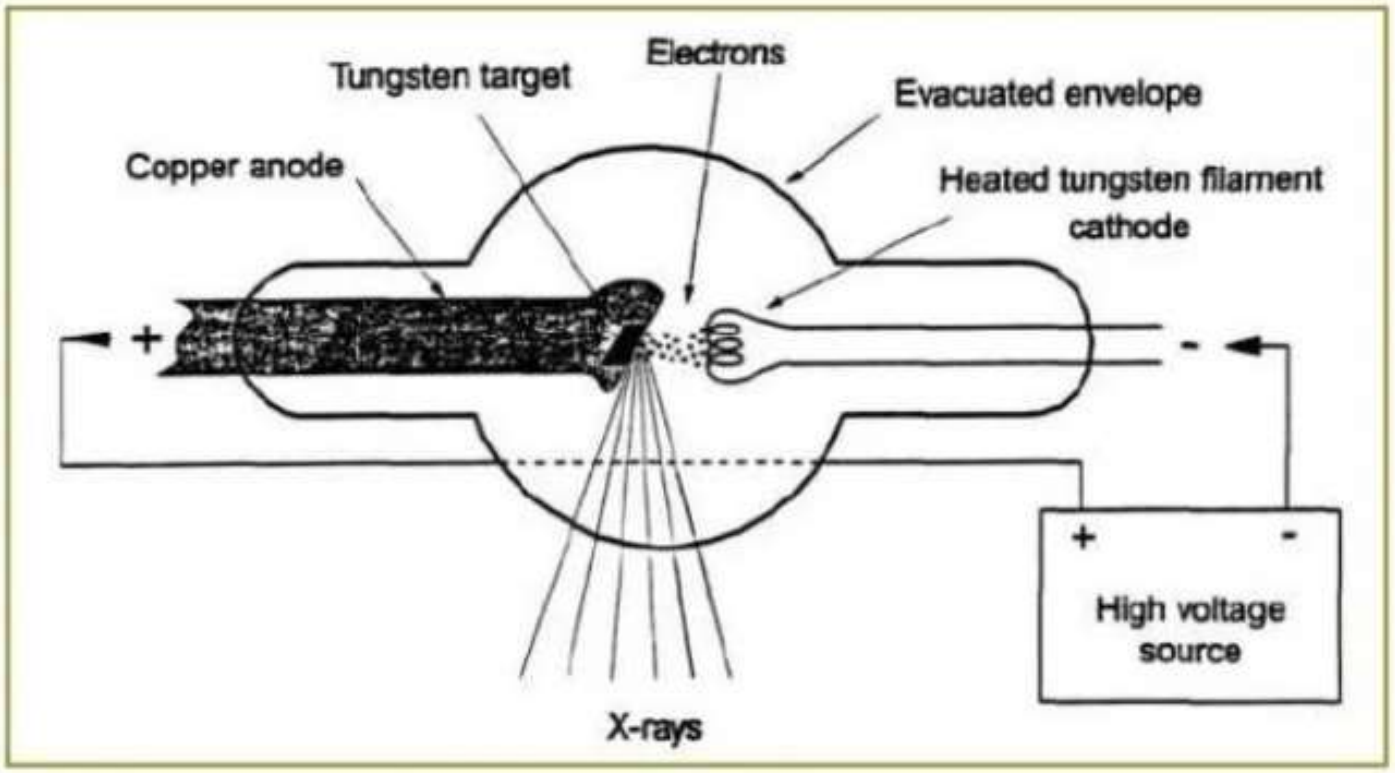
Introduction

- X ray is an electromagnetic wave of high energy and very short wavelength (between ultraviolet light and gamma ray).
- X-ray crystallography is a tool used for determining the atomic and molecular structure of a crystal or material.



Production of X ray

- Power is send to X-Ray tube via cables .
- Ma (milliamperage) is send to filament on cathode side.
- Filament heats up electron “ boil off ”
- Negetive charge
- Positive Voltage (KVP) is applied to ANODE.
- Negetive Electrons = attracted across the tube to the positive ANODE.
- Electrons “ Slam into” anode suddenly stopped .
- X-Ray photons are created .



- Elecron beam is focused from the cathode to the anode target by the focusing cup.
- Electron interact with the electrons on the tungstuns atoms of target material.
- Photons send to through the window port towards the patient.

X-ray diffraction methods

These are generally used for investigating the internal structures and crystal structures of various compounds .They are as follows,

- Laue's photographic method.
  - a) Transmission method.
  - b) Black reflection method.
- Rotating crystal method .
- Powder method .

X-Ray Diffraction Methods

Von Laue

Orientation
Single Crystal
Polychromatic
Beam, Fixed
Angle single θ

Rotating Crystal

Lattice constant
Single Crystal
Monchromatic
Beam, Variable
Angle θ
Varied by
rotation

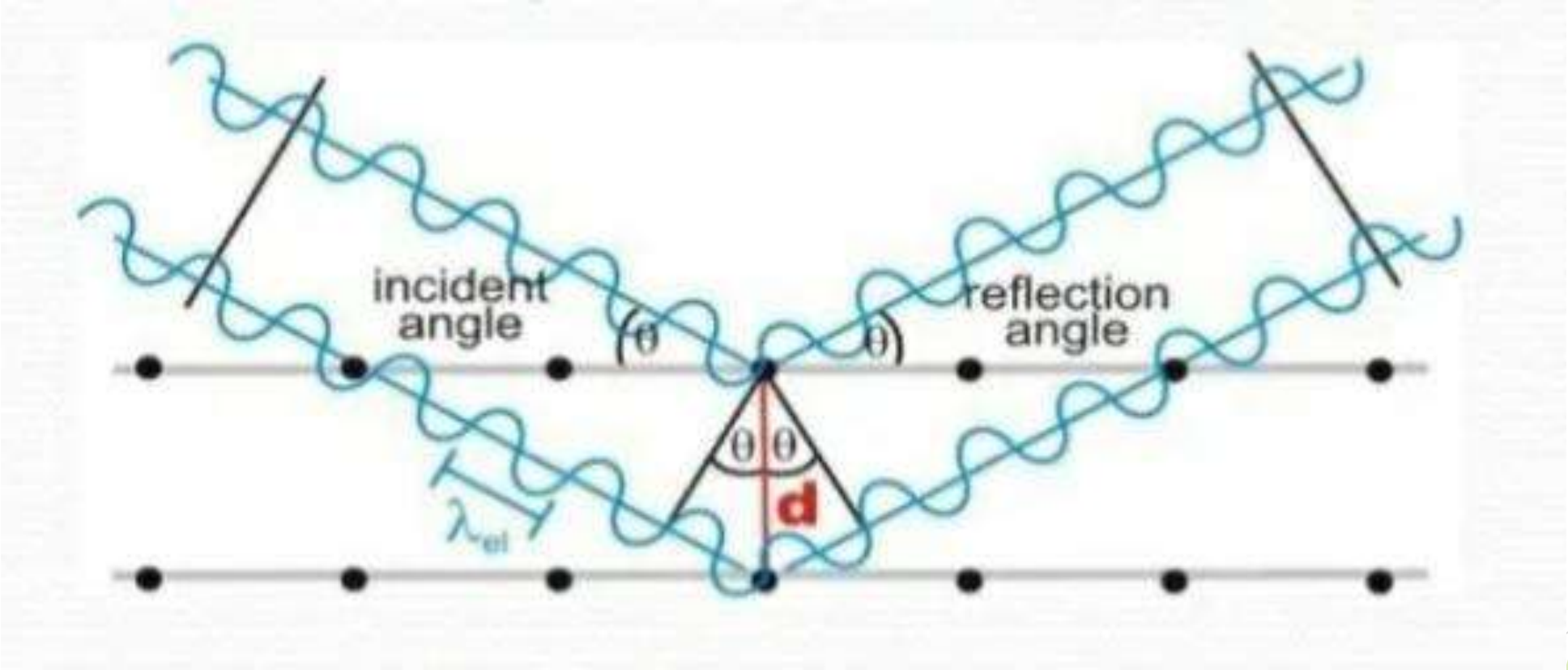
Powder

Lattice
Parameters
Poly Crystal
Monchromatic
Beam, Variable
Angle Many θs
(orientations)

Bragg's law:

$$n\lambda = 2d\,sin\Theta$$

- Here d is the spacing between diffracting planes, Θ is the incident angle, n is any integar, and λ is the wavelength of the beam.
- These specific directions appear as spots on diffraction pattern called reflections.
- Thus X-ray diffraction results from an electromagnetic wave impinging on a regular array of scatters.


incident
angle
(θ
θ)
reflection
angle
θ θ
d
λel

Thank you