PPT Bank by SHN

1. Electron transport chain
2. Fatty Acid synthesis
3. Physical chemical properties of lipid
4. Urea cycle
5. Germ plasm
6. Animal biodiversity
7. Colorimeter spectrophotometer

CREDIT 02-UNIT 03: ANIMAL GENETIC RESOURCES

INTRODUCTION

Those animal species that are used, or may be utilized, for food and agriculture production, as well as the populations that make up each of them. Wild and feral populations, landraces and main populations, standardized breeds, chosen lines, variations, strains, and any preserved genetic material, all of which are now designated as Breeds, can be found within each species.

ANIMAL GENETIC RESOURCES

1. Animal Genetic Resources include all species, breeds and strains that are of economic, scientific and cultural interest to agriculture, now and in the future.

2. Common species include sheep, goats, cattle, horses, pigs, buffalo and chickens, but many other domesticated animals such as camels, donkeys, elephants, reindeer, rabbits and rodents are important to different cultures and regions of the world.

3. Animal domestication began some 12000 years ago when people began selecting animals for food, fibre, work power and other agricultural uses.

4. Approximately 40 percent of the total land available in developing countries can be used only for some form of forage production.

5. Animals account for 19 percent of the world‘s food directly

6. In addition, livestock serve as very important cash reserves in many of the mixed farming systems.

7. A very few nations of the world are bestowed with huge resources and India is one of them. India is aptly placed in one of the 12 mega-biodiverse countries of the world contributing about 11.6 percent to the world livestock population, accounting for immense contributions from the livestock sector to its GDP (4.1%).

8. the low management inputs exhibiting unique traits of productivity, feed utilization, disease resistance and adaptability.

9. The Indian government and the agencies concerned are steadfast in the process of making a fool proof and perfect breeding policy.

10. By establishing breeding units, national research centres and other such initiatives along with various other missions taken up at ground level, the only aim is to better the productivity and conservation of indigenous livestock resources.

11. called insurance value, emerges from conserving the breed for the future and enabling response to the future unpredicted events, like climate change and production environment change.

12. Many local breeds posses characteristics which enables them to cope with different production system, often harsh environment, hot or cold climate, rough terrain or high elevations, which can only be manged by local breeds (FAO; 2015).

Categorized of Breeds –

1. Breed: either a sub-specific group of domestic livestock with definable and identifiable external characteristics that enable it to be separated by visual appraisal from other similarly defined groups within the same species, or a group for which geographical and/or cultural separation from phenotypically similar groups has led to acceptance of its separate identity.

2. Locally Adapted Breeds –

Indigenous Breeds, also termed autochthonous or native breeds and originating from, adapted to and utilized in a particular geographical region, form a sub-set of the Locally Adapted Breeds.

3. Recently Introduced Breeds –

whose importation was within the last 5 or so generations for the species concerned, and which were imported over a relatively short period of time.

4. Continually Imported Breeds –

whose local gene pool is regularly replenished from one or more sources outside your country

5. Exotic Breeds –

Exotic breeds comprise both **Recently Introduced Breeds** and **Continually Imported Breeds**.

6. Breed at Risk

any breed that may become extinct if the factors causing it s decline in numbers are not eliminated or mitigated. Breeds may be in danger of becoming extinct for a variety of reasons.

7. Extinct Breed –

This situation becomes absolute when there are no breeding males or breeding females remaining.

Population

The genetics of the population is concerned with the genetic of all individuals it comprises, and with the transmission from generation to generation of samples of the genetic variability associated with this population.

Domestic animal diversity DAD

- Spectrum of genetic differences within each breed and across all breads within each domestic animal species together with the species differences of interest for food and agriculture production
- Assessment- 1. description 2. Analysis 3. Reporting of DAD status 4. Trends and causes 5. art and capacity to manage the diversity as well as country needs and priorities for effective management.

Adaptive management- positive management.

Need to release the food security and sustainable agricultural and rural development.

by expanding knowledge, understanding and awerness of multiple goods and services provided by these resources.-

Management of DAD

- Capacity building
- Mainstreaming
- Clearing house mechanism
- Local knowledge
- Domesticated animals
- Management of farm Animal genetic resources
- Characterization of animal genetic resources
- Base-line breed survey
- Adaptive fitness
- Evaluation
- Meaurement of DAD
- Valuation

Capacity Building

- Capacity building involves education and training, technology transfer, organizational infrastructure, development of policy and of financial mechanisms.

Mainstreaming

- The conservation and sustainable use of DAD, including their integration in sectoral and cross-sectorial plans and programmes.

Clearing House Mechanism

- an information exchange platform for AnGR management that reflects the recognition that co-operation and sharing of knowledge, expertise and other benefits among communities is necessary for effective characterization, utilization and conservation of DAD.

Local Knowledge

- Local Knowledge also encompass Indigenous` Knowledge' and `Traditional Knowledge’.

Domestic (ated) Animals

- whose breeding and husbandry are controlled by human communities to obtain benefits or services f rom them.

Farm Animal

- encompasses all technical, policy, and logistical operations involved in understanding (characterization), using and developing (utilization), maintaining (conservation), accessing, and sharing the benefits of animal genetic resources.

Characterization of Animal Genetic Resources

Base-line Breeds Survey: summary data describing the identification and observable characteristics, location, uses and general husbandry of the AnGR for each species used in the country for food and agricultural production.

Adaptive Fitness: a genetically determined complex of characteristics which enhance a breed's ability to reproduce and survive in a particular production environment. Also referred to as **Adaptation**.

Evaluation: measurement of the characteristics that are important for production and adaptation, either of individual animals or of populations, most commonly in the context of comparative evaluation of the traits of animals or of populations.

Meaurement of DAD

- **Valuation:** description of the extent to which market values of AnGR reflect their `real' or `fair' value, accounting for all goods and services they may provide to current and future generations of humankind. In the case of market failures, market pric es will differ from the value that society attaches to AnGR. The primary motivation for valuing AnGRs is to assist policy development and management decisions.

Conservation of farm animal genetic resources

- In situ conservation
- Exsitu conservation
- Gene bank
- Gene pool

In situ Conservation of Farm Animal Genetic Diversity:

- all measures to maintain live animal breeding populations, including those involved in active breeding programmes in the agro-ecosystem where they either developed or are now normally found, together with husbandry activities that are undertaken to ensure the continued contribution of these resources to sustainable food and agricultural production, now and in the future.

Ex situ Conservation of Farm Animal Genetic Diversity

- conservation of genetic material within living animals but out of the environment in which it developed (*Ex situ in vivo)*, or external to the living animal in an artificial environment, usually under cryogenic conditions including, *inter alia*, the cryo-conservation of semen, oocytes, embryos, cells or tissues *(Ex situ in vitro)*.
- **Genebank**: the physical location for conservation of collections of well identified genetic material in the form of live animals, *in situ* or *ex situ (as conservation herds or flocks)*, or *ex situ* stored semen, oocytes, embryos, cells or tissues. Also referred to as Genomebank.
- **Gene pool**: the total genetic information in all the genes in a breeding population at a given time.

Utilization of Farm Animal Genetic Resources

- the use and development of animal genetic resources for the production of food and agriculture.
- adaptive fitness to the environment concerned,
- The wise use of AnGRs is possible without depleting domestic animal diversity.
- It requires careful definition of breeding objectives, and the planning, establishment and maintenance of effective and efficient animal recordingand breeding strategies

principle working and instrumentation, application s of Colorimeter

By
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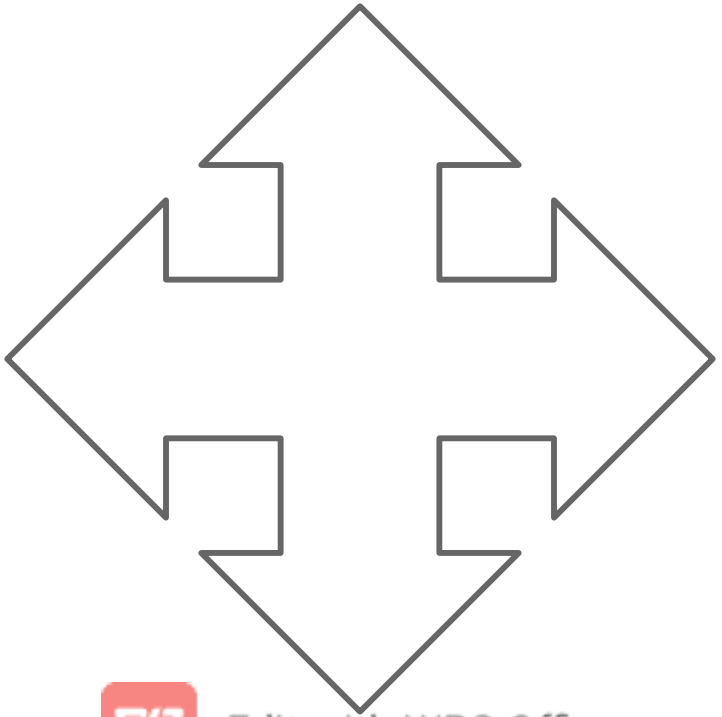
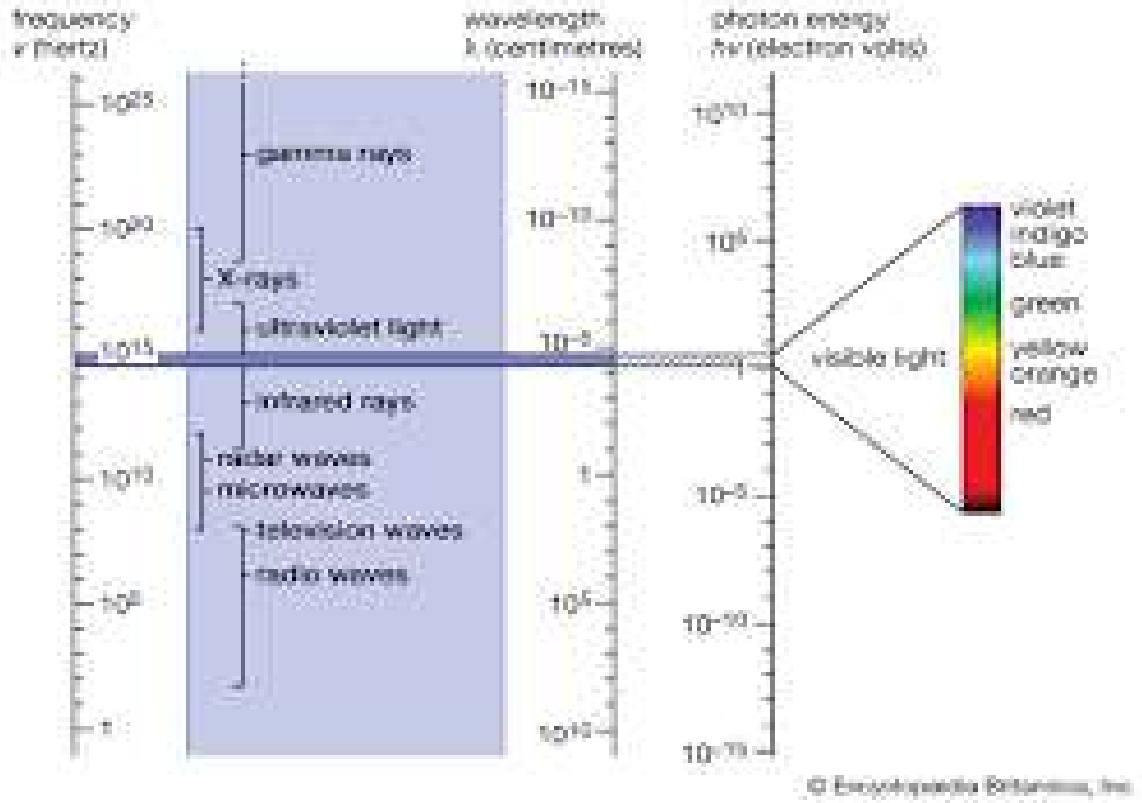
Autonomous.

Colorimeter



Electromagnetic spectrum refers to the full range of all frequencies of electromagnetic radiation and also to the characteristic distribution of electromagnetic radiation emitted or absorbed by that particular object. Devices used to measure an electromagnetic spectrum are called spectrograph or spectrometer.



HISTORY

It is not something that was discovered, it was invented. One of the most popular designs is the Duboscq colorimeter which was invented by Jules Duboscq in 1870.

DISCOVERER OF COLORIMETER

Principle-

A colorimeter is a light-sensitive device used for measuring the transmittance and absorbance of light passing through a liquid sample. The device measures the intensity or concentration of the color that develops upon introducing a specific reagent into a solution.

The colorimeter is based on Beer-Lambert's law, according to which the absorption of light transmitted through the medium is directly proportional to the medium concentration.

Terms:/Parameters

Transmittance : The passing of light through a sample

Absorbance: Amount of light absorbed by a sample (the amount of light that does not pass through or reflect off a sample)

%Transmittance: The manner in which a spectrophotometer reports the amount of light that passes through a sample

Absorbance units: A unit of light absorbance determined by the decrease in the amount of light in a light beam

Absorbance spectrum: A graph of a sample's absorbance at different wavelengths

Lambda$_{max}$ The wavelength that gives the highest absorbance value for a sample

Lambert's law

- When a ray of monochromatic light passes throughan absorbing medium its intensity decreases exponentially as the length of the absorbing medium increases.

$$I = I_0 e^{-k_1 l}$$

Beer's law :

- When a monochromatic light passes through an absorbing medium its intensity decreases exponentially as the concentration of the absorbing medium increases.

$$I = I_o e^{-k_2 c}$$

BEER–LAMBERT'S LAW

(Beer–Lambert–Bouguer law)

- Relates the absorption of light to the properties of the material through which the light is travelling.

BEER'S LAW

- _When monochromatic light (light of a specific wavelength) passes through a solution there is usually a quantitative relationship between the solute concentration and the intensity of the transmitted light
- The amount of light absorbed by the a medium (solution/ sample) is proportional to the concentration of the absorbing material or solute present.
- Thus the concentration of a coloured solute in a solution may be determined in the lab by measuring the **ABSORBANCY OF LIGHT AT A GIVEN WAVELENGTH**

BEER–LAMBERT'S LAW (Beer–Lambert–Bouguer law)
....contd

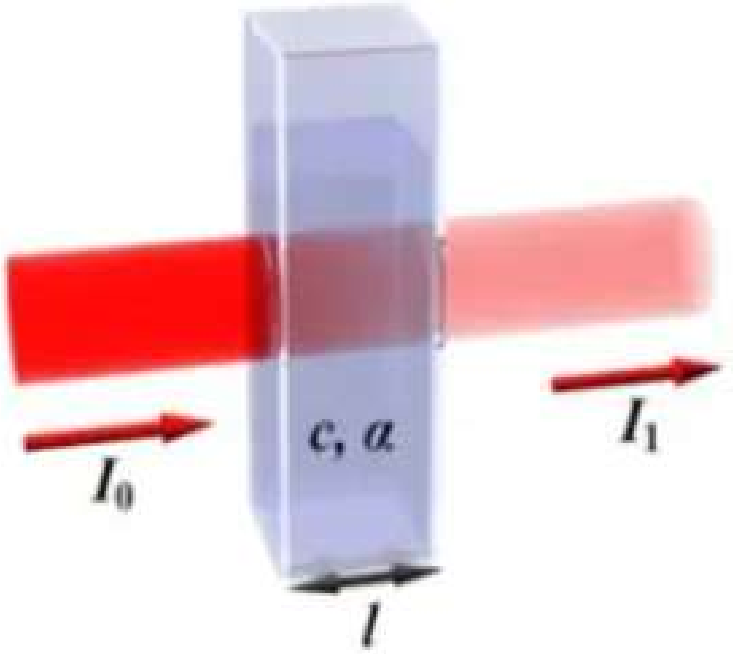
LAMBERT'S LAW

Lambert described how intensity changes with distance in an absorbing medium.

- The amount of light absorbed by the a medium (solution/ sample) at a given wavelength is proportional to thickness of the absorbing layer: path length of the light

Beer – Lambert Law

States that the Absorbance (O.D) of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length.



$$T = \frac{I}{I_0} = 10^{-a\ell} = 10^{-\varepsilon \ell c}$$

The fraction of the incident light absorbed by a solution at a given wavelength is related to

a. thickness of the absorbing layer (path length) and
b. concentration of the absorbing species

Transmittance

Defined as the ratio of the intensity of light emerging from the solution (I) to that of incident light entering (Io)

- There is a logarithmic dependence between the transmission (or transmissivity), *T*, of light through a substance and
- The product of : the absorption coefficient of the substance, α, and the distance the light travels through the material (i.e. the path length), *ℓ*.

- **The ABSORPTION COEFFICIENT:** (α) =
  Molar absorptivity (extinction coefficient) of the absorber, (*c*)
  the concentration (c) of absorbing species in the material

$$T = \frac{I}{I_0} = 10^{-\alpha \ell} = 10^{-\varepsilon \ell c}$$

intensity(power) of the incident light intensity(power) of the transmitted light ; *ℓ* . thickness of the absorbing layer (path length) and cross section of light absorption by a single particle;

$$T = \frac{I}{I_0}$$

T- Transmittance

I_0 - Original light intensity

I- Transmitted light intensity

$$\% \text{ Transmittance (T)} = 100 \times \frac{I}{I_0}$$

$$\text{Absorbance (A)} = \text{Log} \frac{I}{T}$$

(OPTICAL DENSITY)

$$= \text{Log} \frac{I_0}{I} = KCL$$

$$\frac{I_0}{I}$$

By definition of the Beer - Lambert Law.

$$\alpha =$$

$$A = \alpha$$

$$\mathbf{A = ECL}$$

A = Transmission/Transmissivity ; expressed in terms of Absorbance (numerical number only)- (OPTICAL DENSITY)

E = Molar Extinction Coefficient of the absorber ( )- Extinction Coefficient of a solution containing 1g molecule of solute per 1 liter of solution

L= length of light path through the solution

Beer-Lambert's Law

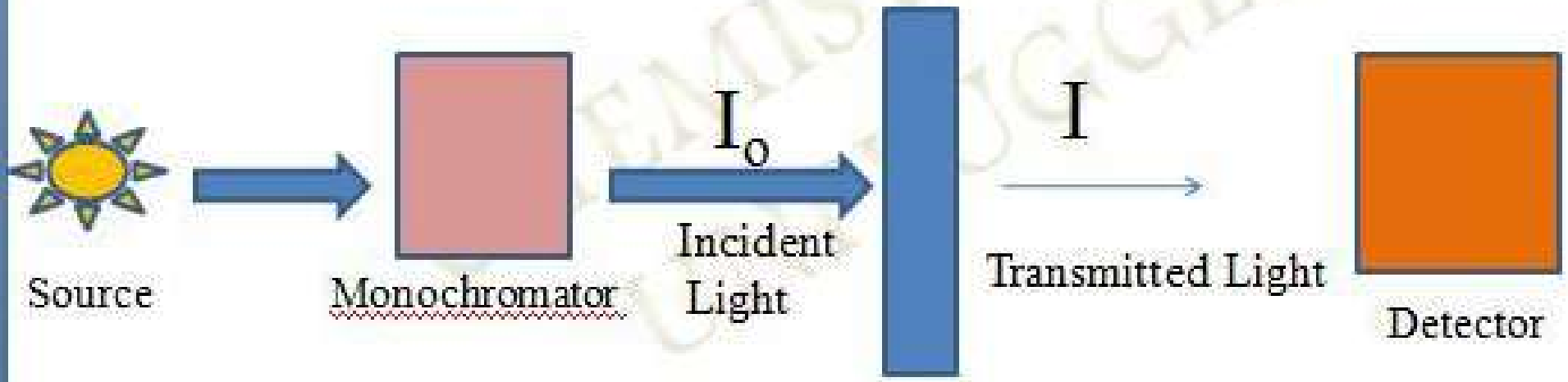
- Absorbance is directly proportional to concentration of the solution.

$$A = \varepsilon cl = \log(I_o/I)$$

where, c= concentration (mol/litre)

l = length of light path through the cell (cm)

ε = molar absorption coefficient (L mol^{-1} cm^{-1})



Working and instrumentation

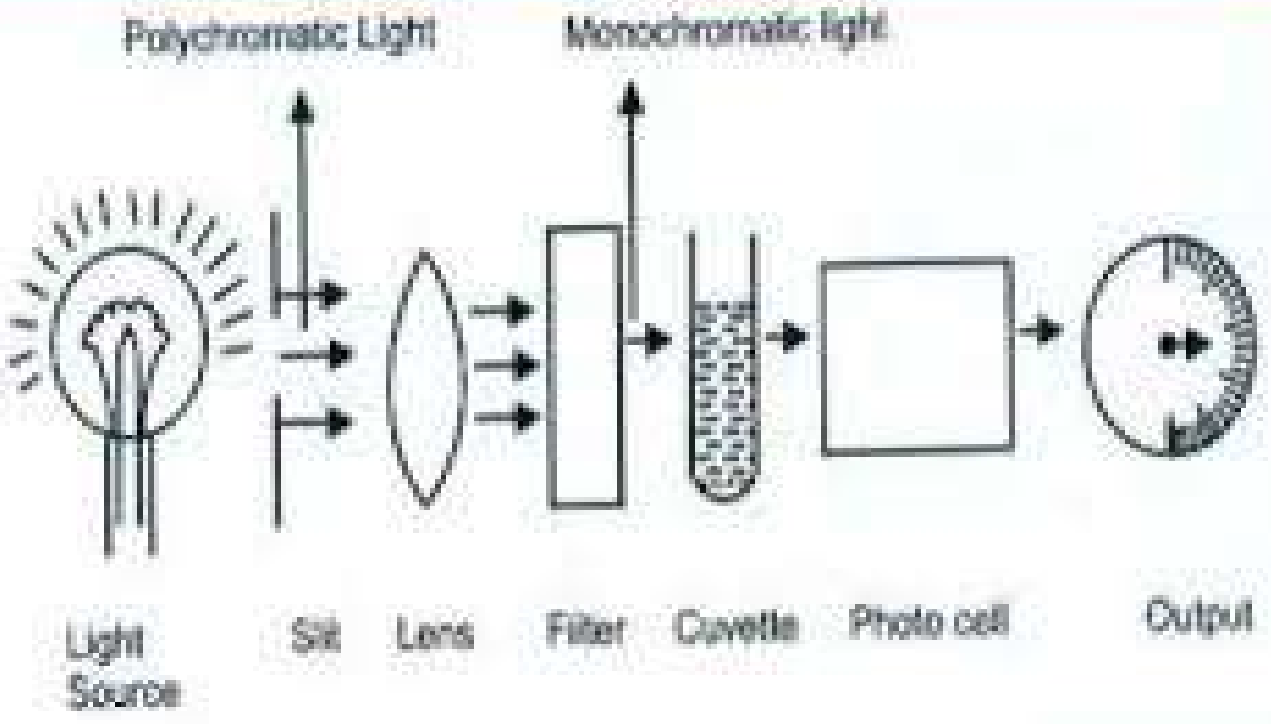


Fig. 27.1: Parts of the colorimeter

1.**Light source**-Colorimeters have three main components:
A light source, usually a tungsten or xenon lamp

2. **Light dispersion devices-** disspersion devices causes different wavelengths of light to be dispersed at different angles

   Two types of dispersion devices, Prisms and holographic gratings are commonly used in U.V. Visible spectrometer.

   Prisms are simple and inex[ensive but the resulting dispersion is angularly non-linear moreover, the angle of dispersion is temperature sensitive.

3. **Slit**- It is adjustable which allows only a beam of light to pass through , it prevents unwanted or stray light.

4. **Condensing lenses-** parallel to beam of light

5. **Filter-** made up of colored glass . Filters are used for selecting light of narrow wavelength.

Filters will absorb light of unwanted wavelength and allow only to monochromatic light to pass through.

complementary to the color of solution

Complementary filters for coloured solutions.

The selected filters has the color to the complementary to that of the color of unknown solution

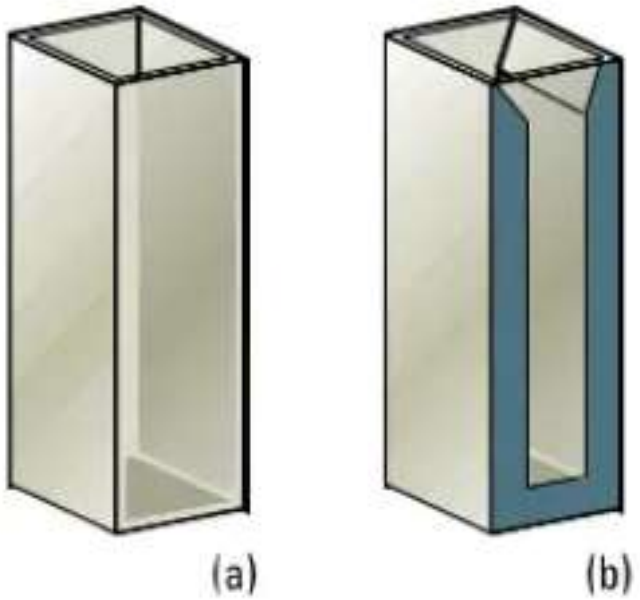
Si. No.	Color of the Solution	Colour Absorbed	Wavelength of Absorption
1.	Yellow to Green	Violet	400 nm – 435 nm
2.	Yellow to Orange	Blue	435 nm – 490 nm
3.	Red	Blue to Green	490 nm – 500 nm
4.	Purple	Green	500 nm – 560 nm
5.	Violet	Yellow to Green	560 nm – 580 nm
6.	Blue to Green	Yellow to Orange	580 nm – 650 nm
7.	Bluish Green	Red	650 nm – 700 nm

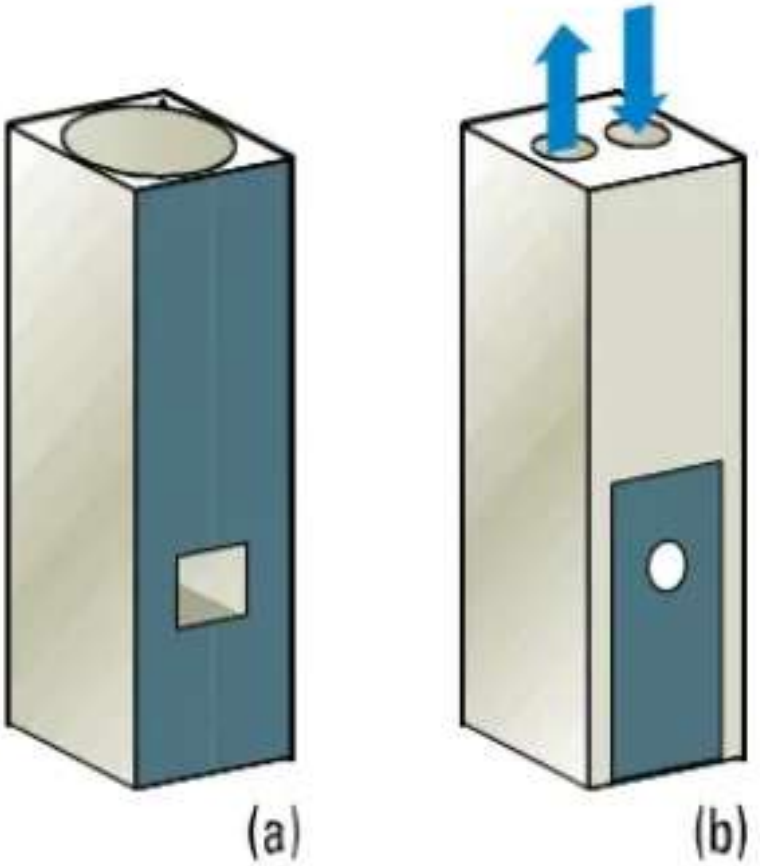
6. Cuvette or sample holder-

CUVETTES (SAMPLE CONTAINERS)

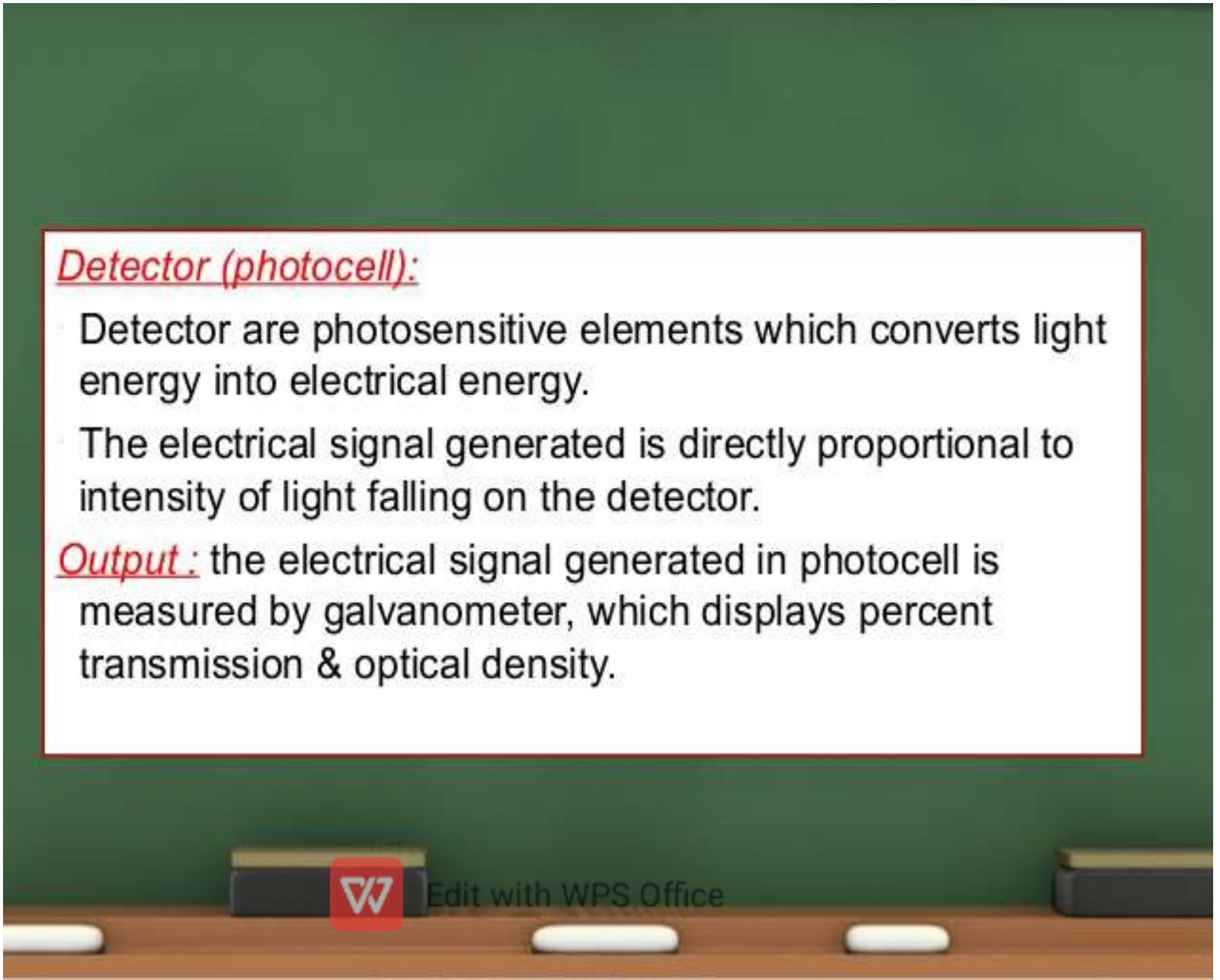
- The containers for the sample- usually plastic or quartz:
- Reference solution must be transparent to the radiation which will pass through them.
- Quartz or fused crystalline silica cuvettes for UV spectroscopy .
- Glass cuvettes for Visible Spectrophotometer
- NaCl and KBr Crystals for IR wavelengths

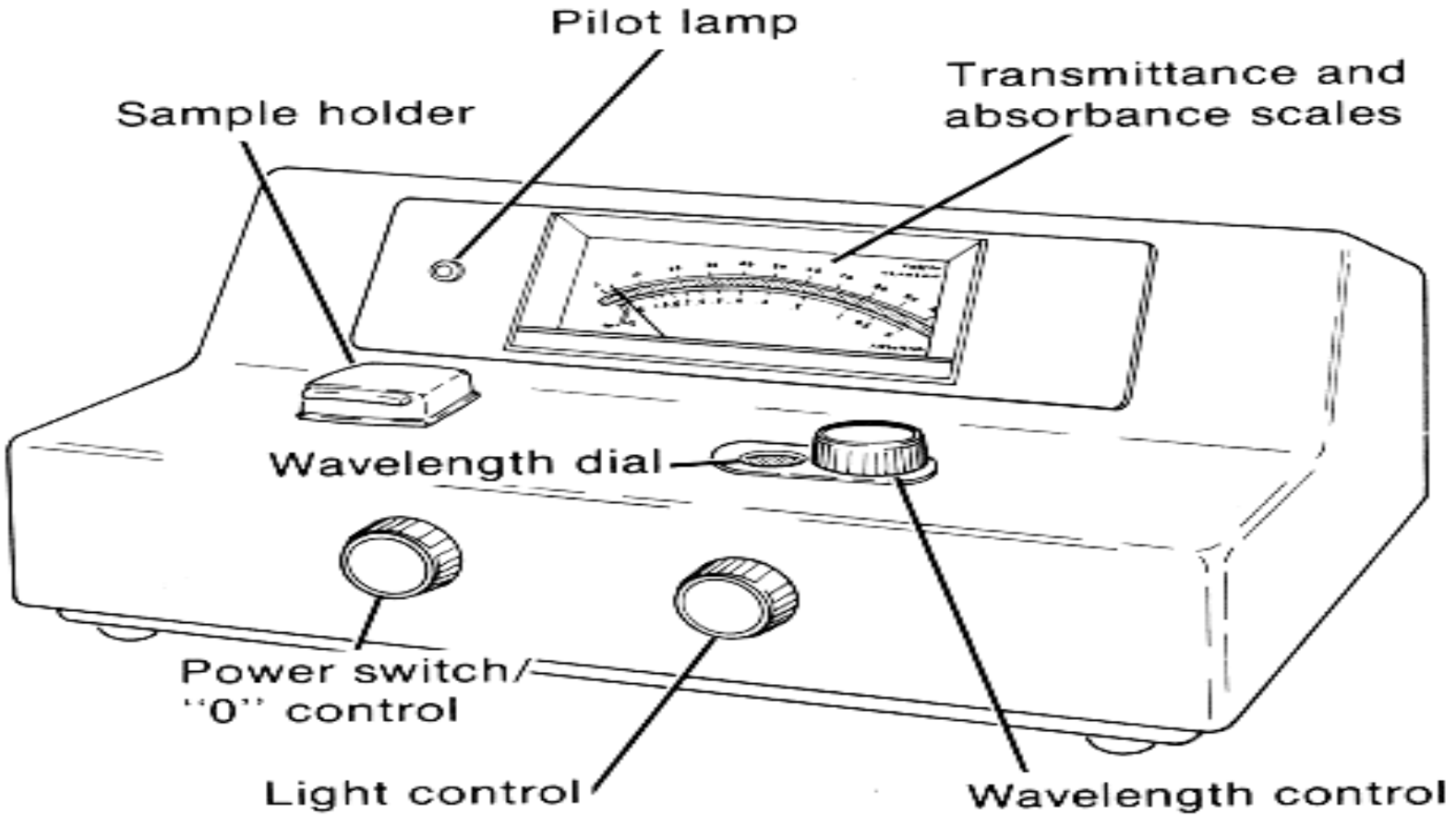
Cell Types I AND II





Detector and Output





Applications -

1. they are used to test for water quality, by screening for chemicals such as chlorine, fluoride, cyanide, dissolved oxygen, iron, molybdenum, zinc and hydrazine.

2. They are also used to determine the concentrations of plant nutrients (such as phosphorus, nitrate and ammonia) in the soil or hemoglobin in the blood

3. To identify substandard and counterfeit drugs.

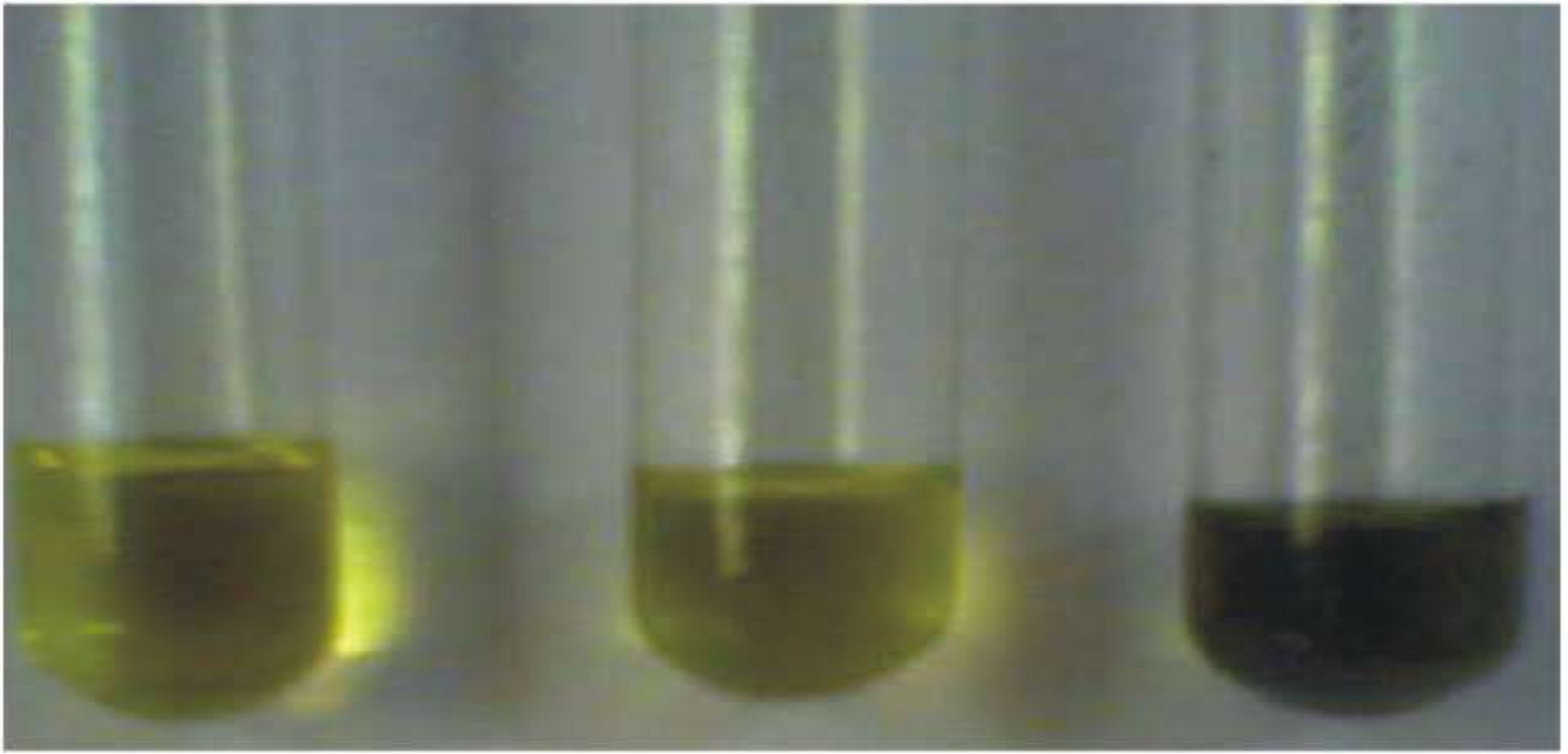
4. In addition, they are used by the food industry and by manufacturers of paints and textiles. In these disciplines, a colorimeter checks the quality and consistency of colors in paints and fabrics, to ensure that every batch comes out looking the same.

Glucose estimation by DNSA method

Biuret test for proteins

Protein estimation by biuret method

Ribose reacts with orcinol reagent gives green colour on heating



Spectrophotometer

What are Spectroscopy and Spectrophotometry??

- Light can either be ***transmitted*** or ***absorbed*** by dissolved substances
- Presence & concentration of dissolved substances is analyzed by passing light through the sample
- Spectroscopes measure electromagnetic ***emission***
- Spectrophotometers measure electromagnetic ***absorption***

❖ INTRODUCTION

- Spectrophotometer is an instrument which measures light absorption as a function of wavelength in the UV as well as visible regions.

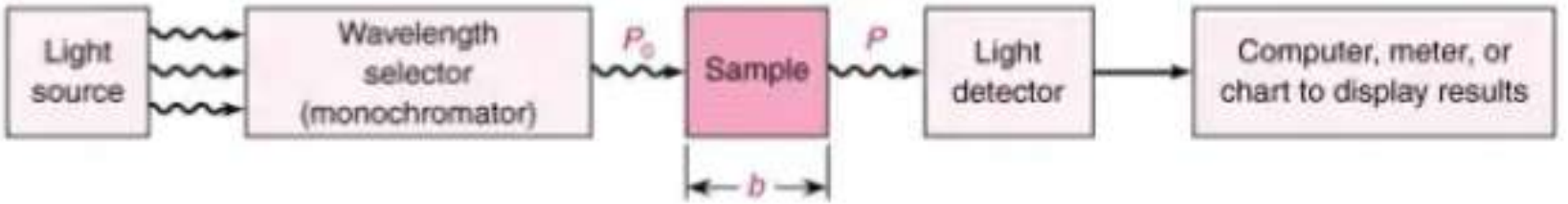
- It also follows essentially the laws of light absorption viz the beer-lambert's law.
- Unlike colorimeters in spectrophotometers the compound can be measured at precise wavelength.

Types of Spectrometry

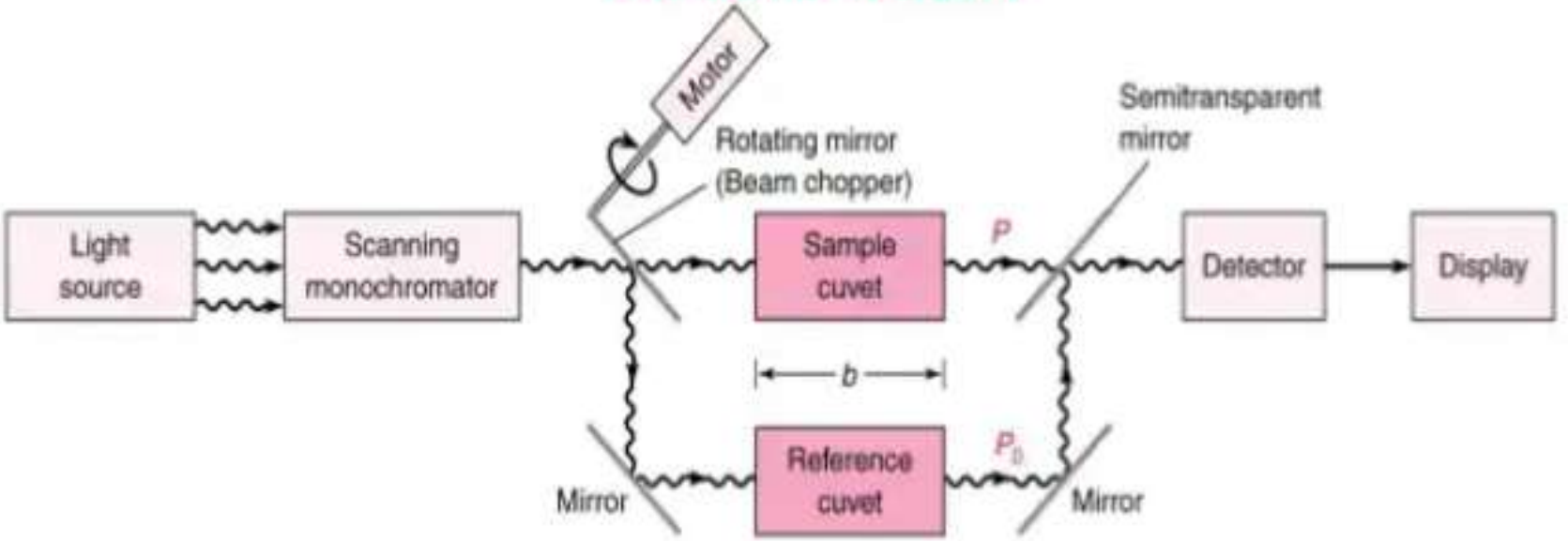
- Mass Spectrometry
  - Measurement along the spectra of mass
- Spectrophotometry
  - Measurement along the spectra of light
- Spectroscopy
  - Interaction of matter and radiated energy
  - Reflectance

The Spectrophotometer

Single-beam



Double-beam



A spectrophotometer is an instrument used to measure absorbance at various wavelengths. It is similar to calorimeter except that it uses prism or diffraction grating to produce monochromatic light. It can be operated in UV (Ultraviolet) region, Visible spectrum as well as IR (Infrared) region of the electromagnetic spectrum.

prism or diffraction grating to produce monochromatic light. It can be operated in UV (Ultraviolet) region, Visible spectrum as well as IR (Infrared) region of the electromagnetic spectrum.

⇒ **Absorption of light** – Light falling on a colored solution is either absorbed or transmitted. A colored solution absorbs all the colors of white light and selectively transmits only one color. This is its own color.

PRINCIPLE OF SPECTROPHOTOMETER

Spectrophotometer is based on the photometric technique which states that When a beam of incident light of intensity I_0 passes through a solution, a part of the incident light is reflected (I_r), a part is absorbed (I_a) and rest of the light is transmitted (I_t)

Thus,

$$I_0 = I_r + I_a + I_t$$

⇒ In photometers (colorimeter &

Beer's Law

⇒ This law states that the amount of light absorbed is directly proportional to the concentration of the solute in the solution.

$Log_{10}\ I_0/I_t = a_s c$

where,

a_s = Absorbency index

c = Concentration of Solution

Lambert's Law

⇒ The Lambert's law states that the amount of light absorbed is

Lambert's Law

⇒ The Lambert's law states that the amount of light absorbed is directly proportional to the length and thickness of the solution under analysis.

$A = \log_{10} I_0/I_t = a_s b$

Where,

A = Absorbance of test

a_s = Absorbance of standard

b = length / thickness of the solution

the solution

The mathematical representation of the combined form of Beer-Lambert's law is as follows:

$$Log_{10}\ I_0 / I_t = a_s bc$$

If *b* is kept constant by applying Cuvette or standard cell then,

$$Log_{10}\ I_0/I_t = a_s c$$

The absorbency index a_s is defined as

$$a_s = A/cl$$

Where,

c = concentration of the absorbing material (in gm/liter).

Where,

c = concentration of the absorbing material (in gm/liter).

l = distance traveled by the light in solution (in cm).

In simplified form,

The working principle of the Spectrophotometer is based on Beer-Lambert's law which states that the amount of light absorbed by a color solution is directly proportional to the concentration of the solution and the length of a light path through the solution.

$A \propto cl$

and the length of a light path through the solution.

$A \propto cl$

Where,

A = Absorbance / Optical density of solution

c = Concentration of solution

l = Path length

or,

$A = \in cl$

$\in$ = Absorption coefficient

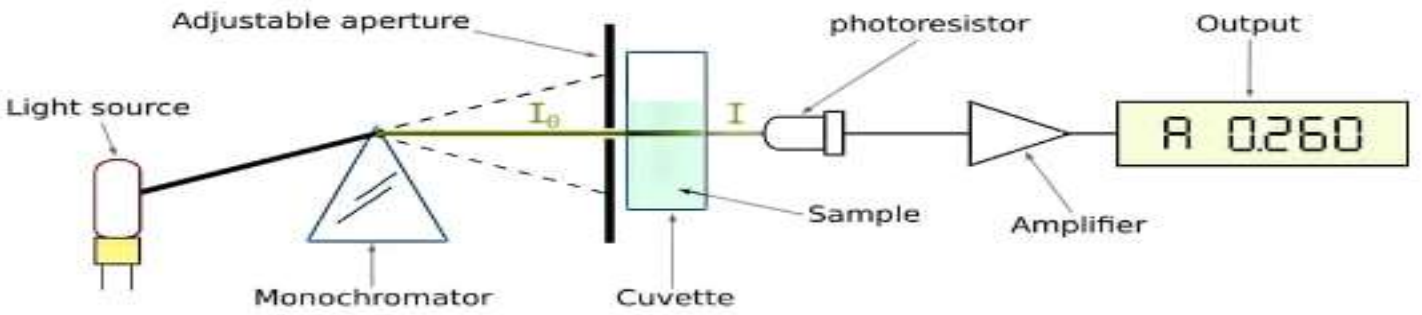
Working and instrumentation

TYPES OF SPECTROPHOTOMETER

Spectrophotometer is of 2 types –

- Single beam spectrophotometer
- Double beam spectrophotometer

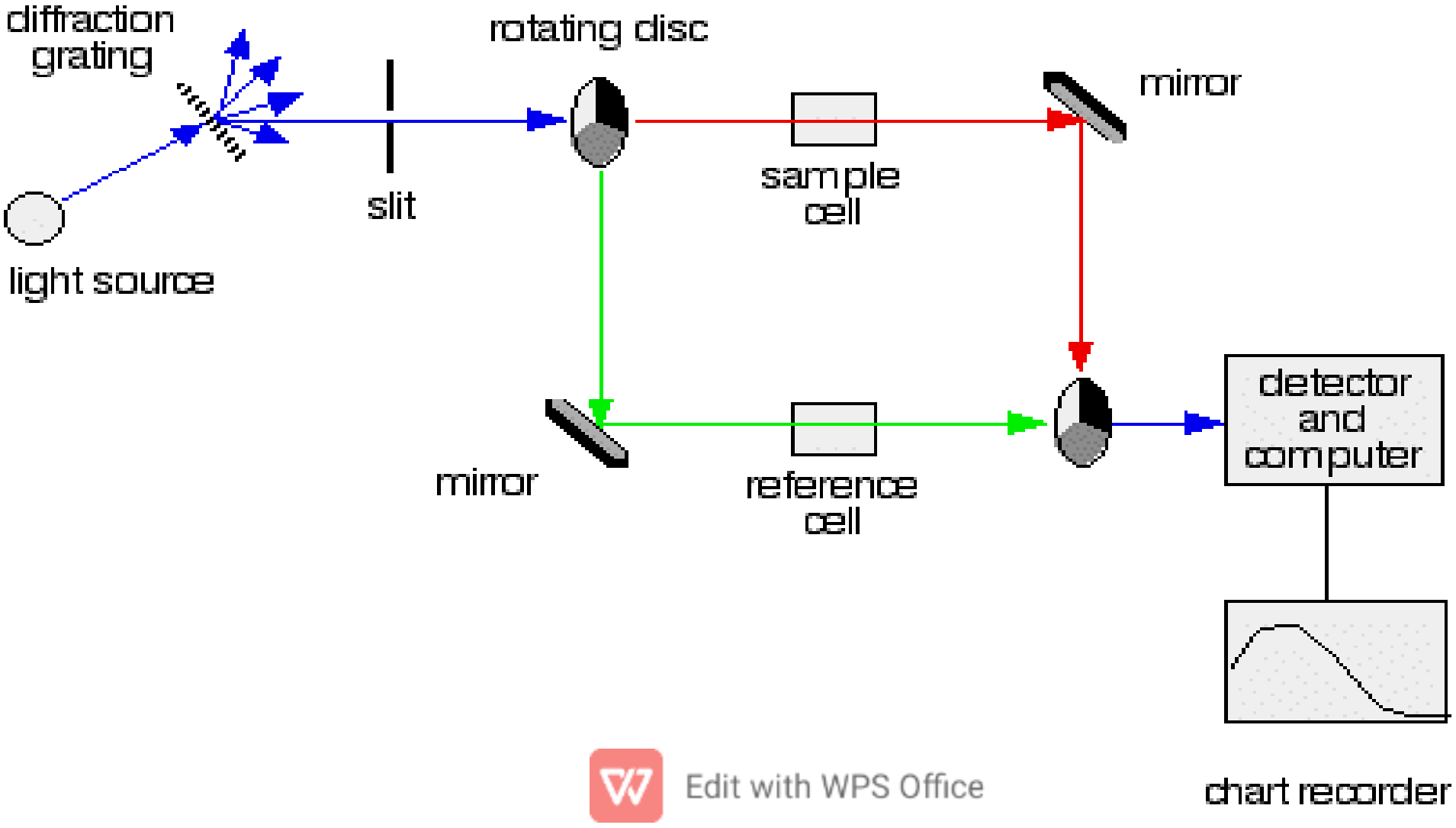
Single beam spectrophotometer operates between 325 nm to 1000 nm wavelength using the single beam of light. The light travels in one direction and the test solution and blank are read in the same.



SINGLE BEAM SPECTROPHOTOMETER

Double beam spectrophotometer operates between 185 nm to 1000 nm wavelength. It has two photocells. This instrument splits the light from the Monochromator into two beams. One beam is used for reference and the other for sample reading. It eliminates the error which occurs due to fluctuations in the light output and the sensitivity of the detector.

Double beam spectrophotometer



PARTS OF SPECTROPHOTOMETER

There are 7 essential parts of a spectrophotometer

Light source — In spectrophotometer three different sources of light are commonly used to produce light of different wavelength. The most common source of light used in the spectrophotometer for the visible spectrum is a tungsten lamp. For **Ultraviolet radiation,** commonly used sources of are the hydrogen lamp and the deuterium lamp. Nernst filament or globar is the most satisfactory **sources** of **IR (Infrared) radiation.**

Monochromator – To select the particular wavelength, prism or diffraction grating is used to split the light from the light source.

Sample holder – Test tube or Cuvettes are used to hold the colored solutions. They are made up of glass at a visible wavelength.

Beam splitter – It is present only in double beam spectrophotometer. It is used to split the single beam of light coming from the light source into two beams.

Mirror – It is also present only and double beam spectrophotometer. It is used to the right direction to the splitted light from the beam splitter.

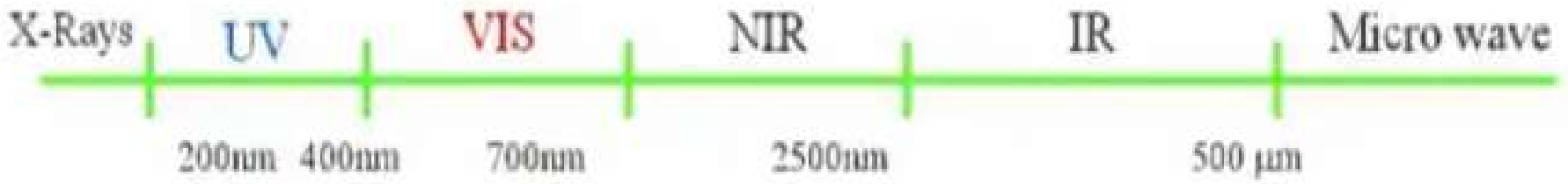
Photodetector system – When light falls on the detector system, an electric current is generated that reflects the galvanometer reading.

Measuring device – The current from the detector is fed to the measuring device – the galvanometer. The meter reading is directly proportional to the intensity of light.

Light Sources:

- This provides a sufficient amount of light which is suitable for making a measurement.
- The light source typically yields a high output of *polychromatic light* over a wide range of the spectrum.

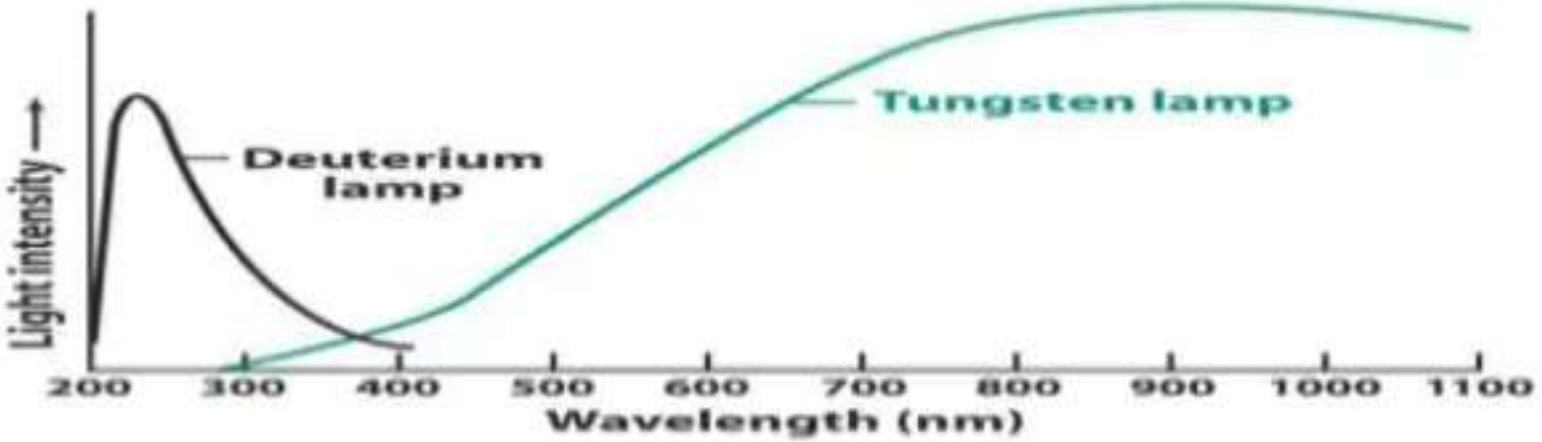
Electromagnetic spectrum:



- Types of light sources used in spectrophotometers include: Incandescent lamps and lasers.

Incandescent Lamps:

- *Tungsten Filament Lamp*: The most common source of visible and near infrared radiation (at wavelength *320 to 2500 nm*)
- Deuterium lamp: Continuous spectrum in the ultraviolet region is produced by electrical excitation of deuterium at low pressure. (*160nm-375nm*)



- Hydrogen Gas Lamp and Mercury Lamp, Xenon (wavelengths from *200 to 800 nm*): high-pressure mecury and xenon arc lamps are commonly used in UV absorption measurements as well as visible light.
- Globar (silicon carbide rod): Infra-Red Radiation at wavelengths: *1200 - 40000 nm*
- NiChrome wire (*750 nm to 20000 nm*); ZrO_2 (*400 nm to 20000 nm*): for IR Region

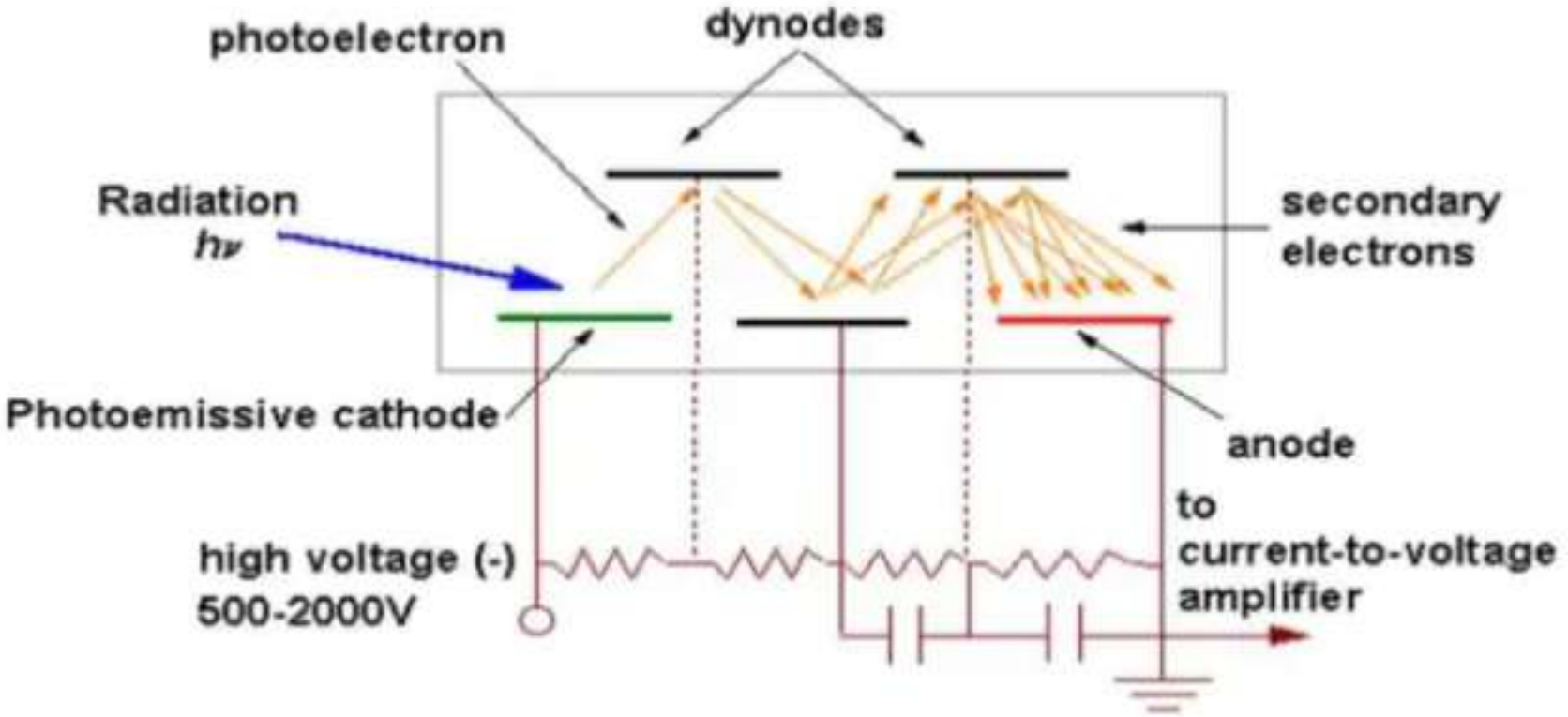
Laser Sources:

- These devices transform light of various frequencies into an extremely intense, focused, and nearly non-divergent beam of *monochromatic* light
- Through selection of different materials, different wavelengths of light emitted by the laser are obtained.
- Used when high intensity line source is required
- Unique properties of laser sources include:
  - Spatial coherence: a property that allows beam diameters in the range of several microns
  - Production of monochromatic light
  - Have pulse widths that vary from microseconds to (flash lamp-pulsed lasers) to nanoseconds (nitrogen lasers), to picoseconds or less (mode-locked lasers)

Photodetectors:

- These are devices that convert light into an electric signal that is proportional to the number of photons striking its photosensitive surface.
- The photocell and phototube are the simplest photodetectors, producing current proportional to the intensity of the light striking them
- The Photomultiplier tube (PMT) is a commonly used photodetector for measuring light intensity in the UV and Visible region of the spectrum. They are extremely rapid, very sensitive and slow to fatigue.

- The PMT consists of:
  - A *photoemissive cathode* (a cathode which emits electrons when struck by photons)
  - Several *dynodes* (which emit several electrons for each electron striking them)
  - An *anode* – Produces an electric signal proportional to the radiation intensity
  - Signal is amplified and made available for direct display
  - A sensitivity control amplifies the signal
  - Examples: Phototube (UV); Photomultiplier tube (UV-Vis); Thermocouple (IR); Thermister (IR)



- Other photodetectors include: Barrier layer cells (photovoltaic cells), Photodiodes,
- Photodiodes are made of photosensitive semi-conductor materials like silicon, gallium, arsenide etc which absorb light over a characteristic wavelength range e.g 250nm to 1100nm for silicon. They are capable of measuring light at a multitude of wavelengths.

Display or Readout Devices:

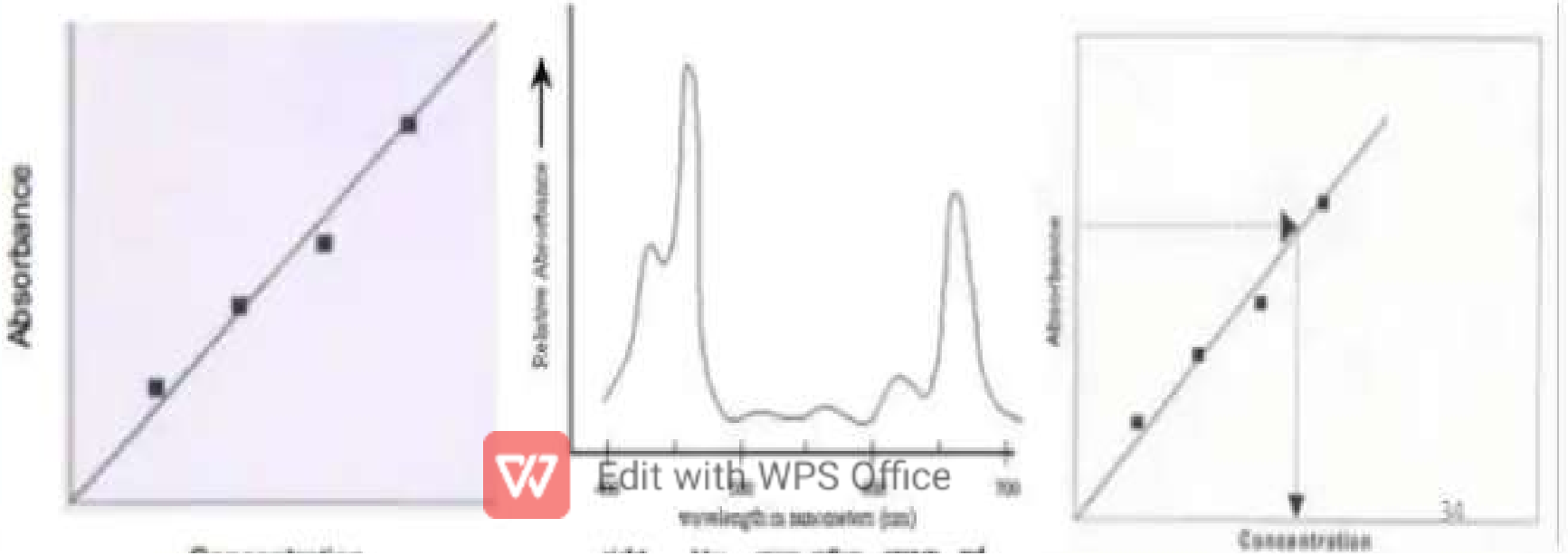
- Electrical energy from the detector is displayed on a meter or readout system such as an analog meter (obsolete), a light beam reflected on a scale, or a digital display, or LCD
- Digital readout devices operate on the principle of selective illumination of portions of a blank of light emitting diodes (LEDs), controlled by the voltage signal generated.
- Compared to meters, digital read out devices have faster response and are easier to read

Applications of spectrophotometer

APPLICATIONS:

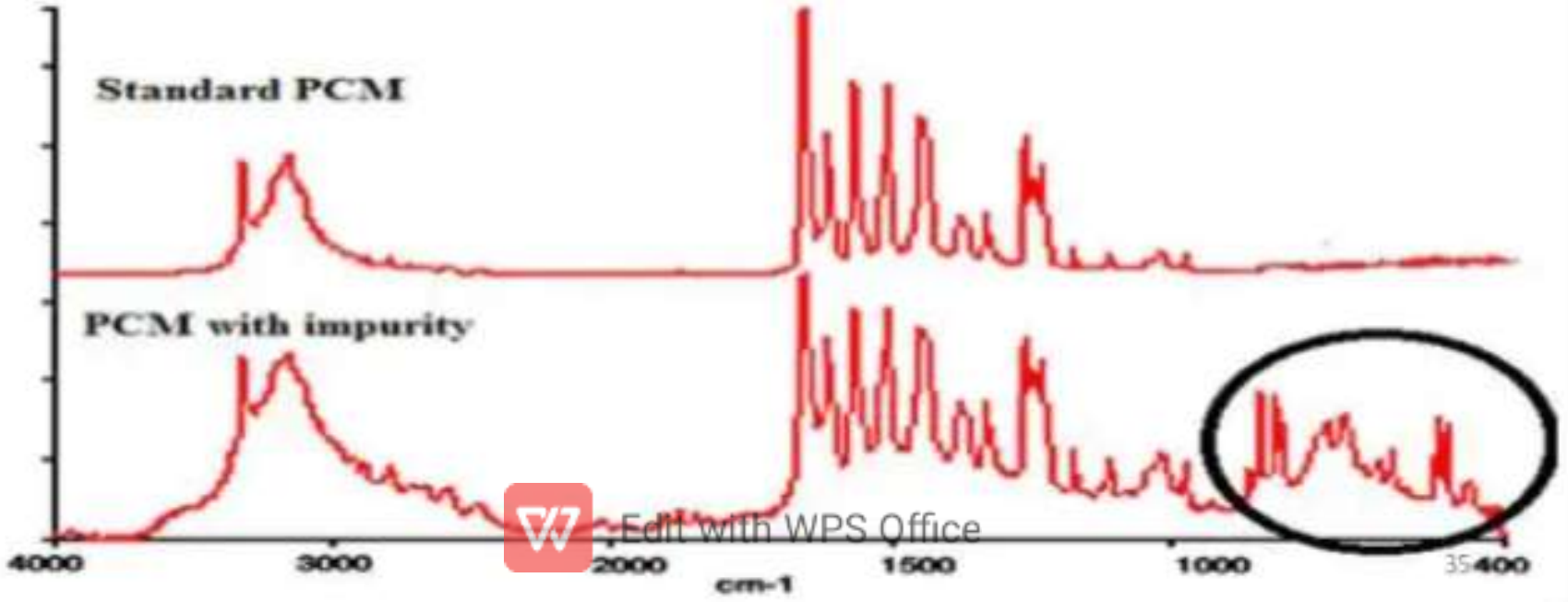
1. Measurement of Concentration:

- Prepare samples
- Make series of standard solutions of known concentrations
- Set spectrophotometer to the λ of maximum light absorption
- Measure the absorption of the unknown, and from the standard plot, read the related concentration



2. Detection of impurities:

- UV absorption spectroscopy is one of the best methods for determination of impurities in organic molecules
- Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material



3. Elucidation of the structure of Organic Compounds:

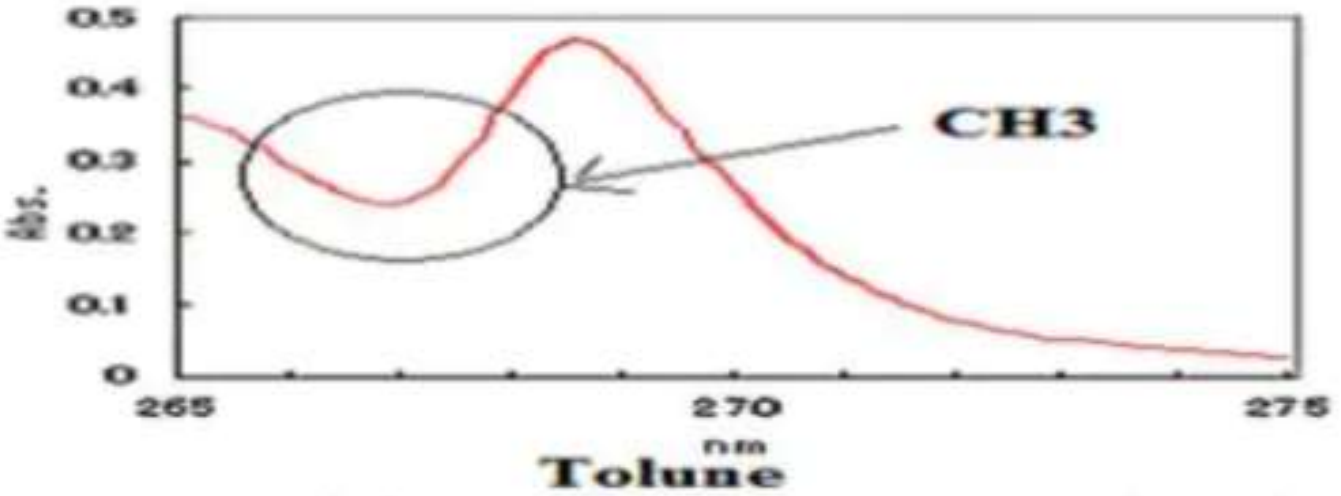
- From the location of peaks and combination of peaks UV spectroscopy elucidate structure of organic molecules:
  - the presence or absence of unsaturation,
  - the presence of hetero atoms

4. Chemical Kinetics:

- Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed

5. Detection of Functional Groups:

- Absence of a band at particular wavelength regarded as an evidence for absence of particular group



6. Molecular weight determination:

- Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds.
- For example, if we want to determine the molecular weight of amine then it is converted in to *amine picrate*

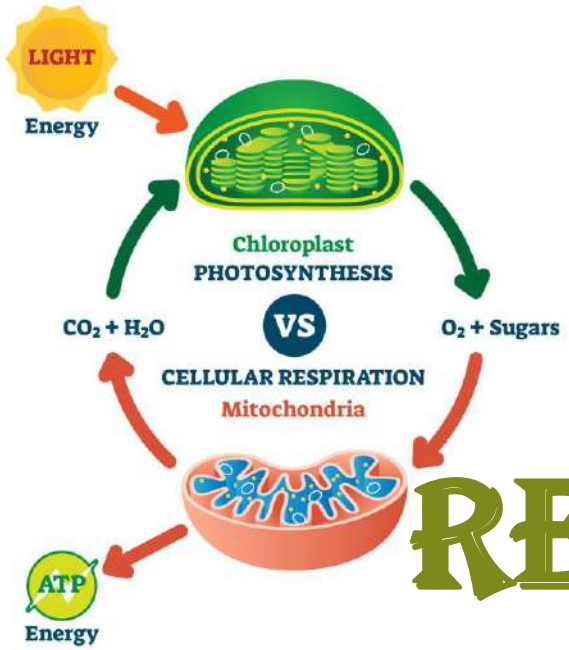
SPECTROPHOTOMETRY

- A photometer (a device for measuring light intensity)
- Measure intensity as a function of the color, or more specifically, the wavelength of light
- Tungsten or xenon flashlamp as the source of white light
- **Tungsten lamp for measurements in visible region(360-900nm)**
- **Hydrogen /deuterium lamp for UV region(200-380nm)**

COLORIMETRY

- The measurement of color
- Any technique used to evaluate an unknown color in reference to known colors
- It determines color based on the red, blue, and green components of light absorbed by the object or sample,
- Colored light beam through an optical filter, which transmits only one particular color / band of wavelengths of light to the photodectector

Thank you



RESPIRATION

Presented by

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Content of topic

a. Aerobic respiration

b. Flow of electron reducing power in ETC.

c. Complexes of Electron Transport Chain

d. Redox potential

e. Components of ETC.

f. Mechanism of ATP generation- Chemiosmotic hypothesis ATP synthase complex

AEROBIC RESPIRATION

The physical and chemical processes (such as breathing and diffusion) by which an organism supplies its cells and tissues with the oxygen needed for metabolism and relieves them of the carbon dioxide formed in energy-producing reactions

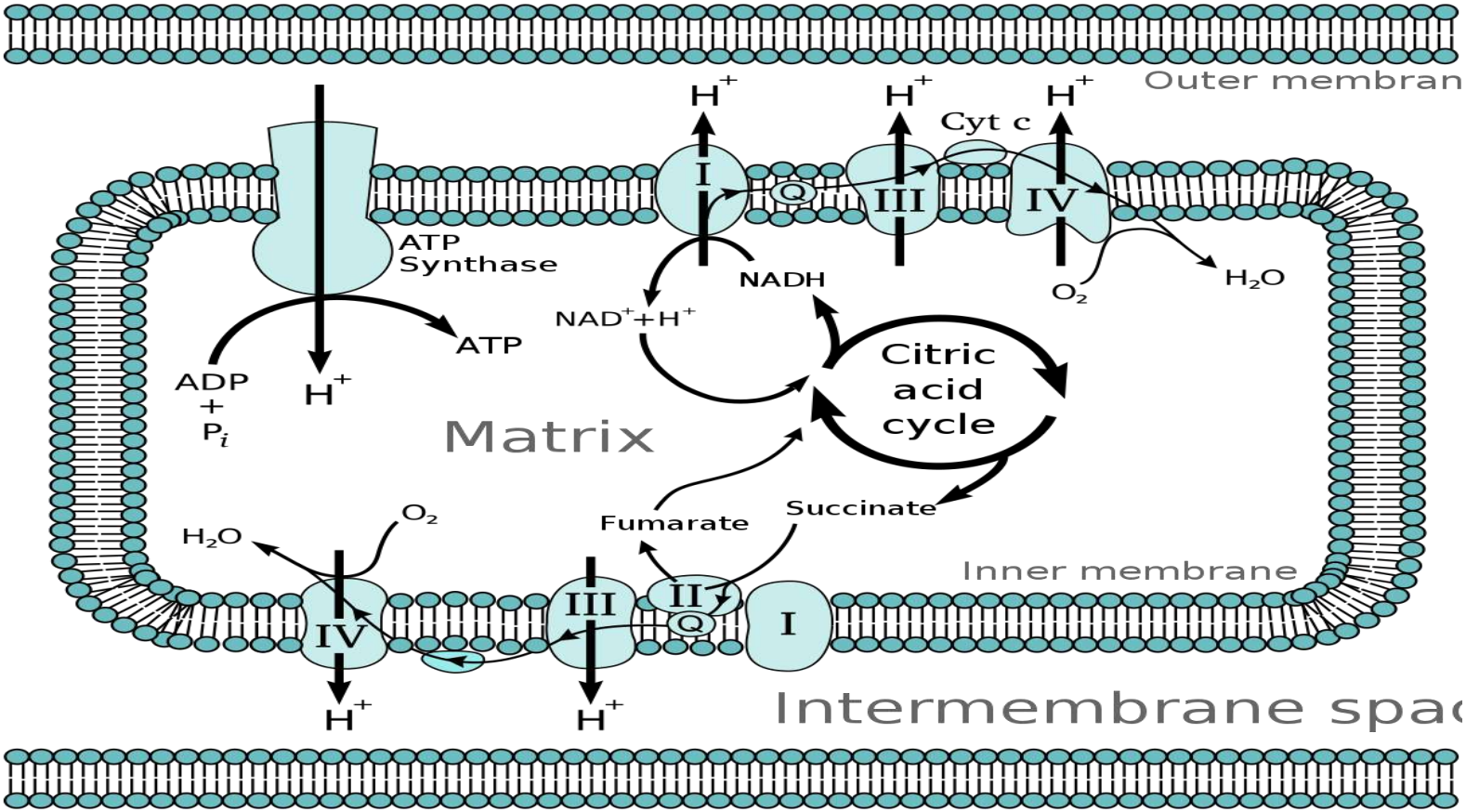
humans use oxygen as the terminal electron acceptor for the ETCs in our cells. This is also the case for many of the organisms we intentionally and frequently interact with (e.g. our classmates, pets, food animals, etc). We breath in oxygen; our cells take it up and transport it into the mitochondria where it is used as the final acceptor of electrons from our electron transport chains. That process - because oxygen is used as the terminal electron acceptor **- is called aerobic respiration.**

many organisms can use a variety of compounds including nitrate (NO_3^-), nitrite (NO_2^-), even iron (Fe^{3+}) as terminal electron acceptors. When oxygen is NOT the terminal electron acceptor, the process is referred to as **anaerobic respiration.**

CELLULAR RESPIRATION

Any of various energy-yielding oxidative reactions in living matter that typically involve the transfer of oxygen and production of carbon dioxide and water as end products

Flow of electron reducing power in ETC



Flow of electron reducing power in ETC

An **electron transport chain**, or **ETC**, is composed of **a group of protein complexes in and around a membrane**.

Which helps energetically couple a series of exergonic/spontaneous red/ox reactions to the endergonic pumping of protons across the membrane to generate an electrochemical gradient.

This electrochemical gradient creates a free energy potential that is termed a **proton motive force** whose energetically "downhill" exergonic flow can later be coupled to a variety of cellular processes.
As previously mentioned, the ETC is composed of a series of protein complexes that undergo a series of linked red/ox reactions. These complexes are in fact multi-protein enzyme complexes referred to as **oxidoreductases** or simply, **reductases**. The one exception to this naming convention is the terminal complex in aerobic respiration that uses molecular oxygen as the terminal electron acceptor. That enzyme complex is referred to as an **oxidase**.

Red/ox reactions in these complexes are typically carried out by a non-protein moiety called a **prosthetic group**. The prosthetic groups are directly involved in the red/ox reactions catalyzed by their associated oxidoreductases.

In general, these prosthetic groups can be divided into two general types: those that carry both electrons and protons and those that only carry electrons.

The electron and proton carriers

•**Flavoproteins (Fp)**, these proteins contain an organic prosthetic group called a **flavin**, which is the actual moiety that undergoes the oxidation/reduction reaction. $FADH_2$ is an example of an Fp.

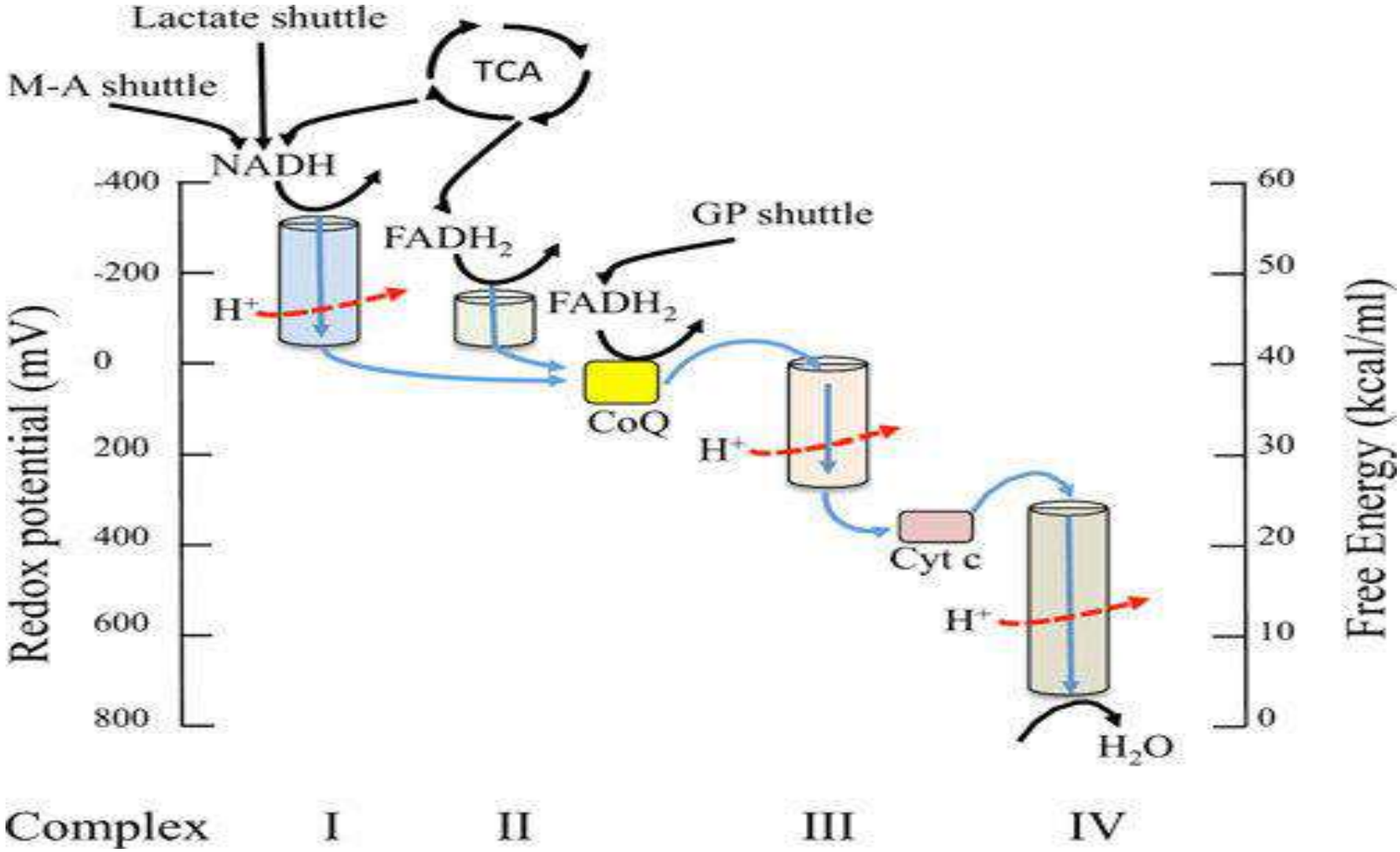
•**Quinones** are a family of lipids, which means they are soluble within the membrane.

•It should also be noted that NADH and NADPH are considered electron ($2e^-$) and proton ($2\ H^+$) carriers.

Electron carriers

•**Cytochromes** are proteins that contain a heme prosthetic group. The heme is capable of carrying a single electron.

•**Iron-Sulfur proteins** contain a nonheme iron-sulfur cluster that can carry an electron. The prosthetic group is often abbreviated as **Fe-S**


Lactate shuttle
M-A shuttle
TCA
NADH
GP shuttle
FADH2
FADH2
H+
CoQ
H+
Cyt c
H+
H2O
-400
-200
0
200
400
600
800
Redox potential (mV)
60
50
40
30
20
10
0
Free Energy (kcal/ml)
Complex
I
II
III
IV

c. Complexes of Electron Transport Chain

1.The process in which electrons are transferred from NADH and FADH2 to oxygen and the energy released in this oxidation- reduction reaction is used to synthesize ATP from ADP is known as Oxidative-phosphorylation.

2.This is high energy yielding reaction, G=52.5 and occur at inner mitochondrial membrane through a series of complexes. Movement of electrons via an array of electron carriers is integrated with translocation of protons (H+) from the mitochondrial matrix to the inter-membrane space.

3. Movement of protons builds the proton-motive force and is responsible for ATP synthesis. There are four complexes through which electrons pass to oxygen and several metal ions or prosthetic groups which are compactly and specifically connected with these complexes

4. In electron transport chain, electrons enter in chain from NADH, move from complex I to complex III and then to complex IV, by passing complex II. The electrons which enter from citric acid intermediate succinate are transferred to FADH2, then to complex III and IV, bypassing complex I.

Complex I (NADH-COQ reductase) –
Electrons from NADH enter into the chain and transfer to COQ by this complex which consists of 40 polypeptide chains.

Firstly, electrons are transferred from NADH to FMN, a cofactor related to FAD, and afterward through FeS carrier finally to Coenzyme Q (COQ).

There is a drop in electric potential-360 mv and the energy released ΔG= -16.6 Kcal/mol.

CoQ is also known as Ubiquinone and acts as a carrier that transports electrons from complex I to complex III.

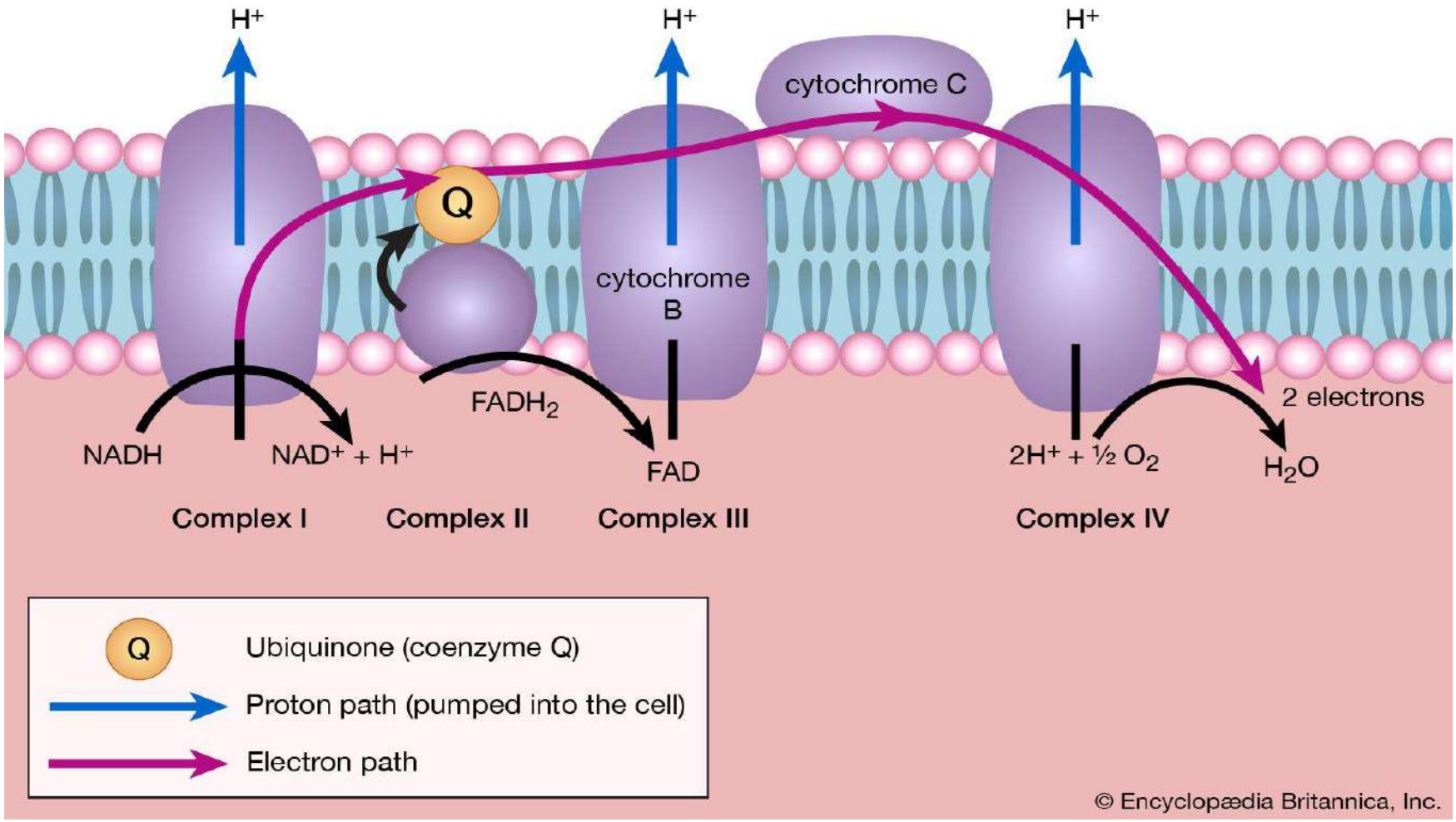
It is the only prosthetic group which is not protein bound.

The oxidized quinone from CoQ can accept e- to form semiquinone.

COQ accepts e- released from complex I or complex II and donates to complex III.

The movement of each pair of electrons by CoQ is linked with the transport of 2 protons from the matrix to intermembrane space fluid.

Electron transport chain



Succinate-CoQ Reductase (complex II)-

is a distinct protein complex, consisting of four polypeptides. Succinate dehydrogenase is one of the four subunits of complex II, involves in the citric acid cycle in the conversion of succinate to fumarate. The two e- released in this conversion are transported to FAD and then to the Fe-S cluster and then to CoQ and finally from CoQ to complex III and complex IV.

CoQH2- Cytochrome c Reductase (complex III) -

consists of ten polypeptides and in this electrons are transferred from cytochrome b to cytochrome c. This is an energy-yielding reaction, $\Delta G = -10.1$ Kcal/mol. Cytochrome c is a water-soluble peripheral protein that diffuses into intermembrane space. Both cytochrome c and COQ act as mobile electron shuttles and transfer electrons between the complexes.

Cytochrome c Oxidase (complex IV) –

Electrons are transferred from cytochrome c to cytochrome c oxidase after reduction of cytochrome c by complex III (see In complex IV, electrons are further transferred, from copper ions (cu2+) to cytochrome a and a3 and then to the final acceptor oxygen and generate water molecule. Two protons are translocated across the mitochondrial membrane during the transportation of each pair of through the complex IV

d. Redox potential

The oxidation–reduction potential or simply redox potential
It is a quantitative measure of tendency of a redox pair to lose or gain electrons.
The redox pairs assigned specific standard redox potential (E0 Volts) at pH 7.0 and 25^0c

Oxidant	Reductant	E_0' (V)
succinate	α-ketoglutarate	−0.67
NAD^+	NADH	−0.320
FAD	$FADH_2$	−0.22
CoQ (ubiqinone)	$CoQH_2$ (ubiquinol)	0.045
cytochrome *b* (Fe^{3+})	cytochrome *b* (Fe^{2+})	0.077
cytochrome *c* (Fe^{3+})	cytochrome *c* (Fe^{2+})	0.254
cytochrome *a* (Fe^{3+})	cytochrome *a* (Fe^{2+})	0.29
O_2	H_2O	0.8166

e. Components of ETC

Complex	Name	No. of Proteins	Prosthetic Groups
Complex I	NADH Dehydrogenase	46	FMN, 9 Fe-S cntrs.
Complex II	Succinate-CoQ Reductase	5	FAD, cyt b_{560}, 3 Fe-S cntrs.
Complex III	CoQ-cyt c Reductase	11	cyt b_H, cyt b_L, cyt c_1, Fe-S_{Rieske}
Complex IV	Cytochrome Oxidase	13	cyt a, cyt a_3, Cu_A, Cu_B

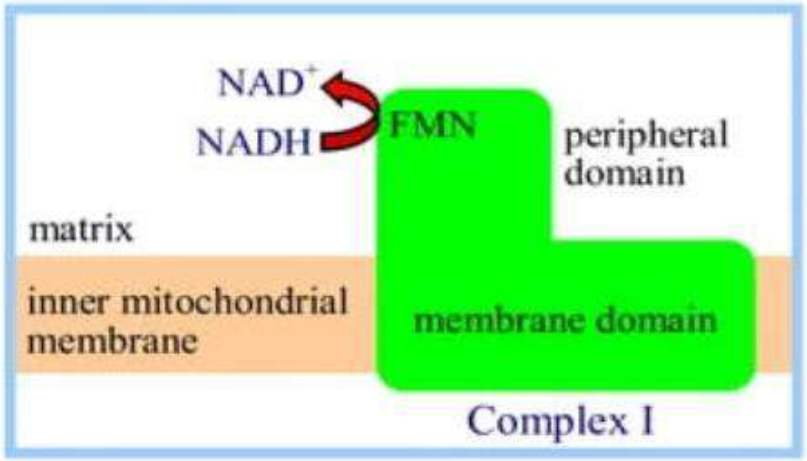
Complex 1 – NADH dehydrogenase

Complex I catalyzes oxidation of NADH, with reduction of coenzyme Q.

$$NADH + H^+ + Q \rightarrow NAD^+ + QH_2$$

It includes at least **46 proteins**, along with prosthetic groups **FMN** & several **Fe-S centers.**

Pumps 4 protons across the mitochondrial membrane.



The initial electron transfers are:

$NADH + H^+ + FMN \rightarrow NAD^+ + FMNH_2$

$FMNH_2 + (Fe\text{-}S)_{ox} \rightarrow FMNH\cdot + (Fe\text{-}S)_{red} + H^+$

After Fe-S is reoxidized by transfer of the electron to the next iron-sulfur center in the pathway:

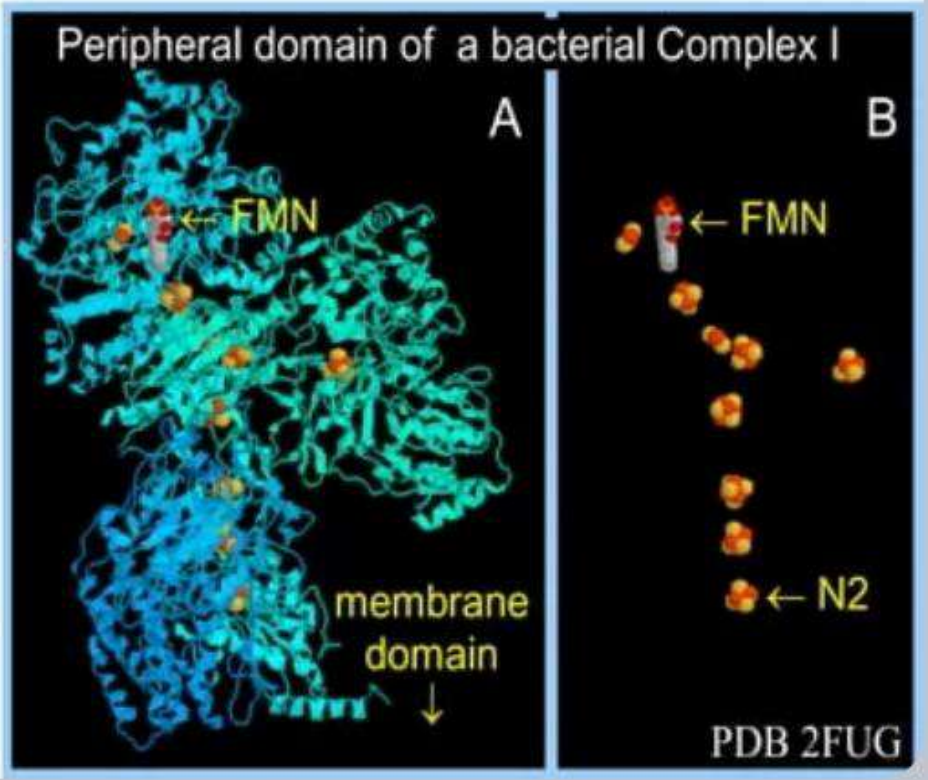
$FMNH\cdot + (Fe\text{-}S)_{ox} \rightarrow FMN + (Fe\text{-}S)_{red} + H^+$

Iron-sulfur centers are arranged as a **wire**, providing a pathway for e^- transfer from **FMN** through the protein

N2, the last Fe-S center in the chain, passes e^- one at a time to the mobile lipid redox carrier **coenzyme Q**.

A proposed **binding site for CoQ** is close to N2 at the interface of peripheral & membrane domains.

Coenzyme Q accepts 2 e^- and picks up 2 H^+ to yield the fully reduced QH_2.



Co enzyme Q (ubiquinone)

- It is a benzoquinone linked to a number of isoprene units.
- **Coenzyme Q** (CoQ, Q, Ubiquione) is very **hydrophobic**. It dissolves in the hydrocarbon core of a membrane.
- 3 redox states-

1. Fully oxidised- Ubiquinone Q
2. Partially oxidised- Semiquinone
3. Fully reduced- Ubiquione

- Only electron carrier that is not a protein bound prosthetic group.

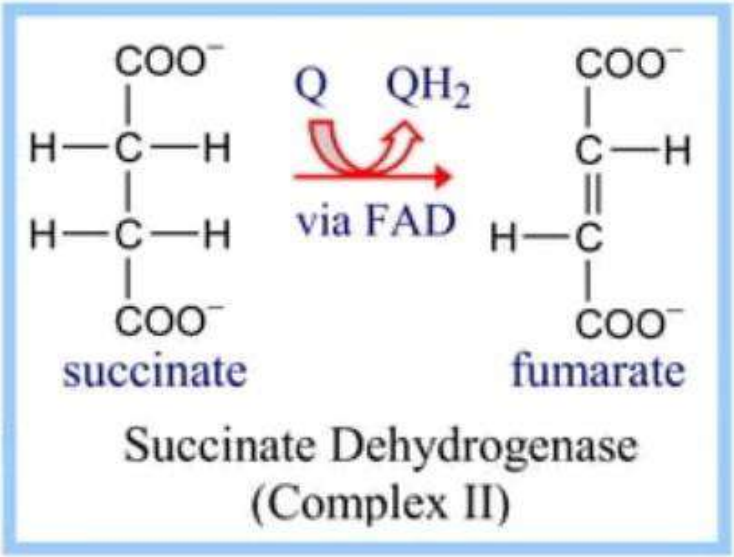
Complex 2- Succinate dehydrogenase

- **Succinate Dehydrogenase** of the Krebs Cycle is also called **complex II** or Succinate-CoQ Reductase.
- Inner mitochondrial membrane bound protein.
- **FAD** is the initial e^- acceptor.

•FAD is reduced to **$FADH_2$** during oxidation of succinate to fumarate.

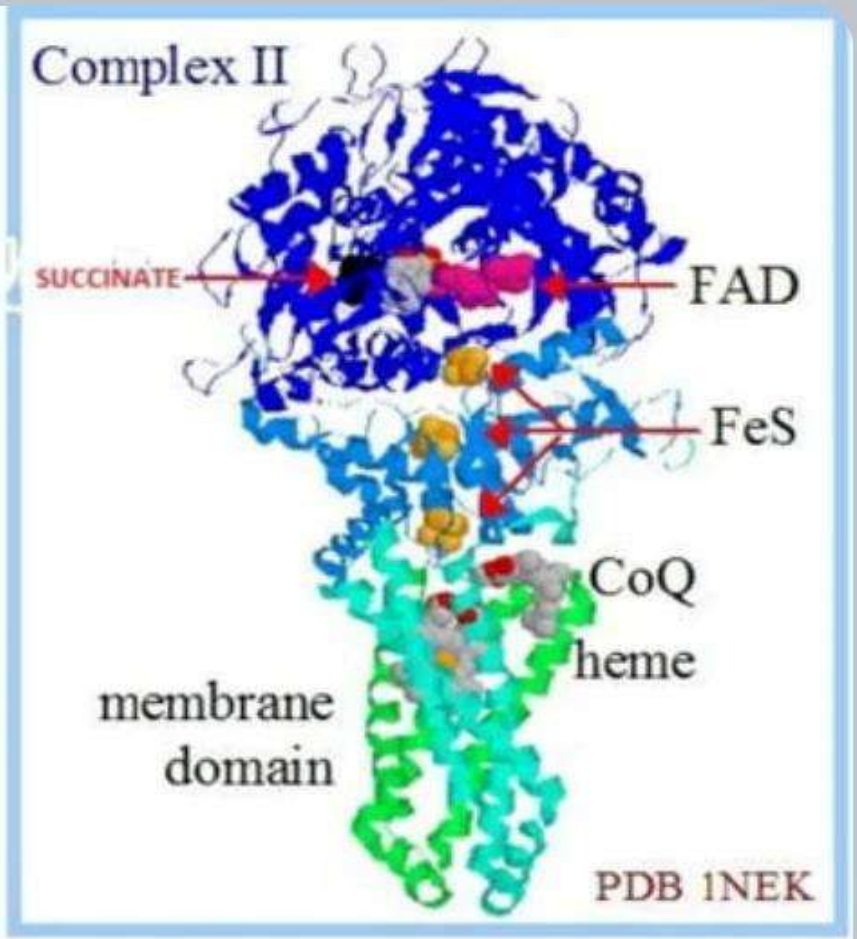
•$FADH_2$ is then reoxidized by transfer of electrons through a series of 3 iron-sulfur centers to CoQ, yielding **QH_2**.

•It does not pump any proton during transport of electron across the inner mitochondrial membrane.



Succinate Dehydrogenase (Complex II)

- X-ray crystallographic analysis of *E. coli* complex II indicates a **linear** arrangement of electron carriers within complex II, consistent with the predicted sequence of electron transfers:



FAD → $FeS_{center\ 1}$ → $FeS_{center\ 2}$ → $FeS_{center\ 3}$ → CoQ

Complex 3- Coq-Cyt Reductase

- **Complex III** accepts electrons from coenzyme QH_2 that is generated by electron transfer in complexes I & II.
- Concominantly, it releases two protons into transmembrane space.
- Within complex 3,the released electrons are transferred to an iron sulfur center and then to two b-type cytochromes or cytochrome c_1.
- Finally the two electrons are transferred to two molecules of the oxidised form of cytochrome c. two additional protons are translocated from mitochondrial matrix across the intermembrane space. This transfer of protons involves the proton motive ***Q cycle***.

Complex 4- Cytochrome Oxidase

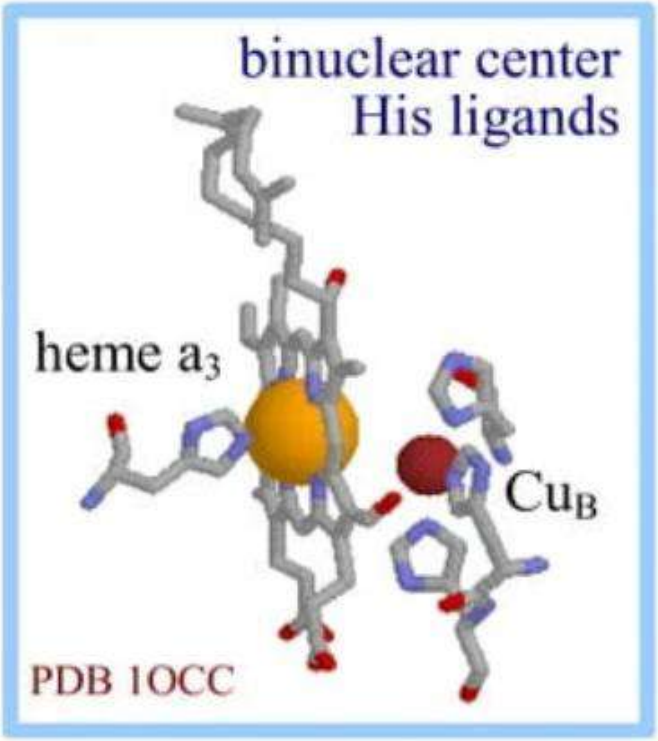
- It catalyses the transfer of electrons from reduced cyt c to molecular oxygen.
- Contains 13 subunits
- 2 heme groups i.e. heme a & heme a_3
- 3 copper ions arranged as 2 copper centers designated as Cua & Cub.
- Cua contain 2 copper ions linked by 2 bridging disulfide residues.
- Cub is coordinated by 3 histidine residues.
- Two protons per pair of electron are pumped across the membrane and another two protons are transferred to molecular oxygen to form water.

- **Metal centers** of cytochrome oxidase (complex IV):
- heme a & heme a_3,
- Cu_A (2 adjacent Cu atoms) & Cu_B.

- O_2 reacts at a **binuclear center** consisting of heme a_3 and Cu_B.

•Electrons enter complex IV one at a time from cyt c to **Cu_A**.

•They then pass via **cyt a** to the **binuclear center** where the chemical reaction takes place.



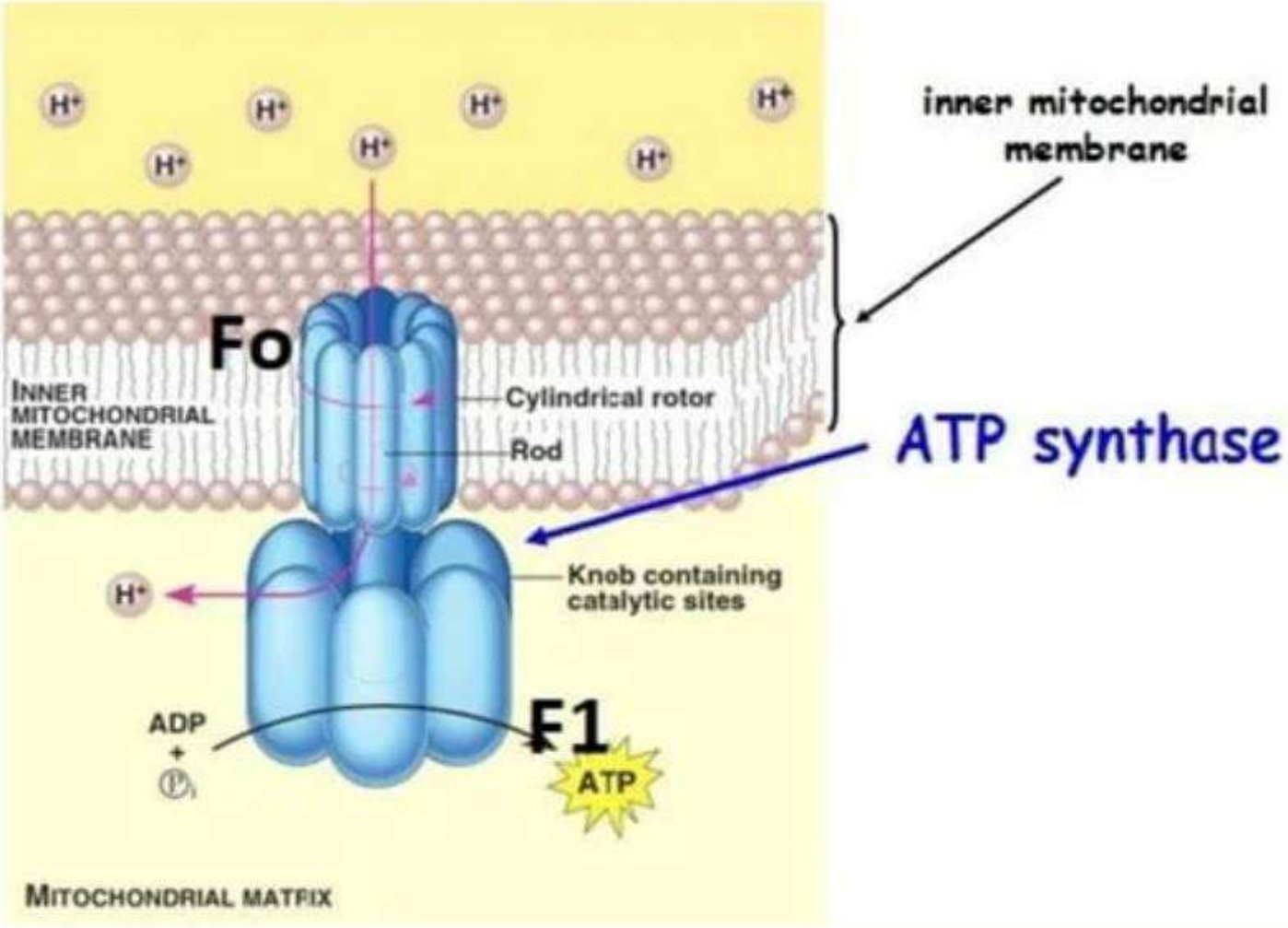
e^- transfer: cyt c → Cu_A → cyt a → heme a_3/Cu_B → O_2

Complex 5- ATP Synthase

- Mitochondrial ATP synthase consist of two multisubunit components F_0 and F_1 which are linked by a slender stalk.
- F_0 is a elecrically driven motor that spans the lipid bilayer foming a channel through which protons can cross the membrane.
- F_0 provides channel for protons.

- F_1 harvest the free energy derieved from proton movement down the electrochemical gradient by catalyzing the synthesis of ATP.
- F_1 Phosphorylates ADP to ATP.

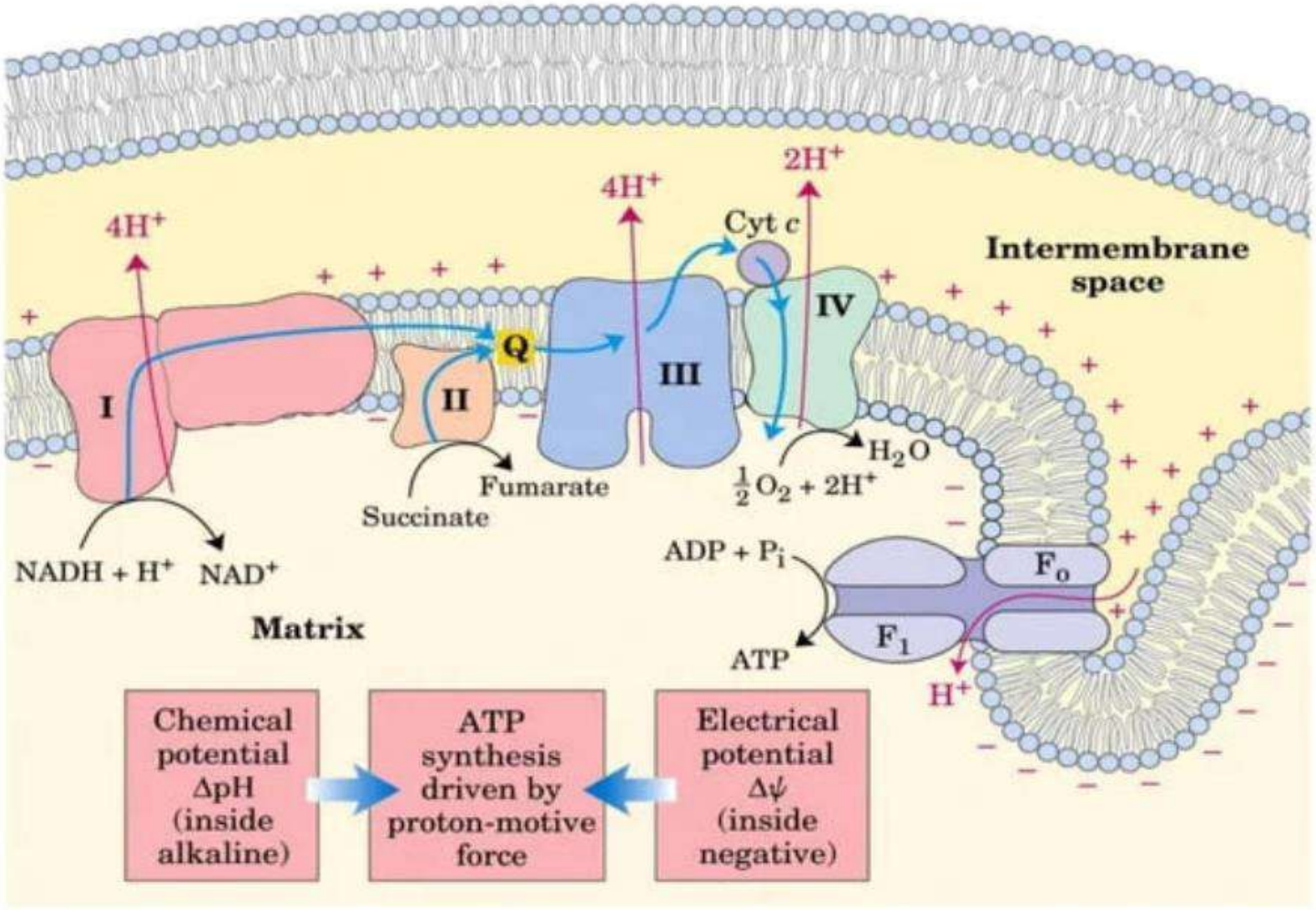
ATP SYNTHASE



CHEMIOSMOTIC HYPOTHESIS

- Proposed by **PETER MITCHELL** in 1961.
- This hypothesis couples electron transport to ATP generation.
- Mitchell suggested that ATP is generated by use of energy stored in the form of proton gradient across biological membranes rather than by direct chemical transfer of high energy groups.
- Complex 1 and 4 appear as proton pump which transport protons across the membrane due to conformational change induced by electron transfer.
- In Complex 3 protons are carried across the membrane by Ubiquione.
- Complex 1 and 3 pump four protons per pair of electrons.
- Complex 4 pumps two protons per pair of electrons transported and other two protons are combined with oxygen to form water.

Summary


4H+
4H+
2H+
Cyt c
Intermembrane space
I
II
Q
III
IV
Fumarate
Succinate
H2O
½ O2 + 2H+
NADH + H+
NAD+
Matrix
ADP + Pi
ATP
Fo
F1
H+
Chemical potential ΔpH (inside alkaline)
ATP synthesis driven by proton-motive force
Electrical potential Δψ (inside negative)

Summary of ATP synthesis

PATHWAY	NADH	FADH2	ATP
GLYCOLYSIS	2	0	2
KREBS CYCLE	8	2	2
TOTAL	10	2	4
TOTAL ATP	25	3	4

1 NADH

$$10\ H^+ \times \frac{1\ ATP}{4\ H^+} = 2.5\ ATP$$

1 $FADH_2$

$$6\ H^+ \times \frac{1\ ATP}{4\ H^+} = 1.5\ ATP$$

Total ATP from Mitochondrial matrix

Pyruvate dehydrogenase

NADH .....2.5 ATP

Krebs

3 NADH X 2.5 ATP/NADH .....7.5 ATP

$FADH_2$ X 1.5 ATP / $FADH_2$.....1.5 ATP

GTP X 1 ATP / GTP .....1.0 ATP

(from a separate reaction)

Total

.....12.5 ATP

(Per glucose = X 2 = 25 ATP)

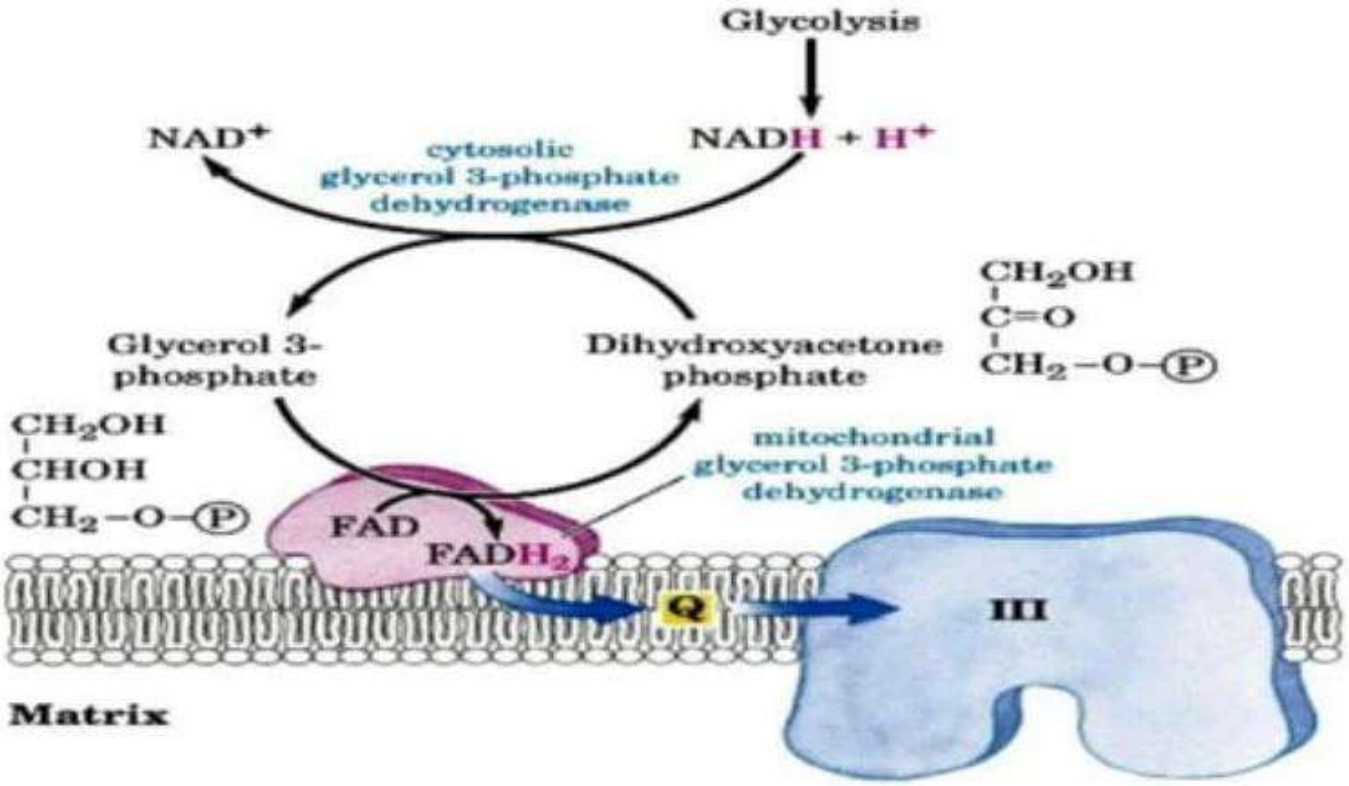
What about NADH from glycolysis?

- NADH made in cytosol
- Can't get into matrix of mitochondrion

- 2 mechanisms
  1. In muscle and brain
     Glycerol phosphate shuttle
  2. In liver and heart
     Malate / aspartate shuttle

Glycerol Phosphate shuttle

In muscle and brain, the glycerol-P shuttle brings cytoplasmic NADH to the ETS

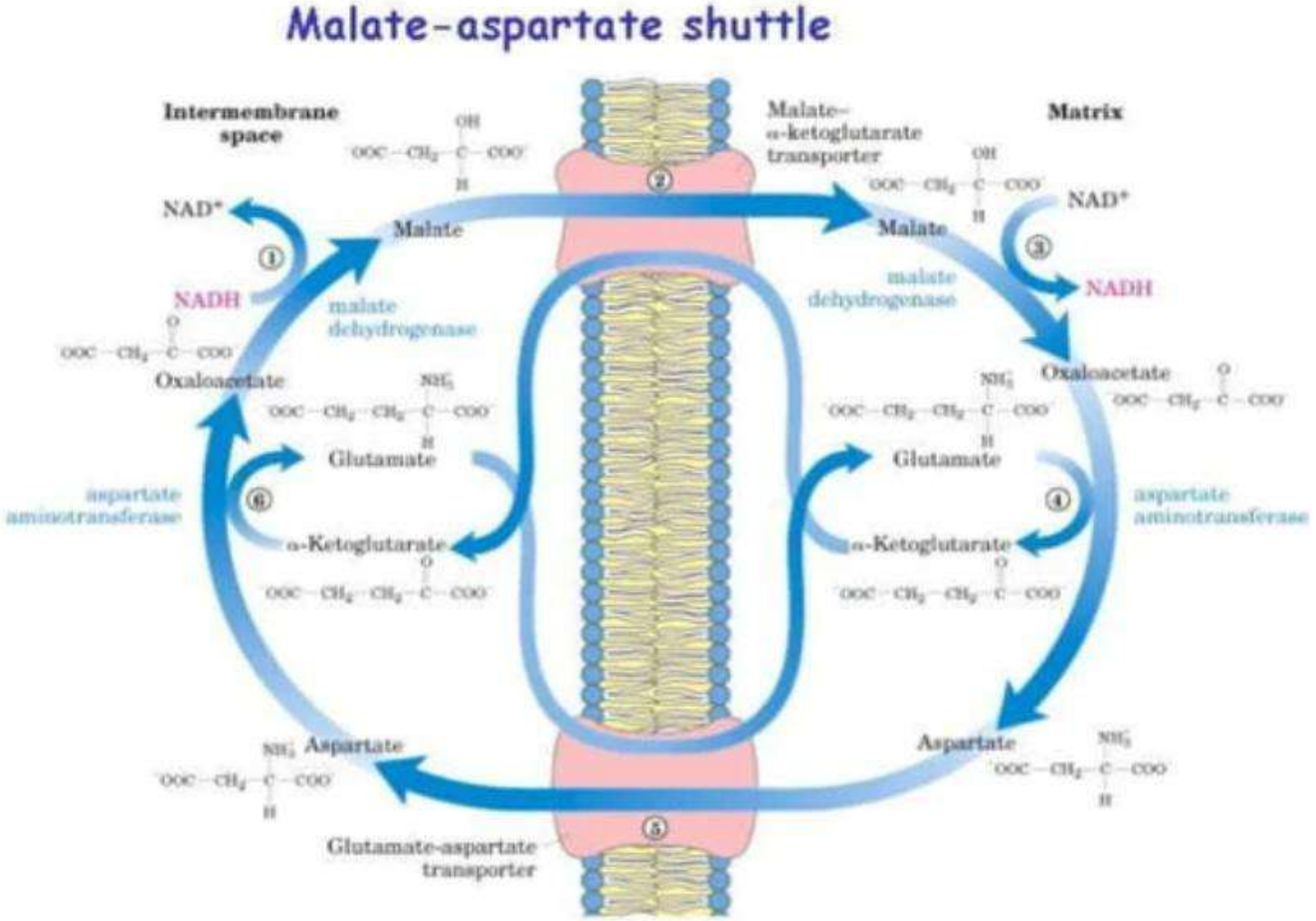


Glycerol phosphate shuttle

- In muscle and brain
- Each NADH converted to $FADH_2$ inside mitochondrion
  - $FADH_2$ enters later in the electron transport chain
  - Produces 1.5 ATP
- Gycerol phosphate shuttle
  - 2 NADH per glucose -→ 2 $FADH_2$
  - 2 $FADH_2$ X 1.5 ATP / $FADH_2$.....3.0 ATP
  - 2 ATP in glycoysis .....2.0 ATP
  - From pyruvate and Krebs
    - 12.5 ATP X 2 per glucose .....25.0 ATP

Total = 30.0 ATP/ glucose

Malate – Aspartate Shuttle



Malate – Aspartate Shuttle in Cytosol

- In liver and heart
- NADH oxidized while reducing oxaloacetate to malate
  - Malate dehydrogenase
- Malate crosses membrane

Total ATP per glucose in liver and heart

- Malate – Aspartate Shuttle
  - 2 NADH per glucose -→ 2 NADH
  - 2 NADH X 2.5 ATP / NADH.....5.0 ATP
  - 2 ATP from glycolysis.....2.0 ATP
  - From pyruvate and Krebs
    - 12.5 ATP X 2 per glucose .....25.0 ATP

Total = 32.0 ATP/ glucose

INHIBITORS OF ETC

- **ROTENONE** – Complex 1
- **AMYTAL** – Complex 1
- **Piericidin** – competes with CoQ
- **Antimycin A** – Complex 3
- **Cyanide**, **Azide**, **Carbon monoxide** – Bind with complex 4 and inhibit transfer of electrons to oxygen

Uncouplers of ETC

- 2,4 Dinitrophenol
- Dicoumarol
- Carbonyl cyanide p-flouromethoxyphenylhydrazone(FCCP)

Site of ETC - Mitochondria

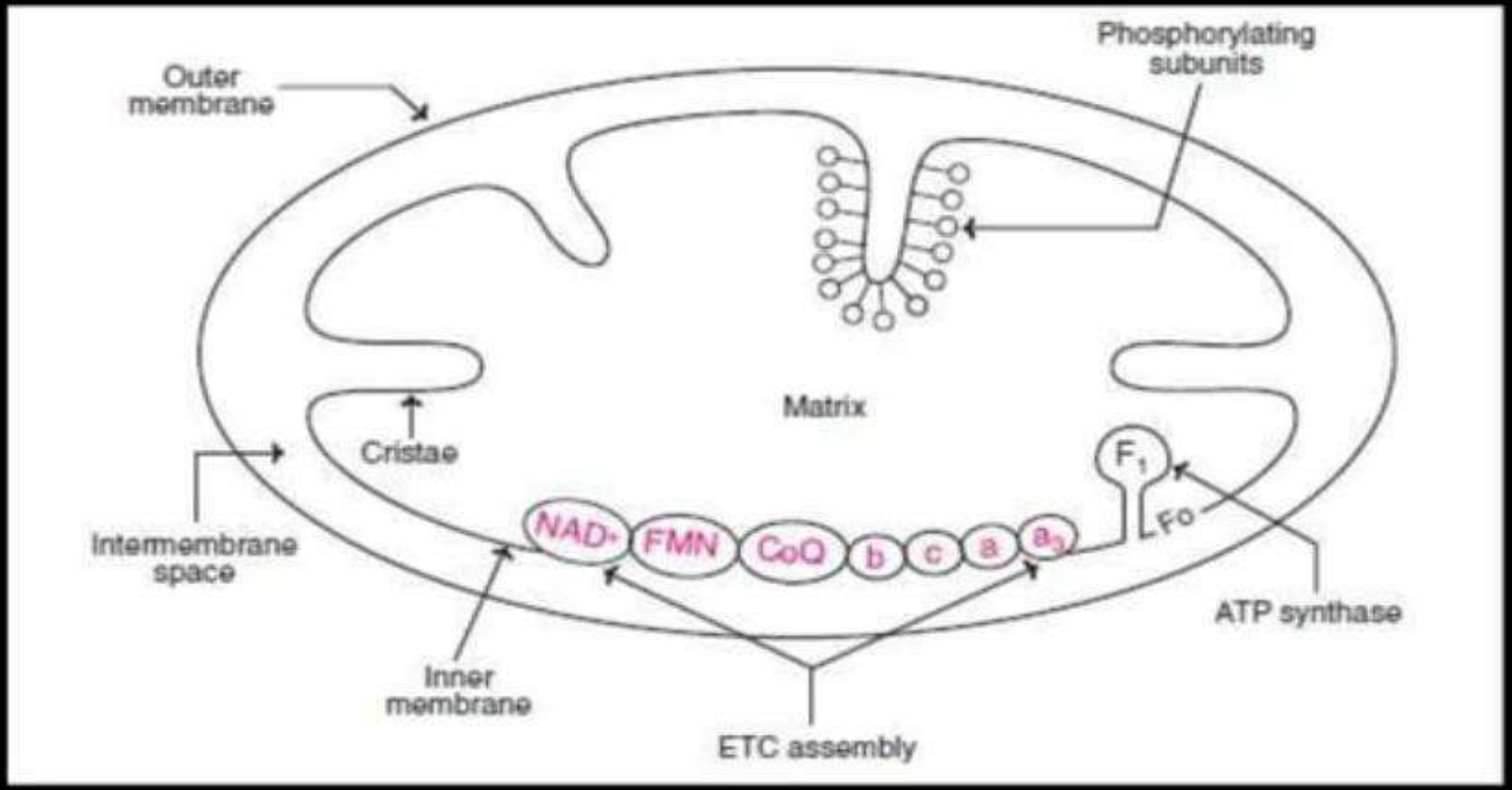


Fig 1 : Mitochondria

Carriers in ETC

- Hydrogen carrier :
  1. Flavoprotien
  2. Ubiquinone or Co-enzyme Q
- Electron carrier :
  1. Iron sulphur protein
  2. Cytochrome

HYDROGEN CARRIERS

1) FLAVOPROTEIN

- Protein tightly bound to FAM & FAD are called f**alvoprotein.**
- It is capable of accepting or donating one or two e^-.

2) Ubiquinone or Co-enzyme Q

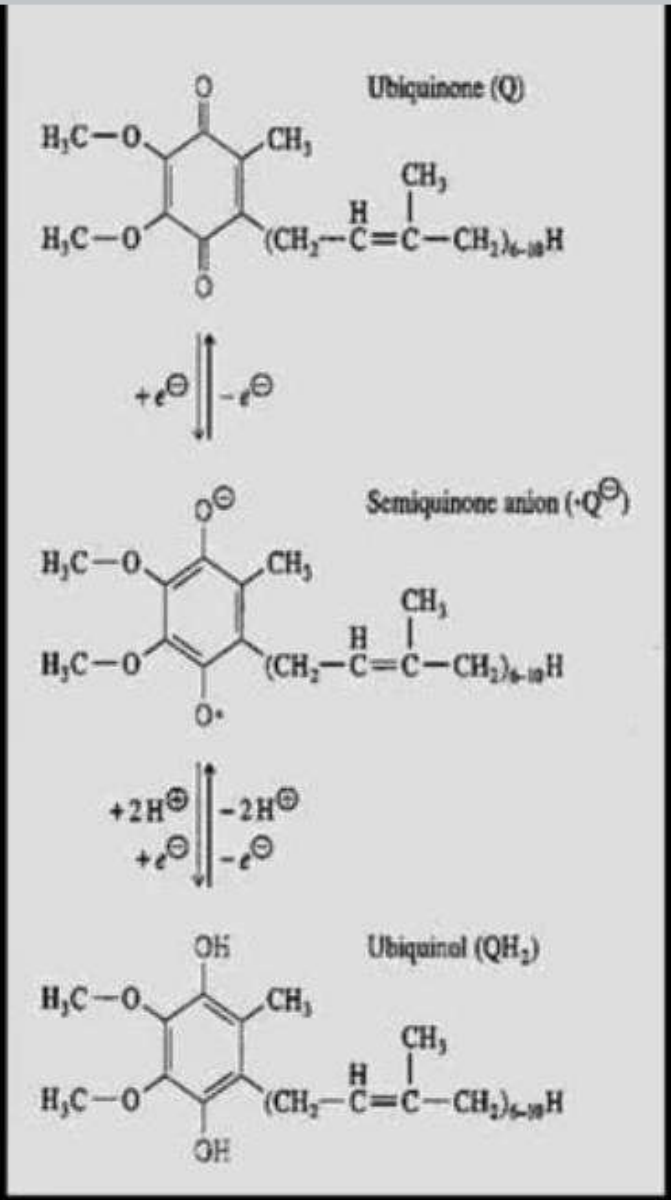


Fig 3 : Ubiquinone

- Is a lipid-soluble benzoquinon with a long isoprenoid side chain.
- Accept one electron to become the semiquinone radical (QH) or two electrons to form ubiquinol (QH2).
- It plays a central role in coupling electron flow to proton movement.

ELECTRON CARRIER

1) Iron sulphur proteins :

- Containing Fe atom co-ordinate to sulphur of either Cys residue of protein or also with inorganic sulphur atoms.
- The Fe atoms are oxidised or reduced.
- There are 8 different Fe-S protein that function in mitochondrial e^- transfer.

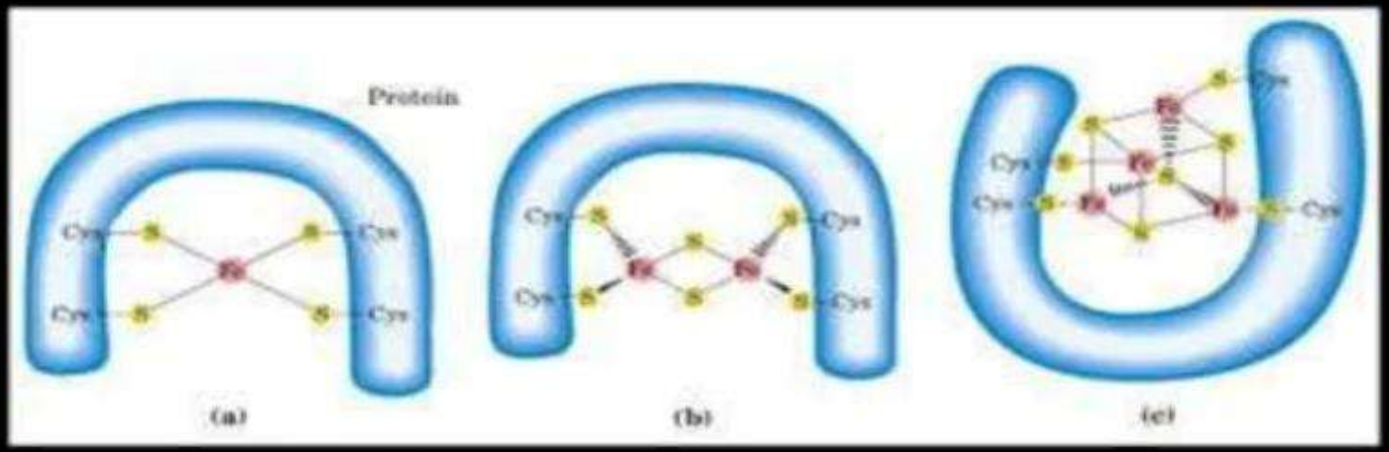


Fig 4 : Fe-S protein

2) Cytochromes :

- A proteins with a Fe-containing heme prostatic group, absorb light in visible range.
- Major respiratory Cytochromes- *b, c or a.*
- In ETC-

T
T
T
C cell
c

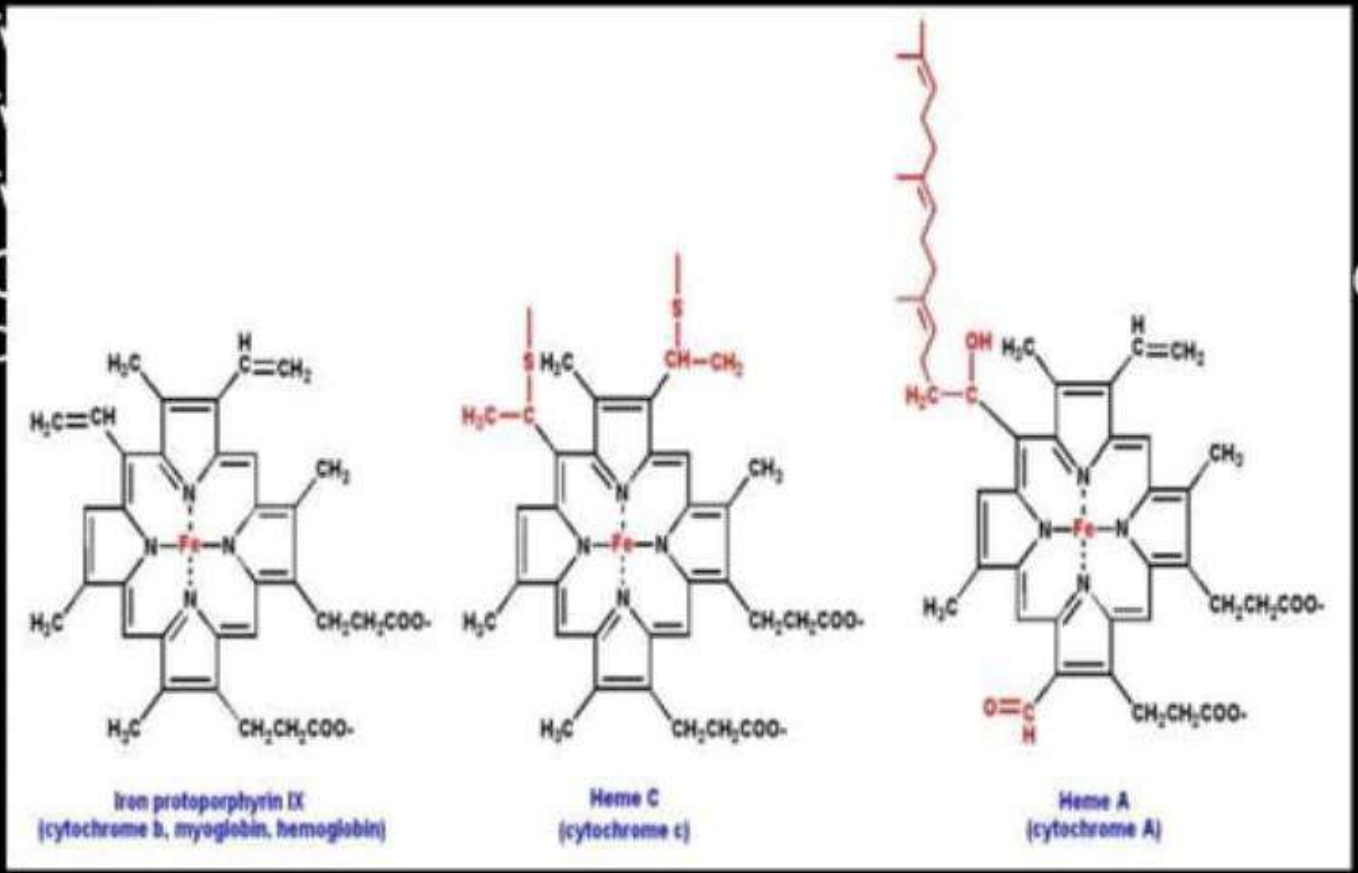


Fig 5 : Cytochrome b,c,a

INHIBITORS OF ETC

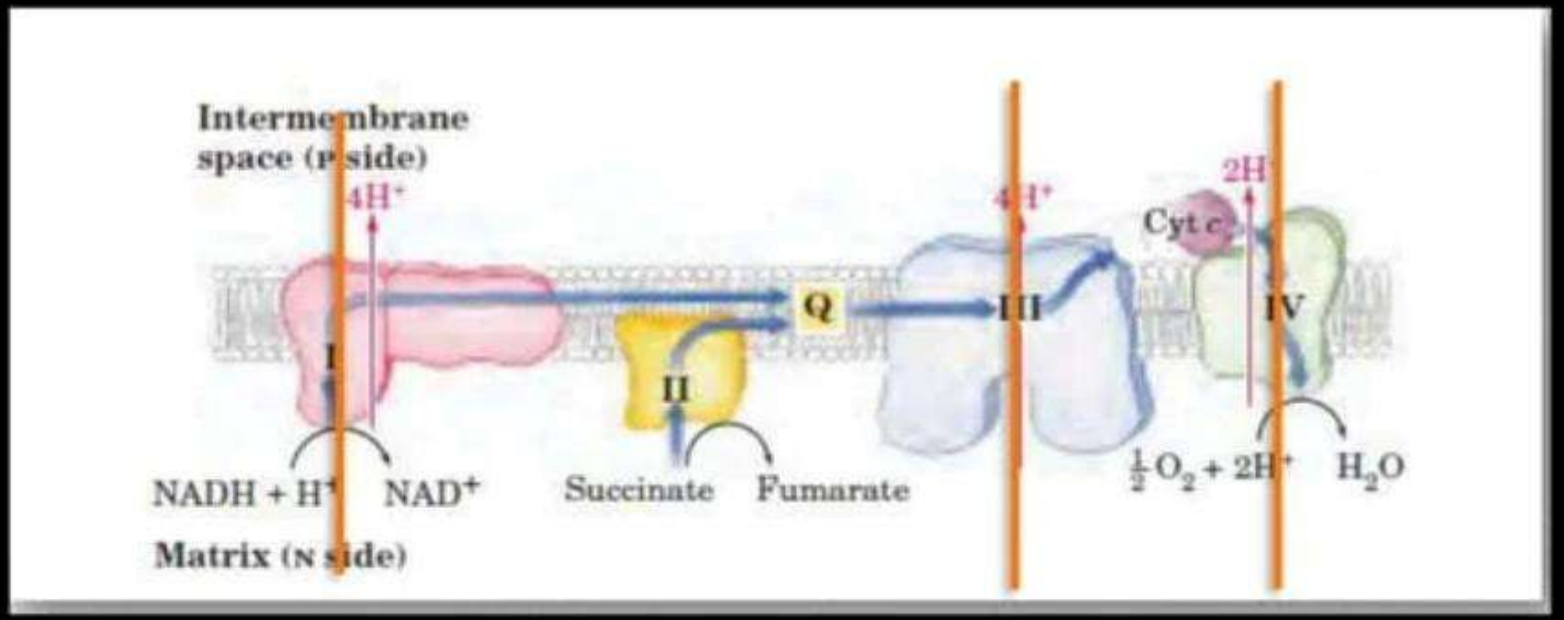


Fig 13 : Inhibitor oh ETC

BLOCKING ETC

COMPLEX I

COMPLEX III

COMPLEX IV

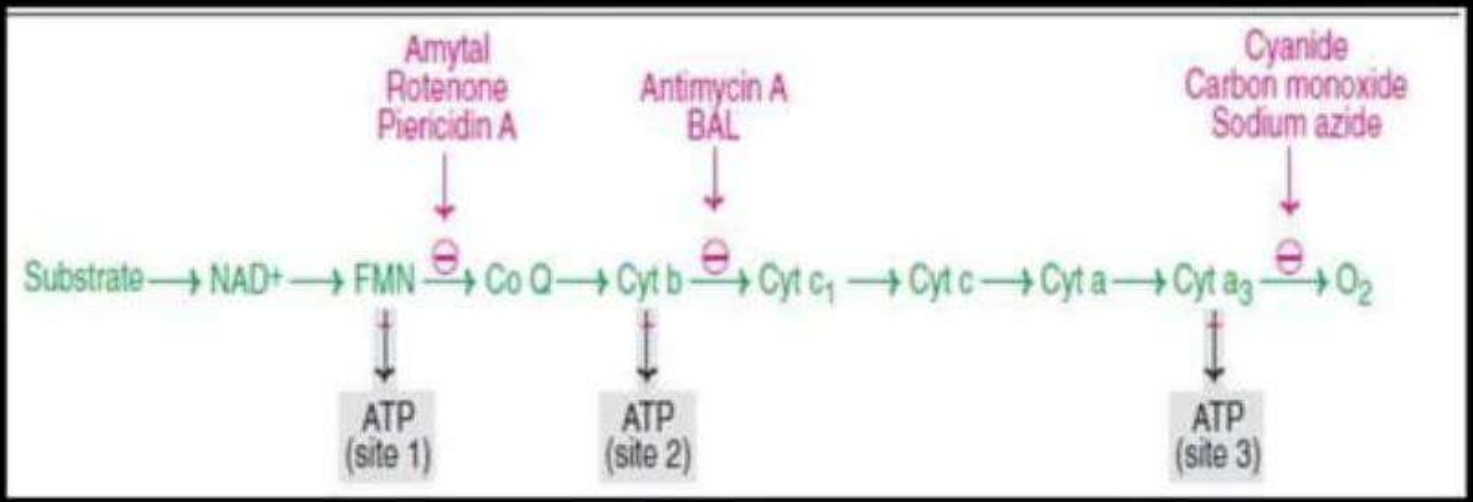
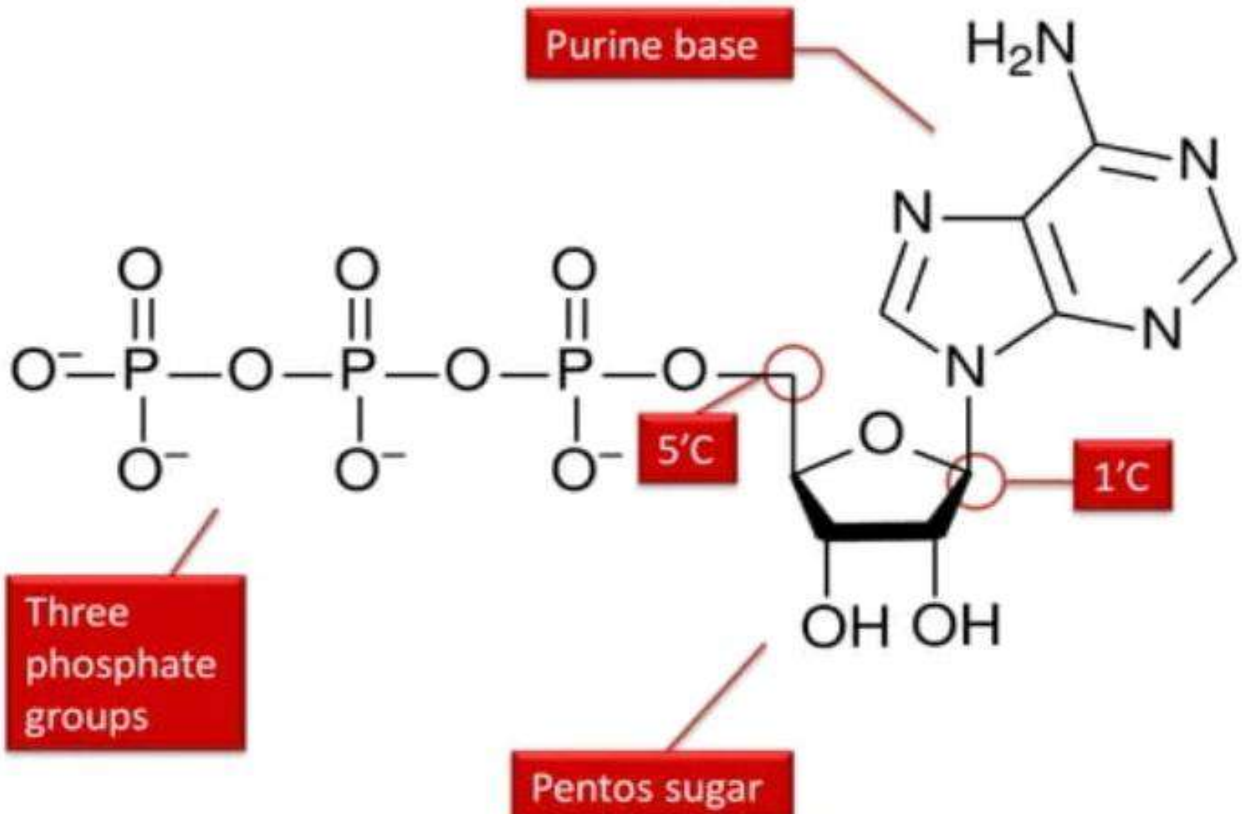


Fig14 : Blocking site of ETC

Structure



Purine base
H₂N
N
N
N
N
N
O⁻
P
O
P
O
P
O
O
O
O
O⁻
O⁻
O⁻
5′C
O
1′C
OH
OH
Three phosphate groups
Pentos sugar

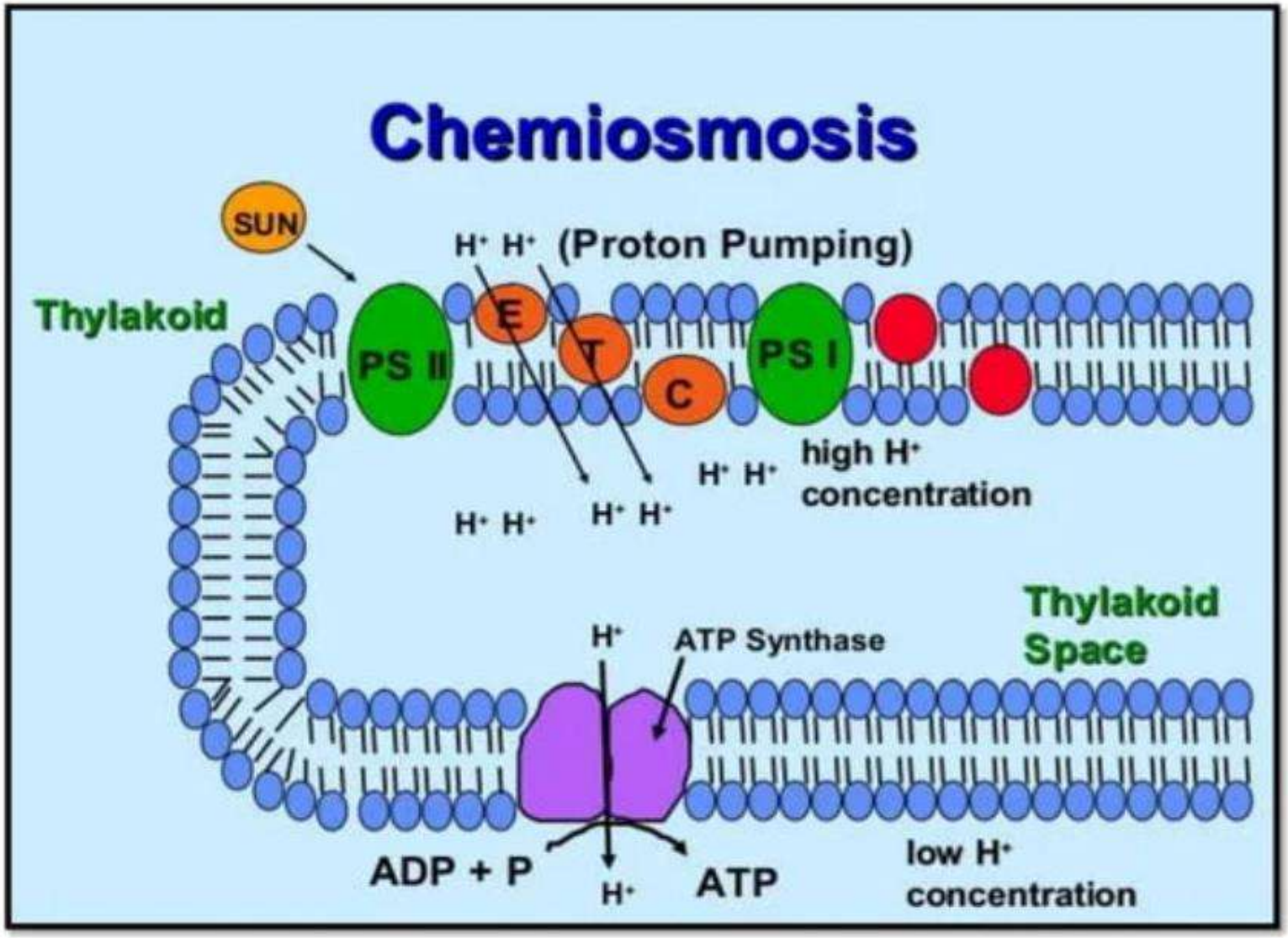
ATP

- ATP is the most commonly used "energy currency" of cells from most organisms. It is formed from adenosine diphosphate (ADP) and inorganic phosphate (P_i), and needs energy.
- The overall reaction sequence is: ADP + P_i → ATP, where ADP and P_i are joined together by ATP synthase
- ATP synthase utilizes the energy stored in this electrochemical gradient to drive nucleotide synthesis.

ATP Synthase

ATP synthase—also called F_oF_1 ATPase is the universal protein that terminates oxidative phosphorylation by synthesizing ATP from ADP and phosphate.

ATP Synthase is one of the most important enzymes found in the **mitochondria of cells**



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Structure

- ATP synthase is composed of at least 8 subunit types, whose stochiometry is denoted with subscripts: (a_3, b_3, g, d, e, a_6, b_2, c_{12}), which combine into two distinct regions.
- The **F1 portion** is soluble and consists of a hexamer, a_3b_3. This hexamer is arranged in an annulus about a central shaft consisting of the coiled-coil g subunit.
- The **Fo portion** consists of three transmembrane subunits: a_6, b_2 and c_{12}.

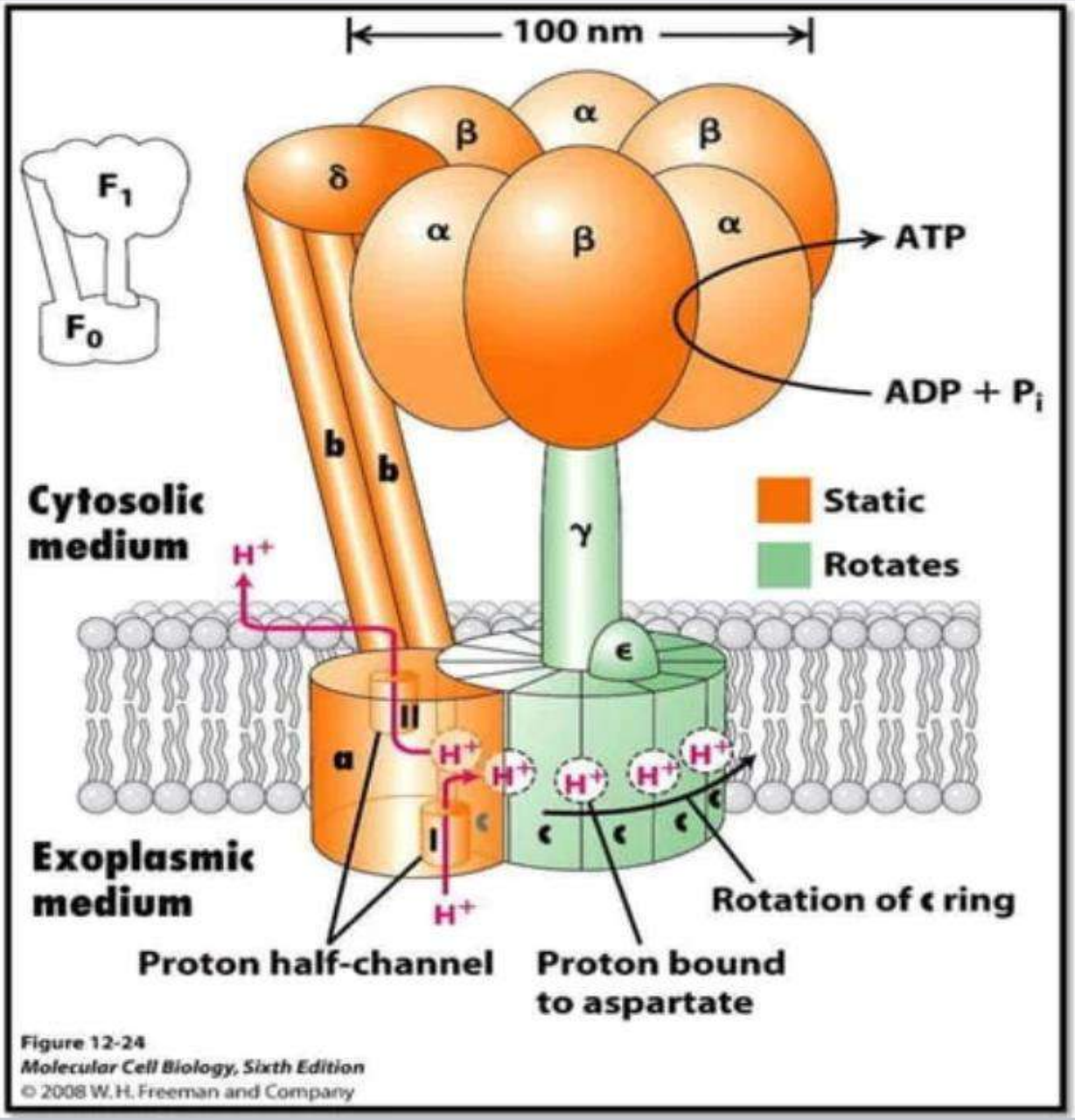
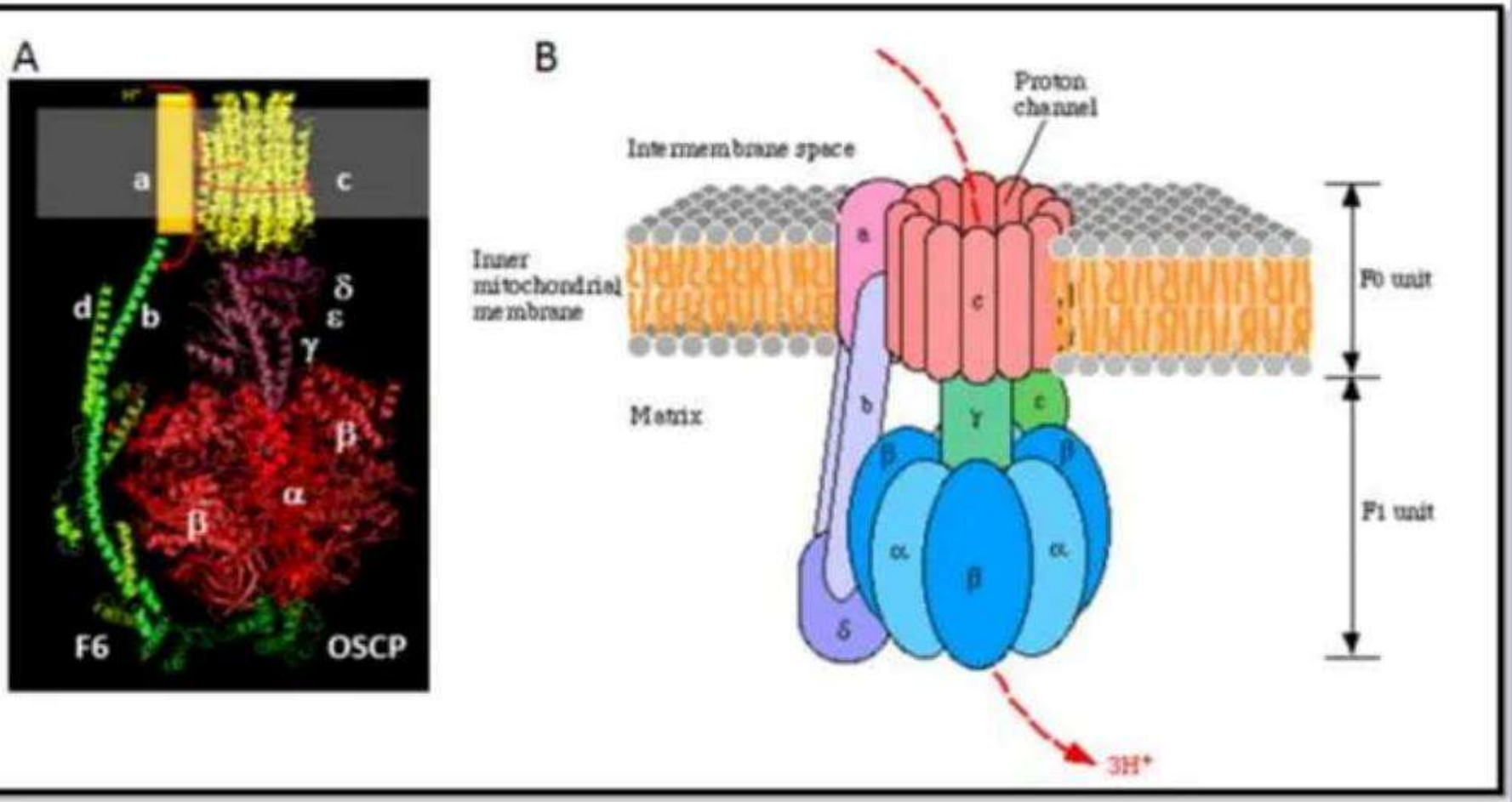


Figure 12-24
Molecular Cell Biology, Sixth Edition

Working

ATP synthesis does not require the input of energy, but the release of the newly synthesized ATP does require energy.

The movement of protons through the F_0 channel causes the γ subunit to rotate which drives a conformational change in the structure of the β-subunit resulting in the binding of substrates (ADP and Pi) and the release of the product ATP.



www.diapedia.org


Thank You!

Biosynthesis of Fatty acid

S.H. Nadaf

Vivekanand College Kolhapur

Autonomous

- De novo synthesis of fatty acids occurs in liver, kidney, adipose tissue & lactating mammary gland.
- Enzymes are located in cytosomal fraction of the cell.
- It is called as extramitochondrial or cytoplasmic fatty acid synthase system.

- **Major fatty acid synthesized de novo is palmitic acid ($_{16}$C saturated fatty acid).**
- **It occurs in liver, adipose tissue, kidney, brain & lactating mammary glands.**
- **Acetyl CoA is the source of carbon atoms.**

- **NADPH provides reducing equivalents – NADPH is produced from HMP shunt & malic enzyme reaction.**
- **Every molecule of acetyl CoA delivered to cytoplasm, one molecule of NADPH is formed.**
- **ATP supplies energy.**

- **NADPH provides reducing equivalents – NADPH is produced from HMP shunt & malic enzyme reaction.**
- **Every molecule of acetyl CoA delivered to cytoplasm, one molecule of NADPH is formed.**
- **ATP supplies energy.**

Stages

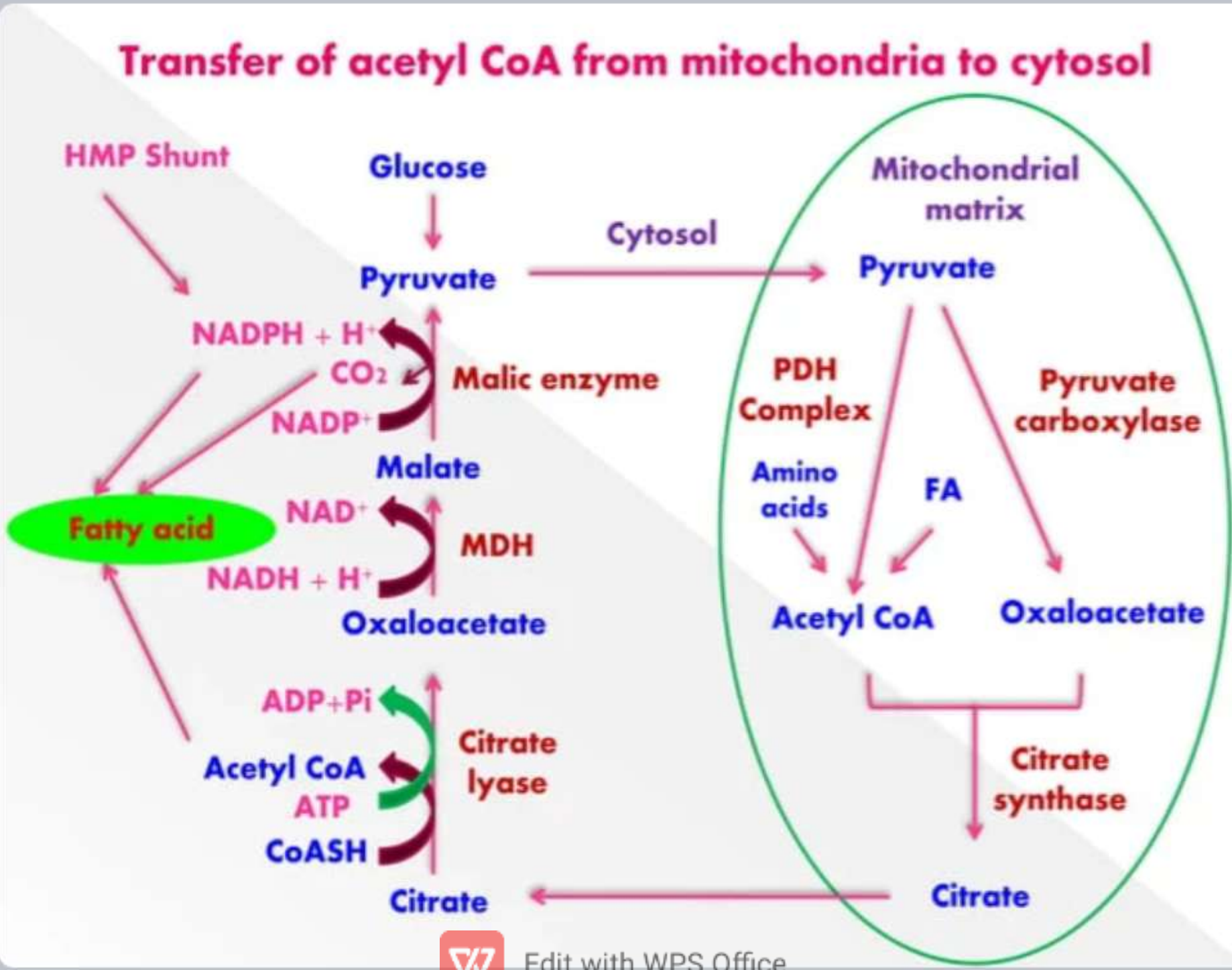
- Production of acetyl CoA & NADPH
- Conversion of acetyl CoA to malonyl CoA
- Reactions of fatty acid synthase complex.

Production of acetyl CoA & NADPH

- Acetyl CoA is the starting material for de novo synthesis of fatty acids.
- Acetyl CoA is produced in the mitochondria by the oxidation of pyruvate, fatty acids, degradation of carbon skeleton of certain amino acids & from ketone bodies.
- Mitochondria are not permeable to acetyl CoA.

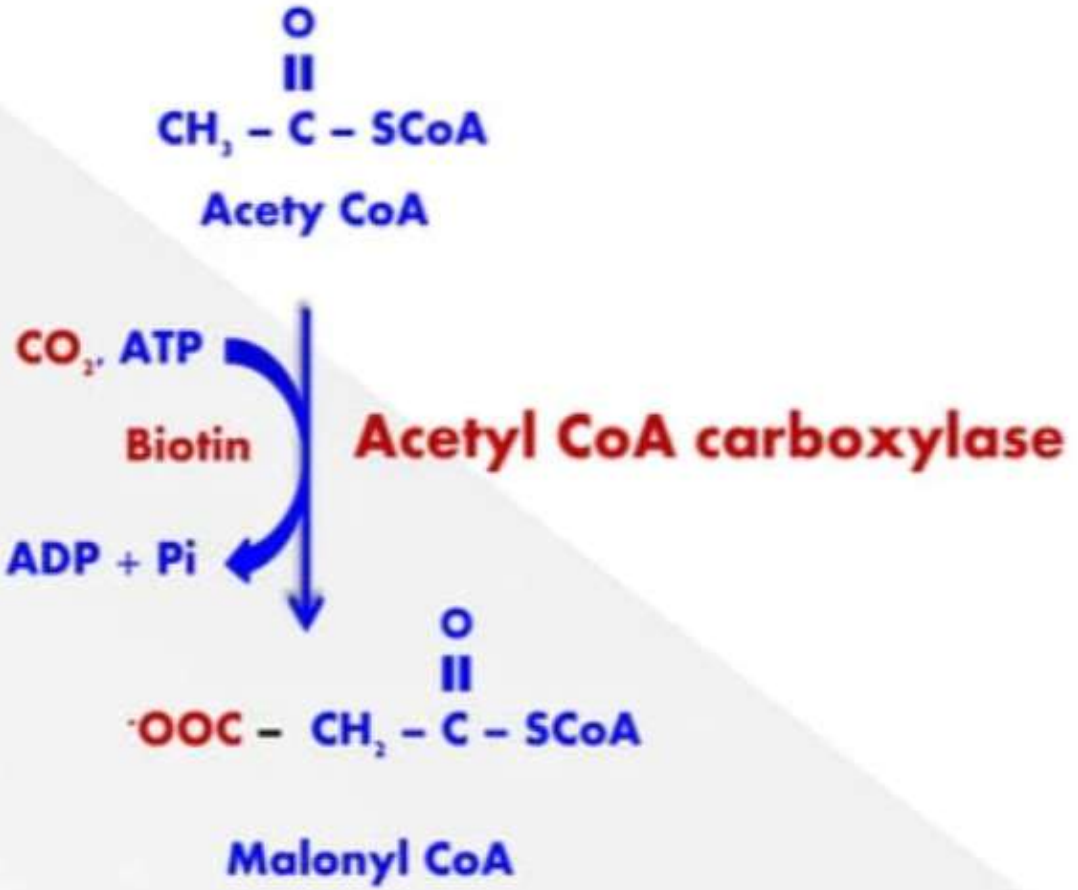
- An alternate or a bypass arrangement is made for the transfer of acetyl CoA to cytosol.
- Acetyl CoA condenses with oxaloacetate in mitochondria to form citrate.
- Citrate is freely transported to cytosol by tricarboxylic acid transporter.

- In cytosol it is cleaved by ATP citrate lyase to liberate acetyl CoA & oxaloacetate.
- Oxaloacetate in the cytosol is converted to malate.
- Malic enzyme converts malate to pyruvate.
- NADPH & CO_2 are generated in this reaction.
- Both of them are utilized for fatty acid synthesis



- **Advantages of coupled transport of acetyl CoA & NADPH**
- **The transport of acetyl CoA from mitochondria to cytosol is coupled with the cytosomal production of NADPH & CO_2 which is highly advantageous to the cell for optimum synthesis of fatty acids**

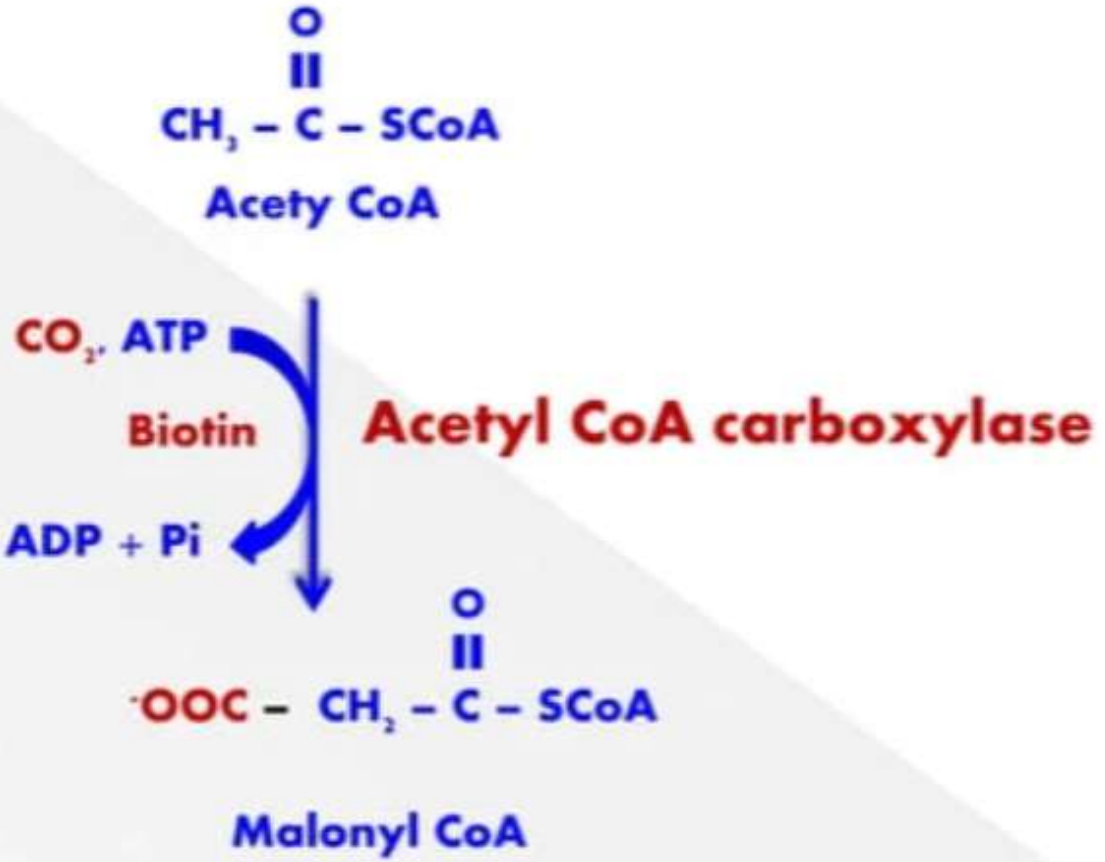
Conversion of acetyl CoA to Malonyl CoA



Formation of malonyl CoA

- Acetyl CoA is carboxylated to malonyl CoA by the enzyme acetyl CoA carboxylase.
- This is an ATP-dependent reaction & requires biotin for CO_2 fixation.
- The mechanism of action of acetyl CoA carboxylase is similar to that of pyruvate carboxylase.
- Acetyl CoA carboxylase is a regulatory enzyme

Conversion of acetyl CoA to Malonyl CoA



Reactions of fatty acid synthase complex

- Fatty acid synthase (FAS) - multifunctional enzyme.
- In eukaryotic cells, fatty acid synthase exists as a dimer with two identical units.
- Each monomer possesses the activities of seven different enzymes & an acyl carrier protein (ACP) bound to 4′-phosphopantetheine.
- Fatty acid synthase functions as a single unit catalyzing all the seven reactions.

Reactions of fatty acid synthase complex

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- Fatty acid synthase functions as a single unit catalyzing all the seven reactions.

Advantages of Multi-enzyme complex

- Intermediates of the reaction can easily interact with the active sites of the enzymes.
- One gene codes all the enzymes; all enzymes are in equimolecular concentrations.
- The efficiency of the process is enhanced.

FAS Complex

- **First domain or Condensing unit:**
- **It is initial substrate binding site.**
- **The enzymes involved are β-keto acyl synthase or condensing enzyme (CE), acetyl transferase (AT) & malonyl transacylase (MT).**

Second domain or Reduction unit

- It contains the dehydratase (DH), enoyl reductase (ER), β-keto acyl reductase (KR) & acyl carrier protein (ACP)
- The acyl carrier protein is a polypeptide chain having a phospho-pantotheine group, to which acyl groups are attached in thioester linkage.
- ACP acts like CoA carrying fatty acyl groups.

Third domain or releasing unit

- It is involved in the release of synthesized fatty acid in the cytosol.
- Major fatty acid synthesized is palmitic acid.
- It contains thio-esterase(TE) or de-acylase.

Reactions

- The two carbon fragment of acetyl CoA is transferred to ACP of fatty acid synthase, catalyzed by the enzyme - acetyl CoA-ACP transacylase.
- The acetyl unit is then transferred from ACP to cysteine residue of the enzyme.
- The ACP site falls vacant.

- The enzyme malonyl CoA-ACP transacylase transfers malonate from malonyl CoA to bind to ACP.
- The acetyl unit attached to cysteine is transferred to malonyl group (bound to ACP).
- The malonyl moiety loses CO_2 which was added by acetyl CoA carboxylase.
- CO_2 is never incorporated into fatty acid carbon chain.

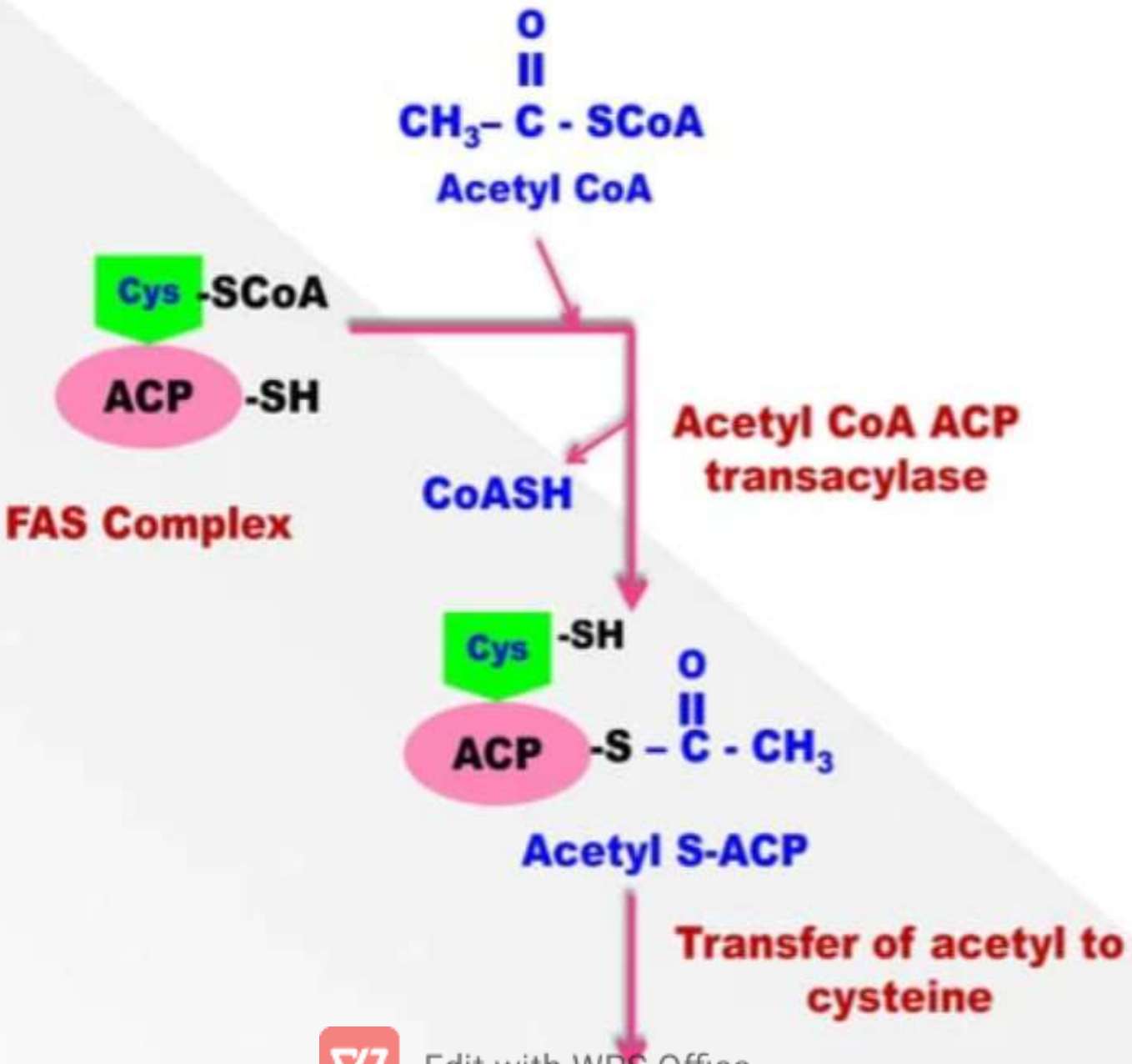
- The decarboxylation is accompanied by loss of free energy which allows the reaction to proceed forward.
- It is catalyzed by β-ketoacyl ACP synthase.
- β -Ketoacyl ACP reductase reduces ketoacyl group to hydroxyacyl group.
- The reducing equivalents are supplied by NADPH.
- β -Hydroxyacyl ACP undergoes dehydration.

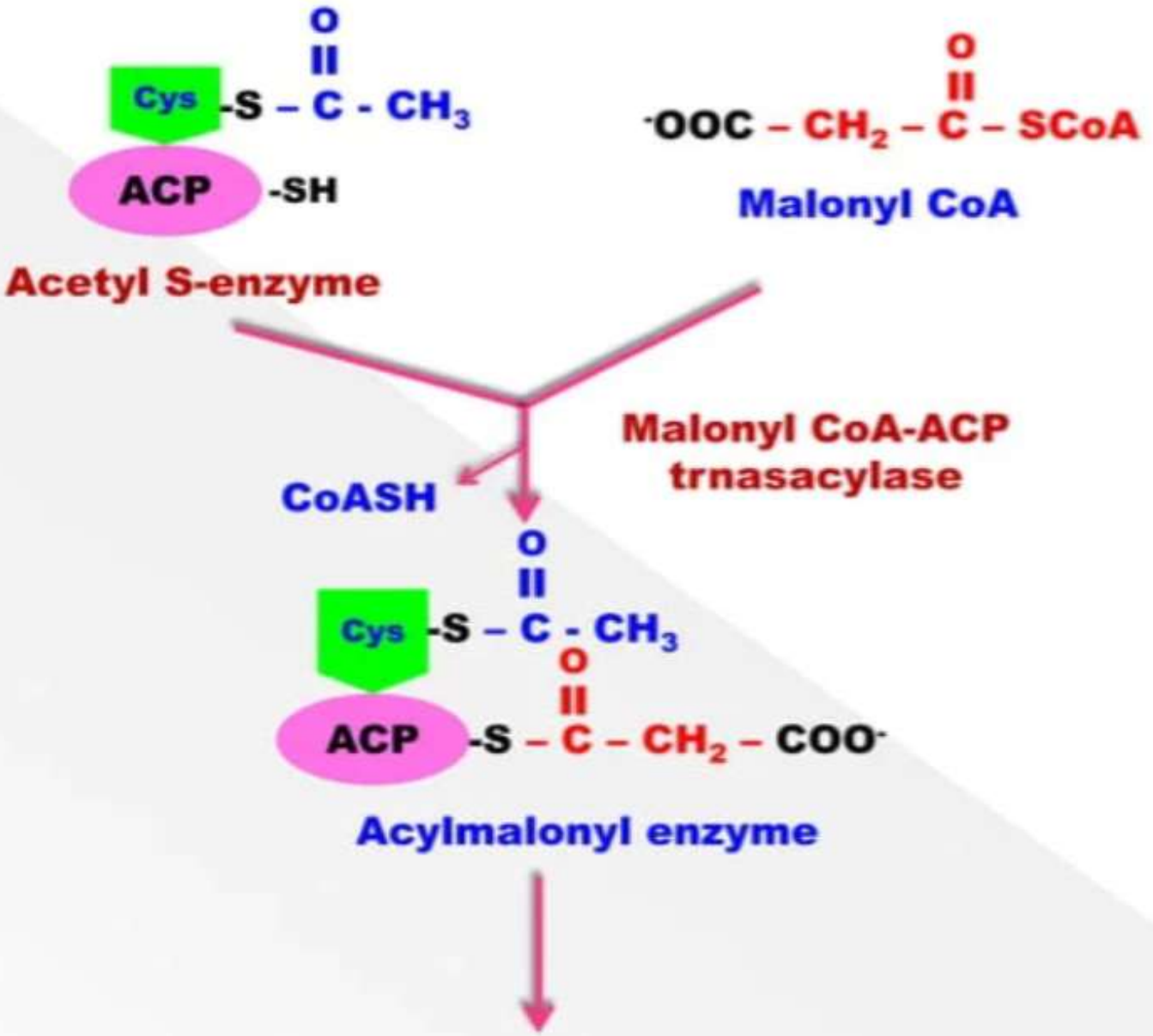
- A molecule of water is eliminated & a double bond is introduced between α & β carbons.
- A second NADPH-dependent reduction, catalysed by enoyl-ACP reductase occurs to produce acyl-ACP.
- The four-carbon unit attached to ACP is butyryl group.

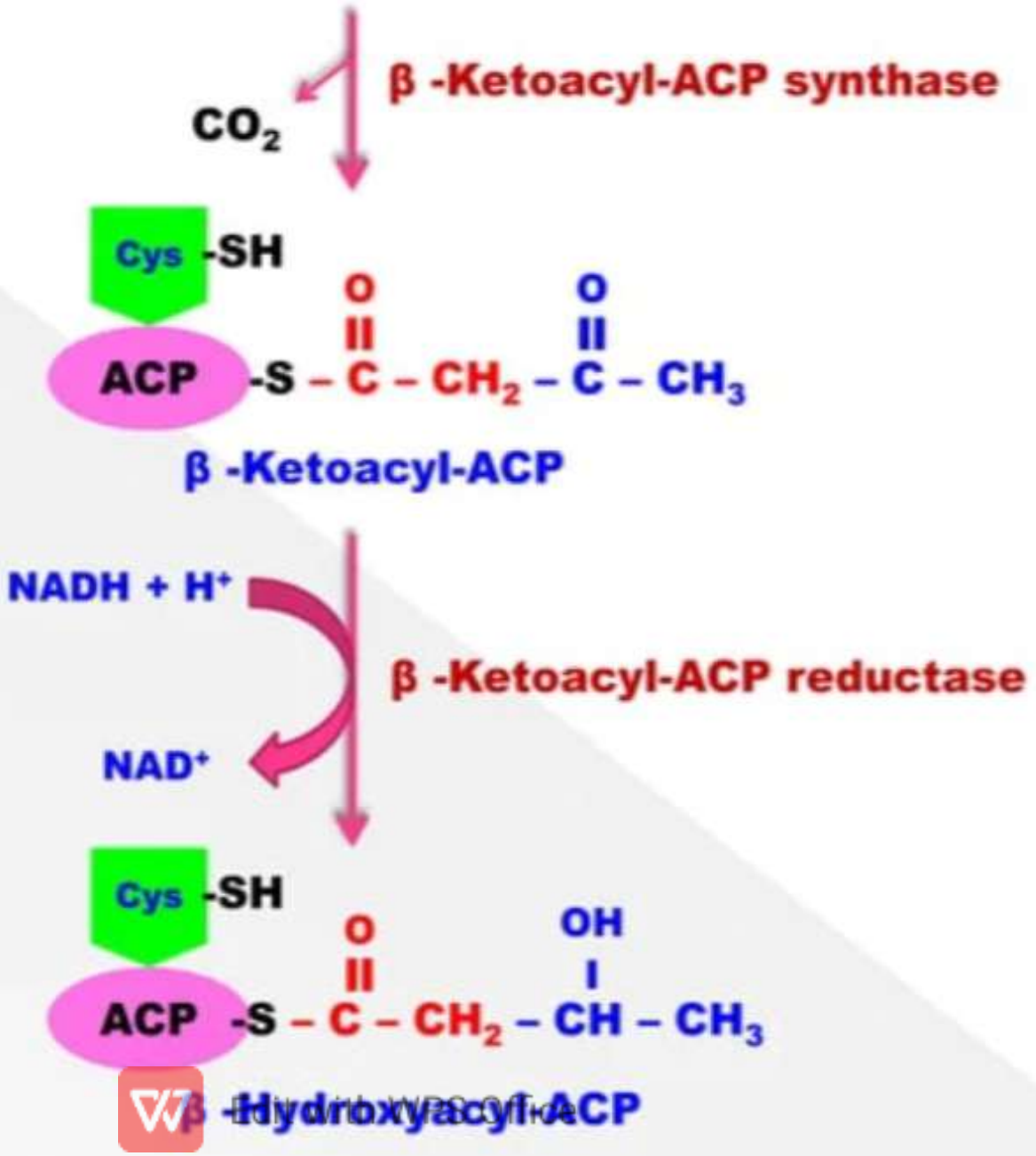
- The carbon chain attached to ACP is transferred to cysteine residue & the reactions of malonyl CoA-ACP transacylase & enoyl-ACP reductase are repeated 6 more times.
- Each time, the fatty acid chain is lengthened by a two-carbon unit (obtained from malonyl CoA).

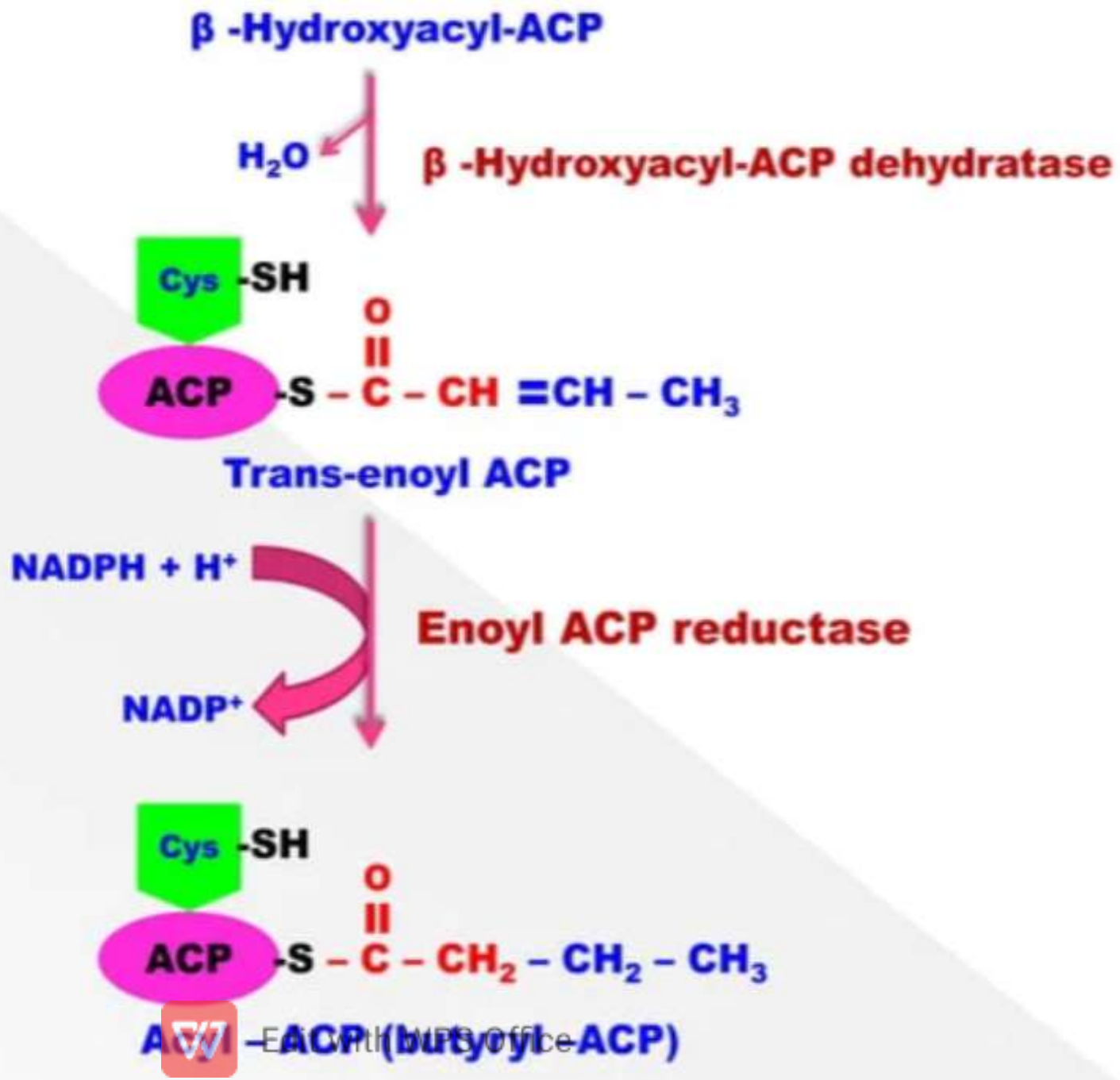
- At the end of 7 cycles, the fatty acid synthesis is complete & a 16-carbon fully saturated fatty acid-namely palmitate-bound to ACP is produced.
- The enzyme palmitoyl thioesterase separates palmitate from fatty acid synthase.
- This completes the synthesis of palmitate

De novo synthesis of fatty acids

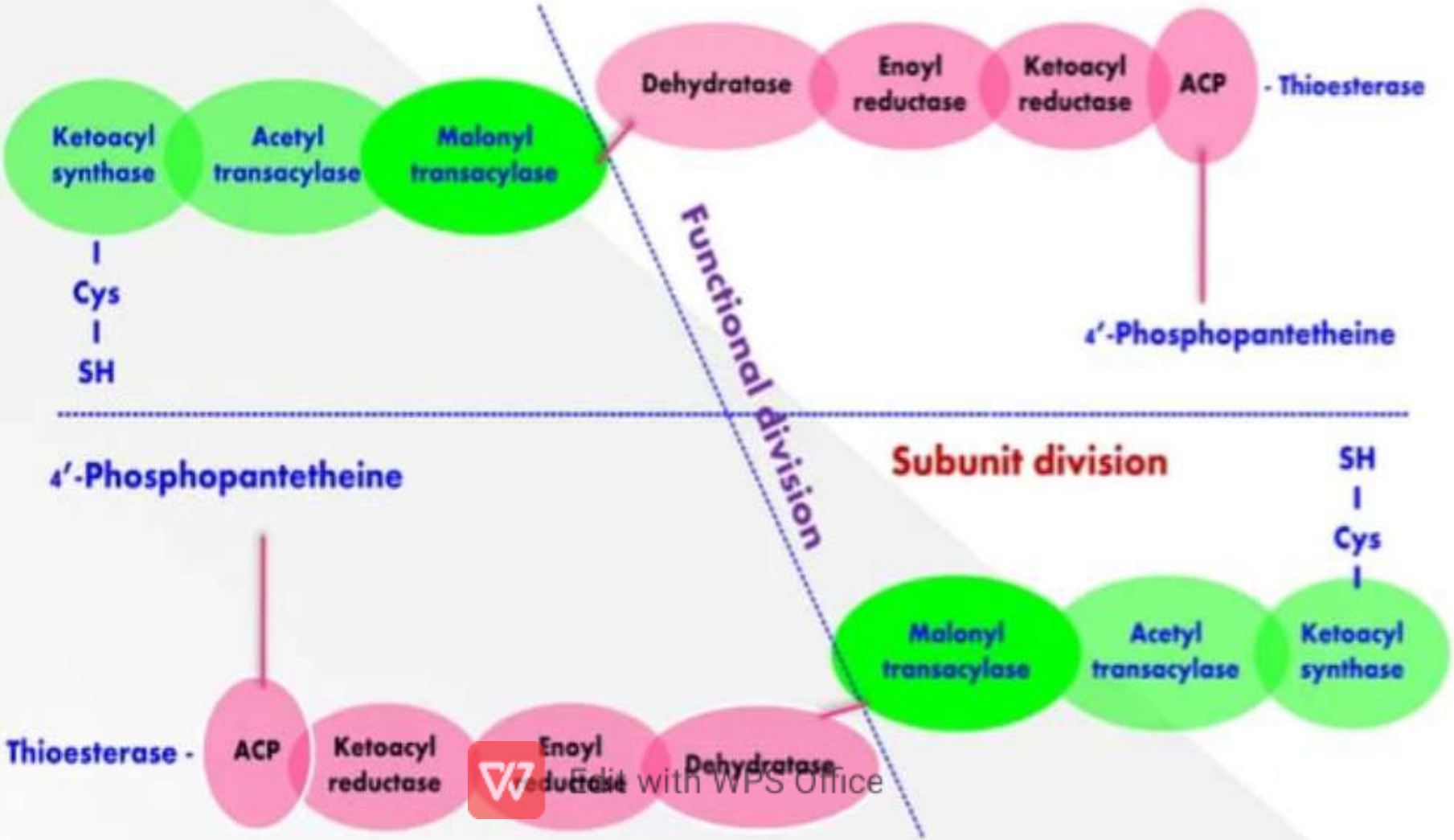





β -Ketoacyl-ACP synthase
CO_2
Cys -SH
ACP -S – C – CH_2 – C – CH_3
β -Ketoacyl-ACP
NADH + H^+
β -Ketoacyl-ACP reductase
NAD^+
Cys -SH
ACP -S – C – CH_2 – CH – CH_3
OH
β -Hydroxyacyl-ACP


β -Hydroxyacyl-ACP
H_2O
β -Hydroxyacyl-ACP dehydratase
Cys -SH
ACP -S – C – CH =CH – CH_3
O
Trans-enoyl ACP
NADPH + H^+
Enoyl ACP reductase
$NADP^+$
Cys -SH
ACP -S – C – CH_2 – CH_2 – CH_3
O
Acyl –ACP (butyryl-ACP)

Fatty acid synthase - multienzyme complex



- The -SH group of phosphopantetheine of one subunit is in close proximity to the -SH of cysteine residue (of the enzyme ketoacyl synthase) of the other subunit.
- Each monomer of FAS contains all the enzyme activities of fatty acid synthesis.
- Only the dimer is functionally active.

- The functional unit consists of half of each subunit interacting with the complementary half of the other.
- FAS structure has both functional division & subunit division
- The two functional subunits of FAS independently operate & synthesize two fatty acids simultaneously

Significance of FAS complex

- The FAS complex offers great efficiency that is free from interference of other cellular reactions for the synthesis of fatty acids.
- There is a good coordination in the synthesis of all enzymes of the FAS complex.

Regulation of fatty acid synthesis

- Fatty acid production is controlled by enzymes, metabolites, end products, hormones and dietary manipulations.
- Acetyl CoA carboxylase:
- This enzyme controls a committed step in fatty acid synthesis.

- **Acetyl CoA carboxylase exists as an inactive protomer (monomer) or an active polymer.**
- **Citrate promotes polymer formation & increases fatty acid synthesis.**
- **Palmitoyl CoA & malonyl CoA cause depolymerization of the enzyme, inhibits the fatty acid synthesis.**

GERMPLASM

by
S.H.Nadaf

GERMPLASM

- **Germplasm is** the genetic material of germ cells.

- The sum total of hereditary material i.e. all the alleles of various genes, present in a crop species and its wild relatives is referred to as **Germplasm**.

- This is also known as genetic resources or gene pool or genetic stock.

- **Germplasm** are living genetic resources such as seeds or tissues that are maintained for the purpose of plant breeding, preservation, and other research uses.

IMPORTANT FEATURES OF GERMPLASM

- **Genetic pool** represents the entire genetic variability or diversity available in a crop species.
- Germplasm **consists** of land races, modern cultivars, obsolete cultivars, breeding stocks, wild forms and wild species of cultivated crops.
- **Germplasm includes** both cultivated and wild species and relatives of crop plants.
- Germplasm is **collected** from centers of diversity, gene banks, gene sanctuaries, farmer's fields, markers and seed companies.
- Germplasm is the **basic material** for launching a crop improvement programme.
- Germplasm may be **indigenous** (collected within country) or **exotic** (collected from foreign countries)

Molecular Marker Types

1. Direct Markers

- **This kind of molecular marker is one which is located directly within the gene of interest.**
- These kinds of markers are the most uncommon and are thus the most difficult to find.

2. Linked Markers/indirect markers

- **This marker are located very close to major genes of interest.**
- Linked markers are near the gene of interest and are not part of the DNA of the gene.

Molecular Markers/DNA markers

- A molecular marker refers to a unique sequence of nucleotides found on a strand of DNA.
- Genetic markers are not the target genes but act as signs or flags, they are located close to gene of interest
- Also called as "gene tags"
- Molecular markers are devoid of environmental effect and highly reproducible
- DNA markers have been used in evolutionary studies, crop improvement and more recently in tagging genes coding for argonomically important traits.

A molecular marker should have some desirable properties.

- ✓ It must be polymorphic as it is the polymorphism that is measured for genetic diversity studies.
- ✓ Co-dominant inheritance. The different forms of a marker allow discrimination of homo and heterozygote.
- ✓ A marker should be evenly and frequently distributed throughout the genome.
- ✓ It should be easy, fast and cheap to detect.
- ✓ It should be reproducible.
- ✓ Absence of non-allelic interaction.

(1) LEAF TISSUE SAMPLING

(2) DNA EXTRACTION

(3) PCR

(4) GEL ELECTROPHORESIS

(5) MARKER ANALYSIS

FUNDAMENTAL OF MARKER ASSISTED SELECTION

- Marker assisted selection (MAS) refers to the indirect selection for a desired plant phenotype based on the banding pattern of linked molecular marker.
- **It refers to the identification of the genomic region that are involved in the expression of the trait of interest through molecular markers.**

- They are transmitted by the standard laws of inheritance from one generation to the next.

Following are types of molecular markers:

- ✓ Restriction fragment length polymorphism($RFLP_s$)
- ✓ Random amplified polymorphic DNA ($RAPD_s$)
- ✓ Amplified fragment length polymorphism ($AFLP_s$)
- ✓ Sequence tagged microsatellite sites ($STMS_s$)

Getmplasm evaluation

Main aim

- Phenotyping genebqnk acessions
- Steps agronomic performance-- yield--reaction to biotic and abiotuc stresses such as drought
- Or pests
- Special b biochemical techniques DNA based method
- Collection management
- Query answering
- Genetic resources research

- The initial evaluation may be
- Screening techniques
- Control or check line(s)
- The key to successful utilization

Generally, genebank managers, breeders and other specialists work together to develop the set of traits that provide the basic description of a species diversity into a more usable form for further use in plant breeding.

Descriptors lists different
classes of their expression (characterization) or how to measure the range of their
variation (evaluation).

- species - specific.
- Provides an international format and a universally understood 'language' for PGRFA data.
- Molecular markers
- e.g. – evolutionary studies,
- – for assessing interrelationships among accessions and among geographic
- groups of accessions,
- – for estimating genetic diversity, and
- – for identifying duplicates.

Descriptor states may be

- – a numeric value such as a weight, a length, or an output from a sensor;
- – a code within a scale, such as a 1 to 9 rating for disease severity or a rating for shade and intensity of color; or
- – a qualifier, such as absence or presence of a trait.
- The name, state, and scale for any descriptor are agreed upon by researchers involved
- in germplasm characterization and evaluation..
- The descriptors could be – qualitative or – quantitative
- **Qualitative descriptors** – typically morphological, physiological and molecular
- (biochemical and DNA) traits
- **Quantitative descriptors** – subject to environmental factors, e.g. yield and
- components, host plant resistance and stress tolerance.

Characterization:

The main aims of germplasm characterization are to:

• describe accessions and establish accessions‘ diagnostic characteristics

• classify accessions into groups using sound means

• assess interrelationships among accessions or among traits and among
geographic groups of accessions

• estimate the extent of variation in the genebank collection

• identify duplicates in a collection

FOOD SECURITY AND AGROBIODIVERSITY

- The main reasons are:
- • Continued population growth
- Changing diets, including a shift from plant to animal products and rising
- consumption of animal products in emerging and developing countries
- The associated increase in fodder production for livestock
- Competition for land from crops grown for biofuels.

WHAT ARE THE DIFFERENT LEVELS OF FOOD SECURITY?

- **Availability –**
- **Access –**
- **Utilisation –**
- **Stability -**

HOW IS FOOD SECURITY MEASURED?

- 1. Estimating calories per capita.
- 2. Household income and expenditure surveys.
- 3. Measuring individual‘s dietary intake.
- 4. Measuring individual‘s height, weight and body composition.
- 5. Reports of individual‘s experience of food security.

WHAT ARE THE MAJOR CAUSES OF FOOD INSECURITY?

- **Conflict**
- **Climate change**
- **Population Growth**

WHAT HAPPENS WHEN THERE IS FOOD INSECURITY?

- **Malnutrition**
- **Stunting**
- **Mental Health Issues**

WHAT IS THE MOST FOOD INSECURE COUNTRY?

- 10. **Nigeria** –
- 9. Afghanistan
- 8. **Lesotho**
- 7. Sierra Leone
- 6. Liberia
- 5. **Mozambique**
- 4. **Haiti**
- 3. **Madagascar**
- 2. **Timor-leste**
- 1. Chad

WHO IS MOST AT RISK OF FOOD INSECURITY?

- **Women**
- **Small-scale farmers**
- **Urban poor**

HOW CAN WE PREVENT FOOD INSECURITY?

- **Building climate change resilience**
- **Address inequalities of hunger**
- **Conflict-sensitive relief**

WHAT IS THE ROLE OF THE GOVERNMENT IN FOOD SECURITY?

- Governments can play a key role in improving the food security of its citizens. A great example of this can be seen in **Kenya.** Concern are working in partnership with the Kenyan government, who have adopted a CMAM (Community-based Management of Acute Malnutrition) Surge approach and implemented its ideals into government -run health systems.
- Through this approach, seasonal spikes in malnutrition are anticipated so that relief can be provided quickly and effectively where needed. This pro-active approach leads to the improvement of food security for those most vulnerable.

WHAT IS THE ROLE OF PRIVATE ORGANISATIONS IN FOOD SECURITY?

- Private organisations can have an enormous impact on food insecurity. A great example of this is Concern‘s partnership with Kerry Group, who are helping to support our RAIN (Realigning Agriculture to Improve Nutrition) programme.
- The **RAIN programme** is making lasting improvements to food security in Tahoua Region of Niger, West Africa. Currently in the final year of the four-year programme, the work being done in Niger builds on the success that Concern and Kerry Group had achieved with the same programme in Zambia.

Agrobiodiversity

- Agrobiodiversity is actively managed by male and female farmers;
- • many components of agrobiodiversity would not survive without this human interference; local knowledge and culture are integral parts of agrobiodiversity management;
- • many economically important agricultural systems are based on _alien‘ crop or livestock species introduced from elsewhere (for example, horticultural production systems or Friesian cows in Africa). This creates a high degree of interdependence between countries for the genetic resources on which our food systems are based;

- • as regards crop diversity, diversity within species is at least as important as diversity between species;
- • because of the degree of human management, conservation of agrobiodiversity in production systems is inherently linked to sustainable use – preservation through establishing protected areas is less relevant; and
- • in industrial-type agricultural systems, much crop diversity is now held ex situ in gene banks or breeders‘ materials rather than on -farm.

THE ROLE OF AGROBIODIVERSITY

- Experience and research have shown that agrobiodiversity can:
- • Increase productivity, food security, and economic returns
- * Reduce the pressure of agriculture on fragile areas, forests and endangered species
- Make farming systems more stable, robust, and sustainable
- * Contribute to sound pest and disease management
- * Conserve soil and increase natural soil fertility and health
- * Contribute to sustainable intensification
- * Diversify products and income opportunities

- Reduce or spread risks to individuals and nations
- * Help maximize effective use of resources and the environment
- * Reduce dependency on external inputs
- * Improve human nutrition and provide sources of medicines and vitamins, and
- * Conserve ecosystem structure and stability of species diversity.

Lipid physical and chemical properties

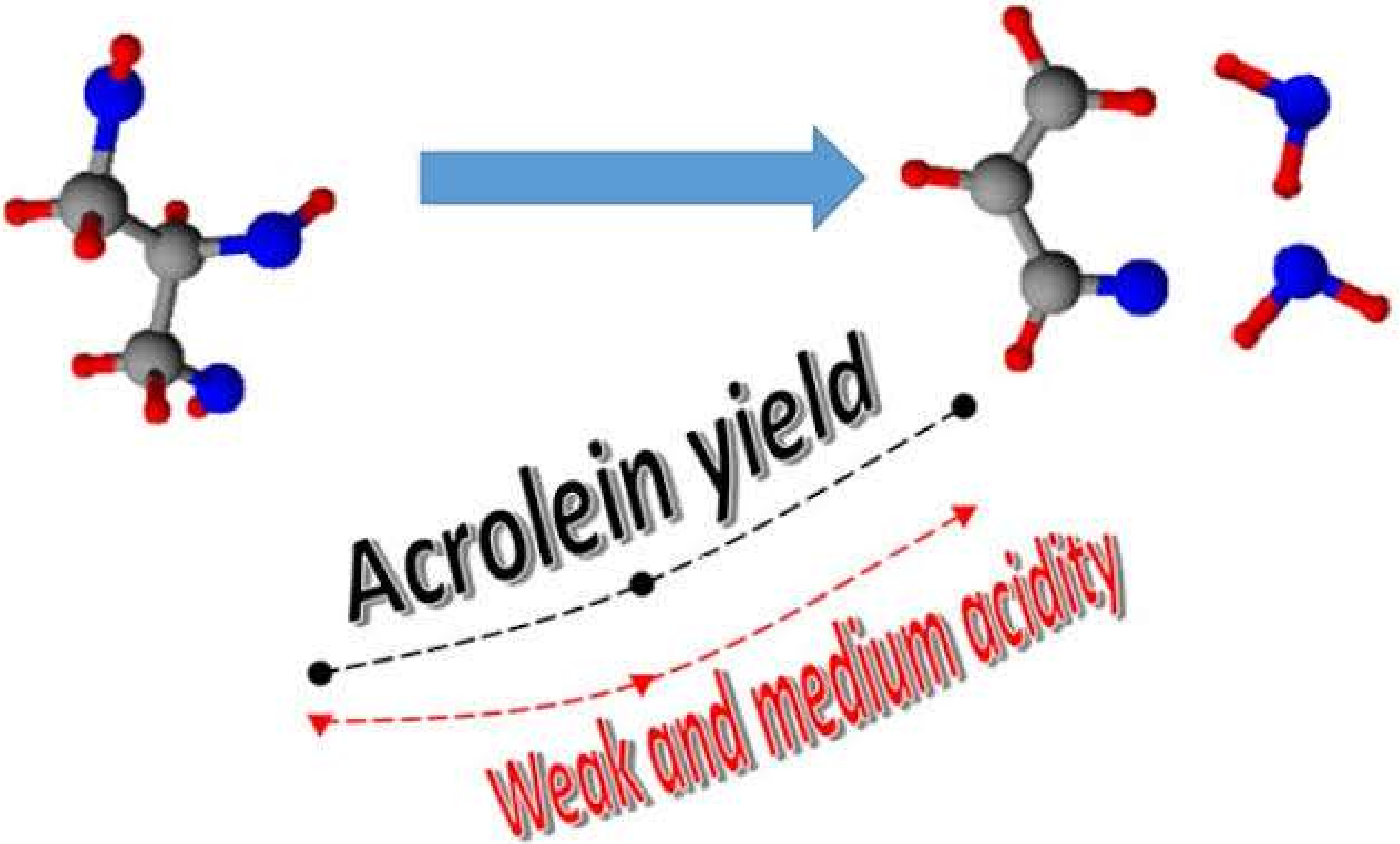
B Sc I Optional

Physical properties of fat and oils:

1. Freshly prepared fats and oils are colorless, odorless and tasteless.Any color, or taste is due to association with other foreign substances, e.g., the yellow color of body fat or milk fat is due to carotene pigments(cow milk).
2. Fats have specific gravity less than 1 and, therefore, they float on water.
3. Fats are insoluble in water, but soluble in organic solvents as ether and benzene.
4. Melting points of fats are usually low, but higher than the solidification point,

Chemical Properties of Fats

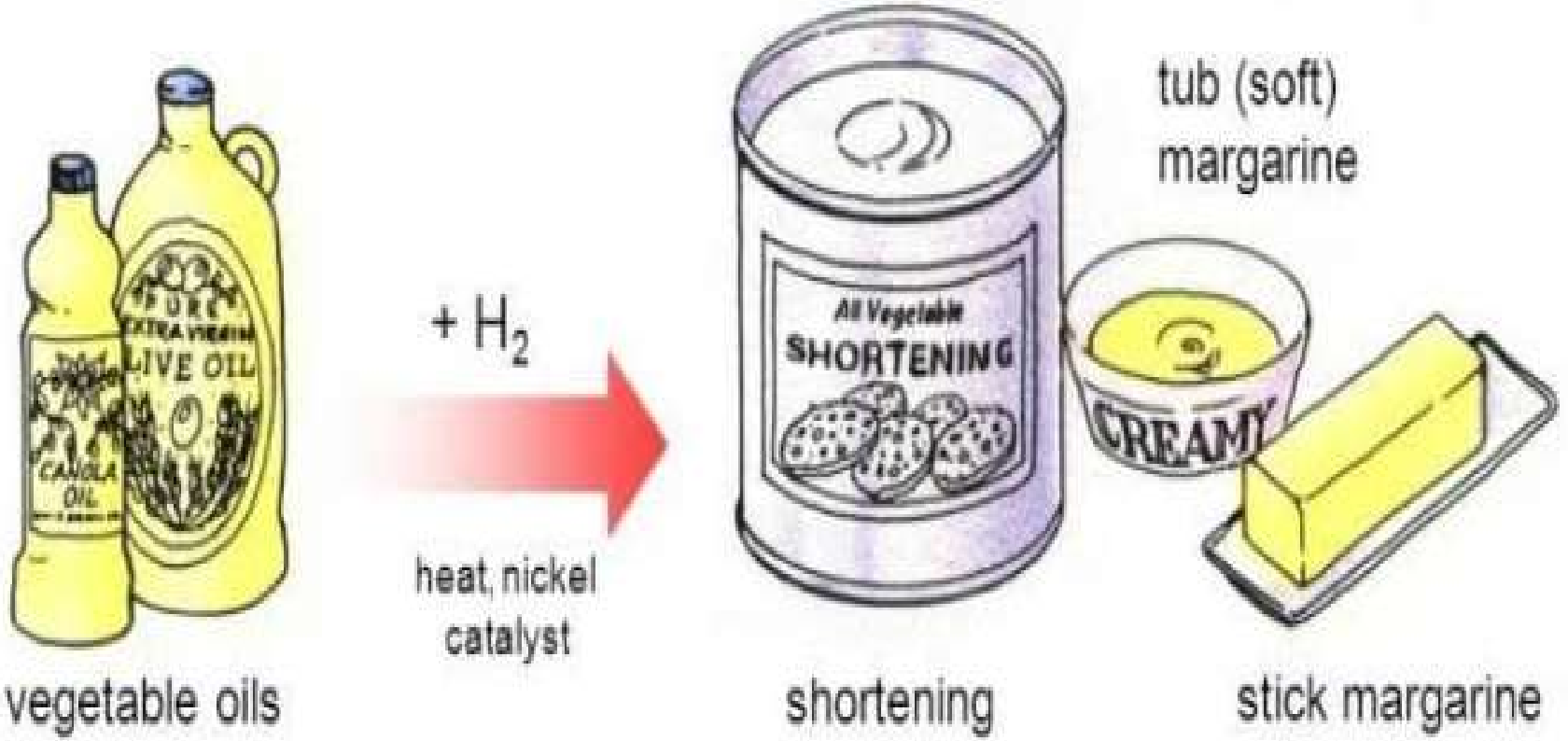
- Acrolein Formation – glycerol → heat + potassium bisulphate
- Hydrogenation – unsaturated fats (+nickel-catalyst) – saturated fats ("hardening") e.g vegetable oil – commercial cooking oil
- Saponification – hydrolysis of fat by alkali (glycerol + alkali salts = soap)
- Rancidity – chemical change resulting in unpleasant odor and taste on storage when fats are exposed to light, heat, air and moisture.
  - E.g. Ascorbic acid (Vitamin C) and Vitamin E are antioxidants (prevents rancidity)



Acrolein (2-propenal) is ubiquitously present in (cooked) foods and in the environment. It is formed from carbohydrates, vegetable oils and animal fats, amino acids during heating of foods, and by combustion of petroleum fuels and biodiesel.

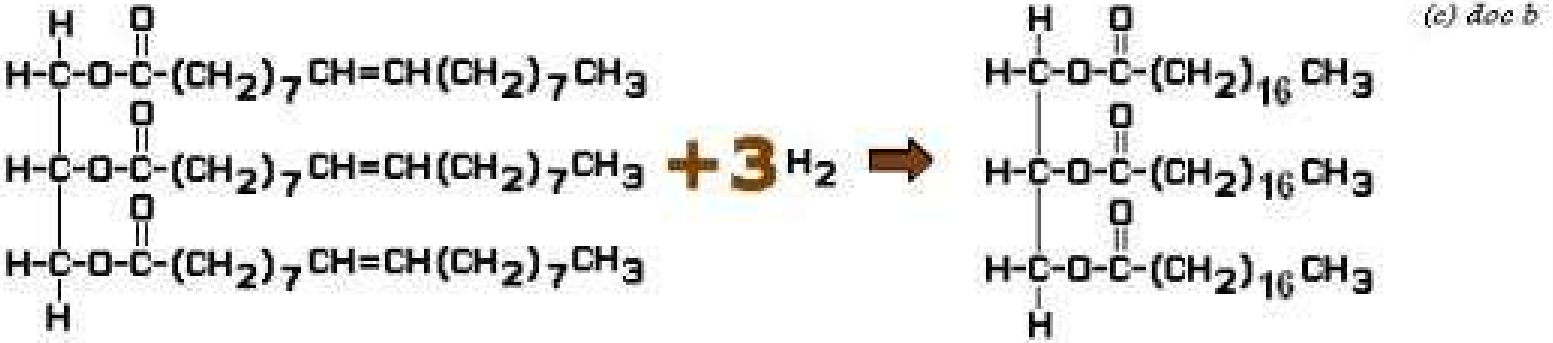
Chemical reactions responsible for release of acrolein include heat-induced dehydration of glycerol, retro-aldol cleavage of dehydrated carbohydrates, lipid peroxidation of polyunsaturated fatty acids, and Strecker degradation of methionine and threonine. Smoking of tobacco products equals or exceeds the total human exposure to acrolein from all other sources. The main endogenous

Hydrogenation



Chemical Properties of Lipids

- Hardening Of Oils
  - Unsaturated triglycerides (Oils) are liquid at room temp.
  - Hydrogenation – H_2 is passed in presence of Metal catalyst
  - Saturated Triglycerides (Fats) are produced which are semisolids

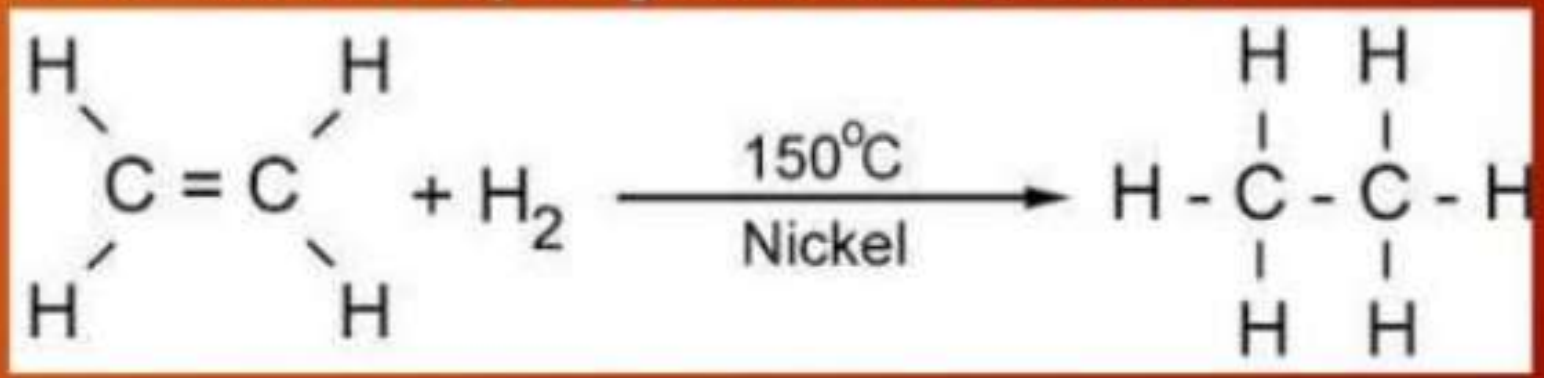


What is Hydrogenation?

- Hydrogenation is a process that reduces unsaturated fatty acid content of triglycerides by attaching hydrogen atoms at the point of unsaturation in the presence of catalyst, usually Nickel.
- Hydrogenation accomplishes two things-:
  - 1.It increases the melting point of the oil or fat.
  - 2.Resistance to oxidation and flavor deterioration.
- Hydrogenation process depends on several parameters:
  - Pressure, Temperature, Type of catalyst, Speed of agitation etc.

MECHANISM

- The mechanism involved in fat hydrogenation is believed to be the reaction between unsaturated liquid oil and atomic hydrogen adsorbed on a metal catalyst.
- It is similar to hydrogenation of alkenes.

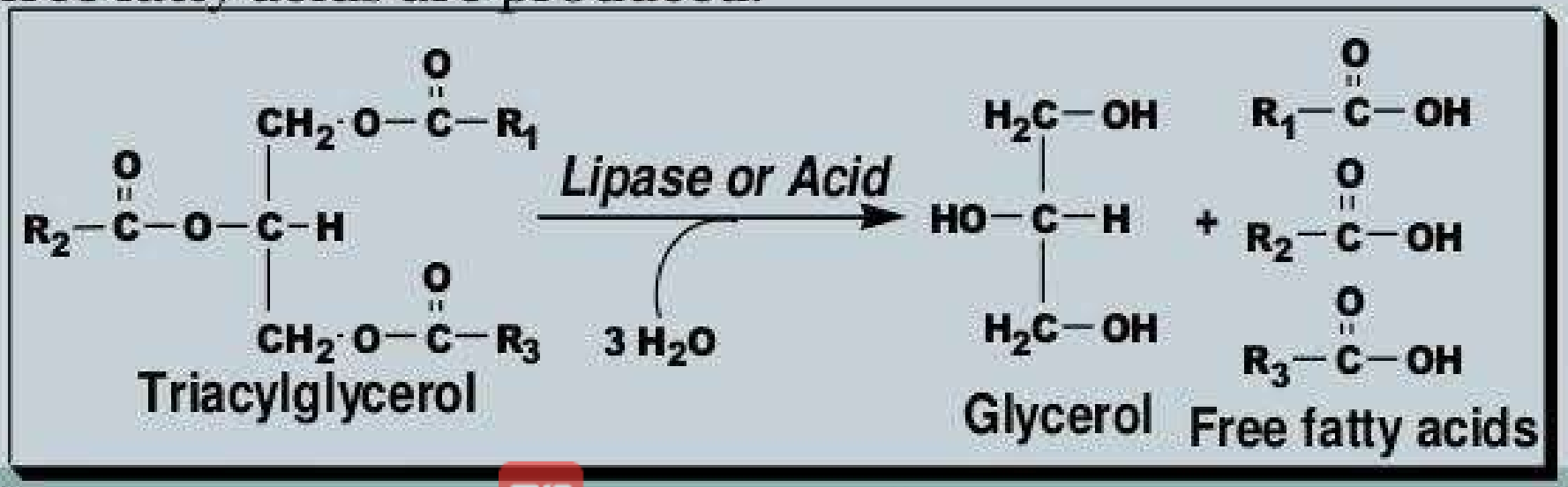


Chemical Properties of fats and oils:

1-Hydrolysis:

- They are hydrolyzed into their constituents (fatty acids and glycerol) by the action of super heated steam, acid, alkali or enzyme (e.g., lipase of pancreas).

- During their enzymatic and acid hydrolysis glycerol and free fatty acids are produced.



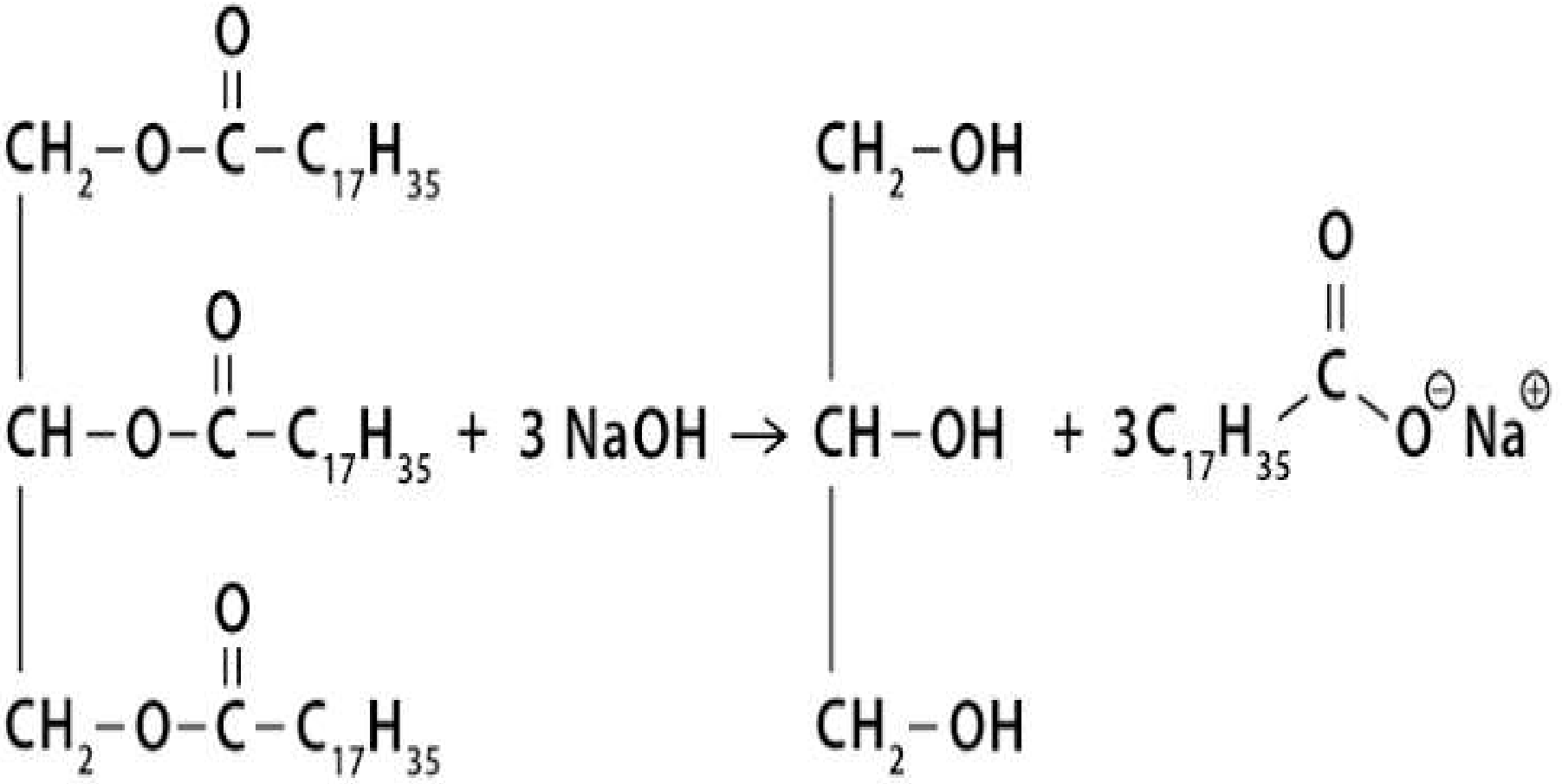
Ethanoic acid Ethanol Ethyl ethanoate Water

Saponification: When an ester reacts with sodium hydroxide, sodium salt of acid and alcohol is formed.

$$CH_3COOC_2H_5 + NaOH \rightarrow CH_3COONa + C_2H_5OH$$

Ethyl ethanoate — Sodium hydroxide — Sodium ethanoate — Ethanol

Saponification Example



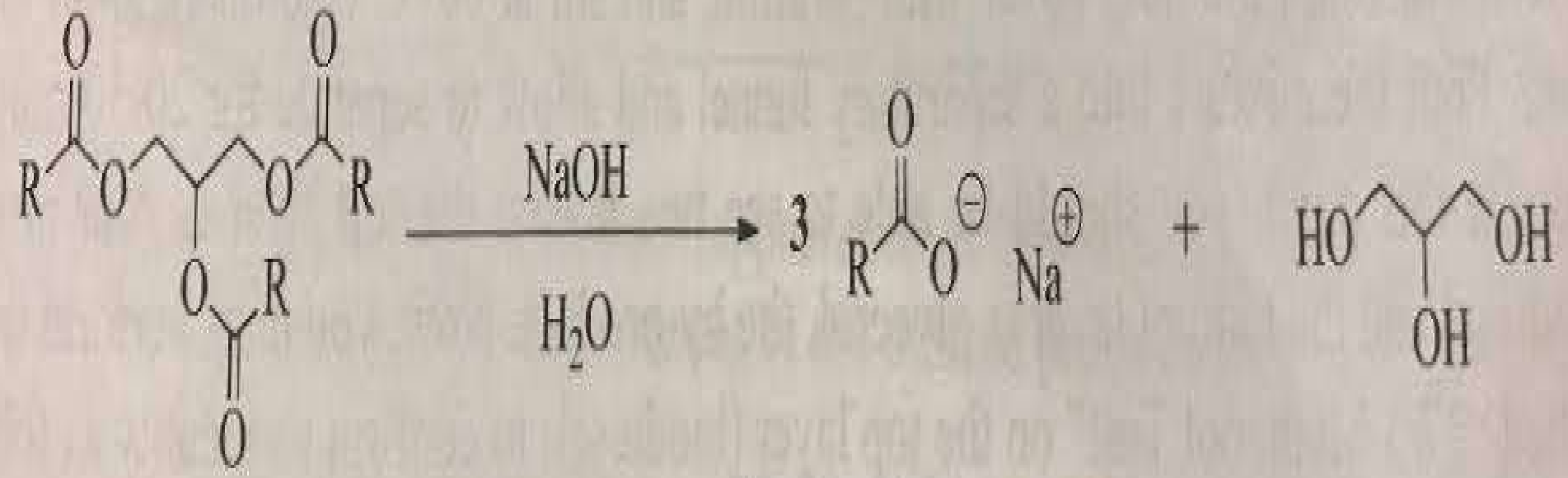
Glyceryl tristearate

Sodium hydroxide

Glycerol

Sodium stearate

Saponification



Triacylglycerol
Triglyceride
(Vegetable oil or Animal Fat)

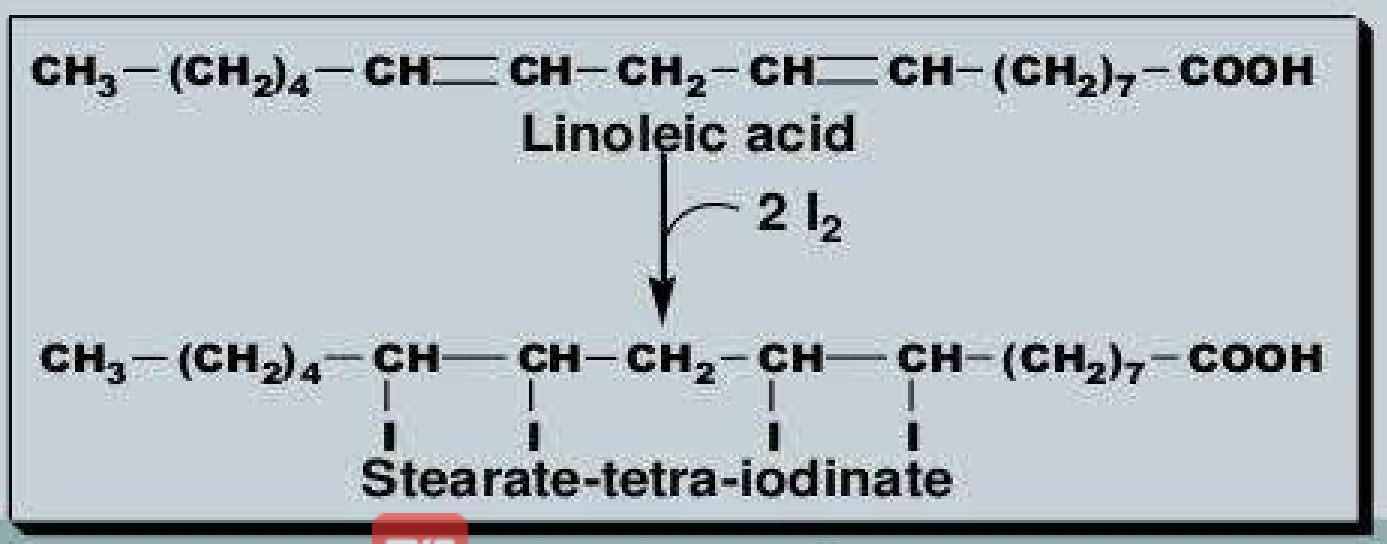
Fatty Acid Carboxylate
(Soap)

Glycerol

3-Halogenation

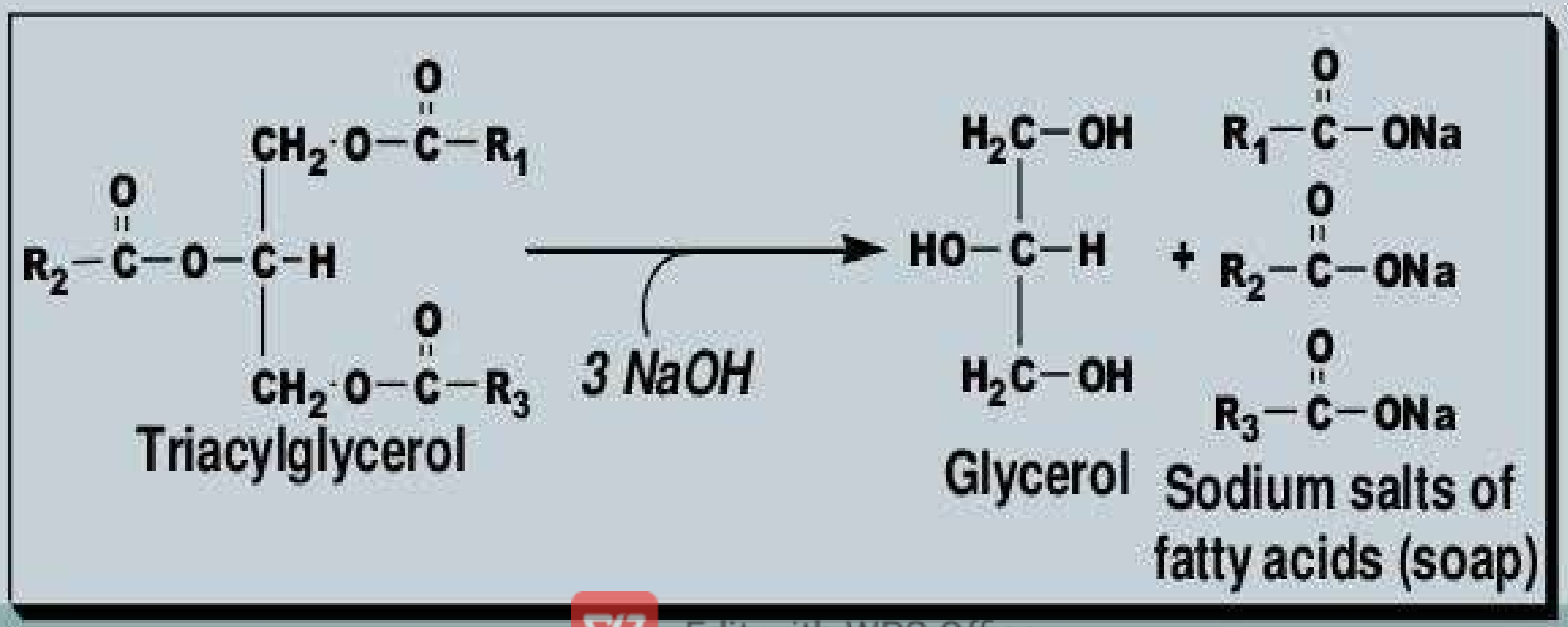
- Neutral fats containing unsaturated fatty acids have the ability of adding halogens (e.g., hydrogen or hydrogenation and iodine or iodination) at the double bonds.

- It is a very important property to determine the degree of unsaturation of the fat or oil that determines its biological value



2-Saponification

- Alkaline hydrolysis produces glycerol and salts of fatty acids (soaps).
- Soaps cause emulsification of oily material this help easy washing of the fatty materials



RANCIDITY

What is rancidity?

- Rancidity is the development of unpleasant smells in fats and oils, which are often accompanied by changes in their texture and appearance.
- Two types of rancidity:

  Hydrolytic rancidity

  Oxidative rancidity (auto-oxidation)

Types of Rancidity

There are 3 types/pathways of rancidity:

1. **Oxidative Rancidity**

- Known as autoxidation
- It is due to the auto-oxidation of PUFA present in triacylglycerols by the atmospheric O_2 on free radicals.
- The end product is the formation of aldehyde epoxide and peroxide.

2. Hydrolytic Rancidity

- Known as hydrolysis/enzymatic oxidation.
- It is due to the contamination of fat by lipase leading to the formation of diacyl & triacylglycerols with free fatty acids.
- The end product is the formation of aldehyde epoxide and peroxide.

Measuring Rancidity

Rancidity is most commonly detected by taste or smell, but it is also accompanied by a marked increase in the acid value of the fat, which is tested by using two basic laboratory tests:

- Peroxide Value (PV) for primary oxidation products
- Anisidine Value (AnV) for secondary oxidation products.

Hydrolytic rancidity	Oxidative rancidity (auto-oxidation)
Caused by the breaking down of a lipid into its component fatty acids and glycerol. $C\text{-}O\text{-}CO\text{-}R + H_2O \rightarrow C\text{-}O\text{-}H + HO\text{-}CO\text{-}R$	Occurs due to the oxidation of fatty acid chains, typically by the addition of oxygen across the C=C bond in unsaturated fatty acids.
Occurs more rapidly in the presence of enzymes such as lipase, and with heat and moisture.	The process proceeds by a free radical mechanism catalysed by light in the presence of enzymes or metal ion.
The water present in the food and the high temperature will increase the rate of hydrolysis to fatty acids.	The complex free radical reactions will produce a wide variety of products, many of which have unpleasant odours or tastes.
The free fatty acids have an unpleasant smell giving a rancid smell and taste to milk and butter that have been stored for too long. Longer chain acids are less volatile, so the smell is less noticeable.	In highly unsaturated lipids, such as fish oils, oxidative rancidity can be a major problem.

Factors causing Rancidity

Temperature - Rancidity rate increases with increase in mperature.

Duration - For the more time you keep a thing attended or without using it, it has more ances to turn rancid.

Oxygen - Oxygen promotes the decomposition food.

Light - In the presence of oxygen, light omotes the decomposition of unsaturated fatty acids.

Microorganisms and fungi (molds) - They are the most common ason for the food to become rancid . They use their enzymes on the od material and destroy its chemical mposition.

Moisture - Moist air helps the microbes in eir activity upon the food material.

Trace elements - Trace elements like Fe d Zn also increase the rate of rancidity.

ACID VALUE

Refers to the number of mg of KOH needed to neutralize the free acids in 1 g of oil.

- High acid values occur in oils which are rancid.
- Pharmaceutical oil must not have any acidity..

Significance

Acid value is the measure of hydrolytic rancidity. In general, it gives an indication about edibility of the lipid.

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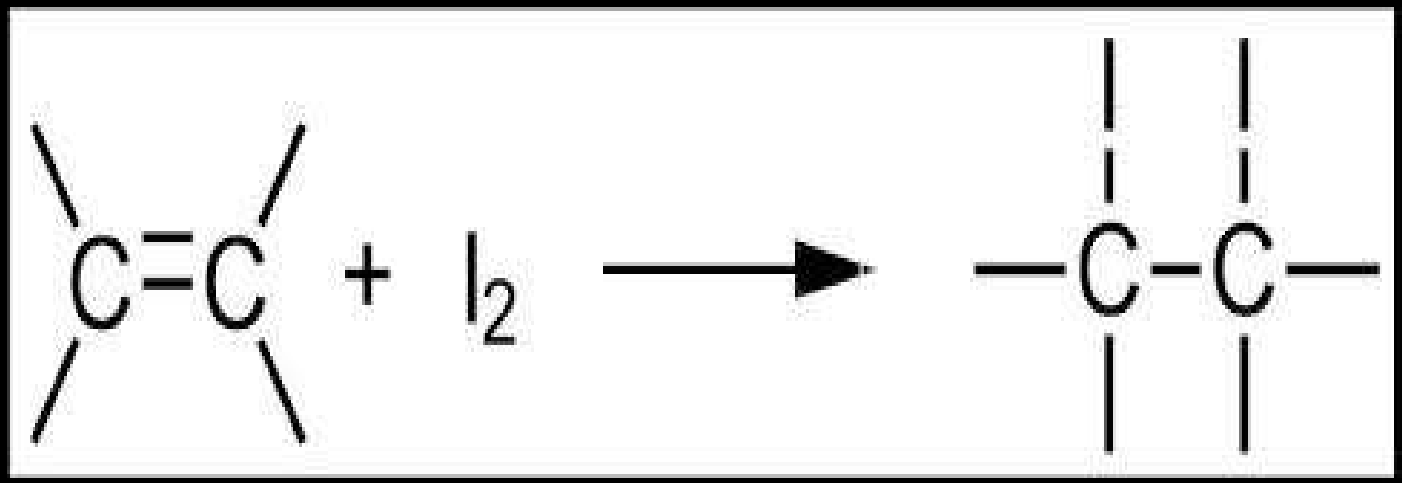
Iodine number

The addition of iodine (I_2) to unsaturated fats can be used to determine the number of carbon to carbon double bonds in the fat.



One mole of double bonds reacts with one mole of I_2

The iodine number is defined as the number of grams of iodine that reacts with 100g of fat.


Thank
you!!
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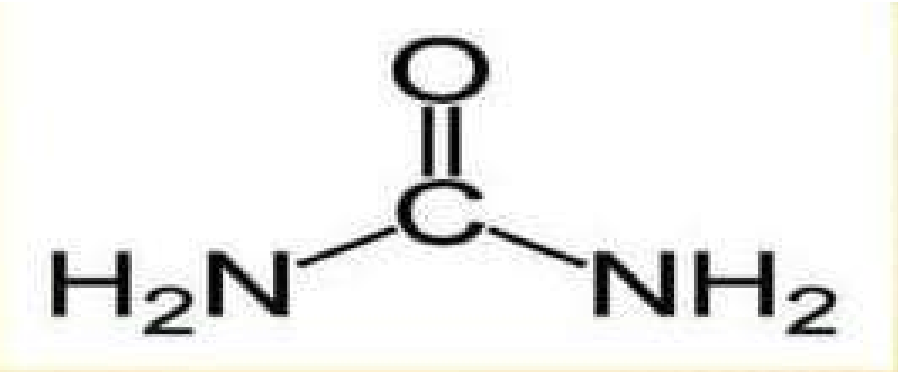
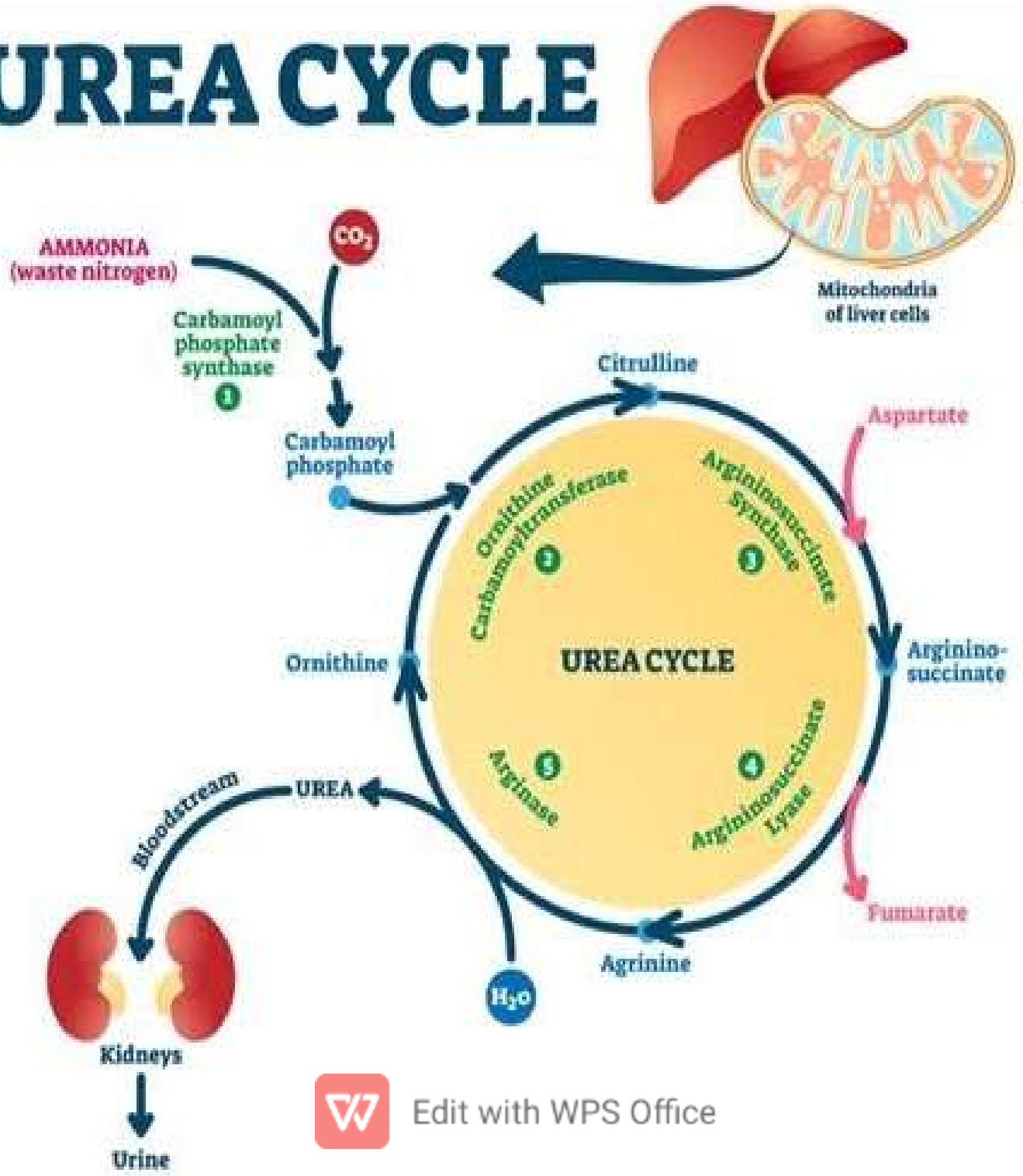


UREA CYCLE

Significance of Urea Cycle

- Toxic ammonia is converted into non-toxic urea.
- Synthesis of semi-essential amino acid-arginine.
- Ornithine is precursor of Proline, Polyamines.
- Polyamines include putrescine, spermidine, spermine.
- Polyamines have diverse roles in cell growth & proliferation.

UREA CYCLE



Steps in the urea cycle are

Step 1: Formation of carbamoyl phosphate

Step 2: Formation of citrulline

Mitochondria

Step 3: Synthesis of Argininosuccinate

Step 4: Synthesis of Arginine

Step 5: Release of urea and Ornithine

Cytosol

Urea Cycle

- The urea cycle is the first metabolic pathway to be elucidated.
- The cycle is known as Krebs–Henseleit urea cycle.
- Ornithine is the first member of the reaction, it is also called as Ornithine cycle.
- Urea is synthesized in liver & transported to kidneys for excretion in urine.

- The two nitrogen atoms of urea are derived from two different sources, one from ammonia & the other directly from the a-amino group of aspartic acid.
- Carbon atom is supplied by CO_2
- Urea is the end product of protein metabolism (amino acid metabolism).

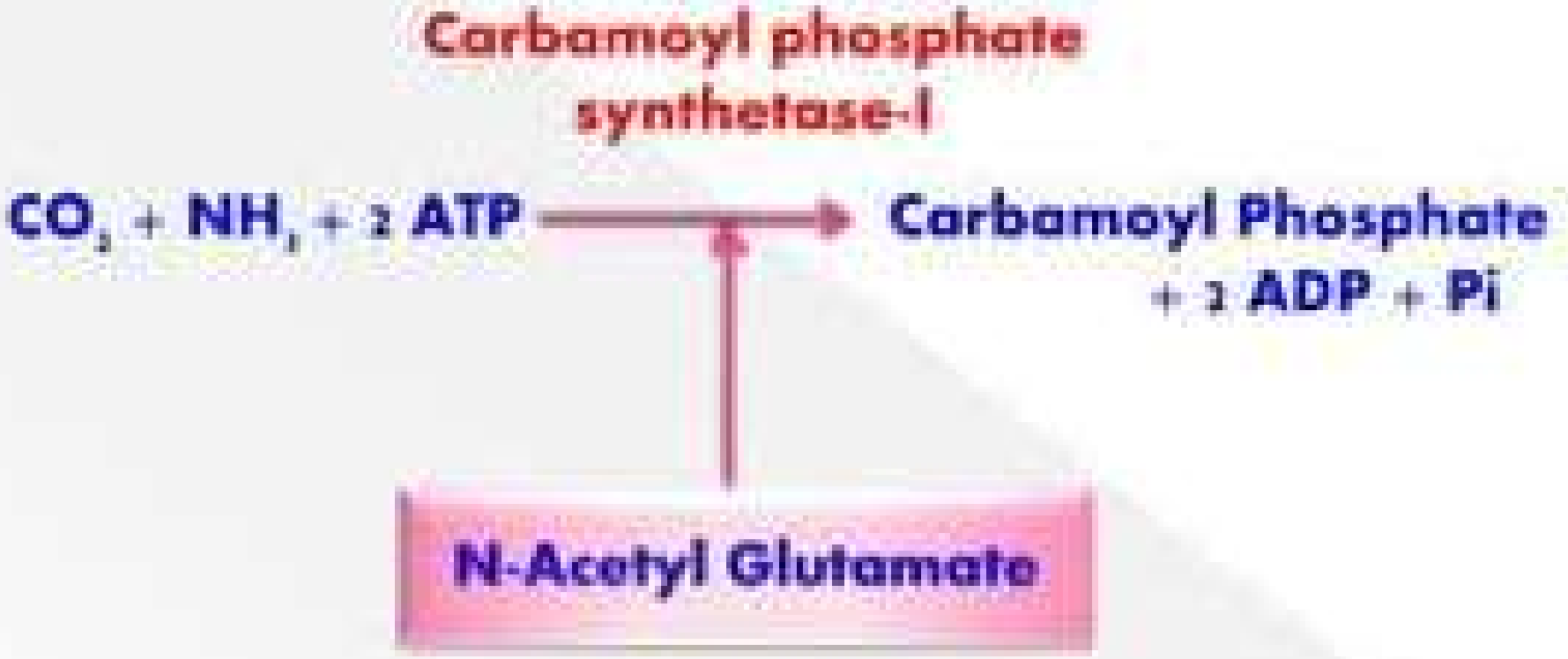
- Urea accounts for 80-90% of the nitrogen containing substances excreted in urine.
- Urea synthesis is a five-step cyclic process, with five distinct enzymes.
- The first two enzymes are present in mitochondria while the rest are localized in cytosol.

Step: 1 Formation of carbamoyl phosphate

- Carbamoyl phosphate synthase I (CPS I) of mitochondria catalyses the condensation of NH_4^+ ions with CO_2 to form carbamoyl phosphate.
- This step consumes two ATP & is irreversible.
- It is a rate-limiting.

- **CPS I requires N-acetylglutamate for its activity.**
- **Carbamoyl phosphate synthase II (CPS II) - involved in pyrimidine synthesis & it is present in cytosol.**
- **It accepts amino group from glutamine & does not require N-acetylglutamate for its activity.**

Step: 1 Formation of carbamoyl phosphate



Carbamoyl Phosphate Synthetases

CPS-I

- Mitochondria
- Uses NH_3
- Urea Cycle
- Activated – NAG

CPS-II

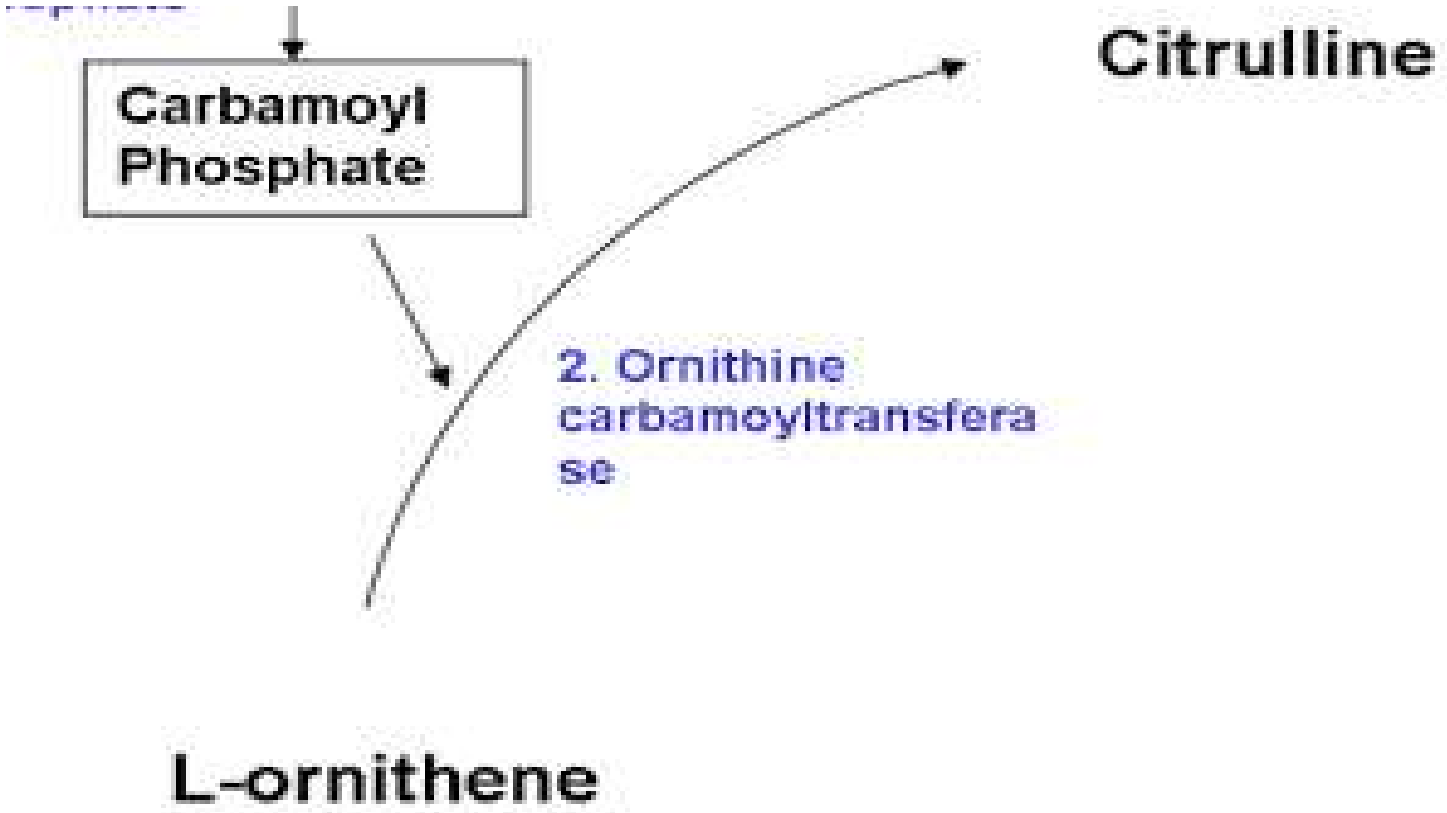
- Cytosol
- Uses Glutamine
- Pyrimidine biosynthesis
- Inhibited - CTP

Step 2: Formation of Citrulline

- The second reaction is also mitochondrial.
- Citrulline is synthesized from carbamoyl phosphate & ornithine by ornithine transcarbamoylase.
- Ornithine is regenerated & used in urea cycle.

- **Ornithine & citrulline are basic amino acids. (Never found in protein structure due to lack of codons).**
- **Citrulline is transported to cytosol by a transporter system.**
- **Citrulline is neither present in tissue proteins nor in blood; but it is present in milk.**

Step 2: Formation of Citrulline



Step -2

Step 2: Formation of Citrulline

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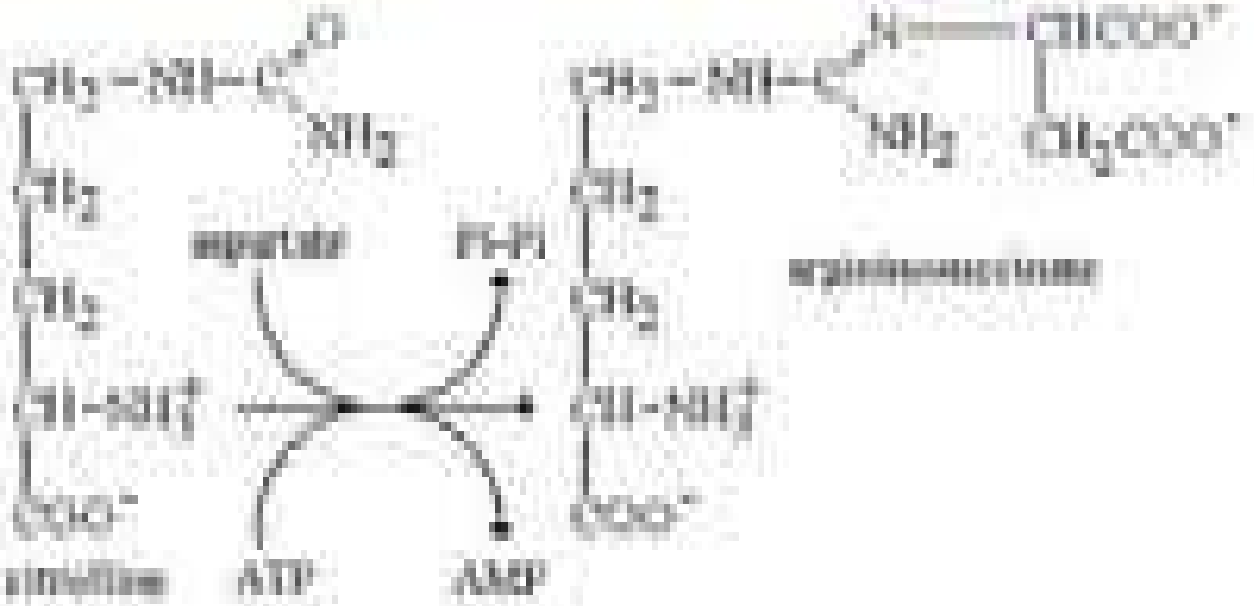
Step 2: Formation of Citrulline

Step 3: Formation of Arginosuccinate

- Citrulline condenses with aspartate to form arginosuccinate by the enzyme Arginosuccinate synthetase.
- Second amino group of urea is incorporated.
- It requires ATP, it is cleaved to AMP & PPi
- 2 High energy bonds are required.
- Immediately broken down to inorganic phosphate (Pi).

Step-3- Formation Of Arginosuccinate

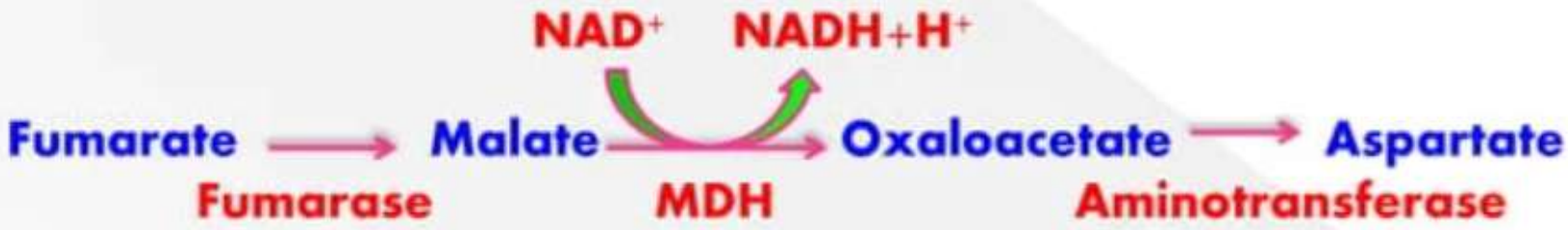


- Production of argnino-succinate is an energetically expensive process, since the ATP is split to AMP and pyrophosphate.
- The pyrophosphate is then cleaved to inorganic phosphate using pyrophosphatase , so the overall reaction costs two equivalents of high energy phosphate per mole.
- The reaction requires ATP and involves intermediate formation of citrullyl-AMP. Subsequent displacement of AMP by aspartate then forms Argininosuccinate.

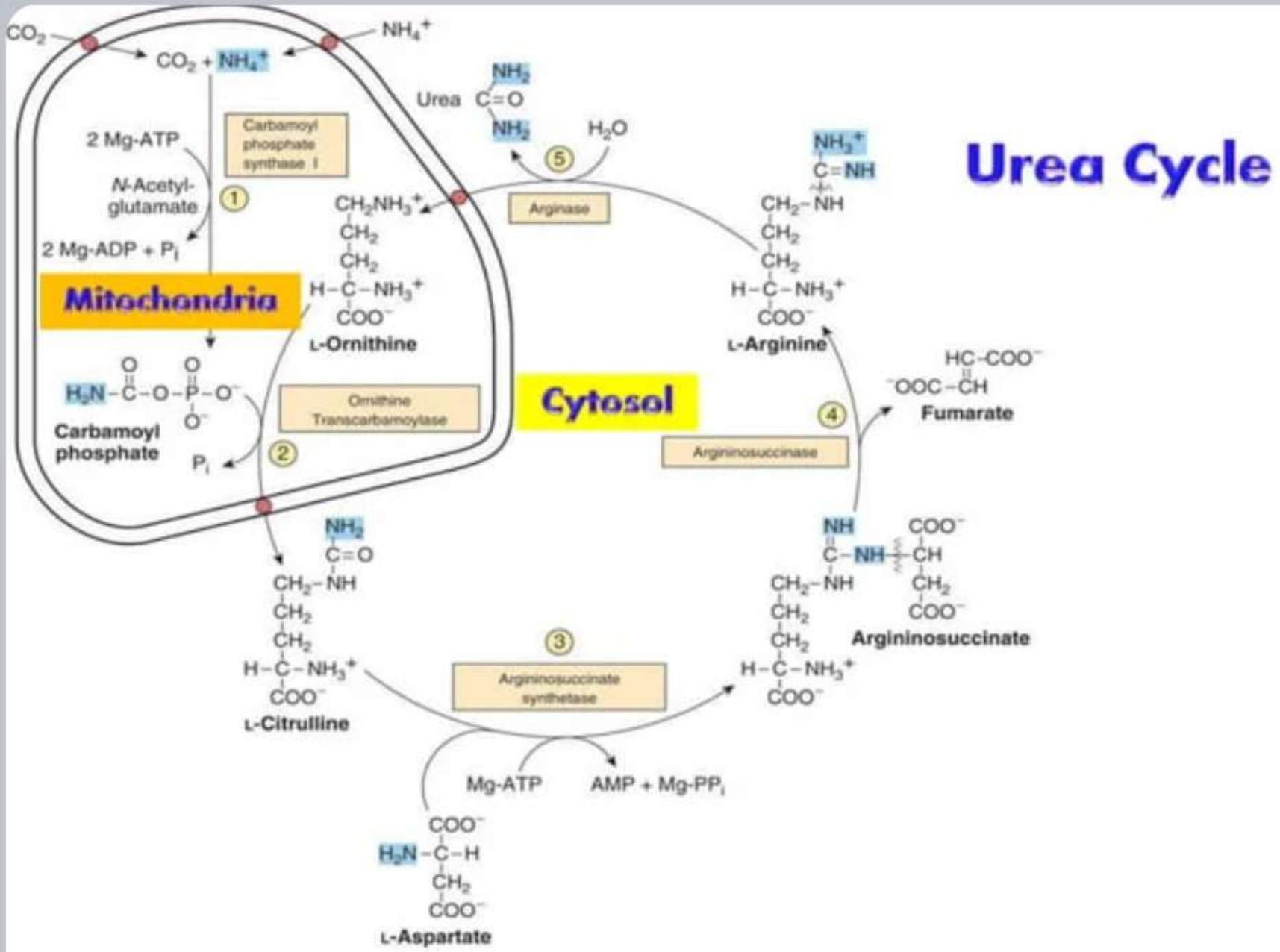
Step:4 Formation of Arginine or cleavage of Arginosuccinate

- The enzyme Argininosuccinase or argininosuccinate lyase cleaves arginosuccinate to arginine & fumarate (an intermediate in TCA cycle)
- Fumarate provides connecting link with TCA cycle or gluconeogenesis.

- The fumarate is converted to oxaloacetate via fumarase & MDH & transaminated to aspartate.
- Aspartate is regenerated in this reaction.



- Arginase is mostly found in the liver, while the rest of the enzymes (four) of urea cycle are also present in other tissues.
- Arginine synthesis may occur to varying degrees in many tissues.
- But only the liver can ultimately produce urea.



Step 5: Formation of Urea

- **Arginase is the 5th and final enzyme that cleaves arginine to yield urea & ornithine.**
- **Ornithine is regenerated, enters mitochondria for its reuse in the urea cycle.**
- **Arginase is activated by Co^{2+} & Mn^{2+}**
- **Ornithine & lysine compete with arginine (competitive inhibition).**

- Malate when oxidised to oxaloacetate produces 1 NADH equivalent to 2.5 ATP.
- So net energy expenditure is only 1.5 high energy phosphates.
- The urea cycle & TCA cycle are interlinked & it is called as "urea bicycle".

Energetics of Urea Cycle

- The overall reaction may be summarized as:
- $NH_3 + CO_2 + Aspartate \rightarrow Urea + fumarate$
- 2ATPs are used in the 1st reaction.
- Another ATP is converted to AMP + PPi in the 3rd step, which is equivalent to 2 ATPs.
- The urea cycle consumes 4 high energy phosphate bonds.
- Fumarate formed in the 4th step may be converted to malate.