Unit 1 ❑ Aims and scope of comparative physiology

Structure

1.1 General physiological functions and principles
1.2 Validity of comparative approach
1.3 Organisms and cell physiology
1.4 Suggested questions

1.1 General physiological functions and principles

The science of physiology is the analysis of function in living organisms. Physiology is a synthesizing science which applies physical and chemical methods to biology. The term physiology originated from the greek word 'Physiologikos' which means 'discourse on natural knowledge.' French physician Jean Fernel introduced the term in 1552. Physiology is thus a branch of science that deals with normal functions of the body.

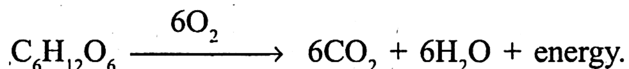
In the science of physiology, there is nothing supernatural about life. All the living processes of an organism can be explained on the basis of physico-chemical changes and structural pecularity. The progress of ancillary subjects like biology, microscopic anatomy, physics, chemistry, have been very useful in understanding the working processes of the living organisms. All physiological processes are governed by basic laws of physics and chemistry. Accordingly to the changes in the environment, functional alterations also occur and thus the survival is made possible. Hence, it is held that Physiology is a tripod science, its three legs being Anatomy, Physics and Chemistry. To know physiology, one has to study the subject with a sound basic knowledge of these three subjects. Blood flow through cardio-vascular system is governed by the 'Laws of fluid dynamics', exchange of fluids between different body compartments is regulated by hydrostatic pressure and osmotic pressure ; transfer of information signal involving transaction and development of action potential ; exchange of gases, and solutes obey the laws of diffusion. A study of all these physical phenomenon in the body comes under the head Biophysics. Biochemistry is the branch of science that explains the functions of the body on a chemical basis. All biochemical reactions including energetics are all governed by chemical laws and principles of thermodynamics. A study of structure of an animal comes under the study of Anatomy. The study of gross structure is called macroscopic anatomy, while the study of fine structures is called microscopic anatomy. Histology, cytology, histochemistry and cytochemistry all come under the study of microscopic anatomy. Medical statistics is a branch of science that deals with evaluation of experimental

data. These are used to confirm the authenticity of any observed change in the functions of the body.

In short, the whole purpose of Physiology is to explore, with the help of modern techniques, the normal functioning of the living organisms—their principle, their mechanism and their control.

A short summary of physiological study

The structural and functional unit of a living body is a cell. A collection of similar cells having same origin and performing same function but held together by intercellular substance is called a tissue. There are unicellular and multicellular animals in the world. When a multicellular animal is analyzed in terms of cell, it is complicated and is seen to be made up of huge number of cells of various shape and size, and a large amount of intercellular substance. But if the animal body is analyzed in terms of tissue, it is found to be made up of basically four types of tissues like, epithelial, connective, muscular and nervous. The term 'organ' is used to denote a structure being made up of those four types of tissues and is responsible for a particular function. For example, liver is an organ made up of hepatic cells which are epithelial cells ; it has connective tissue fibres forming septa, walls of blood vessels contain smooth muscle cells i.e. muscular tissue ; it has nerve supply, so it possess nervous tissue. Liver has many functions including secretion, excretion and metabolism. Hepatectomised animal will not survive. Finally, many different organs join together to constitute a 'system' responsible for a particular function. For example, the excretory system, responsible for excretion is made up of organs like kidney, ureter, urinary bladder and urethra. A multicellular animal has many systems in it like skeletal, muscular, cardiovascular, respiratory, excretory, endocrine, reproductive, nervous etc. There is a question why so many systems are present in an animal body including humans. A critical analyses revealed that every-one of them is necessary for the living organism. The ultimate aim of any living organism is to survive in the world. For survival one has to do work. For doing work energy is necessary. Hence, every living organism must have a mechanism to yield energy for doing work. Food is the source of energy. The items of food are carbohydrate, lipid, protein (calorigenic food); vitamins, minerals and water. Of these, the catorigenic foods gives energy, hence the name. When these foods are oxidized energy is released. For example—



$$C_6H_{12}O_6 \xrightarrow{6O_2} 6CO_2 + 6H_2O + \text{energy.}$$

thus, a molecule of glucose, when oxidized to CO_2 and H_2O, energy is liberated. It is seen from the equation that, to achieve this, oxygen is supplied by respiratory system. So this system is required. Next about the food, monosacclaride, say glucose, is utilised by the cell ; but the carbohydrate food that we eat is not monosacharide. They are either polysacharide like starch or glycogen or disaccharide like sucrose or

lactose. Hence, in the body there should be a system to convert these poly- and di-saccharides to monosaccharides so that they can be utilized in the body for not only supplying energy but also for forming any carbohydrate like substances in the body. This conversion of complex food to simplest substances is called digestion, which is carried out by digestive system. Hence, this system is also necessary.

Next comes the distribution of food. Digestion occurs in the digestive tract, the end products of digestion are produced in the small intestine. From here the end-products are to be transported to the cells all over the body. This requires a transport system. This is done by cardio-vascular system. Hence, the animals cannot go without this system. When the foods are utilized by the body by way of metabolism, many intermediate products are formed which are harmful to the body and need elimination to outside the body, e.g. urea. Thus a system has to be developed to cause excretion i.e. excretory system. To maintain a structure, a skeletal system is required ; for movement, mascular system is necessary. All these different systems must be properly regulated. For such regulation, endocrine system and nervous system have been developed.

1.2 Validity of comparative approach

For practical purposes, physiology has been divided into three categories, like—i) Cellular physiology, (ii) Physiology of special groups, and (iii) Comparative physiology.

An understanding of comparative animal physiology requires some background knowledge in general zoology, animal morphology, biochemistry and cellular physiology.

Comparative physiology is the study of organ function in a wide range of groups of organisms. Comparative animal physiology integrates and co-ordinates functional relationships that occur in more than one group of animals. It is concerned with the ways in which diverse organisms perform similar functions. Study of the comparative physiology reveals that, generally dissimilar organisms may show striking similarities in functional characteristics as well as in responses to the same environmental stimulus. On the other hand, closely related animals frequently react differently to their surroundings. Light, temperature, O_2 tension and hormone balance are used or are considered as variables for each function. Comparative physiology uses in addition to these, species or animal type as a variable for each type of function. Comparative animal physiology use kind of animal as an experimental variable which is a unique kind of biological generalization. Comparative environmental and behavioral physiology constitutes a bridge between molecular and organismic biology.

The most important function of comparative physiology is to put man into perspective in biological history and phylogenetic relationships.

To achieve the goal, the plan for the study of comparative physiology is made to examine the relation between components of the environment and the whole organism and to analyze the interactions in terms of organs and cell physiology.

For the applied biologist, comparative physiology has practical application in describing the physiology of economically important animals. For the ecologists, it helps the understanding of restriction of plants and animals to particular habitats. For the medical physiologists, the comparative viewpoint places man in his proper biological perspective. For the general biologist, it provides meaning to natural variation, and general principles which can be reached only with kind of organism as a variable.

1.3 Organisms and cell physiology

1.3.1 Diffusion

Particles, molecules and ions have a tendency to spread uniformly in the entire available space by their incessant random movements. This tendency to spread is called diffusion. Though they are moving at random in all directions, a greater number of particles move from a region of higher concentration to the area of lower concentration, than in reverse direction. Hence, there occur a net diffusion from higher to lower concentration and the two concentrations become equal. In this state equal number of particles move in all directions and the net diffusion is zero.

Fick's First law of diffusion states that the rate of diffusion (flux) of a solute particle is directly proportional to the magnitude of concentration gradient and it occurs down the concentration gradient.

Graham's law states that the diffusional flux (J) of a gas varies inversely with the square root of its density (P) and molecular weight (M).

Diffusion coefficient (D) denotes the diffusibility of the particle or gas. In case of solid the 'D' is the mass of solute diffusing across 1 sq cm area in 1 sec, down a concentration gradient of unity. Diffusion coefficient of a gas is the volume of gas that diffuses across 1 sq. cm. area per second, down a partial pressure difference (ΔP) of unity.

Diffusion is directly proportional to :

(i) Concentration gradient / Pressure gradient / electrical gradient.

(ii) Solubility in the medium

(iii) Temperature of the medium

(iv) Cross-sectional area through which diffusion is taking place.

Diffusion is inversely proportional to :

(i) Distance to be travelled / thickness of the membrane

(ii) Diameter of the diffusing particle

(iii) Viscosity of the medium

(iv) Shape of the particle—spherical particle diffuse easily than elongated particle.

(v) Charge of the particle and charge of the pore. If the charges are same, diffusion will be less, if the charges are opposite, diffusion will be more.

Biological application

1. Absorption of certain substances like pentoses, some minerals, some water soluble vitamins from intestine is carried out by diffusion. Same is the case of renal reabsorption of urea.
2. Water, water soluble substances pass largely by diffusion through water filled pores of the cell membrane. This is dependent on the pore size and size of the diffusing particle. Pore size is about 0.8 nm. Hydrated K^+ ion is 0.4 nm in diameter, hydrated Na^+ ion has 0.5 nm diameter. Hence, movement of K^+ is two times faster than the movement of Na^+. Glucose and galactose molecules have a diameter of 0.85 nm. Hence, they cannot pass via pores. They pass via membranes after combining with some carrier.
3. There are voltage gated ion channels in the membrane. When these channels open, ions move by diffusion along electrochemical gradient.
4. Exchange of respiratory gases occur by way of diffusion. The partial pressure of O_2 is higher in alveolar air and lower in the deoxygenated blood. So O_2 enters blood from the lungs. Alternatively, the partial pressure of CO_2 is higher in deoxygenated blood and lower in the alveolar air. So, CO_2 diffuses from blood to alveolar air. In the same way, due to difference of partial pressure, O_2 flows from the oxygenated blood to the tissue cell and CO_2 diffuses from the tissue cells to the blood.

   During hyperventillation, alveolar size is increased. Hence, the surface area is increased and wall thickness of the alveoli is decreased. So, rate of diffusion of O_2 and CO_2 is increased.
5. The alveolar surface area per unit body weight is larger in children than in adult humans. So, the resting O_2 uptake from the alveoli is higher in children than in adults.
6. There is a difference of partial pressure of O_2 and CO_2 between alveolar air and inspired air. So, O_2 and CO_2 diffuse down their respective pressure gradients between the terminal bronchioles and alveoli. This is how the alveolar air has its O_2 renewed and CO_2 partially removed.

7. When the cell size is increased, the surface volume ratio is decreased. This decreases diffusion of gases and solute across the plasma membrane, per unit volume of the cell. Enhanced cell-size also decreases rate of diffusion.
8. Replacement of cutaneous respiration across the general body surface by gill respiration and pulmonary respiration enhances the respiratory surface. So, respiratory exchange increases with the evolution of gills and lungs.

1.3.2 Osmosis

The movement of solvent from solvent side to the solution side at or from dilute solution to a concentrated solution, when the two are separated by a semipermeable membrane is called osmosis. Semi permeable membrane is that membrane which allows only the solvent molecules to pass through and not the solute molecules.

Osmotic pressure (OP) is the pressure which has to be applied on the solution side to stop the osmotic inflow into it from pure solvent.

Van't Hoff's laws of osmotic pressure

1. It is directly proportional to the molar concentraion of the solute so long as the temperature remains constant.

$$\pi = k_1 c$$

(where, π = Osmotic pressure, k_1 = constant, c = molar concentration.)

2. Osmotic pressure of a solution is directly proportional to the absolute temperature (T), so long as its concentration remains constant.

$$\pi = k_2 T \text{ (where, } k_2 = \text{constant)}$$

3. Van't Hoff - Avogadro law :

Identical numbers of moles of different solutes produce an identical osmotic pressure, when dissolved in the same volume of the solvent at the same temperature.

Osmotic pressure (OP) is expressed as atmosphere, mm of Hg or dynes per sq. cm (dynes cm^{-2}).

1 mole of a nonionized solute is equivalent to 1 osm (osmole). Osmolarity of a solution is its solute concentration in osmoles per litre of the solution. A solution of one mOsm of any solute in a litre possess an osmotic pressure of 19.3 mm Hg at 38 °C. Osmolatity of a solution is its solute concentration in Osm per kg of solvent.

It can be determined by—

1. Osmometer.

2. Barkley—Hartley method.

3. Freezing point method. This method depends on the direct proportionality between the osmotic pressure and the depression of freezing point of a solution. Depression of Freezing point is measured by Beckmann Thermometer.

$\Delta t = K_f M$ [where, M = Molal concentration of solute.

or, $M = \frac{\Delta t}{K_f}$ Δt = Depression of freezing point of the solution.

K_f = Cryoscopic constant]

The freezing point of one Molal solution is called cryoscopic constant (K_f) / molal freezing point.

The K_f for water is – 1.858°C

$\pi = CRT$

π = Osmotic pressure in atmosphere.

where C = Molal concentration

R = Molar gas constant (0.082 litre atmosphere)

T = Temperature in Absolute scale.

A sample of urine freezes at –0.56°C. Calculation of its OP at 37°C

$$\text{Molal conc. } C = \frac{-0.56}{-1.858} = 0.3$$

The sample will have OP at 37°C ($\pi = CRT$)

$0.3 \times 0.082 \times 310 = 7.6$ atms.

or, 7.6×760 mm Hg.

Osmotic work

When substances are transferred from lower concentration to higher concentration, osmotic work must be performed upon them. But when substances pass from higher to lower concentration, osmotic work is done by them.

Relation between osmotic work and concentration change is given by the equation—

$$W_{min} = NRT \ln \frac{C_2}{C_1} \quad \text{or, } 2.3\ NRT \log \frac{C_2}{C_1}$$

[W_{min} = Minimum osmotic work in small calories involved in the transfer of N moles of substance from a molal concentration of C_1 to a molal concenfration C_2.

R = Gas constant = 1.987 cal/mole/degree

T = Absolute temperature

ln = Natural log

2.3 is the factor for converting natural log to the log to the base 10.

Calculation of osmotic work to be done to transfer or secrete 3.545 g of Cl^- from plasma urine at 37°C, when the Cl^- concentrations in plasma and urine are 0.1 and 0.2 Mol respectively.

Cl^- conc. in Plasma .... 0.1 Molal C_1

Cl^- conc. in Urine .... 0.2 Molal C_2

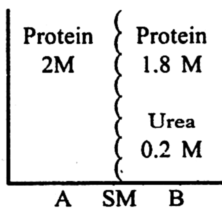
Osmotic work to be done to transfer / secrete 3.545g of Cl^- at 37°C in urine.

N = 0.1, R = 1.987, T = 310

$$
\begin{aligned}
W_{min} &= NRT\ 2.3 \log \frac{C_2}{C_1} \\
&= 0.1 \times 1.987 \times 310 \times 2.3 \log \frac{0.2}{0.1} \\
&= 141.7 \log 2 \\
&= 141.7 \times 0.301 \\
&= 42.65 \text{ cal}
\end{aligned}
$$

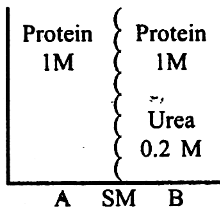
Osmoticity and tonicity

The osmoticity of a solution depends on the total solute concentration, both diffusible and nondiffusible. Whereas, tonicity of a solution depends on the concentration of nondiffusible solute only. Hence, two solutions may be isosmotic, but may notbe isotonic.



Solution A is isosmotic with solution B,
But solution A is hypertonic to solution B.

Similarly, two solutions may be isotonic but may not be isosmotic.



Solution A and B are isotonic, but the Solution B is hyperosmotic to Solution A.
SM = Semipermeable membrane.

Solvent always flows from by hypotonic to hypertonic solution. In biological system, tonicity is considered because biological membrane are not strictly semipermeable. They allow some solutes to pass.

Biological application of osmosis

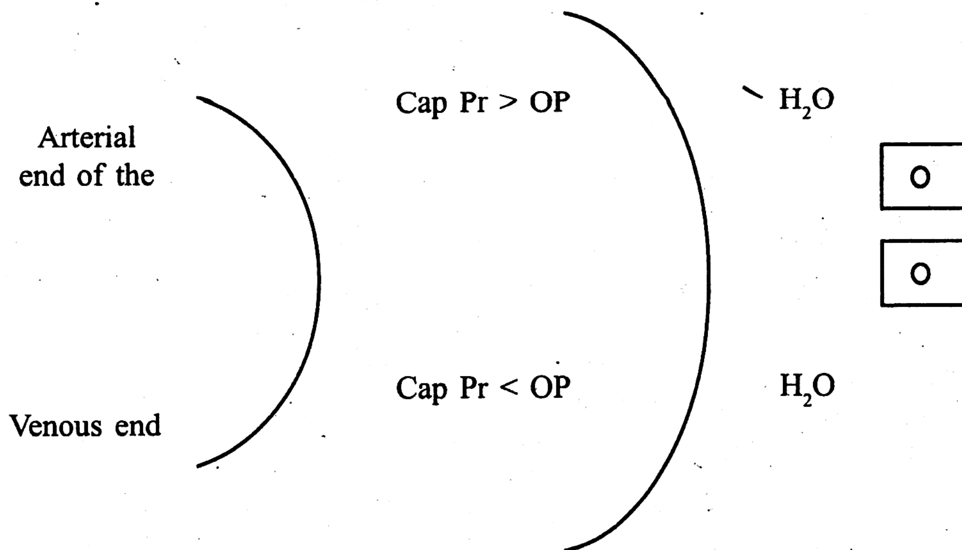
1. **Hemolysis, crenation, plasmolysis :** RBC is hemolysed or animal cell ruptures if placed in hypotonic solution. This happens because solvent flows from hypotonic solution to RBC or cell. Consequently, they swell and burst at a certain degree of swelling. Similarly, these cells or RBC will shrink (crenated)

if placed in hypertonic solution. It is due to osmotic outflow of water from the hypertonic solution. Plant cells lose water when placed in hypertonic solution. So, the cell membrane collapses and withdraws from the cell wall. This is called plasmolysis. Plant cells swell if placed in hypotonic solution, but they do not burst due to rigidity of cell wall.

2. **Osmotic distension of RBC :** Osmotic pressure of RBC fluid is 1.5 atm., higher than plasma, due to higher electrolyte concentration in RBC than plasma. So, RBC remain slightly distended, but they do not rupture. However, due to genetic disorder, Vit-E deficiency, or selenium deficiency and other defects, RBC may not be able to withstand such distension and rupture.

   RBC of camel are more resistant to osmotic distension. Camel can drink more than 100 litres of H_2O in 10 minutes. Blood becomes temporarily highly hypotonic, but RBCs do not rupture. It has been observed that RBC may be distended upto two times its volume, but hemolysis does not occur.

3. Osmotic pressure of plasma is higher than tissue fluid. It is due to plasma proteins. Total osmotic pressure of plasma is about 5453 mm Hg and that of ECF is 5430 mm Hg. The difference of 23 mm Hg is due to plasma proteins. It is called colloidal osmotic pressure of plasma. This osmotic pressure, hydrostatic pressure i.e. capillary pressure and pressure of the tissue fluid play an important role in the exchange of body fluid across the blood capillaries. Owing to pressure differences, fluid passes out from arterial end of blood



capillaries to the tissue fluid and body fluid enters from the tissue fluid into the capillaries at the venous end. These forces also govern the flow of fluid between any two compartments.

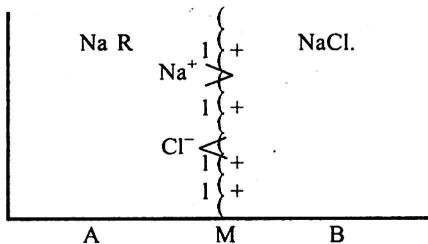
In Kwashiorkor, (a form of childhood malnutrition), hepatic cirrhosis and nephrosis, plasma protein concentration is decreased. So, colloidal osmotic pressure is decreased. This decreases water retention by the plasma and so edema develops.

4. Osmotic pressure plays a vital role in the absorption of water from intestine and kidney tubules.

5. Water absorption by plant roots is also governed by osmotic pressure. Root hair cells have higher osmotic pressure than surrounding soil-fluid. So, water enters into root hair cells. Water moves from one cell to the next cell by cell to cell osmosis and thus other cells are also distended. Hence, the cells become turgid and rigid and they stand erect on watering.

1.3.3 Donann membrane equilibrium

When a non diffusible ion in present in a solution, the distribution of diffusible ions across the membrane will be unequal. This was observed by Donann and it is called Donann membrane equilibrium.



In the compartments A and B, NaCl solution is present. The two compartments are separated by a semi-permeable membrane. Which allows NaCl to pass through but not the nondiffusible ion R. When R is not present the ion distribution in the two compartments is :

Na^+ A = Na^+ B

Cl^- A = Cl^- B

$Na^+ \times Cl^-$ A = Na^+ B $\times$ Cl^- B.

But when the nondiffusible ion (R) is added the distribution of diffusible ions become unequal :

Na^+A > Na^+ B

Cl^- A < Cl^- B.

Na^+ A $\times$ Cl^- A = Na^+ B $\times$ Cl^- B

The unequal distribution depends on the nature of nondiffusible ion. In a situation, as described above, where the nondiffusible ion is negative there will be more positive diffusible ion in the compartments that contains the nondiffusible ion.

Because of such unequal distribution of diffusible ion, there will be a pH difference, a potential difference, and an osmotic pressure difference on the two sides

of the membrane. If we consider the distribution of H^+ and OH^- then there will be more H^+ in the compartment that contains negative nondiffusible ion. Hence, there will be a pH differnce in the two compartments. The fluid in the compartment where the nondiffusible negative ion is present will have a lower pH (acidic) compared to the other compartment. Because of the presence of negatively charged hemoglobin in RBC, the pH of RBC fluid is less than plasma. The membrane is permeable to Na and Cl. Concentration of Na^+ in A is more than Na^+ concentration in compartment B. The concentration of Cl^- is less in compartment A than in the compartment B. Thus Na ions in A will try to move from compartment A to compartment B and Cl^- will try to move from compartment B to compartment A. This can only be prevented by developing an opposite electrical gradient. Hence, the side of the membrane where the non-diffusible negative ion is present is negatively charged and the other side of the membrane is positively charged. Donnan Phenomenon plays an important role in the development of resting membrane potential. It can be shown by calculation that total solute concentration in the compartment that contains the non diffusible ion is more compared to the other compartment.

In the diagram of the concentration of NaR is taken as 'a' and the concentration of NaCl as 'b' then at equilibrium, the concentration of NaCl in the two compartments will be :

Concentration of Na^+A will be (a+x) and Cl^- A will be x. Whereas concentration of Na^+ b will be b-x and Cl^- will be b-x. Since the product of the concentration of Na^+ and Cl^- will be equal in the two compartments. The equation can be written as $(b-x)^2 = (a+x)x$. From the equation the value of x in terms of a and b will be $x = \frac{b^2}{a+2b}$. Thus the amount of NaCl (x) that has moved from compartment B to compartment A is inversely proportional to the concentration of non diffusible ion in compartment A. When the concentration of NaCl (b) will be large relative to the concentration of nondiffusible ion (a) the value of x will be more. If specific arithmetic number is assigned to a as 1 mole and b as 2 mole then specific value of x will be obtained.

A	M	B
Na^+ a+x mol, Cl x mol, R a mol.		Na^+ (b-x)mol, Cl (b-x) mol

if a = 1 mol
and b = 2 moles

$$x = \frac{b^2}{a + 2b} = \frac{4}{1+4} = \frac{4}{5} \text{ or } 0.8 \text{ mol.}$$

A	B
Na^+....1.8	Na^+...1.2
Cl 0.8	Cl 1.2
R......1.0	
3.6 mol	2.4 mol

Thus the total solute concentration in compartments A is 3.6 mols and in compartment B is 2.4 mols. Hence the osmotic pressure of the solution in compartment A will be more than in compartment B.

The Donnan principle operates to regulate the distribution of electroyte ions across the membrane of living organism. However, the plasma membrane is not a strictly semipreamble membrane. It has selective permeability property. Thus the membrane may be impermeable to some diffusible ion and in that case such ions do not move following Donnan phenomenon. However this may produce Donnan effect. Thus Donnan effect is there but its quantitative evaluation is difficult. Deviation from Donnan Phenomenon has also been found. The distribution of cations, Na^+, K^+, Ca^{2+} an Mg^{2+} between plasma and lymph follows Donnam principle. They are high in plasma than in lymph. Plasma has a higher concentration of negatively charged nondiffusible ion protein. The difference in the composition of glomerular filtrate and plasma also follows Donnan principle. The glomerular filtrate contains less amount of positive diffusible ion and more amount of negative diffusible ion compared to plasma. This is also due to Donnan effect because of negatively charged plasma protein. Peculiarly, however, the concentration of Cl^- is higher in lymph than in plasma as required by Donnan principle, but the concentration of HCO_3^- in the plasma is little higher than in lymph which is contrary to Donnan principle.

1.3.4 pH and buffer

pH is negative logarithm of concentration of hydrogen ion (CH) to the base 10.

pH = –log CH

It is a measure of the H^+ concentration of a solution. It is determined by pH meter.

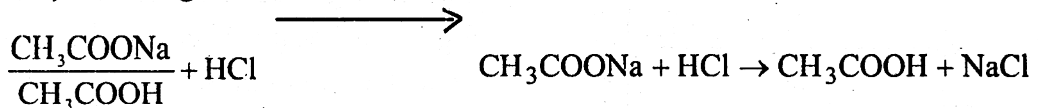
Buffer is a mixture of weak acid and its salt or weak base and its salt.

Henderson— Hasselbach equation of a buffer solution is given below.

$pH = pKa + \log \frac{Salt}{Acid}$. pKa is the dissociation constant of the buffer acid. In a buffer solution with equal amount of salt and acid. pH = pKa

Each buffer has a pH and a capacity.

The pH of a buffer depends on the pKa and salt ratio. The capacity of a buffer depends on the salt/acid ratio and on its amount. The buffers can not prevent the change of pH but it can resist the change of pH when any acid or base is added to it. For example when any strong acid is added to a buffer sol the salt of the buffer react with the strong acid added. As a result, equivalent amount of buffer acid is produced. Because buffer acid is a weak acid the pH is not changed much. However, as the salt/acid ratio is altered, there should be a new salt/acid ratio, so pH will definitely be changed.



$$\frac{CH_3COONa}{CH_3COOH} + HCl \longrightarrow \qquad CH_3COONa + HCl \rightarrow CH_3COOH + NaCl$$

Thus the buffer present in our body fluid help to resist the change of pH during production of metabolic intermediates. The buffer of our body has a high salt/acid ratio e.g. $\frac{HCO_3^-}{H_2CO_3} = \frac{20}{1}$. Such a buffer is good for handling acids. Important buffers present in our body fluid are bicarbonate, phosphate and protein buffers.

1.3.5 Poiseuille's Law

This law states the relationship between pressure gradient, resistance and volume flow.

Poiseuille and Hagen equation is $Q = \frac{P_1 - P_2 \pi (r)^4}{8\eta L}$

Where, Q = volume flow in ml/sec.

$P_1 - P_2$ = pressure difference between 2 points (dynes/cm^2.)

r = radius of the tube in cm.

L = length of the tube in cm.

η = viscasity of the fluid in poise.

It is revealed from the equation that

1) It pressure gradient is doubled, the flow will be doubled.

2) If the length of the tube or viscosity is doubled, the flow will become half.

3) If the radius of the tube is doubled. The flow will increase 16 times.

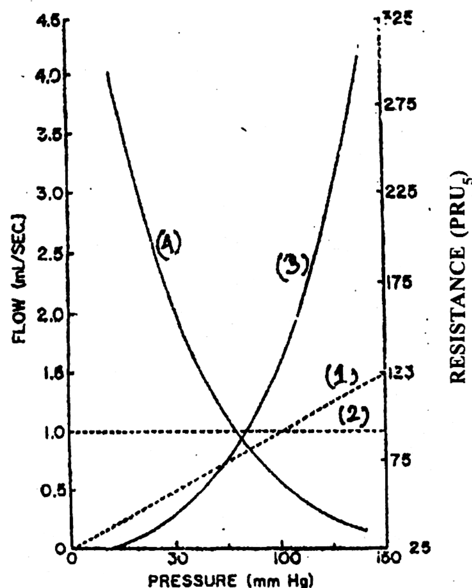


Fig 1.1 : The relationships of flow, pressure and resistance in nondistensible tubes using Newtoning fluids (dotted lines) at constant temperature (Poiseulle's law) and blood in vessels (solid lines).

In Poiseuille's experiment the fluid was a constant visocity fluid (Newtonian fluid) the tube was a rigid tube.

This law is applicable in closed vascular system but it is not obeyed in toto, because the vascular tubes are not rigid but distensible.

With a Newtonian fluid flowing through a rigid tube the pressure flow curve (Fig 1.1) is linear (1) because the resistance is not changed as pressure is increased (2). But with flow of blood in a vascular system it is not so (3) because the resistance is changed (4). It is very high at low pressure. When there is no flow (closing pressure). At high pressure the resistance is almost constant so flow becomes more linear.

1.4 Suggested Questions

1. Why respiratory exchange increased with the evolution of gills and lungs ?
2. How alveolar air gets its O_2 renewed and CO_2 partially removed ?
3. Why resting alveolar O_2 uptake is higher in children than the adults ?
4. Why rate of diffusion of respiratory gas is increased in hyperventillation ?
5. What is diffusion ? Name the factors which favour the rate of diffusion/ oppose the rate of diffusion.
6. State the Fick's law of diffusion and Graham's law of diffusion.
7. What is diffusion coefficient ?
8. Describe the role of diffusion in the transfer of respiratory gases.
9. Describe why evolution of gills and lungs is advantageous compared to cutaneous breathing.
10. Gill and lung respiration is better than cutaneous respiration—Justify.
11. What is Donnan membrane equilibrium ? State the role of Donnan phenomenon on osmotic pressure difference across the plasma membrane ?
12. Why pH of RBC fluid is less than plasma ?

13. Write the Henderson-Hasselbotch equation for a buffer solution.
14. Mention the factors on which the pH and capacity of a buffer depends.
15. Describe the role of pressure gradiant and resistance on the volume flow in a closed vascular system.
16. What is osmosis ?
17. What is osmotic pressure ?
18. State the laws of osmotic pressure.
19. State the role of osmotic pressure in the exchange of fluid across the blood capillaries.

Unit 2 ❑ Thermoregulation

Structure

2.1 **Regulation of body temperature in homeothermic animals specially mammals**

2.2 **Temperature regulation in poikilotherms**

2.3 **Hibernation**

2.4 **Suggested questions**

2.1 Regulation of body temperature in homeothermic animals specially mammals

Animal organisms have been classified into two groups depending on their capability of regulation of body temperature namely, homeothermic and poikilothermic animals.

Homeothermic Animals : The animals which maintain their body temperature more or less constant inspite of changes in the temperature of the environment are called homeothermic animals. They are also called warm blooded animals. Birds and mammals belong to this category.

Poikilothermic animals : The animals which do not maintain a constant body temperature and it varies as the environmental temperature is changed are called poikilothermic animals. They are also called cold blooded animals. Fish, amphibia, reptiles and invertebrates belong to this group.

In homeothermic animals a balance is maintained between heat gain and heat loss mechanisms present in them and thus the body temperature is kept constant. This is achieved by the operation of a complex neuro-hormonal mechanism regulated by the temperature controlling centre located in the hypothalamus.

Birds and mammals have a normal body temperature ranging between 40^0 – 43^0 C and 36^0 – 39^0 C respectively.

Normal body temperature in human subject

The normal body temperature of man ranges between 35·8 – 37·3^0 C when measured by introducing clinical thermometer into the mouth cavity (oral temperature). The temperature recorded from arm pit is called axillary temperature. It is slightly lower (37^0 C) than oral temperature. The rectal temperature is slightly higher than oral temperature (about 37^0 C). The superficial temperature i.e. skin or surface temperature ranges between 29·5^0 C and 33·9^0 C. The average temperature in deeper

tissue is called core temperature. It is always more than oral or rectal temperature. It is about 37·8^0 C (100^0 F).

The body temperature is slightly more in children than adults. It shows diurnal variation. It is about 1^0 C less in the morning and it reaches maximum value in the afternoon (1^0 C more than normal). The body temperature is increased in exercise, emotion and after meals. It is slightly less in females than males and the temperature rises sharply immediately after ovulation (0·5^0 C – 1^0 C). It is reduced 0·5^0 C in sleep.

A constant rectal temperature can be maintained in a nude human subject at air temperature or 0 – 1^0 C for 1–2 hours. In dry air maximum tolerance to high temperature has also been observed e.g. 200^0 C in a nude subject and 260^0 C in heavily dressed man.

Heat gain and heat loss mechanisms of the body

The body will gain heat from the environment if the body temperature is less than the environment and vice versa by way of conduction, convection and radiation. Thermogenesis and thermolysis : Heat production/gain is thermogenesis, heat loss is thermolysis in the body. However, the physiological processes of heat gain mechanism are shivering and nonshivering thermogenesis and vasoconstriction. On the other hand, physiological processes of heat loss from the body are vasodilation, sweating and fenting. The mechanism by which the body temperature is normally adjusted is known as thermotaxis.

Temperature regulating centre

A balance is maintained between thermogenesis and thermolysis and thus the body temperature is maintained. There is a temperature regulating centre in the hypothalamus which maintains this balance and thus help maintain homeothermy. If this part is destroyed the animals become poikilothermic.

On the basis of ablation and stimulation experiments it was shown that the anterior hypothalamus act as hot responsive centre, stimulation of which increases heat loss and dereases heat gain. The posterior hypothalamus acts as a cold responsive centre, activation of which stimulates heat gain mechanism and inhibits heat loss mechanisms.

However, subsequent studies were made with physiological stimuli like cooling and heating the hypothalamic regions by diathermy or by using thermodes. The studies revealed that the temperature detecting centre for both heat and cold are located in the anterior hypothalamus, whereas the centre initiating heat loss or heat gain mechanisms are present in the posterior hypothalamus.

Microelectrode studies on the hypothalamic neurons revealed the presence of different temperature sensitive neurons in the anterior hypothalamus.

(a) Some warm sensitive neurons respond on local heating of hypothalamus and they stimulale heat loss mechanism.

(b) Some respond to local cooling of hypothalamus and they activate heat gain machanism.

(c) There are some neurons whose rate of firing is changed in response to peripheral warming or cooling.

(d) There are neurons which respond to a rise in both hypothalamic and peripheral temperatures.

(e) There are neurons whose firing rate remain unchanged with temperature variation.

Serotonergic and cholinergic neurons are involved in temperature regulation, Mayers and Sharpe observed in monkey with push-pull technique using saline that when the donor is cooled a transmitter is released which can cause shivering in receipient. Similarlv heating the animal causes the recipient to lower body temperature. Subsequently it was observed in 'saline withdrawal' technique that saline withdrawn from donor monkey during cooling contained increased concentration of serotonin. Liberation of noradrenaline was augmented by peripheral warming.

Mayers *et al* reported the presence of a heat conservation and production pathway originating in the anterior hypothalamus is passing through the posterior hypothalamus and there is a heat dissipation pathway originating in the posterior hypothalamus.

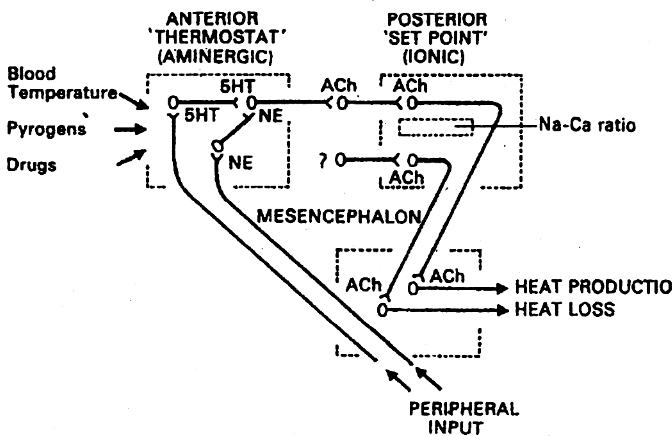


Fig 2.1 : Diagram of a model to account for temperature regulation under normal conditions as well as during a pyrogen-induced fever. Factor which affect the aminergic 'thermostat' in the anterior hypothalamus at given, and the outflow from the posterior hypothalamic 'set point' is mediated by a cholinergic system which passes through the mesencephalon. 5H = 5-hydroxytryptamine ; NE=noradrenalin ; ACh = acetylcholine. (Fro Myers, R. D. (1971). *Pyrogens and Fever.* CIBA Foundation Symposium Elsevier, Amsterdam.)

They proposed a mechanism of action of hypothalamus in the temperature regulation on the basis of experiments in monkey.

Serotonergic neurons in the preoptic region increase their firing in response to cooling. This causes activation of cholinergic pathway to the posterior hypothalamus and heat production is initiated (Fig. 2.1).

The noradrenergic neurons in the anterior hypothalamus are stimulated due to warming. They inhibit the serotonin cholinergic heat production

pathway by noradrenergic blockade of the synapse of serotonin—cholinenrgic junction. Such suppression of the heat production pathway permits the second cholinergic system in the posterior hypothalamus to activate the efferent heat loss pathway.

Set-point in the hypothalamus

Different experimental evidence indicate that there is a 'set point' in the hypothalamus which is like that of a tharmostat. When the temperature goes above the set point heat dissipation is increased and the temperature is lowered and brought back to set point. On the other hand if the temperature goes below the set point heat production occurs, the body temperature is raised and the set point in reached.

The presence of warm and cold sensitive neurons in the hypothalamus indicate that the combined action of these neurons play the role of hypothalamic thermostat. Thus temperature sensitive neurons in the anterior hypothalamus form the basis of a 'set point' mechanism.

It is held that over a physiological range of hypothalamic temperatures both warm and cold sensitive neurons or sensors are active. However a change in temperature will increase the discharge of one type of neuron and will decrease the firing of the other type. Hence, there will be a value of hypothalamic temperature at which the activity of the two sets of sensors will be balanced in terms of body temperature regulating responses which they produce. This will be the setpoint value of hypothalamic temperature. There are hypothalamic neurons whose firing rates remain unaltered by changes in hypothalmic temperature. These neurons or neural firings can provide a 'reference signal'. The difference between the firing rates of these temperature insensitive neurons and the temperature sensitive neurons provide an 'error signal'. The direction and magnitude of the 'error signal' will determine the extent of operation of heat gain and heat loss mechanisms to bring the temperature to the set point level (Fig 2.2).

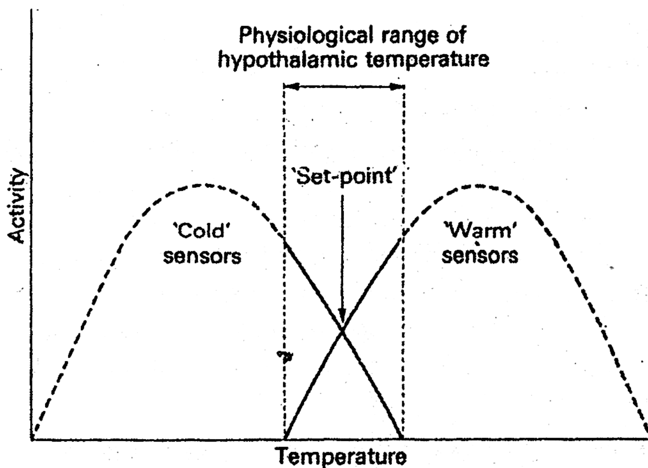


Fig 2.2 : Determination of hypothalamic 'set-point' temperature by the balance of activity in warm and cold-sensitive neurons. The response characteristics of these temperature sensors have been represented by bell-shaped curves. The interrupted portions of these curves are a hypothetical projection based on the suggestion that the temperature-sensitive characteristics of these sensors may be similar to those of the warm and cold receptors in the cat's tongue as described by Hensel and Zotterman.

Basic mechanism of temperature regulation

A change in body temperature, with reference to 'Set point', stimulates thermoreceptors present on the body surface as well as inside the body. These impulses (originate due to stimulation of receptors) reach the hypothalamus via neural pathways and stimulate it. Altered temperature of blood also activates it. It then modifies the heat gain and heat loss mechanisms via posterior hypothalamus, as required and the body temperature is kept constant. It is believed that in the cold the neural impulses are very important part in hot, the altered temperature of the blood is the most effective slimulus. It has been observed that when one hand is immersed in hot water, vasodilation is seen in the other arm. But this does not occur if the blood flow from the arm immersed in hot water is blocked. Similarly, if one hand is immersed in cold, there occur vasoconstriction in the other hand but this does not stop even if the blood flow for this region in blocked.

Temperature regulation in the cold

When the body temperature tends to decrease on exposure to cold environment, the following changes occur to keep the body temperature normal.

The cold receptors present in the body are stimulated, the neural discharge in the nerves attached to this is increased. These impulses and also the decreased temperature of blood stimulates the temperaure detecting centre in the anterior hypothalamus. This results in stimulation of thermogenesis and inhibition of thermolysis.

Thermogenesis is the primary motor centre for shivering and locatd in the dorsomedial portion of the posterior hypothalamus. Impulse discharge from here increase the tone of skeletal muscles throughout the body. The arrector pili muscles also contract. Thus heat production is increased. During maximum shivering body heat production can be increased four to five times normal. Intially there occur uncoordinated muscle twitches, the intensity of which increases until the rhythmic activity of visible shivering appears. It has been, shown that tensing the muscles can raise the heat production to 2–3 times the basal level, shivering starts when the body temperature goes below critical temperature.

Non-shivering thermogenesis also starts at the same time. Impulse discharge via sympathetic fibers reach the adrenal medulla and causes discharge of adreno medullary hormones adrenaline and noradrenaline. Neural impulse also cause release of TRH from hypothalamus which causes release of TSH from anterior pituitary. This hormone stimulates thyroid gland and causes secretion of T_3 and T_4 hormones. The calorigenic effect of these hormones increase heat production. Adrenaline increases glycolyis and fatty acid oxidation. It increases lipolysis specially from brown fat, and the oxidation of the released fatty acid is increased. Beside heat production it helps in heat conservation by preventing heat loss by way of vasoconstriction. Thyroid hormones

specially triiodothyronine (T_3) increases general metabolism and heat production. It is held that calorigenic effect of thyroid hormones is due to increased ATPase activity, oxidative phosphorylation, glycolysis as well as uncoupling of oxidation and phosphorytation. It also increases the calorigenic effect of adrenaline. Beside thermogenesis, there occur vasoconstriction in the body. This is caused by increased sympathetic discharge from posterior hypothalamus. This vasoconstriction decreases heat loss from the body and thus heat is conserved, and body heat production is increased. Along with this, there occur inhibition of sweating and this is another mechanism to prevent heat loss. In this way by stimulating shivering and nonshvering thermogenesis and vasoconstriction and inhibition of sweating, body heat production is increased and heat loss from the body is inhibited. As a result, fall of temperature is prevented and normal body temperature is maintained.

Different exprimental results indicate that :-

(1) The controlling centre for integrating and coordinating various temperature conservation function is contained in the posterior hypothalamus, although it is not thermosensitive.

(2) Critical temperature is the external temperature below which heat production has to be increased by shivering thermogenesis to maintain normal body temperature. It differs with species. In case of a tropical animal it is between 20^0–30^0 C, whereas in arctic animal like huskey dog the basal heat production may not increase even at enviromental temp, below –30^0C. In this respect it is 20^0 C in a nude human subject.

(3) In case of muscle shivering both flexors and extensors are stimulated.

(4) The nerve inpulse goes to the muscle via lower motor neuron being activated by tecto-spinal or rubro-spinal tract and not by pyramidal tract.

(5) Shivering begins when environmental temperature is below critical temperature (20^0 C in case of nude man). The degree of shivering increases as the ambient temperature is decreased.

(6) In respect of body temperature shivering begins when the core temperature goes below the set point (37^0 C in case of man). Shivering is increased as the body temperature goes much below the set point.

(7) Shivering is more if both air and core temperature are low.

(8) When air temperature goes below 27^0C, vesoconstriction occurs.

Temperature regulation in hot environment

When the body temperature tends to increase on exposure to hot environment the following changes occur to maintain the normal body temperature.

The hot receptors present in the body are stimulated. Nerve inpulse in generated in the nerve fibres attached to them. These impulses as well as increased temperature

of blood stimulate the temperature detecting centre located in the anterior hypothalamus. This results in inhibition of thermogenesis and stimulation of thermolysis.

Vasodilation and sweating cause thermolysis. It has been found that when the air temperature goes above 27^0 C the skin vessels dialate. This helps in heat loss from the body to outside.

Sweating : The sweat glands are innervated by sympathetic cholinergic fibres. When body temperature is increased impulse goes to the sweat glands from posterior hypothalamus via this pathway and cause secretion of sweat. Evaporation of sweat takes away the latent heat of vaporisation from the skin surface and thus the body is cooled.

It has been found that above 29^0 C environmental temperature, very little heat can be lost by convection. So, vasodialation does not help much in heat loss from the body. Above 35^0 C environmental temperature, the body loses heat entirely by evaporation. The air temperature at which sweating begins has been found to be about 31^0 C and for those lightly clad is 29^0 C in humans (the average skin temperature is about 34^0 C).

In respect of body temperature, when it goes above the setpoint, sweating begins. It increases as the body temperature is increased. It has been found that the degree of sweating is related to skin temperature and core temperature. When both core and air temperature high the degree of sweating is more. It has been observed that maximum rate of sweat secretion for thermolysis (thermal sweat) may be as high as 1.7 liters/ hour or more. When one liter of sweat is evaporated 580 Kcal of heat is lost from the body

As sweat coures from the blood rapid sweating demands a large cutaneous blood flow and so requires cutaneous vasodialation. This is brought about by :

(a) External heat acting directly on the blood vessels.

(b) Reflexly from cutaneous warm ending..

(c) By the rise of blood temperature acting directly on the hypothalamic centre..

(d) Activity of sweat gland secretion leads to formation of bradykinin which acts as vasodialator.

If the air is humid, sweat cannot be evaporated and so sweating cannot help in heat loss.

The secretion of sweat also show adaptation. If exposure to heat is continued, the sweat secretion is increated, it starts at a lower temperature, i.e. threshold for sweat secretion is decreased. The NaCl content of sweat is decreased by the action of aldosterone secreted in this condition. This prevents salt loss from the body. In case of excessive sweating, water and salt should be ingested to prevent dehydration and salt deficiency.

It has been shown that some birds and mammals allow their body temperatures to vary widely, either regionally in the body or in the whole body for sametime.

Temperature regulation in new born

The new born of all species, including human infants are of smaller size compared to their corresponding adults. Hence, they have greater surface area in relation to their body weight. This poses a great problem in the maintenance of normal body temperature.

A fall in body temperature can be prevented by decreasing heat loss and increasing heat production, cutaneous vasoconstriction has been found to occur in response to cold even in premature babies. The new born has a higher capacity to increase its heat production. A newborn animal or human infant, when exposed to cold hunch themselves and tucks their limbs to reduce effective surface area. Brown adipose tissue plays a very important role in heat production in infants. Fatty acid produced from lipolysis in brown adipose tissue are oxidized within these tissue as well as oxidized in other tissues after being carried there via circulation.

Tempurature regulation in non-sweating animals

In non-sweating animals, evaporative heat loss is achieved by other means. In case of birds panting and gular flutter help in evaporative heat loss from respiratory tract. Gular flutter, however, has been shown to be metabolically less expensive. In respect of heat gain in cold, these animals respond to cold by shivering. Muscular activity appear to be their only means of increasing heat production. Non-shivering thermogenesis has not been demonstrated in them. They lack brown fat and fail to show a thermogenic response to norepinephrine. However, they depend on white adipose tissue for compensation in cold.

When plenty of water is available, some birds may increase their cooling by urinating on their legs. Kangaroo rats have no sweat glands. Evaporation from the lungs is a great source for heat loss in them. However, due to scarcity of drinking water, they do not use much water for heat regulation, rather they are nocturnal and move for food only during night when the environment is cool.

Small animals such as rodents have no true sweat gland and also do not pant. They avoid heat by living in underground burrows.

Behavioral regulation of body temperature

Animals have been found to change their behaviour by regulating their body temperature. In a cold condition the animals including humans are found to curl themselves or remain close together to prevent heat loss. Alternatively, in hot weather the animal stretch themselves to increase the surface area to favour heat loss. The

rodents, specially in deserts have been found to enter into burrows in the daytime to avoid heat. Moreover, they become nocturnal so that this may be able to acquire food at night. This type of temperature regulation has been found to be much documented in several poikilotherms, both vertebrates and invertebrates.

Some use solar energy, while others utilize metabolic heat to raise the body temperature. The terrestrial environment is more prone than aquatic to sudden temperature changes. The most successful terrestrial poikilotherm insects and reptiles have made use of behavioral response to avoid extreme temperature or to elevate temperatures sufficiently for certain activities. Some reptiles have well developed sensory organs for this purpose. The infrared sense organs in the facial pit of rattle snakes can detect a temperature difference of the order of 0·001 to 0·005^0 C. This helps the animal to orient themselves to warm and cool environments. It also helps the animal in detecting a wam blooded and cold pray. The insects use solar or metabolic energy to warm up before flight. Social insects like ants, termites and bees may regulate their temperature in their nest or hives through varied activities.

Temperature regulation in aquatic animals

Water has high thermal conductance and a high heat capacity. Hence, the thermal loss to water is much higher than to air of the same temperature. The cooling power of water may become as high as 100 times as great as for air.

Many whales and seals live and swim in the near freezing water. However, regarding body temperature they are similar to other warm-blooded animal. It is around 36^0–38^0 C. There are three ways by which aquatic animals can cause heat balance in cold condition.

(1) They can live with a lowered body temperature.

(2) They can increase their metabolic rate to increase heat production.

(3) They can increase their body insulation to reduce heat loss.

It has been found that several species of seals and dolphins have resting metabolic heat production twice as high as would be expected from their body size. However, in the harp seal, their metabolic rate remained same in water even when the temperature goes down to the freezing point. (The critical temperature for harp seal in water is below freezing point, but it has not been determined). Thus the effective solution to the problem is to develop effective insulation and this is done in these animals.

They have a thick blubber under the skin that acts as an insulator. In seals, the temperature of the skin surface is identical to that of water but at the depth of about 50 mm (the thickness of the blubber), the temperature is nearly that of core temperature. The seals and whales being such well insulated feel difficulty in heat loss when the temperature of water in increased. In such a situation its skin temperature is increased

to eliminate heat. This is achieved by increasing blood flow through the blubber to the superficial layer of the skin which is well supplied with blood vessels. The cutaneous blood vascular system permits a precise regulation of the amount of heat that reaches the skin surface and thus is lost to the environment. Since the insulator is located internally to the surface of heat dissipation (skin), blood can bypass the insulator and heat loss during heavy exercise or in warm water can be independent of insulator.

In arctic land mammals, furs act as insulator. It is located outside the skin surface. The surface temperature of the body skin under the fur is close to core body temperature. Most of the insulation resides outside the skin surface. In case of polar bear fur is the insulator. But it has also a substantial layer of blubber under the skin. This is very important. When the polar bear swim in cold water, most of the furs get wet, most of the insulation value of furs is lost, the blubber plays an important role in heat conservation. Hence, blubber plays an inportant role in samiaquatic way of life.

In seals and whales, that lack blubber, have flippers are flakes. These appendages are well supplied with blood vessels and can lose substantial amount of heat if required. However, they require heat conservation in the cold which is achieved by developing heat exchanger system. It has been found that in the whale flipper, each artery is completely surrounded by veins. Thus, as warm arterial blood flows into the flipper, it is cooled by the cold venous blood that surrounds it in all sides. The arterial blood therefore reaches the periphery precooled and loses little heat to the water. The heat has been transferred to the venous blood, which is pre-warmed before it reaches the body. This kind of vascular heat exchange arrangement is called counter current heat exchanger, because blood flows in opposite directions in the two vessels.

2.2 Temperature regulation in poikilotherms

Poikilothermic animals attain the temperature of the environment where they live and do not maintain a constant body temperature like homeotherms. But some poikilotherms have some regulation of body temperature and thereby keep their body tempereture above the ambient temperature. Such temperature regulation has been developed to satisfy their requirement to combat thermal stress.

The insects are poikilotherms, but they face thermal problem because of high rate of metabolism during their flight. If the insect is too cold its muscles will not contract suitably for flight. In such a situation, it can increase the temperature of their flight- muscle by contraction similar to shivering in man. On a cold day a butterfly

or moth has been found to vibrate their spread cut wings for several minutes before take-off. At this stage, the temperature of the flight muscle has been found to be raised to about 35^0 C within 6 minutes in an ambient temperature of 20^0 C.

Snakes and lizards have been found to expose themselves to the sun in the early morning and raise their body temperature above the surrounding temperature by sunbasking. A lizard caught in the early morning at Peru (altitude—15,000 ft.), had a body temperature of 31^0 C. Later in the day it avoids sun and in hot days take shelter under rocks or cool burrows. A desert lizard save itself from overheating by behavioral changes because its lethal temperature is about 45^0 C and it will die if it is exposed to such temperature for 10 – 15 minutes.

Active temperature regulation has been observed in snakes. Large python has been found to coil their bodies around their eggs. At a room temperature of 25^0 C, the snakes maintain their body temperature about 5^0 C higher and this is achieved by spasmodic muscle cantraction like shivering in man.

2.3 Hibernation

Sometimes the animals pass through a state of dormancy to overcome adverse conditions. Dormancy is a general term for reduced body activities, including reduced metabolic rate. Dormancy has been variously classified according to its depth and duration e.g. sleep, torpor, hibernation, winter sleep and estivation. All these are specific physiological conditions, attained and maintained to pass over unfavorable conditions.

Torpor : It is a state of inactivity often with lowered body temperature and reduced metabolism that some homeotherms enter into so as to conserve energy stores. Small endotherms, because of their high metabolic rate are subject to starvation during periods of inactivity when they are not feeding. During these periods some animals enter into a state of torpor in which the temperature and mebabolic rate subsides. Daily torpor is practicised by many terrestrial birds. Several species of small mammals also undergo torpor e.g. Shrews.

Estivation : It is a state of dormancy in response to high ambient temperatures and/or danger of dehydration. It is also called summer sleep. Some species of both vertebrates and invertebrates exhibit estivation. Well known as estivators are African lungfish (Protopterus).

Hibernation : It is a state of deep torpor, or winter dormancy, in animals in cold climates, lasting weeks or months. Hibarnation is a well regulated physiological state that permits survival during most unfavourable part of the year. In this state the body temperature is greatly lowered and the metabolism, respiration, heart rate are greatly reduced.

As the ambient temperature goes below the critical temperature the heat production is increased to keep the body temperature normal. To have this, more food is required. In the winter, the food supply is short. So the animal cannot consume adequate food necessary to increase the metabolism so that the body temperature is kept constant and the animals will succumb to cold.

In such a situation, the animals give up homeothermy and pass into the state of hibernation and remains dormant. It remains in this state during the unfavourable condition. When the environmental condition becomes favourable it awakes and becomes active again.

Hibernating animals

It is seen in some mammals are birds. Pearson classified homeotherms into obligatory, stubborn and indifferent. Some members of the stubborn and indifferent homeotherms truly hibernate. Mammals that hibernate are some monotremes insectivores, rodents and bats.

Preparation for hibernation

Hibernation usually occurs annually and the animal prepares itself for hibernation by accumulating food. In hibernating bats circadian rhythm in body temperature and metabolism has been observed and this rhythm has been found to disappear as hibernation continues. Ultradian rhythm, however, has been found to persist for at least four years in hibernating golden-mantled squirrels. The animal gradually enter into hibernation when the ambient temperature is below a critical temperature.

Many mammals and a few birds regularly hibernate in each winter. The body temperature drops almost to the level of surroundings. Heart rate, respirations, metabolism and many other functions are greatly reduced. They show little response to external stimuli with the active life suspended, they can survive a long winter. Most animals that hibernate are of small size. Thus many rodents, hamstars, pocket mice, hibernate. insectivores at high lattitude, (e.g. hedge hog) bats hibernate. Humming birds, insect eating swifts and some mouse also hibernate. After the hibernation period is over, they revert back to prehibernation state. This is called arousal.

State of hibernation

In the state of hibernation the plysiological status of the animal is some what different.

1. The body temperature is very close to the ambient temperature and rises and falls with it. The colon and esophageal temperature may be 2–3^0 C above air temperatures. When air temp is 0^{0}C, animals tend to hold their body temperature at about 2^{0}C and the 0_2 consumption increases.

During hibernation, the thermostat activity of hypothalamus is reset at a low level as 20^{0}C or more below normal. At ambient temperature between 5^0 C – 15^0 C, many hibernators keep this body temperature as little as 1^{0}C above ambient temperature.

During hibernation, the thermoregulatory control operate at a low set point and with a reduced sensivity.

2. Prolonged period of suspended respiration develops, the rate of breathing may be reduced to even one or less per minute. Due to reduced respiratory exchange blood of many hibernators become acidic. This acidosis may further lower enzyme activity due to departure from optimal pH of metabolic enzymes.

3. Heart rate is markedly reduced. In case of active ground squirrel whose heart rate is 200 – 400/minute drops to 7–10/minute in the hibernating state.

The P – T interval of ECG in lengthened. Block of conduction may result in uncoordinated beats, vagal stimulation has been found to show no effect on heart. That hibernants can maintain there physiological functions at a low temperature is an adaptive phenomenon. For example, impulse conduction through nerve is blocked at 9^0C in rat (non-hibernant) but at $3{\cdot}4^0$ C in hamstar (hibernant). Na-pump mechanism is almost completely inhibited at 5^0 C in non hibernants, but in hibernants it still persists.

4. Blood flow is reduced, cardiac output shows a small decrease and that is accompanied with decreased heart rate. Stroke volume, however, remains unchanged, WBC count may be reduced and hematocrit slightly diminished. Clotting time has been prolonged it is due to a decrease in prothrombin. Serum Mg^{++} has been found to be high. Metabolism is reduced by 20 – 100 times. The RQ corresponds to fat.

5. Activity of nervous system does not entirely stop when the body cools. Citellus shows low amplitude cortical waves at 5^0 C and at a brain temperature of $6{\cdot}1^0$C it can still localize sound, erect pinae, vocalize and move.

6. Hibernation can last for weeks or for several months in cold climates. Many hibernators arouse periodically (once a week or every four to six weeks) to empty the bladder and defecation.

7. Some hibernators become temporarily resistant to X-irradiation.

8. It is a state in which dormany or torpor occurs that is much more pronounced than deep sleep.

Hibernating animals revert back to original normal state under favourable condition – the term called arousal. The time taken to go into hibernation is often much higher than the time taken for arousal. In ground squirrel, the time taken for attaining the peak torpid state is about 12 – 18 hours but arousal requires less than 3 hours. The hibernators are usually small. There are no large hibernators. They undergo winter sleep. This is because, they have less need to save fuel. This is again due to their normal BMR is low relative to their fuel stores owing to allometry of metabolism and fuel storage. Secondly, because of large mass and low BMR a prolonged metabolic effort would be required to raise the body temperature to normal level for a very low ambient temperature. For example, a large bear would require

at least 24 – 48 hours to warm up to 37^0 C from a hibernating temp of 5^{0}C. Warming up of such a large mass would also be energetically very expensive.

Arousal from hibernation

The hibernant retains its ability to arouse when the ambient temperature increases appreciably. The animal warms up again and returns to the warm blooded state. Arousal from hibernation is a rapid awakening with warming starting from thoracic region. It is a process of self rewarming and does not require external heating. It is a rather rapid process. The rectal temperature can increase by nearly 20^0 C in an hour. Awakening starts with an explosive outburst of heat production and the peak metabolism is maintained for sometime (e.g. 1/2 hr for marmot) after which the heat production subside gradualy into its basal state.

The rewarning during arousal from hibernation is most expensive.

During arousal, heat is produced by violent muscular shivering and oxidation of fat in the brown adipose tissue. Brown fat or brown adipose tissue are present in smaller or larger patches along the neck and between the shoulders. These cells are filled with fat and with large mitochondria (responsible for brown color— hence the name brown adipose tissue or brown fat.) The tissue has rich blood supply and connected by sympathetic nerves. Here oxidation of fat yields high amount of energy because in these cells uncoupling of oxidation and phosphorylation occurs. It is caused by a protein called thermogenin (MW 32 kDa). Hence, heat is produced at a very high rate. Besides fat it also oxidizes other substrates supplied with blood. The heat production is turned on by noradenaline or by nervous stimulation. This brown fat is found in all hibernating mammals, but it also occurs in many new born mammals including man. It is rather peculiar that they are not seen in a number of birds which regularly hibernate. In most mammals, the brown fat has been found to be lost but it remains in hibernators.

The rewarming occurs differently in different parts of the body. It has been seen that the anterior part of the body that contain vital organs like heart and brain warms much faster than the posterior part. In this respect, rewarming of the heart at the begining is not only essential but must be an initial step because the proper functions of the heart is needed to supply circulation of oxygen for all other organs. The major mass of brown fat are also located in the anterior part. It has been observed that the reheating process in the posterior part begins only when the anterior part has reached near normal temperature.

Control of hibernation

Hibernation – arousal is the cyclic phenomenon operating in hibernating animals. It is regularly controlled. Neuroendocrine involvement has been well documented in this process.

A circadian rhythm has been noticed. The begining of hibernation cycle is usually

associated with a particular time of the year, but it is not necessarily induced by low temperature or adequate food.

Yearly cycle of hibernation is influenced by the duration of the daily light cycle and is also associated with a dark cycle.

A well regulated heat production require a well coordinated Central Nervous System (CNS). e.g. European Hedgehog keep the body temp. at $+5^0$ to $+6^0$ C as the ambient temperature goes below freezing temperature. Aes imilar well regulated torpor has been observed in West Indian humming bird *Eulampis jugularis* which like other humming birds readily becomes torpid. The body temp. of *Eulampis*, when torpid, approaches air temperature but if air temperature drops to below 18^0 C it results for the fall (in body temperature) and keep the body temp at 18 – 20^0C.

It is seen in hamstars that in case of lesion in post-hypothalamus, they fail to enter into hibernation. However in case of lesion in anterior hypothalamus, the ground squirrel may enter into hibernation but they fail to arouse.

Adrenalectomized animals do not hibernate and in hibernation adrenal cortex is depleted. Hibernating animals do not respond by thermogenesis to injection of norepinephrine.

2.4 Suggested questions

1. Explain homeothermic and poikilothermic animals. Discuss the role of hypothalamus in the regulation of body temperature.
2. Write briefly on the regulation of body temperature in poikilotermic animals.
3. How body temperature in regulated in aquatic animals.
4. Describe how body temperature is regulated in hot/cold environment.
5. Comment on temperature regulation in infants.
6. Write a note on behavioral regulation of body temperature.
7. How body temperature is regulated in non sweating animals
8. What is hibernation ? Write a note on the preparation for hibernation.
9. What is arousal ? Describe the process.
10. Write briefly on the neuroendocrine involvement during hibernation.

Unit 3 ☐ Communication among animals

Structure

3.1 Bioluminescence

3.2 Pheromones and other semiochemicals

3.3 Audio signal

3.4 Suggested questions.

3.1 Bioluminescence

The emission of light of visible spectrum by living organism is called bioluminescence.

It is a process in which living organisms convert chemical energy into light.

Luminescent organisms have been observed throughout the ages. Christopher Columbus during his historic voyage across the Atlantic noticed mysterious patches of luminescent light around the water of his ships. Aristotle observed that the flesh of dead fish and damp wood appear to luminescence. Subsequently it was shown that glow in dead flesh was dependent on oxygen. Raphael Dubois was the first to isolate the light producing chemicals from 'clams'. This paved the way for the characterization of the molecular chemical and physiological mechanisms behind the process. Bioluminescence has been observed in thousands of species including bacteria, fungi, and marine animals. Bioluminescence can be defined as the emission of ecologically functional light by living organisms. It is primarily a marine phenomenen with a few exceptions seen in freshwater and terrestrial organisms, light emission plays an important role in the life of bioluminescent animals.

Types of bioluminescent animals

Many organisms produce light with the help of photogenic organs, tissues or cells. This is called self-bioluminescence e.g. fire-flies. Some other organisms emit light which is actually produced by some symbiont bacteria present in their bodies. This is called hetero-bioluminescence e.g. Loligo and some fishes.

Bioluminescent organisms

Although it is basically a marine phenomenon many animals exhibit this. It is seen in Protozoa, Cnidaria, Annelida, Arthropoda, Mollusca, Echinodermata, Protochordata and Fishes. It is not seen in terrestrial vertebrates.

Photogenic devices

In some cases, e.g. protozoa the chemicals and enzyme required for light

production are diffused throughout the cell; in ophiuroids the light producing apparatus is present in photogenic cells present in body surface. In higher organisms definite photogenic organs are present which produces light. These organs have been best studied in insects and fishes. These are paired organs in insects being located in the head and abdominal segments. Typically, each photogenic organ has a layer of photocytes containing mitochondria, required chemicals and enzymes for chemical reaction. These cells are arranged cylindrically at right angles to the translucent cuticle which permits light to pass through it. Behind the photocytes, there is a reflecting surface chiefly consisting of urate granules. They receive oxygen through air tubes or tracheoles (Fig. 3.1).

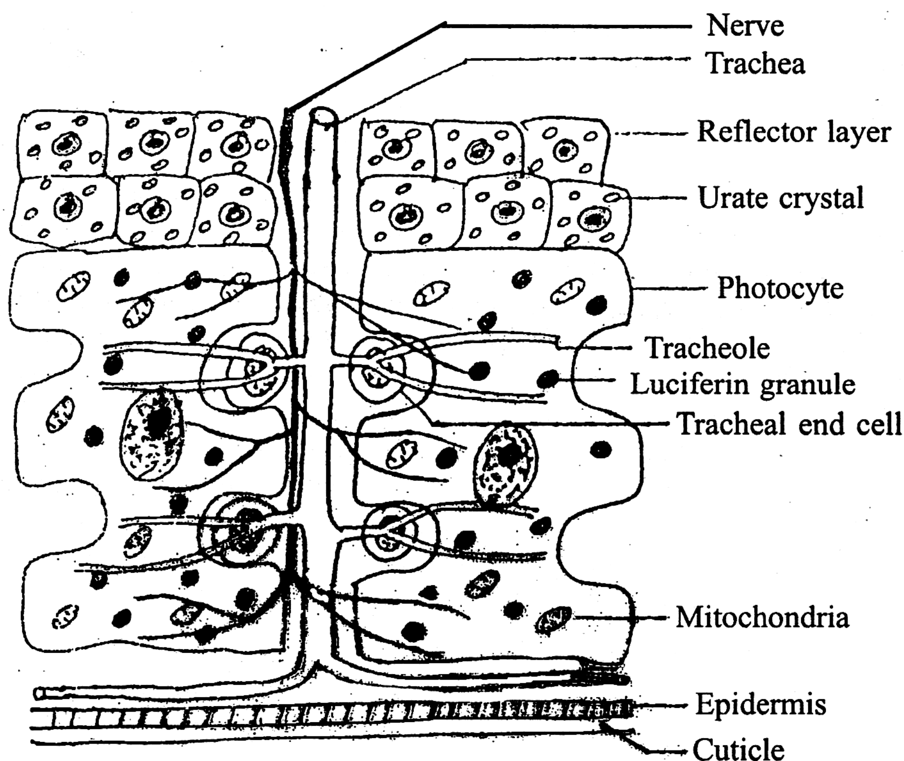


Fig 3.1 : Photogenic organ of a fire-fly. (After Chapman)

A number of fishes, mostly deep sea forms, possess charasteristic luminescent organs called photophores. These organs are probably specialized gland cells of the epidermis. They show considerable variation in their number and mode of distribution on the body. These glanduler structures generally occur along the lateral and ventral sides of the body and head. They may be arranged in one or two rows extending on the sides from head to tail as in *Scopelus* and *Halosauropnis* or they may be located in some limited parts of the body. Besides these organs, a few large and complex

organs may be present on different parts of the body e.g. suborbital organ in *Opostomias micripnuhs, Scopelus benoitii and Pahystomias microdon*. Large photophores present on the elongated first finrays of pectoral, dorsal fins of Angler fishes. In toad fish a large number of photophores are present along the lateral line.

Fishes with luminescent organs have world wide distribution. Majority of them are bathypelagic living at a median depth (500 – 2500 meters) and sometimes migrate to the surface at night. Some species are also found in deeper waters.

Luminescent organs are of two main types. Some species are self luminous. In others the light is produced by symbiotic bacteria. Self luminous photophores are found in some elasmobranchs (Squalidae and Torpidinidae) and in the teleost belonging to the families Stomiatidae and Myctophidae (or Scopelidae). These photophores are simple and complex. The simple photophores consist of a series of radially arranged glandular tubules that receive branches from the adjoining cranial and spinal nerves. In more complex photophores additional structures like a reflecting layer and a lens like structure also develop. The suborbital organ of *Pachystomias microdon* is quite complex in structure. It is a cup like structure and its wall is composed of several concertric layers. Externally there is a layer of black pigment and numerous glandular tubles are present in the cup. A thick layer of light reflecting spicules is present in the cup where axial part is full of a number of radially arranged glandular tubules. The mouth of the cup is occupied by a lens-like structure and the skin forms a covering like an iris diaphragm over it. The organ is supplied by a branch of fifth cranial nerve. Both simple and complex photophores may be present in the same species.

Luminescent organs in which light is produced by symbiotic bacteria are found in a large number of species belonging to different families like *Malacocephalus laevis, Monocentris japonicus, Photoblepharon, Anomalops, Leiognathus* and the Angler fishes.

Structurally these photophores consist of a large number of glandular tubules that secrete luminous bacteria. In some genera e.g. *Malacocephalus*, the highly vascular gland opens by a duct on the ventral surface of the fish in front of the anus *(Malacocephalus)*, species of some genera (*Photoblepharon* and *Anomalops*) possess an elongated luminescent organ below each eye. It consists of numerous long parallel glandular type with rich blood supply. The organ has pores opening to the exterior at the anterior end a 'reflector layer' at the hind end.

Since these luminescent organs produces light for long periods due to bacterial luminescence, mechanical devices have developed to turn the light on and off. In *Anomalops*, the light is cut off by moving the luminescent organ downward by a hinge, so that it comes in contact with a black pigmented tissue. In *Photoblepharon* a fold of black tissue is drawn up like an eye lid over the organ to cut off the light.

Bioluminescence reaction

Reaction occurs between the substrate luciferin and the enzyme luciferase in presence of oxygen. Bioluminesence is an enzymatically catalyzed chemiluminescence. In chemiluminiscence the reaction releases energy but instead of being lost as heat or coupled to some synthetic reaction the energy is used for the specific excitation of a molecule capable of releasing the energy as a photon. The energy of the photon is not fixed, but depends upon the color of the light. The energy, E, is given by the fundamental equation $E = h\nu$. when h is the planck's constant and ν is the frequency. For bioluninescence, where the light is in the frequency range correspoding to wave length between 450-600 nm. the energy involved ranges from about 65 – 45 k cal per mole of photons (an einstein).

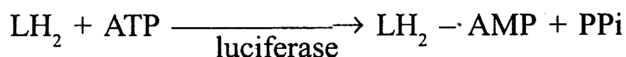
French scientist Dubois coined the term luciferin (color peaning) which is the substrate and the enzyme called luciferase that catalyzes the reaction.

Luciferin and lucifeurase are now used an generic terms to refer to the substrate and the enzyme involved in any bioluminescent reaction. Although different specific molecular species are involved in each different class or group of organisms, the general reaction mechanisms have certain close similarities and thus can be represented by a generalized equation. Luciferin is a heat stable, heterocyclic phenolic compound with a molecular weight of 280 and an emperical formula $C_{15} H_8 N_2 S_2 O_3$. There are different types of luciferins like bacterial luciferin, Dinoflagellate luciferin, Vargulin, Coelenterazines, Firefly luciferin.

Luciferases are heat-sensitive enzyme. Firefly luciferase belongs to acyl adenylate thioester forming super family. The enzyme of different organisms have different amino acid composition and structurs. For exemple pure bacterial luciferase is a simple protein with MW 79 k Da. Fire fly luciferase has a MW of approximately 100 k Dal. with two different subunits and one active centre per molecule.

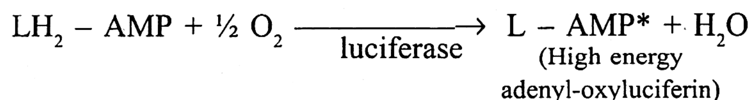
Giese (1973) proposed the following scheme of reaction within photocytes in firefly.

1. At first luciferase catalyzes reaction between luciferin (LH_2) and ATP, the products formed are Adenyl-luciferin and pyrophosphate.



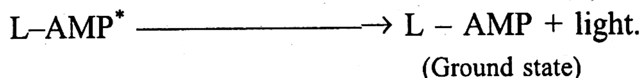
$$LH_2 + ATP \xrightarrow[\text{luciferase}]{} LH_2 - AMP + PPi$$

2. Next, the enzyme catalyzes oxidation of adenyl-luciferin by atmospheric oxygen with the production of high-energy adenyl-oxyluciferin and water



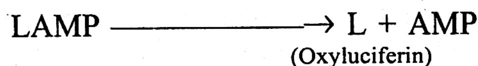
$$LH_2 - AMP + \tfrac{1}{2} O_2 \xrightarrow[\text{luciferase}]{} \underset{\text{(High energy adenyl-oxyluciferin)}}{L - AMP^*} + H_2O$$

3. In the next step, high-energy adenyl - oxyluciferin spontaneously returns to ground state liberating its energy in the form of light.



$$\text{L–AMP}^* \longrightarrow \underset{\text{(Ground state)}}{\text{L – AMP}} + \text{light.}$$

4. Later, adenyloxy-luciferin breaks down into oxyluciferin + AMP



$$\text{LAMP} \longrightarrow \underset{\text{(Oxyluciferin)}}{\text{L}} + \text{AMP}$$

important characteristics of the reaction :

(a) Luciferase acts best at a pH of 7·8

(b) The activity is highest at 23^0 C.

(c) Atmospheric oxygen and ATP are required for their reaction.

(d) Divalent cations like Mg^{++}, Mn^{++} or Co^{++} stimulate luciferase action and the ionic environment probably also controls color of the light produced.

(e) Different types of luciferin – luciferase control the colour.

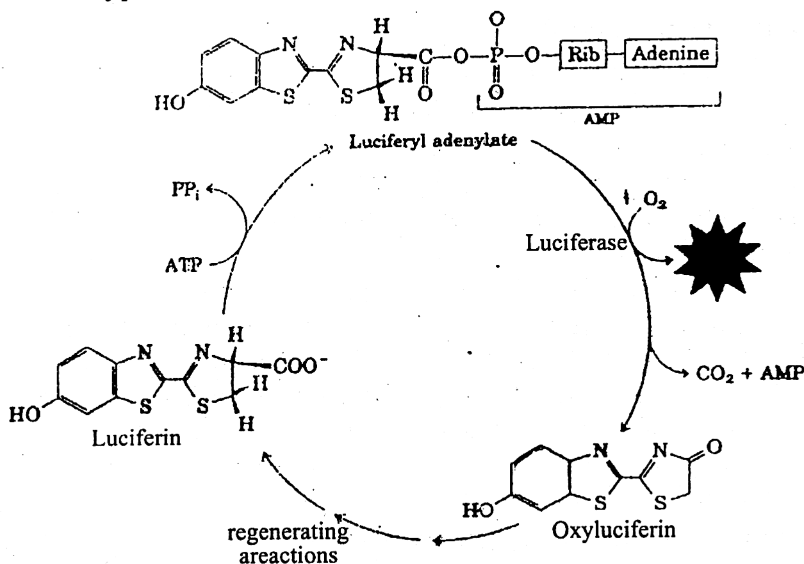


Fig 3.2 : Important components in the firefly bioluminescence cycle.

Physical properties of colours :

(a) The light produced is not hot, it has a temperature below $0{\cdot}001^0$ C.

(b) The light is free of UV and infra red rays.

(c) Intensity of light is low.

(d) Wavelength of light ranges from 5000 – 6000 Å.

(e) The light may be of different colours.

The annelids give blue light. Amongst fireflies, *Fulgora* gives white light, many species produce yellowish green but *Pyrophorus* gives out green light from thorax, and red light from abdomen. Fishes produce blue or bluish-green light. The Dragon fish, however, emits red light. It is known as dragon light. It is peculier that only dragon fish can perceive red light. This allows them to haunt unseen and find prospective mates without alerting their own enemies (Fig. 3.2).

Some organisms emit light continuously but some emit flashes that range in duration from about 1/10th of a second to 10 seconds.

Regulation of bioluminescence

Mechanical, nervous and hormonal processes control light production. In protozoa, mechanical stimulation by waves in the sea stimulates light production. In higher organism nervous regulation exist. In insects, when the nerves supplying photogenic organs are stimulated acetylcholine is produced. It reacts with ATP and CoA and forms acetyl CoA which in turn stimulates luciferase action. In fishes, photophores are under the control of Vth cranial nerve and spinal nerves.

Adrenaline has also been found to stimulate photophore activity.

Importance of bioluminiscence

1. Light flashes help the organism to find out prey, to escape from danger to puzzle and frighten their enemies, to attract opposite sex.

Many deep sea fishes illuminate the surroundings are thus help to find out preys. Many organisms attract their preys by light flashes e.g. deep sea fish, fireflies. Deep sea fishes can frighten their enemies by sudden flashes of light. Flashes of light may act as a mating signal.

2. There are several well known instances of bioluminescence during sexual behaviour. In some cases the light plays a part in the timing of reproduction and in synchronising the activities of males and males. The mating of fireflies often depends on a very accurate signaling system, the males flash their lanterns as they fly approximately 50 cm above the ground if a female sees one of these flashes within 3–4 meters, she may be expected to flash back after an exact interval of 2 sec at 25^0C. This attracts the male in her direction.

3. Luciferin – luciferase system is a highly sensitive test for detection and determination of ATP. In the laboratory pure firefly luciferin and luciferase are used to measure minute quantities of ATP by the intensity of light flash produced. As little as few picomoles (10^{-12} mole) of ATP can be measured by this processes.

In can also be used as a sensor for the determination of intracellular calcium concentration in the micromolar to nanomolar range.

4. Bioluninescence can help in detecting energy problems in human cells. This technique is now used to study abnormalities like ailmens of heart, muscular dystrophy, urological problems etc. It has been found that injection of luciferin and luciferase exhibit different reactions in normal and cancerous cells.

5. In pest management it is used as a tool for mapping the distribution pattern of organisms.

6. The bright luminescence generated from luciferase assay made it ideal for sensitive non-radioactive assay.

7. It helps in the detection of life in an unknown planet. Presence of ATP an indication of life, is ditected by bioluminescence technique in the soil of an unknown planet.

8. This technique is used in the study of genetics. It is used to detect the presence of some gene in a cell and to determine whether the gene is turned on or off. It has been shown that taking a single gene from the jelly fish aequorea and attaching it to the gene of another organism make the cell glow green when that gene is turned on. The jelly fish gene encode a protein called green fluorescent protein (GFP). When the jelly fish is disturbed, Ca^{++} bind to aequorin and produces blue light in the absence of GFP, but in presence of GFP green light is produced. The scientist Charles was able to get GFP to shine green in the absence of Ca^{++} and aequorin by simply shining a blue light on it. This discovery has a broad application in the area of genetics.

3.2 Pheromones and other semiochemicals

The term 'pheromone' is originated from the greek words 'pherin', to transfer, and 'hormone', to excite. It describes a class of chemicals that are communicated between animals of same species and that elicit stereotyped behavioral or neuroendocrine responses. Some pheromones, called releaser pheromones, elicit an immediate response. While others are termed as primer pheromones, which induce long-term changes in behavioral endocrine state.

Pheromone induced responses are mediated primarily through vomeronasal organ (VNO). It is also known as 'Jacobsons's Organ'. It is part of the olfactory system. It is present in a variety of non-human vertebrates as well as in humans. VNO was first discovered by Ruysch (1703). He was a military doctor. He found it in a soldier with a facial wound. It was named as Jacobson's organ after the name of Jacobson, who discovered it in animals. Since 1985, many scientists were able to demonstrate VNO in most humans. VNO is located bilaterally on the anterior third of the floor of the nasal septum. It opens into the nasal cavity by a pit which varies in size from 0.2 to 2 mm. It is situated 1-2 cm. from the posterior margin of the nostril. It is lined by a pseudo-stratified columnar epithelium, 60 μm in depth that lie on a thick basement membrane.

The VNO neuro-epithelium contains three cell types—

(i) Small polygonal dark staining basal cells measuring about 6 μm in diameter, called Basal cell.

(ii) Tall cylinder/columner cells with densely staining cytoplasm called Dark cell.

(iii) Tall columner calls like the dark cells, but lightly stained, hence called Light cells.

All these cells differ from surrounding respiratory epithelium in the nose by having no cilia.

Compounds occurring naturally on the human skin were found to cause a local depolarisation when applied directly to the VNO. This depolarisation had the characteristic of a receptor potential. Subsequently it was shown that these compounds were 16-androstenes and estrenes. These compounds did not produce response from olfactory epithlium. Moreover, olfactory stimulant (e.g. cineole) has no effect on VNO. A pheromone called vomeropherin (pregna-4, 20-diene-3, 6-dione (PDD), caused evoked potential in VNO and also changed gonadotropin pulsatility in males, resulting in a reduced level of LH and testosterone. PDD also decreased respiratory frequency, increased heart rate and also caused event related changes of EES pattern.

Stern and McClintock (1998) have shown that odourless axillary compound from the armpits of women in the late follicular phase of their menstrual cycle accelerated the preovulatory surge of LH of recepient women and shortened the menstrual cycles. Axillary compounds of the same donor collected later in the menstrual cycle (at ovulation) showed the opposite effects—they delayed the LH surge of the recepients and lengthened their menstrual cycle. Savic et al (2001) showed that 'androstadienone' (a human, in particular male secretion) caused activation of hypothalamus of women (gender-specific action), but not in men. It also activates anterior part of the Inferior Prefrontral Cortex (PFC) and the Superior Temporal Cortex (STP) (in addition to olfactory area). The PFC and STP have been implicated in aspects of attention, visual perception and recognition and social congnition.

A pheromone can act as a reinforcing agent or a one-trial conditioning agent in which the presence of a pheromone converts a second odour (that of the partner or infant) in conditioned stimulus. Mice can distinguish one another by odour. This odour is genetically determined and partly specified by the H-2 major histocompatibility complex (MHC) gene located on chromosome 17. Genes located on chromosome Y also regulate production of some odours. The human equivalent of MHC locus is HLA (Human Leucocyte Antigen). There are odourous substances secreted in the mouse urine. These odours many play a part in pregnancy block (Bruce effect), aggression and other mouse social behaviours. It is held that, in human, axillary odours have chemical differences which makes the discrimination possible. Some of these individual specific odours may be under the control of HLA genes. Studies have shown that women prefer those male odours that have HLA types different from their own. However, this preference is reversed if they use oral

contraceptives. Schaal (1980), reported that, mother could recognise their own newborn infant from the smell of a previously worn T-shirt. Infant also prefer breast or axillary pads from their own mothers, distinguishing the odour from other kin.

Receptors or at least their own RNA, for phenomones have been found in the human olfactory epithelium. However, presence of pheromonen receptor protein expressed in the surface membrane of an olfactory receptor neurone and the response of this receptor protein to a ligand (a potential pheromone), have not been convincingly observed as it is in case of olfactory receptors.

Androstenone is the male human pheromone, that helps to attract women. In practice, it is blended with favourite cologne to produce a cologne odour that will attract women. Similarly, androstenol is the female human pehromone, that helps to attract men. It has been observed that once men detect the female human pheromone scent, they subconsciously become more attracted, more receptive an more willing to offer attention. Regarding the mechanism of action, it is held that the chemical scent triggers the part of the brain, where the sexual attraction feeling starts. Once, the powerful feeling of sexual attraction sets in, it moves a person generally more attentive and responsive to the person, who is the source of that sexual attraction.

In humans, pheromone production is primarily linked with secretion of apocrine glands of skin, other glandular secretions and the moist areas of the body like axillae, mouth, feet and genitals. Freshly produced apocrine secretions are odourless. They are transformed to odourous products by microorganisms. The type and density of cutaneous microorganisms on different areas of the body interacting with skin and other glandular secretions give rise to a variety of odours from various body sites.

Types of pheromones

The pheromones are of different types like sex attractant pheromone, fear pheromone, aggresive pheromone, marker pheromoen etc.

1. Sex attractant pheromone : In case of Gypsy moth, the female secretes pheromones which is perceived by thousands of males with the help of their antenae which bear olfactory epithelium. Sensation followed the releaser pathway to manifest behavioral changes. This pheromone can attract thousands of males from a distance of half a mile. This has also been found in humans. It has been possible to synthesize it in the laboratory for use in pest control. Similar female sex attractant pheromones are also secreted by male animals, e.g. a secretion from salivary gland of male boar, a secretion from preputial gland of musk deer.

2. Fear pheromone : It has been observed that if an animal is frightened, fear pheromones are produced. If such a 'frightened animal' is put in a cage having normal animals, the latter are also frightened. These pheromones are secreted in the urine of frightened animals. If a smaple of such urine is applied to a normal individual in a small quantity, the normal animal get frightened.

3. Aggressive pheromone : It is particularly found in male mammals. If some adult and sexually mature bandikoots put in a single cage, they become aggressive to each other. They start fighting and eventually might die. This pheromone is secreted from prepuital gland.

4. Marker pheromone : Some animals secrete marker pheromones which help them to identify their territory. It is secreted from sebaceous gland of female blacktail deer and from anal gland of tigress.

3.3 Audio signal

The production and perception of sound is not only a means for communication it is also important for detection of environment and navigation.

Some animals have both sound production and sound detection systems, some may have either. Many animals can hear but cannot communicate with sound. A source of sound can serve as a warning even in those animals which cannot produce sound but is capable of hearing. In cases where sound is used for communication the production is often restricted to one sex, usually male and the principal function of sound is related to male attraction and maintenance of teritory. Animals use various types of sounds for various purposes but all are related to better survival. Insects can produce and perceive a wide variety of sounds which are used for communication often with the opposite sex.

Among invertebrates, communication through sounds are limited to a few groups of insects, especially Orthoptera, Hymenoptera, Cicadidae and perhaps some Crustaceans. The first two groups produce sound by stridulation, rubbing a toothed structure accross a ribbed plate. This results in the production of a burst of pulses extending upto 40 – 50 KC / sec. Cicadas produce sound by vibrating a thin section of cuticle, some decapod crustaceans make sound by thumping the substrate with their pinchers. Hearing is more widespread and is accomplished most commonly by a modified tracheal structure, the tympanic membrane. This is called tympanic organ. This may be located in the legs in Orthoptera, Arachnida and Crustacea in the thorax or abdomen as in Lepidoptera and Homoptera or in the antennae as in Diptera. The number of receptor neurons also vary for 2 in case of Moth to 70 or more in the Locust, but they all respond to the same frequencies determined by the properties of the tympanic membrane.

In these cases there may not be any frequency discrimination but sensitivity vaires and intensity discrimination is possible. The principal function of these organs is to detect a sound, its recognition apperently by the number of bursts per secound and its localization. Some insects respond to sounds in human audio range. They also respond to ultra high frequency sound as are produced by bats. Sound reception and

its processing help a moth to escape its predator bat. Cicadas sing species-specific songs conisting of complex pattern of clicks and rasps. Courtships and rivalry calls of male crickets can be identified as species specific, each produced by a patterned sequence of muscle contractions. Male lycosid spiders produce courtship and threat sounds by scraping the palps, the frequency of the courtship sounds increases when a female displays legwaving. Some species of bees signal in the hive, the location of feeding places; honeybees also produce sound as a part of communication from foragers to other workers; the sounds are produced by skeletal movements caused by contraction of wing muscles at 250 Hz. Duration of each sound has been found to be related to the distance between the hive and the food – duration is short if the distance is less and long if the distance is more. Sound vibrations can be received by Johnshon's organ present in flies. Vibrations in substrate water and air are important to insects as warning signals, aids to locomotor and posture control and communication. Sounds serve many orthopterans for social communication. Coding of sound in insects is mostly dependent on temporal patterning of pulses by amplitude and not much by frequency and harmonies as happens in case of vertebrates.

Vertebrates

Among the vertebrates, the most sophisticated use of sound for communication is performed by birds and mammals. However, other vertebrates also use sound in different functions.

In case of aquatic animals, fish have a variety of sound receptors like skin, lateral line, and three labyrinthine chambers. They use the first two to detect displacement and low frequencies. Several teleost fishes produce drumming granting or scraping sounds others, produce sound by vibrating there air bladder schooling calls and the sound possibly serve simple communicative functions specially in intraspecific alarm and in reproductive behaviour.

The sounds are mostly produced by contraction of muscles along the swim bladder or between the pectoral girdle and the swimbladder. A few kinds of fish produce sound by resonating the swim bladder. Elasmobranchs lack swim bladder, yet they respond to low frequency sound even after their lateral line is damaged. However, usefulness of sound is limited because most fishes have poor hearing capability. They respond only to low frequency upto about 1000 cps at high intensity. This is because they do not have a specialized cochlea or any means to concentrate acoustic energy on the otolith.

Some fishes have Weberian ossicles (Weberian apparatus) linking saccules and airbladder. Airbladder acts as a tympanic membrane, pick up vibrations in water. These fishes hear sounds upto several thousand cycles/sec and are several thousand times more sensitive than fish without weberian ossicles. Frogs emit mating calls which are species specific. Cetaceans can detect direction of sound in water. A

porprise (Tursiops) with its eyes covered has been found to emit sound pulses 1 – 1·5 msec duration, repeated about 16/sec while cruising ; and accelerating to 190/sec when near a reflecting object such as a fragments of fish. The whales emit sound which may constitute songs lasting several minutes and which are repeated many times. An individual whale may be distinguished by its song, the intensities are high. In the layer of water frequented by whales, the songs may carry for many miles.

Terrestrial vertebrates

The terrestrial vertebrates have found much more complicated uses of sound. Sound is produced by devices which are used in passing air via respiratory passages. The nature of the sound ranges from simple hiss of reptiles to the resonating vocal sacs of amplihia, the syrinx of birds and the larynx and vocal cords of mammals (with lips and tongue helping in man). However most of the anurans in amplibia do not make sounds, among the reptiles only the lizard of the family gekkonidae utilize sound for communication. Even in birds and mammals, nearly all can hear well, though there are some which are usually mute. Animals show maximum sensitivity within a restricted range of frequency. Frogs show maximum sensitivity between 3,000 to 4,000 cps. In the bull-frog response is greatest in the range of 100 – 200 cps, which is used as a mating call. Snakes and most lizards have less well developed ears and respond only to local sounds of a few hundred cps.

The hearing mechanism in birds and mammals are highly developed. Behavioral and electrophysiological studies revealed that they have same absolute sensitivity, differential frequency and intensity determination and dynamic range. The principal mechanism of sound perception in birds and mammals have been described. When the sound wave strikes the tympanic membrane, it vibrates. These vibrations are amplified and transmitted through the ear ossicles in the middle ear to the oval window of the internal ear. This causes vibrations of the basilar membrane. The auditory receptors present here are excited (hair cells in the organ of corti). As a result, nerve impulse is generated in the auditory nerve (cochlear division of VIIIth nerve) by auditory transduction. These impulses reach the auditory cortex and thus perception of hearing occurs. The perception of sound involves perception of pitch, intensity and direction of sound.

In mammals (except bats) cochlea is coiled. It has 2·75 turns in man, 3 turns in cat, 4 turns in pig and guineapig. Frequency perception range in man is 16 Hz to 20 KHz with maximum sensitivity between 1KHz – 3KHz. Some dogs can hear upto 35KHz and rats and guineapigs can hear upto 40 KHz. Primates hear better at low frequency than mammals such as opossum and hedgehog.

Significance of audio signal

In general, sound is a basis for species recognition, communication, predator detection and echolocation. Insects produce and perceive a wide variety of sounds

which are used for communication often with the opposite sex. Many fish produce sounds that are used for communication. Frogs emit mating calls which are species-specific. Baby mice and other rodents call their mother particularly in cold using ultrasonic calls of 60-90 Kc/sec.

A general function of calls in song birds is for the establishment of territory and for the attraction of a mate. Song-patterns have species characteristics that are genetically determined. Crows are gulls, have a variety of calls like— alarm, distress, assembly, chorusing are others. Cave dwelling birds use echolocation. Vocal signal is the best way for communication in humans. Vocal signals are used by them not only for communication, but also for scolding, quarrelling etc. Humans also use sound in the field of industry and medical diagnostic centres. Ultrasound is used not only for diagnosis, but also for treatment.

Infrasound

Recently it has been discovered that homing pigeons can detect very low frequency sound as low as 0.05 Hz (1Hz is 1 cycle per sec ; 0.05 Hz corresponds to 1 cycle per 20 seconds). This response to infrasound is lost or reduced if middle or internal ear is damaged. Such responsiveness is very important in birds because infrasound is produced during thunderstorms, earthquakes, and wind over mountain ranges. Since infrasound are attenuated much less in air (attenuation of sound is inversely related to the square of the wavelength), it travels over a long distance and thus can be detected hundred or even thousand kilometres away. This helps the birds to determine the direction.

The elephant can communicate with each other using infrasound. Because the infrasound undergoes very little attenuation in air, these may be audible for communication specilly in a forest habitat. Some animals show abnormal behaviour prior to earthwake and this may be related to perception to infrasound.

Perception of infrasound and its use for correcting direction and to make communication has been observed in birds and elephants.

Echolocation

The power to localize an object using the echo of a sound is called Echolocation. It is a complicated process. It is comparable with audiolocation devices and rader device invented by man. Animals that echolocate use echoes of sound they produce to locate the objects in its path. Some animals both aquatic and terrestrial are capable for echolocation. It is particularly well developed in bats but exists also in other animals notably whales, dolphins, shrews and a few birds.

Origins of echolocation

The ability to detect and understand the environment is essential for survival. Special sensory devices like vision, hearing, taste and smell can be used to know the environment. For locating an object in the environment vision/light and hearing

sound are very much helpful and in the dark, echolocation is the only correct device available for this purpose. Hence, animals which have to move in the dark have developed echolocation devices to locate an object in its pathway and this helps in capturing a prey in navigation.

Basic principles of echolocation

The animal produces a sound which strikes an object in its pathway and produces an echo. The echosound reflected from the object is detected by the auditory apparatus, analyzed and thus the position of the object is ascertained.

Echolocation in different animals

Sound waves travel at a slower rate in air compared to water. The intensity of sound is more quickly attenuated in air compared to water. High frequency sounds are more rapidly attenuated. The higher the frequency of a sound the shorter is its wave length. There is an inverse relationship between frequency of sound waves and the size of the object that can be detected by its echo. Hence, detection of small-sized object requires high frequency sound. Frequncy also influences directionality of hearing. High frequency sound is capable of detecting the direction of sound more accurately.

In view of these different properties of sound the members of animal kingdom adapted different mechanisms for echolocation depending on their habitat and requirement. All animals do not echolocate and different types of echolocating mechanisms have been observed. Echolocation has been found to be well developed and studied extensively in some animals including bats in terrestrial habitat and cetacians in aquatic habitat.

The bats emit echolocation signals through mouth (oral emitters) or nose (nasal emitters). Most microchiropteran are oral emitters. Only members of Nycteridae, Megadermatidae Rich Rhinolophidle and Phyllostomidae are nasal emitters. Usually high frequency and high intensity sounds are emitted. The detailed mechanisms of echolocation show variation in different genera of bats. Most bats emit 20 – 100 KHz frequency sound and the an intensity of 110 decibel (in bronze bat). Narrow (CF) and broad band (FM) signals are used. CF helps in detecting the object but cannot exactly localize it, this is done with the help of FM.

Insectivorous bats capture an insect by three phases of acoustical orientation. In the first phase sound pulses (100 – 20 KHz) with an interval of 50 msec is sent. When the prey is detected pulses are sent with shorter intervals and this is the second phase. In third on final phase a buzz like sound is emitted, the frequency is decreased duratian of pulse is decreased (0.5 msec) and the interval between two signals is also reduced. Finally the bat scoops up the insect with its wings or in the webbing between its hind legs guiding the insect to its mouth.

The quality, frequency, duration and number of occurrence of sound impulse per second show much variation in different genera of bats. The nature of the sound may

be 'click' type or 'buzz' type. In vespertiliomidaes the duration of pulse ranges from 1– 4 msec. The frequency may be upto 120 kc/sec but drops slowly. Rate of pulse discharge is less than 10/sec at rest but rises above 100/sec while hunting or avoiding obstacles. In horseshoe bats, the frequency of pulse is 85 – 100 kc/s, duration is high 40 – 100 msec. The pulse repetition rate is slow and it is less than 10/sec. If the size of the object is close to the wave length of sound wave, the reflection of sound is better. Thus the frequency of sound wave emitted by bats depends on the size of the object it has to be detected. To locate small size object higher frequency is required (the wave length of a sound of 30 KHz is about 11·5 mm which is roughly the size of a small moth)

There are bats which can avoid or dodge wires with a diameter of 0·5mm. This happens when the wavelength of sound is 5 mm. It has been observed that bats will not be able to avoid hitting a wire when its diameter is less than 1/10th of the wave length of sound.

Regarding detection of distance several theories have been proposed. It is proposed that distance is detected from the time difference between the transmission of ultrasound and the reception of its echo. Mohra proposed that distance is understood from the loudness of the object pye opined that the cochlea can understand the difference between two types of notes— the emitted note and the received echo note. Auditory nerves carry the information about the difference between these two notes to the brain. From this difference between these two notes, bats estimate the distance of object. It has been observed that vespertilioned bats can delect insects at a distance of 50 cm – 1 metre in the dark by echolocation. On the other hand the horseshoe bats can detect insects even at a distance of 6 meters. That echolocation is effected through auditory apparatus has been observed. Spallenzam did experiments with blind folded bats and observed that they can capture insects in the dark and the can return to their place of residence for a distance. Griffin noted that blind bats can capture insects which do not produce any sound and they can detect and chase pebbles and cotton spitballs thrown into the air.

The intensity of sound emitted for echolocation is very high and can be compared to the sound of jet engine which can damage the ear, but this is necessary because the intensity of sound drops faster in the air than water. It can damage the ear. A deafening mechanism has been observed in bats to handle the situation. When the sound is emitted the tensor tympani muscles attacted to tympanic membrane and the stapedius muscle attached to stapes contract. As a result the malleus is pulled inward away from the tympanic membrane and the stapes is pulled outward away from the oval window. In this way transmission of sound wave to the internal ear is reduced. However if this reduced phase is continued the reception of reflected sound will be disturbed. But this does not happen because these two muscles immediately after contraction relax and thus the ear osscicles get back to their specific position and sound transmission standard becomes normal.

The bats change their sound frequency during capturing prey of different sizes. They emit sound to 10 kc/sec (wave length 34 mm) to capture large sized prey and sound over 130 kc/sec with wave length of 5 mm to capture small-sized prey. By using echolocation, a bat can capture two seperate mosquitoes or fireflies in about 0·5 sec. The fish eating bats of Trinidad can even use echolocation to find and capture their under-water prey by detecting the ripples that are produced on the surface of water when a fish swims just under the surface of water.

Different experimental evidence indicate that a number of morphological and neuronal modifications help the bats in detecting the echoes. The snout in covered by couplex folds and the nostrils are spaced to produce a megaphone effect. The ear develop large pinnae which help to capture echoes. The eardrum and ear ossicles are small and light which provide high fidelity at high sound frequencies. Contraction of the muscles of ear ossicles reduce the sensitivity of the ear during the emission of high intensity sound; immediately the muscles relax and the capability of sound wave transmission reverts back to normal. (This is common in the ear of mammals). The bones housing the middle and inner ear are insulated from the rest of the skull by blood sinuses, connective tissue and fatty tissue. This reduces direct transmission of sound from the mouth to the inner ear. The auditory centres of the brain occupy a very large portion of the brain to receive the auditory signals and through the process of neural computation, construct from the auditory cues, a spatial representation of the external world.

Bat-moth predator—prey interaction

Experimental results indicate a remarkable series of adaptations by certain moths in response to predation by bats. Nocturnal of some families e.g. Nocteridae have developed sensitive auditory system to receive the frequencies emitted by bats. When these moths detect the approach of a bat, they alter their levels of flight, when the bats become nearer, the moths fly irregularly. When the bats become very close, the sound is very loud and repitition rate is also high. The moths dive directly on the ground. The moths themselves produce ultrasonic sound to detect the attacking predator. Some moths develop a noise-making organ on each side of the thorax. When the moths are disturbed, these organs produce trails of clicks with prominent ultrasonic components. It has been shown in laboratory experiments that flying bats turn away from their targets when confronted with moth-produced pulses. Hence, these pulses protect moths from the bats. The bats sometimes, in case of capturing moths, abandon echolocation for detecting the prey ; but instead listen to sounds produced by the prey. Echolocation signals of bat helps the moth to detect the foraging bats. Moths have been shown to be able to detect the cries of bats at a distance of 30 meters.

Echolocation in birds

At least two species of birds have been shown to use echolocation. The oil birds

(Steatornis) of South America and the cave swiftlets *(Collocalia)* of South-east Asia are not closely related but both live and nest in deep caves. The best known are the oilbirds or guacharos. They have been found to fly freely in the dark caves without hitting the walls or other obstacles. They use sound of 7000 Hz., which are audible by man. The nature of the sound they produce is like that of ticking of a typewriter. It has been observed that if ear of these birds are plugged, they fail to orient themselves in the dark. However, they are able to fly in a lighted room using their eyes.

Echolocation in aquatic animals

Echolocation has been observed in marine species. Tony shrews use a variety of sounds (30-60 kcps) to explore strange pieces of unfamiliar objects. Both dolphins and whales use echoes to avoid colliding with objects and with the ocean bottoms as well as for finding food. It is very useful in locating food deep under-water when the visibility is very low or zero. A trained dolplin has been found to locate a dead fish in the tank which cannot be seen in man. The dolphins produce sound by vibrations in the nasal sac system near the nasal plugs. That the source of sound is not larynx but nasal has been confirmed using imaging tools such as CAT, MRI, and RET scans. The nasal sac system consists of a series of muscular valves and compliant sacs associated with the blow holes. The muscles associated with these air sacs contract synchronously with the echolocation clicks, while the muscles around the larynx do not. A pair of small, dorsal fatty projection with a lip-like structure, called 'museau de singe' (also called monkey lips) control the passage of air through this system. Cranford hypothesized that the passage of pressurized air past the liplike structure (museau de singe) produce sounds in much the same manner as the glotis in man. It has been suggested that sound waves produced by the nasal sacs are focussed in the forward direction through a structure called melon (which is situated anterior to the monkey lips). It is a lens shaped fatty structure that gives a dome shaped profile to the forehead of many odontocetes. The lipid composition of melon has been analyzed and its acoustic properties suggest that it may serve as an 'acoustic lens' to focus outgoing energy. The echolocation capability of sperm whale has been inferred on the basis of data from other odontocetes. The click of a sperm whale consists of pulses. Clicks lasts for roughly 10 – 20 msecs, and the clicks are rapeated from less than one click per second to 40 per second. The sonar clicks are produced in the front of the sperm whale's had by pnematic action of the 'museau de singe' like that of dolphins. The sperm whale head acts as a sound reflector. Experimental evidence indicate that diving sperm whales use trains of clicks for echolocation of prey.

Some small cetaeceans that inhabit turbid water have tiny eyes and presumably are dependent on echolocation.

Ambient noise imaging (ANI)

It refers to the use of sound to see underwater. Taylor et al., using powerful computers and models inferred that dolphins might be able to detect using ANI. These models predict that dolphins use ANI to see useful images for tens of meters underwater. It has been suggested that dolphins and perhaps other marine mammals, have a whole new way of seeing with sound.

Multiple uses of Echolocation calls

Echolocation helps the animal to detect the direction, distance, size and texture of the objects in its environment. It is seen in birds and mammals. It is mostly developed in two groups of mammals. The microchiropteran bats are some cetaceans like porpoises and dolphins. Two groups of birds also use it.

Echolocation signals are used by bats for communication, as well as for orientation and locating prey. By modifying signal design some bats exploit habitats. Echolocation calls display considerable geographic variation within a species. *L. cinereus* in Arizona had calls with a minimum frequency 53·8% higher than the minimum frequency of calls in Menitoba (26·0 kHz versus 16·9 kHz). Eight of 12 species studied showed a difference of 3 kHz in different geographic location. Under certain conditions same bats add an extra pulse to the echolocation calls. It has been observed that when one bat is very close to other bats, the former lowers its call frequency and add a warning 'houk' to the signal.

Echolocation calls communicate several kinds of information. Same rely on signals of conspecifics to locate day roosts, mating sites, hibernation sites and feeding areas. The solitary forager *E. maculatum* in contrast reacted aggresively to the playbacks of calls of a conspecific individual. This bat either attacks the speaker or abruptly moves away.

Echolocation calls provide vocal signatures. This is the basis for mother-young recognition in a number of species. In a captive colony individuals recognize each other by listening to echolocation calls. Vocal signatures are individual interactions among foraging bats. A number of species are known to alter their signal design when changing from foraging in open areas to these closer to obstactes.

3.4 Suggested questions

1. What is audio signal ? Write briefly on its different uses.
2. Give an account of transmission and reception of audio signal.
3. What is echolocation ? Write briefly on the echolocation in aquatic animals.
4. Why high frequency sound is used for echolocation ?
5. Comment on the utility of CF and FM band in the echolocations.
6. Write briefly on bat-moth predator-prey interaction.

7. What is Ambient Noise Imaging (ANI)?
8. What is biioluminescence ? Write a note on its regulation .
9. Describe the reactions of bioluminescence with a note on the enzymes involved in the process.
10. Write a short account on the importance of bioluminescence.
11. What are phenomones ? Write a note on vomeronasal organ.
12. Write briefly on the different types of phenomenes.

Unit 4 ❑ Contractile elements, cell and tissues among different phylogenic groups

Structure

4.1 **Muscle Structure and function correlation.**

4.2 **Movements—amoeboid, ciliary and flagellar.**

4.3 **Specialized organs (eg : electric organs and tissues)**

4.4 **Suggested questions**

4.1 Muscle structure and function correlation

Muscle cells are specialized cells. They use ATP energy to generate force or to do work. Because work can take many forms like locomotion, pumping of blood or peristalsis, several types of muscles have evolved. The three basic types of muscles are skeletal muscle, cardiac muscle and smooth muscle.

Skeletal muscles are attached to bones, spans a joint and thus helps in joint movement and locomotion. They are voluntary. Their activity is controlled by nervous system. They play a key role in numerous activities like maintenance of posture, locomotion, speech, respiration etc.

Structure

Individual cells are of variable length. They may be as long as 25 cm, but their diameter ranges from 10-80 μm. Each muscle cell is covered thinly by a connective tissue layer called endomysium. Individual muscle fibres are then grouped together and get a covering of another connective tissue called perimysium. These are called fesciculus. Finally fasciculi are grouped together and they are covered by another connective tissue sheath, known as eipmysium. This epimysium covered structure is the muscle. At the ends of the muscle, the connective tissue fibres join to form tendon which are attached to the bones. All the muscle fibres are not of uniform length and extend the entire stretch. The fibres of shorter length become attached to the connective tissue inter lacing the muscle fibres. More than 600 muscles have been identified in the human body. Some are small and consists of few hundred fibres, but large muscles may contain several hundred fibres.

Each muscle fiber is multinuclated, bounded by the plasma membrane called sarcolemma. The protoplasm within is called sarcoplasm or myoplasm. Besides common organelles, the sarcoplasm contain myofibrils and sarcoplasmic reticulum. The myofibrils run along the length of the cell. They show alternate dark (Anisotropic) and light (Isotropic) bands. Proper alignment of the bands give rise to transverse bands and the longitudinal disposition of the myofibrils that gives rise to longitudinal

striations. These two together gives the muscle fibre a cross-sectional appearance. At the middle of the light band there is a dark line called Z-line. Thus a myofibril becomes made up of Z-line bonded units. These are called sarcomere. Average length is 2 μm. Each sarcomere is bonded at both sides by Z-line. At the centre there is A-band and on either side there is I-band. The A-band is made up of thick filament, called myosin. The I-band contains the filament called actin filament. At the middle of the H-zone, there is a fine dark line called M-line. The actin filaments extend from the Z-line to the border of the H-Zone. Thus there is an area where both A-band and I-band overlap, or myosin-actin overlap. This overlapping zone is called O-band. Besides actin many other proteins are associated with thin filament. These

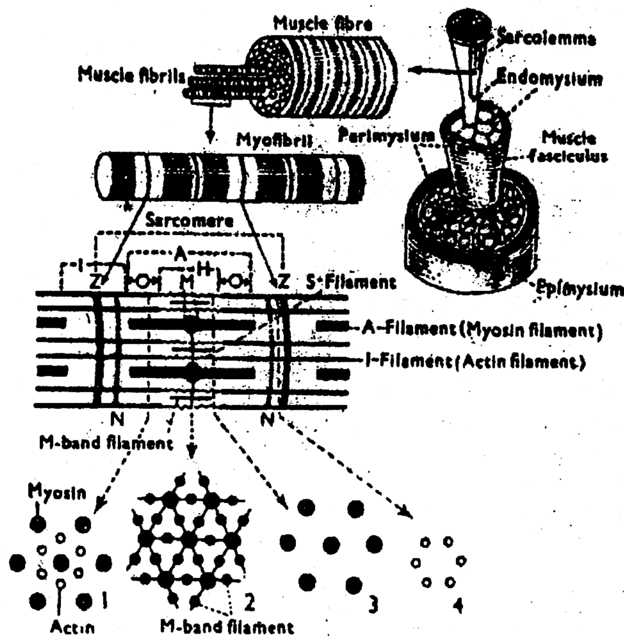


Fig 4.1 (a) : Anatomical organisation of skeletal muscle from gross to moelcular level (diagrammatic).

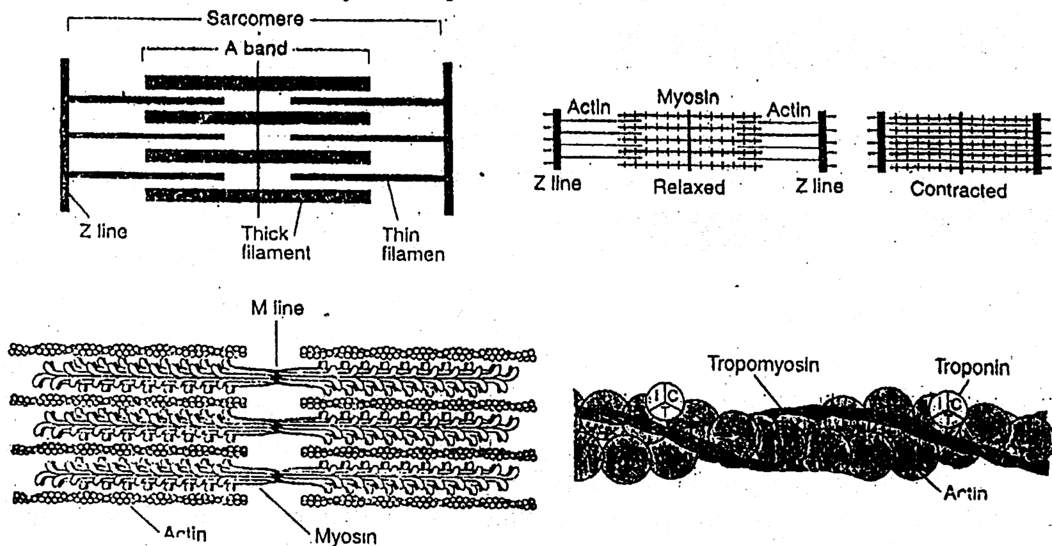


Fig 4.1 (b) : Top left : Arrangement of thin (actin) and thick (myosin) filaments in skeletal muscle. **Top right :** Sliding of actin on myosin during contraction so that Z lines move closer together. **Bottom left :** Detail of relation of myosin to actin. Note that myosin thick filaments reverse polarity at the M line in the middle of the sarcomere (Modified from Alberts B et al : *Molecular Biology of the Cell, 2nd 3d. Garland, 1989)* **Bottom right :** Diagrammatic representation of the arrangement of actin, tropomyosin, and the three subunits of troponin (I, C, and T).

include (a) Troponin, complex made up of 3 subunits, called troponin I, attached to actin, troponin T attached to tropomyosin and troponin C that attaches with Ca^{2+}. (b) tropomyosin, (c) tropomodulin, (d) a-actinin, (e) cap Z protein, (f) titin. Titin extends from Z-line to middle of the sarcomere and attached with myosin. It helps in proper alignmentof actin and myosin. Tropomodulin is located at the end of this filament towards the centre of the sarcomere and may participate in the setting of the length of this filament. α-actinin and capZ protein serve to anchor thin filament to the Z-line. Additional proteins present in the thick filament are myomesin and C-protein. These participate in the bipolar organisation or packing of the thick filament or both. Desmin binds Z-line to plasma membrane. Nebulin extends along the length of thin filament and may participate in the regulation of the length of thin filament (Fig-4.1).

Muscle contraction involves participation of (i) actin, myosin, troponin, tropomyosin system and (ii) sarcotubular system.

(i) The contractile proteins actin and myosin as well as other regulator proteins like tropomyosin and troponin are specifically arranged in a sarcomere and take part in muscular contraction.

Actin exists in two forms G-actin and F-actin. F-actin is arranged as a two-stranded hotical filament. Tropomyosin covers the actin filament. The actin molecule has myosin binding site. After every seven actin molecule, troponin is present. Troponin-I remains bound with actin, and troponin T with tropomyosin, and troponin C can bind with Ca^{2+}. The head of the cross bridge remains very close to the actin molecule. It has ATP-ase activity, ATP binding site and actin binding site. But it cannot bind with actin because myosin binding site of actin remains covered by tropomyosin.

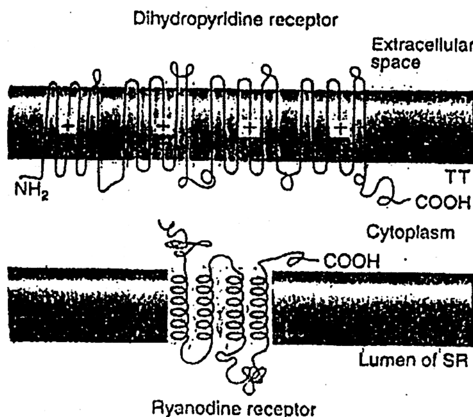


Fig 4.2 : Showing DHP and RYR receptors.

(ii) The Sarcotubular system essentially consists of T-tubule which is an invagination of sarcolemma, and longitudinal tubules running parallel to myofibrils. These tubules end in a dialated structure near the T-tubule, called cistern. One T-tubule and two longitudinal tubules with two cisterne form a triad system. In the T-tubule there is dihydorpyridine receptor (DHP receptor) and on the cisterni there is ryanodine receptors which is a calcium channel. Ca2+ remain stored in the cisterni by combining with calsequestrene. In each sarcomere there are two triads each located at the A-I junction (Fig. 4.2).

In the resting state intracellular Ca^{2+} concentration is low, about 10^{-7} moles/litre. ADP+P ; are attached to myosin head (cross bridge). On stimulation, muscle action

potential develops. It passes along the sarcolemma and goes deep into the cell via T-tubule, and it excites DHP receptor. When it is stimulated, the RYR receptor is excited through protein-protein interaction (Fig 4.3).

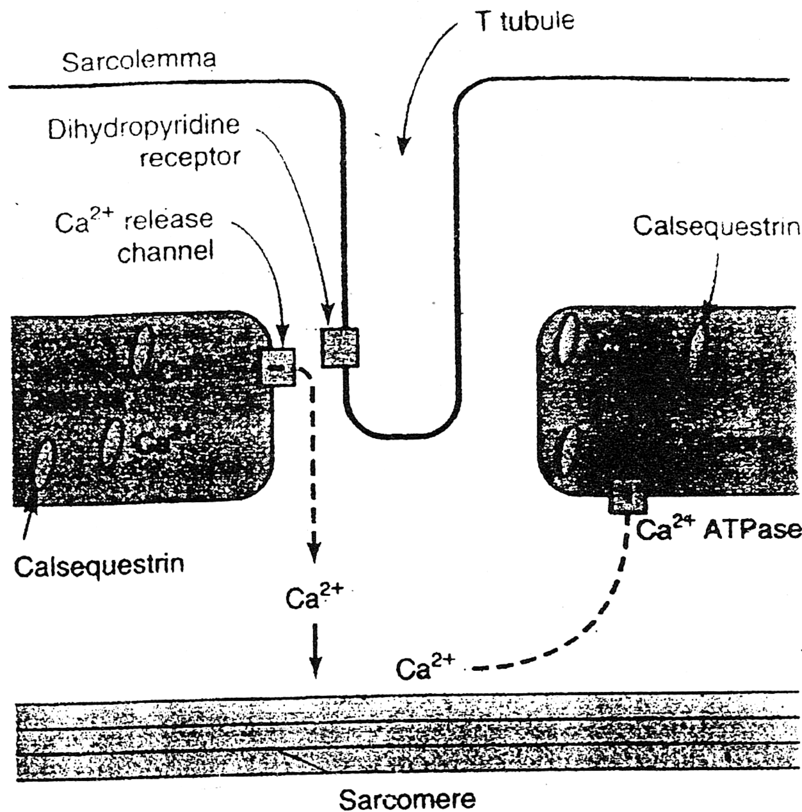


Fig 4.3 : Diagram of the relationship among the sarcolemma (plasma membrane), a T tubule, and two cisternae of the sarcoplasmic reticulum of skeletal muscle (not to scale). The T tubule extends inward from the sarcolemma. A wave of depolarization, initiated by a nerve impulse, is transmitted from the sarcolemma down the T tubule. It is then conveyed to the Ca^{2+} release channel (ryanodine receptor), perhaps by interaction between it and the dihydropyridine receptor (slow Ca^{2+} voltage channel), which are shown in close proximity. Release of Ca^{2+} from the Ca^{2+} release channel into the cytosol initiates contraction. Subsequently, Ca^{2+} is pumped back into the cisternae of the sarcoplasmic reticulum by the Ca^{2+} ATPase (Ca^{2+} pump) and stored there, in part bound to calsequestrin.

Excitation of RYR receptor causes opening of Ca^{2+} channel and Ca^{2+} is released. It binds with troponin C, and causes a conformational change of it. This leads to a movement of tropomyosin. As a result, the myosin binding site of actin molecule is exposed. Next, myosin head gets attached to actin. When actin attaches to myosin, the myosin ATPase activity is increased. So ADP and Pi leaves myosin and actin-myosin interaction occurs. The myosin head binds and so the actin molecule is pulled towards the centre of the sarcomere. ATP then gets bond with myosin and due to this binding actin can no longer remain bound with myosin. Actin is removed and myosin head again goes back to its previous state and gets ready to bind with another actin

molecule. Meantime, ATP is split into ADP+Pi by the ATPase action of myosin-head. But the ATpase action is low, so ADD+Pi do not leave the myosin head, rather remain attached to it. Actin-myosin recycling occurs as muscle contraction occurs.

Intracellular concentration of Ca^{2+} does not remain high for long time. Immediately after its release, it is taken back into longitudinal tubule by active process. As a result, intracellular Ca^{2+} concentration is decreased. As it goes to normal level, 10^{-7} moles/lit, Ca^{2+} leaves troponin C and goes back to tubule. When troponin C becomes Ca^{2+} free, again a conformational change occurs in troponin molecule. As a result, tropomyosin moves and covers the myosin touching site of actin molecule. Here, actin-myosin interaction does not occur and muscle contraction stops and the muscle relax.

During muscular contraction, the muscle fibre is shrotened. The actin filaments slide into and in between myosin filaments, and during relaxation the actin filaments slide out of the myosin filaments. The structure of sareomere has been observed during rest and during contraction.

Cardiac muscle

The cardiac muscle cells are short cylindrical in shape. Typically they measure about 100 μm long and 10 μm in diameter, they are uninucleated. They are cross striated like skeletal muscle because of specific alignment of thick and thin filaments. Especiality of cardiac muscle is that, the cells are conneted with each other through intercalated discs. These are low resistance partitions like gap-junctions, Junction adherens. Through these, signal is transmitted from one cell to the next, and thus a functional syncitium is established.

The sarcotubular system is well developed like skeletal muscle. It has triad and diad systems. But each sarcomere has one, because it is locked at the Z-line. The ryanodine receptor has a special property leading to a situation called calcium induced calcium release. When action potential passes down the T-tubules, the DHP receptor is activated. As a result voltage gated Ca^{2+} channels present here open, and ECF Ca^{2+} enters into the cell. This calcium binds with RYR receptor. Such Ca^{2+} binding opens the calcium channel present here. This is called calcium induced calcium release. Thus intracellular Ca^{2+} concentration is increased and goes above 10^{-5} moles/lit. Now, Ca2+ binds with troponin C and finally muscle contraction occurs in the same way as happens in skeletal muscle. It has been shown experimentally that if cardiac muscle fibres are placed in a medium muscle contraction will occur if the bathing medium contains Ca^{2+}. But this is not required to cause contraction of skeletal muscle. The mechanism of muscular contraction is also similar to skeletal muscle that is actin regulated or actin based muscular contraction.

Smooth muscle

Smooth muscle cells are uninucleated, fusiform in shape with tapering ends.

These are 400-600 μm long. Their diameter ranges from 2-10 μm. The thick and thin filaments are 10,000 times longer than their diameter and are tightly packed.

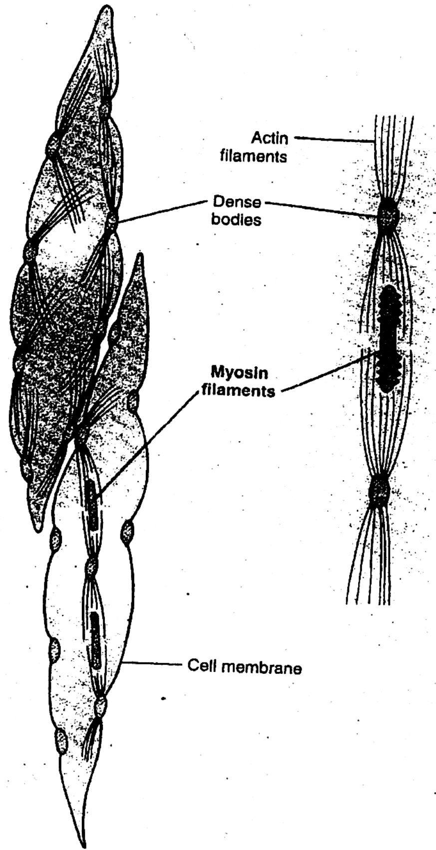


Fig 4.4 : Physical structure of smooth muscle. The upper left-hand fiber shows actin filaments radiating from dense bodies. The lower fiber and the right-hand insert demonstrate the relation of myosin filaments to the actin filaments.

The smooth muscle has been divided into two groups : single unit and multi-unit. In single unit muscle, the muscle cells are electrically coupled, through special junctions. These are gap junctions and adherens junctions, connecting the adjacent cells. Through these, signals (action potential) can pass from one cell to the next. A wave of electrical activity and a wave of contraction occurs and it can be initiated by pacemaker cell (a cell that exhibit spontaneous depolarisation).

Because of syncitial arrangement, they are called cardiac muscle type. In case of multi unit smooth muscle, the individual cells are not electrically coupled, and each cell can contract independently.

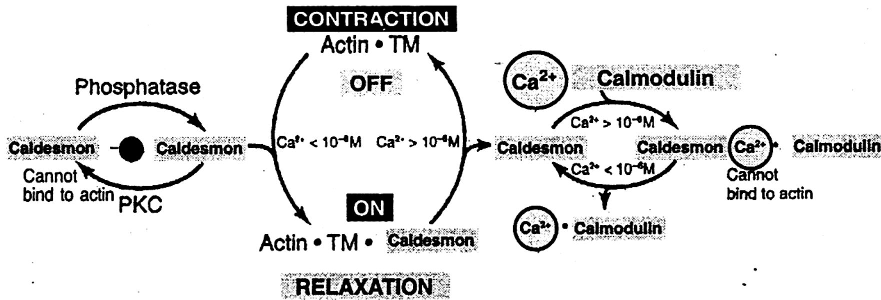
Scrcotubular system is very weakly developed. The sarcolemma shows small invaginations known as caveoli. These represent the rudimentary form of T-tubules of skeletal muscle cells. Just behind caveoli there are small fragments of longitudinal tubules. All these represents rudimentary form of sarcotubular system.

The dispcsition of actin and myosin are also not well organised as seen in skeletal and cardiac muscles. There are dense bodies attached to sarcolemma, some are also present in the sarcoplasm. They represent Z-line of the sarcomere. Actin filaments are seen radiating from dense bodies. Myosin filaments are seen between two dense bodies (Fig 4.4).

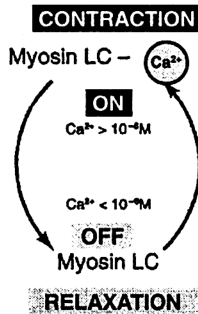
Mechanism of contraction of smooth muscle

In the smooth muscle cells troponin is absent. A number of proteins and enzymes are involved in its contraction. In the resting state, actin-myosin interaction does not occur and the muscle remain in relaxed state. A protein 'caldesmon' present in the

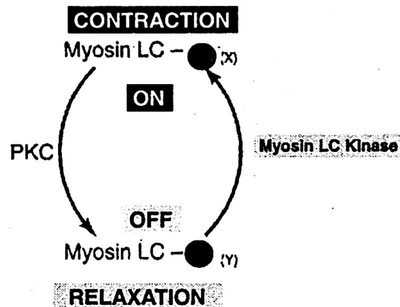
A. Caldesmon regulation of actin



B. Ca^{2+} binding to myosin light chains



D. Regulation of myosin light chains by protein kinase C



C. Phosphorylation of myosin light chains

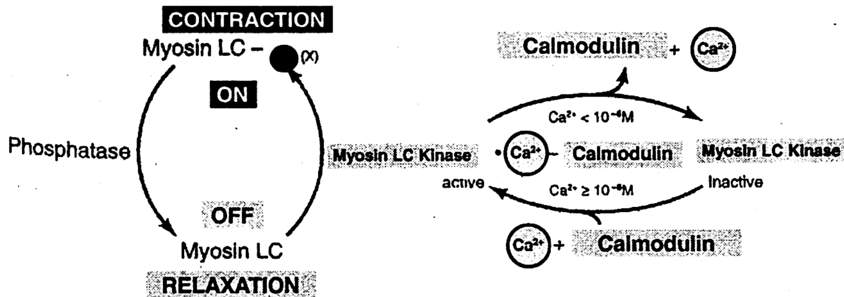


Fig 4.5 : Both actin-and myosin-dependent mechanisms control smooth-muscle contraction and relaxation. (A) Binding of caldesmon to the actin and tropomyosin (TM) of thin filaments prevents contraction. At cytosolic Ca^{2+} levels above 10^{-6} M, formation of the Ca^{2+}/calmodulin complex occurs. Binding of this complex to caldesmon releases it from thin filaments, allowing the muscle to contract. Phosphorylation of caldesmon by protein kinase C (PKC) also prevents it from binding to thin filaments and promotes contraction. (B) Binding of Ca^{2+} to the regulatory light chains of myosin allows actin-myosin interactions and promotes contraction. (C) Phosphorylation of the regulatory light chains by myosin LCkinase, which is activated by Ca^{2+} calmodulin, also promotes muscle contraction. (D) Phosphorylation of the regulatory light chains by protein kinase C, at a site other than that acted upon by myosin LC kinase, inhibits myosin-actin interactions and causes smooth-muscle relaxation. [Adapted from Lodish et al., 1995.]

sarcoplasm binds with the actin and thus actin-myosin interaction is kept prevented.

When intracellular Ca^{2+} concentration is increased and goes above 10^{-5} moles/lit, Ca^{2+} binds with a protein in the sarcoplasm called calmodulin. This Ca^{2+}— calmodulin complex binds with caldesmon and it is released from actin. So, actin-myosin interaction occurs and the muscle contracts. A fall in intracellular Ca^{2+} causes decreased formation of calmodulin-Ca^{2+} complex. So caldesmon becomes free. This now again binds with action and prevents actin-myosin interaction. This results in relaxation of the smooth muscles (Fig 4.5).

Besides this, there are other mechanisms also, that operates or influence the process. There is a regulatory light chain of myosin which remain in two form— phosphorylated and dephosphorylated forms. The dephosphorylated prevents actin-myocin interaction and so the muscle remain in relaxed state.

Calmodulin Ca^{2+} complex activates an enzymes, called myosin light chain kinase (MLCK). This enzyme phosphorylates myosin light chains. The phosphorylated form of myosin light chain cannot prevent actin-myosin interaction and so muscles contract. There is a myosin light chain phosphatase that dephosphory lates myosin light chain. But, it is again phosphorylated immediately by the kinase, so long calmodulin—Ca^{2+} complex is there. However, when Ca^{2+} concentration is decreased, Ca^{2+} ion are removed from calmodulin Ca^{2+} complex. So, kinase is no longer activated. So, further phosphorylation of myosin light chain does not occur and the muscles relax.

PKC can cause phosphorylation of caldesmon and thereby can also prevent actin-myosin interaction. However, it can also phosphorylate the regulatory light chain of myosin and this also inhibits actin-myosin interaction and the muscle relaxes. Both PKC and MLCK phosphory lates myosin light chain (the regulatory light chain of myosin), but the phosphorylation sites are different and the action is also different. MLCK phosphorylation leads to contraction and PKC phosphorylation causes relaxation of the smooth muscle.

Sequence of events in muscular contraction

The general sequence of events in the contraction of three different types of muscles. The skeletal and cardiac muscles undergo action based muscular contraction, whereas the contraction of smooth muscles is myosin regulated.

Sequence :

1. When the muscle fibre is stimulated via neurons (natural stimulus) or otherwise action potential develops.
2. The muscle action potential passes via sarcolemma and enter deep into the muscle cell via T-tubules (in skeletal, and cardiac muscle), and via DHP receptor and RYR receptor escitation causes release of calcium. In smooth muscle, mostly Ca^{2+} comes from ECF due to opening of voltage gated Ca^{2+}

channel present in the plasma membrane of smooth muscle cell.

3. Intracellular Ca^{2+} concentration is increased. It binds with troponin C (in case of skeletal and cardiac muscle cells). This causes a conformational change in troponin molecule. As a result, tropomyosin moves and the myosin binding site of actin is exposed. Actin myosin binding occurs and the muscle contracts. In smooth muscle cells, troponin is absent. Here, Ca^{2+}-Calmodulin complex is formed, that initiates muscles contraction involving caldesmon, MLCK, PKC, muscle phosphatase. (Fig 4.6)
4. As intracellular Ca^{2+} concentration is decreased, Ca^{2+} dissociates from troponin

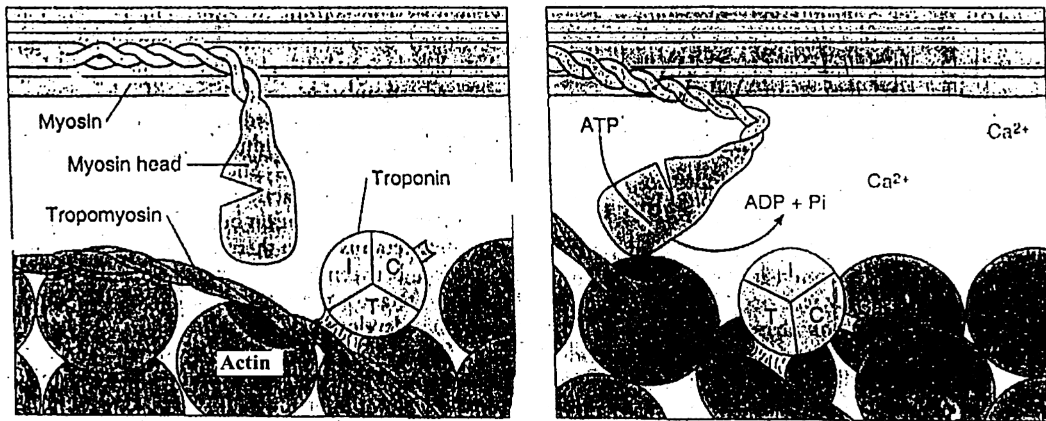


Fig 4.6 : Initiation of muscle contraction by Ca^{2+}. When Ca^{2+} binds to troponin C, tropomyosin is displaced laterally, exposing the binding site for myosin on actin (dark area). The myosin head then binds, ATP is hydrolyzed, and the configuration of the head and neck region of myosin changes. For simplicity, only one of the two heads of the myosin-II molecule is shown.

C and Ca^{2+} calmodulin. This leads to relaxation.

Basically the (i) development of action potential, that is, degree and duration of depolarization and repolarisation, (ii) functioning of the sarcotubular system, that is sensitivity of calcium-associated with degree and duration of calcium release and reuptake, (iii) actin-myosin interaction that is degree and duration of action and myosin attachment and detachment cycle, (iv) energy yielding system in the muscle cells, are the determinants of muscle function. These muscles have been selected based on their structure and physiological propertiesfor placement in specific areas of the body for performing specific function. (Fig. 4.7)

In case of skeletal muscle, the muscle fibers are not inter-connected. So, all or none law is applicable to individual fibere. So, when it contracts, the strength of stimulus can produce summation or tetanus. But in cardiac muscle, the muscle cells are electrically connected resulting functional syncitium. Here all or none law is applicable to whole of heart. The refractory period is short in skeletal muscle. So

summation and tetanus are possible but this does not happen in case of heart, because it is absolutely refractory during systoleand relatively refractory during diastole. The heart cannot afford summation, tetanus or fatigue. Hence, its muscle fibres have such property. The single unit smooth muscle is called cardiac muscle type and multi unit smooth muscle is termed as skeletal muscle type, and their function is also different.

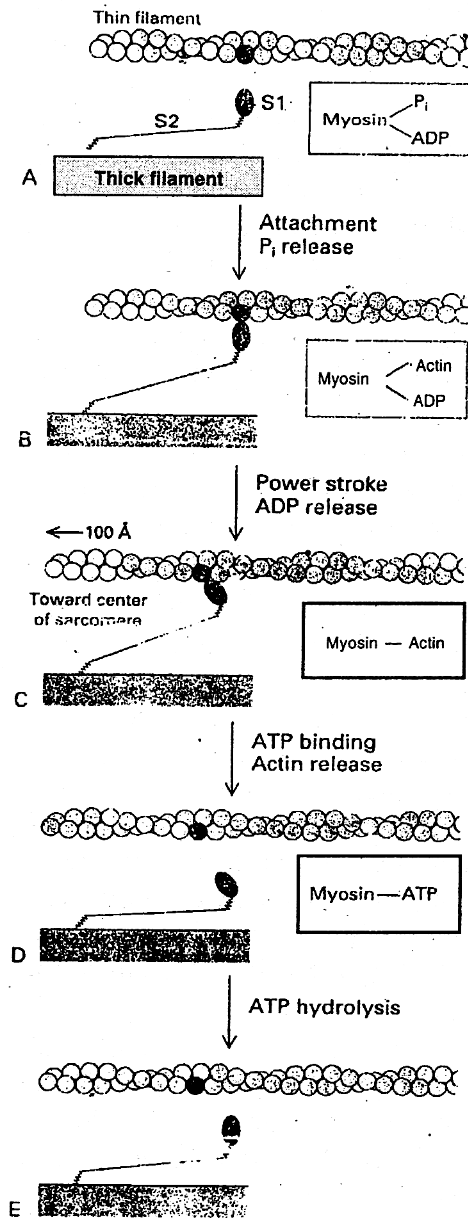


Fig 4.7 : Proposed mechanism for the generation of force by the interaction of an S1 unit of a myosin filament with an actin filament. In the power stroke, the thin filament moves relative to the thick filament when S1 undergoes conformational changes accompaying the release of ADP.

The action potential duration is short in skeleted muscle, but prolonged in cardiac and smooth muscle. Mechanical change is related to electrical change. Hence, the twitch duration is short in skeletal muscle but is is more in caridac muscle and more prolonged in smooth muscle.

Skeletal muscles are attached to skeleton and joints. These muscles take part in movement and locomotion. The muscles are so arranged that they can provide optimum output. Each muscle has origin and insertion and as they contract, the insertion part moves towards the origin. Depending on this property the muscles are arranged accordingly so that the purpose is served. For example, the biceps muscle has origin in the scapula and insertion in the radius, thus in contraction causes flexion of the elbow joint. The muscle of the rib are so arranged that contraction of external inter-costal causes elevation of the ribs, thus increases the antero-posterior diameter of the thoracic cavity. Similarly, contraction of diaphragon muscle causes its downward movement towards the abdominal cavity, and thus superior-inferior diameter of the thoracic cavity is increased. In this way, as the diameter of thoracic cavity is increased, the intra-thoracic pressure is decreased. This is turn decreases intra-pulmonary pressure and so air enters into

the lungs and inspiration occurs.

Force is generated during musculer contraction and this forece is transmitted via tendon to the bones, within the muscle, there are elastic fibres in two forms— (i) parallel elastic component—the fibres lie or run in between muscle fibres. (ii) Series elastic components—these represent the elastic fibres that connect the contractile element with the tendon that is attached to the bone. These are involved in producing isometric and isotonic contractions. Isometric contraction is that where tension is developed but the muscle is not shortened. eg. holding a weight on the palm of a stretched hand. Similarly, an isotonic contraction is that in which muscle stretches but tension is unchanged, eg. lifting a load by flexing the elbow joint. The biceps muscle contract isotonoically as the load is lifted. Because of series elastic element, the force generated during mascular contraction can be transmitted to the bones via the tendon.

Sometimes, the muscles are attached to a long tendon that in turn is attached to the bone. eg. the muscles that cause movement of the finger-joints are present in the lower arm. Such arrangement definitely help in smooth movement of the fingers.

The force generated during muscle contraction is directly related to actin-myosin overlap. It has been shown that the more the overlap, the more the tension development. A study of length-tension relationship revealed that as the length of the fiber is increased the tension developed is dependent of actin-myosin overlap. At longer length, the tension is small and it is zero, when there is no overlap (Fig 4.8). When the length is gradually decreased, the overlap is slowly increased, and the tension gradually rises. It becomes maximum (plateau phase) when there is maximum actin-myosin overlap. A further decrease in length of the fiber again causes a decrease in tension, because overlap of actin retands tension development. Frogs use hip flexor muscles during jumping. It has been found that during jumping the sarcomere length changes from 2.3 μm (at rest) to 1.82 μm at the point of take off, and this length has been found to correspond to the plateau-phase of the length tension diagram of the hip-flexor muscle.

The red and white muscles are used according to the type of contraction required. For quick movement the white type is used and for sustained movement red muscle are used. It has been shown in fish that during steady swimming the sarcomere length of the red muscles coincide with the plateau phase of length-tension curve for this muscle, whereas in case of escape response, this is seen in case of white muscles. This proves which muscles are used for what kind of movement. Biophysical and biochemical characteristics confirm their suitability for doing sustained or quick movement. Thus animal body uses specific muscles for specific purpose.

It has been shown that as the length of the muscle fibre is increased, the tension development during contraction also increases progressively and becomes maximum at a particular length. It is called resting length of the muscle fibre. At this length the

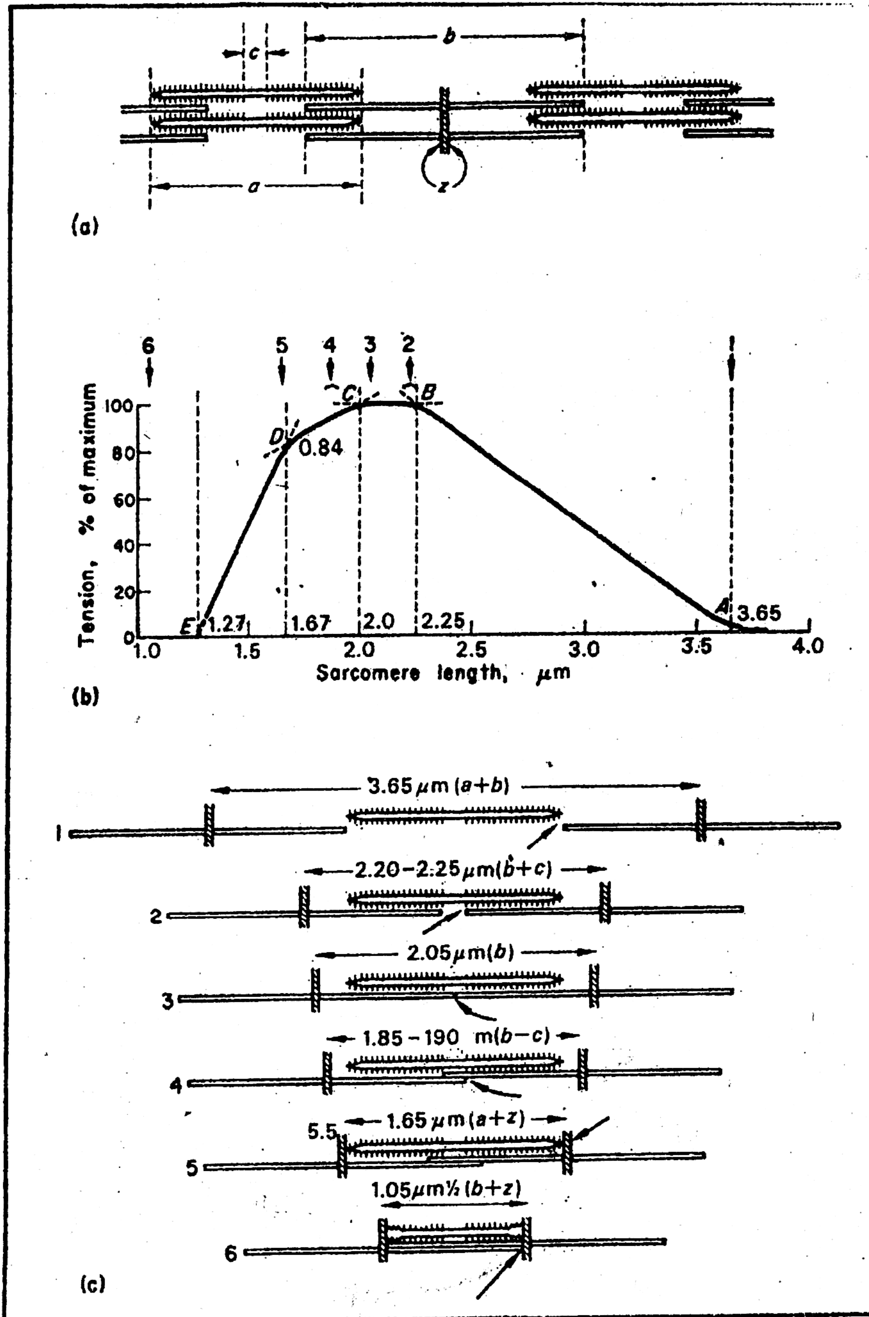


Fig 4.8 : Standard filaments lengths. a = 1.60 µm; b = 2.05 µm; c = 0.15—2 µm; z = 0.05 µm. (b) Tension-length curve from part of a single muscle fibre (schematic summary of results). The arrows along the top show the various critical stages of overlap that are portayed in (c). (c) Critical stages in the increase of overlap between thick and thin filaments as a sarcomere shortens. (Gordon, A.M., Huxley, A.F., and Julian, F. J. (1966). *J. Physiol., Lond.* 184, 170.)

actin-myosin overlap is maximum. If the length is further increased the tension development is decreased. Survey report revealed that the muscles present in the body during rest are at the resting length.

The sound producing muscles (sonic fibres) contract 10-100 times faster than those used for locomotion. Experimental results indicate that this has been made possible by a number of adaptations. These are— (i) an increase in Ca^{2+} kinetics ; (ii) cells are well equipped for aerobic metabolism and the space required for such assembly is made by reducing the number of myofilaments. The animal can afford such reduction in myofilaments in sonic muscles, because the muscles require faster rate and not increased force.

The force or power production during muscular contraction is denoted as V/Vmax, where V is the velocity of muscular contraction at any particular condition and Vmax is the maximum velocity of shortening possible. The power production is considered to be maximum, when the value of V/Vmax is 0.15—0.40. For frog hip flexor muscle during jump V/Vmax is about 0.32. Hence it is said the muscles while lifting the body they try to do it with maximum power.

The skeletal muscles of the vertebrates consist of muscle fibrets of more than one type. Some contain a high proportion of tonic fibers which show steady contraction other muscles contain a high percentage of twitch fibres which are specialized for rapid movements. Such muscle fibres having different properties have been found in the animal body and these are due to biochemical, metabolic and other structural adaptations.

Tonic muscle fibers contract very slowly and do not produce twitches. The motor neuron make contact with the muscle fiber at several points. Hence, action potential is not produced, and in fact they do not require AP to spread excitation. The actin-myosin detach slowly, hence the velocity of shortening is slow. So they are able to generate isometric tension very effectively. They are capable for slow steady contraction. Hence they are used for posture maintenance, where a slow sustained contraction is required. They are found in the postural muscles of amphibians, reptiles, and birds as well as the muscle spindles of all extra ocular muscles of mammals. Slow twitch or type I fibere are characterized by slow to moderate Vmax. Slow Ca^{2+} kinetics. They contract slowly and fatigue slowly. They generate 'All or none' AP. It has one of few end plates. It contains myoglobin. Muscles that contain more of these fibers are also called red muscles. The slow-fatigue is due to presence of large number of mitochondria and rich blood supply. These fibres are suitable for maintaining posture and for moderately fast repititive movements.

Fast twitch oxidative (type IIa) fibres have high Vmax. They acn be activated quickly. They fatigue slowly because they have more mitochondria, can produce ATP quickly by oxidative phosphorylation. They are specialized for rapid repetitive

movements as required in sustained streneous location. For these reasons, they are used for making the flight muscles of wild birds.

Fast twitch glycolytic (type IIb) fibres. These fibers have a high Vmax, very rapid Ca^{2+} kinetics and for the this they get activated and relax quickly. They have few mitochondria. Hence they are dependent on anaerobic glycolysis to genetate ATP. Hence, they fatigue quickly. These are found in breast muscle of domestic fowl, which are never used for flying and cannot produce ssustained activity. Ectothermic vertebrates, such as amphibians and reptiles also make extensive use of glycolytic muscle fibers.

Thus, the animal make up their muscle by different types of muscle fibers according to their requirement. Body not only can improve their muscle mass by growth of individual muscle cell, but they can also improve their power for performance by forming oxidative enzymes to improve aerobic power and by improving glycolytic enzymes to improve anaerobic power. Moreover, by changing to fast twitch fibers and vice versa. This is mediated via changes at genetic level.

Cardiac muscle cells are interconnected functionally. Here, the whole heart obeys all or none law. However, it has been observed that in a quiscent heart repeated stimulation increase the force of contraction for few strokes.This is known as stair case phenomenon or treppe. It is due to increased accumulation of Ca^{2+} that increases the force of contraction of cardiac muscle. At every situation Ca^{2+} is released but in case of repeated stimulation the rate at which it is released can not cope with the removal. So Ca^{2+} accumulates and produce the effect.

It has also been observed that when the cardiac muscle is stretched its force of contraction is increased, as happens in case of skeletal muscle. But the mechanism in cardiac muscle is different at the level of actin-myosin interaction. It has been shown that at saturating Ca^{2+} ion concentration stretched cardiac muscle exhibit greater force of contraction compared to control cardiac muscle. It is not due to difference of overlap of thick and thin filament as happens in skeletal muscle. Evidence suggest that stretch reduces the space between thick and thin filaments (ie., interfilament spacing) as this is associated with the ability of more myosin molecules to interact with actin.

Titin plays an important role in it. Titin binds to both actin and myosin in such a way that when the muscle is stretched, it brings actin more close to the myosin head and they increase the number of myosin heads that interact with actin. It has been found that protolysis of titin attenuated length dependent increase in force. Increased sensitivity to Ca^{2+} is possibly due to decreased interfilament space caused by titin (Fig 4.9).

Increased venous return increases cardiac output. This is possible because of the property of cardiac muscle. As more blood enters the heart (ventricle), the volume of the heart (ventricle) is increased, that is the heart muscle is stretched and this increases the force of contraction of heart muscle. So the blood goes out of the heart.

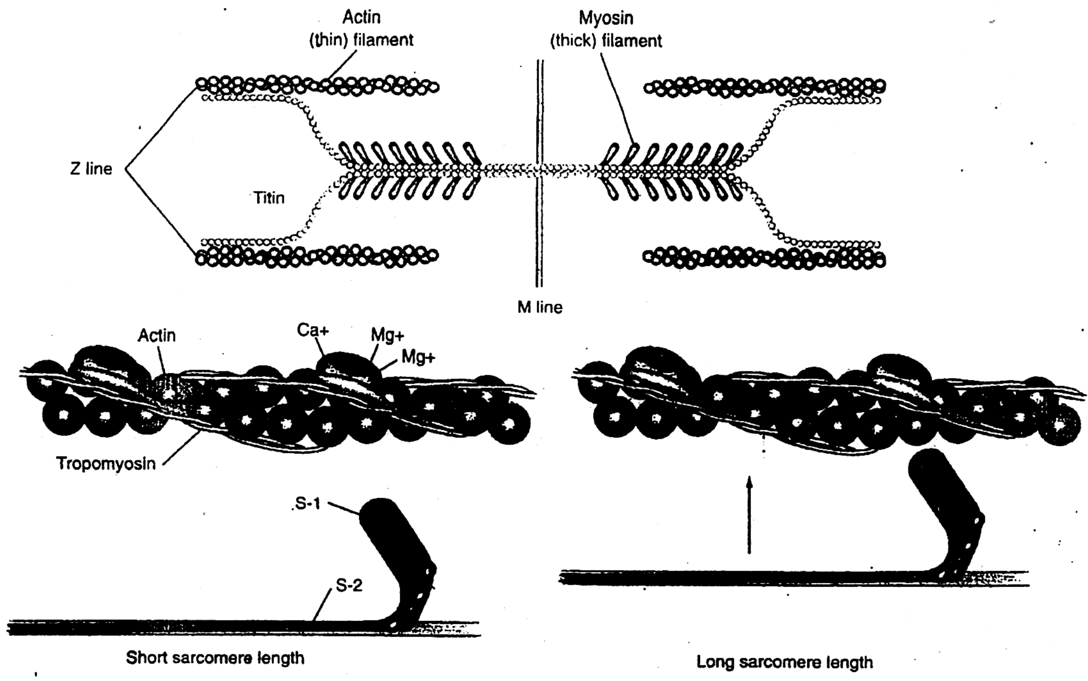


Fig 4.9 : Titin may contribute to the ability of stretch to increase the force of contraction of the heart. Titin binds to both myosin and actin such that stretch of the cardiac muscle may bring the actin filament closer to the myosin head and thus increase the number of myosin heads that interact with actin at a given intracellular [Ca++]. (Redrawn from Moss RL, Fitzsimons DP : Circ Res 90:11-13, 2002.)

In smooth muscle cell actin-myosin recycling occurs. Myosin cross-bridge head attaches itself to the actin, then it is released from actin and then reattaches with next actin in the next cycle. This rate of cycling is very slow. This is because ATPase activity of myosin is very low compared to skeletal muscle. Moreover, in case of tonic contraction, it has been found that, when full contraction has developed, it can be maintained with less ATP utilization. During actin-myosin attachment a great force is generated and this allow the smooth muscle cells to contract as much as 80% of its length (instead of 30% as seen in skeletal muscle). This can cause a great collapsing of the hollow tube.

The response to a sustained or tonic stimulation is a rapid contraction followed by a sustained maintenance of force with reduced cross-bridge cycling rates and ATP consumption. This behaviour is called latch state. It is advantageous for muscles that may need to withstand continuous external force, such as blood vessels, which must be able to withstand blood-pressure. ATP consumption during latch state is less than 1/300 the rate that is necessary for skeletal muscle to maintain the same force. Another important characteristic of smooth muscle cells is length adaptation. It is able to adjust length-tension relationship when chronically stretched or shortened.

This is very helped during filling of urinary bladder. Hence bladder wall is made up of such smooth muscle cells.

It has been observed that a sudden increase in bladder volume causes stretching of the smooth muscle and the pressureof uninary bladder is increased but subsequently within 15 sec to one minute despite continuous stretch the pressure becomes almost back to normal. This has been named as stress relaxation. Because of this, a hollow organ can maintain almost the same pressure in spite of changes in the length of smooth muscle.

The single unit smooth muscles are present in the GI tract. The multi unit smooth muscles can be found in vas deferens and iris muscle.

4.2 Movements—ameboid, ciliary and flageller

Amoeboid movement

This terms derives its name from motion of Amoeba. There are two terms, 'movement' and 'locomotion'. An animal being attached to a site can show a great variety of movement, but the animal does not shift to another place. It is called movement eg, corals and sponges. But when the animal shifts its position from one place to another by movement it is called locomotion. There are animals which have cilia and flagella. These also show movement and movement of these can help the animal to move from one place to another. Thus, ciliary movement and flageller movement are not only related to 'movement', but also to 'locomotion'.

A typical Amoeba consists of an outer layer, the plasmalemma. It is not water wettable and has adhessive property. It slides freely over the next inner layer called ectoplasm (gel-like). The plasmalemma is made up of outer filamentous coat and an inner membrane. The filaments are about 80Å in diameter, and extend outward 0·1 to 1·0 μm. The filamentous coat is made up of 35% lipid, 26% protein and 16% polysacharide.

Beneath the plasmalemma there is a hyaline layer. It is fluid in nature as judged by brownian movement. This layer is very thin in the region of attachment to the substrate. It is often thickened as an hyaline cap at the front of an advancing pscudopodium.

Types of cells that exhibit amoeboid movements

Types of cells that exhibit amoeboid movement are white blood cells, fibroblast, germinal cells in skin, and embryonic cells. Embryonic cells often must migrate long distances from their site of origin to new areas during development of special structures.

Process of amoeboid movement

The amoeboid movement involves cytoplasmic streaming, changes in cell shape and extension of pseudopodia. When an amoeba moves, its cytoplasm flows into

newly formed arm like extensions of the cell (pseudopodia). The pseudopodia gradually extend and enlarge so that the entire cell occupies the space where previously only a small psendopodium began to form. As the cell moves, new pseudopodia are formed in the direction of the movement, while the posterior parts are withdrawn.

The outer layer of the Amoeba is the stiff gel-like layer called ectoplasm. As the pseudopodium is formed, the more liquid endoplasm streams into it, and a new ectoplasm is formed on the surface. In the rear part of the advancing cell, the ectoplasmic gel is converted to a more liquid endoplasmic sol by a sol-gel transformation.

'Rolling and walking' are the two types of movements that have been described. The rate of movement also show much variation. It may be as slow as 1350 μm/sec. (e.g. *Plasmodium* of acellular slime mold) 5-6 cm/hr in migrating Plasmodium. Freely crawling amoebaᴗ move at the rate of 0.5 to 4.5 μm/sec.

In feeding, those amoeba, which travel by small pseudopodia, form food cups. These cups are also motile, encircle the food particle, and their distal ends join and the food is taken into a vacuole.

Mechanism of amoeboid movement

The exact mechanism is not fully understand, but involvement of certain processes have been known from different experiments. Involvement of membrane, nucleus, microtubules of microfilament system, and contractile proteins have been studied.

Total surface area of the membrane is greatly increased when an amoeboid cell changes from a nearly spherical shape to a multipodal form, it then decreases as pseudopodia are retracted. Three theories were proposed for this, but experimental evidence suggest that it is because of the membrane is a fluid or plastic surface that slides freely over the ectoplasm. Essentiality of the nucleaus has been established. Enucleated amoeba soon lose the organised progressive cytoplasmic flow that results in movement.

Involvement of microtubules are microfilaments in amoeboid movement has been well documented. They play a role in movement either by processes like sliding mechanism in mascular contraction or development of shear forces.

The birefringece seen in amoeba suggest an orderly assay of macromolecular structure. The presence of structures like actin and myosin have been well documented in amoeba.

Amoeboid cells have been found to respond to an electrical field and show biopotentials. In an electrical field, *Amoeba proteins* shows solation on the cathodal side and pseudopodia advance in that direction.

Amoeboid movement is caused by contraction of cytoplasm. It is held that at the point of attachment there is less space between the plasmalemma and the ectoplasm than elsewhere. It has been suggested that contact between plasmalemma and ectoplasm

in the *Amoeba proteus* initiates contraction. According to Allen such contraction takes place in the 'fountain zone' and the Amoeba is 'pulled along'. However, Jaha and Bovae proposed that the site of contraction is 'tail process', and the Amoeba is pushed forward.

Actin is largely conserved. It is of similar structure and occur as the filaments in the cell. Myosin from various sources are more diverse, but all bind to actin and causes ATP—hydrolysis with liberation of energy. On this basis, it has been suggested that both cytoplasmic steaming and the formation of pseudopodia may depend on the interaction between actin and myosin.

Control of amoeboid movement

Chemotaxis is the most important initiator of amoeboid movement. Movement occurs either towards the source of chemotactic agent (positive chemotaxis) or away from it (negative chemotaxis). How chemotaxis control the movement is not clear. But it has been observed that the cell surface exposed to the chemotactic agent, develop membrane changes that cause pseudopodial protrusion.

Cilia and flagella

Each cilium has the appearance of a sharp pointed straight or curved hair that projects from the surface of a cell. It is an outgrowth of a structure that lies immediately beneath the cell membrane called the basal body of the cilium. It is of shorter length.

Flagella on the other hand are membrane bound extensions of the cell. A basal body called kinetosome anchor the flagellum with the cytoplasm.

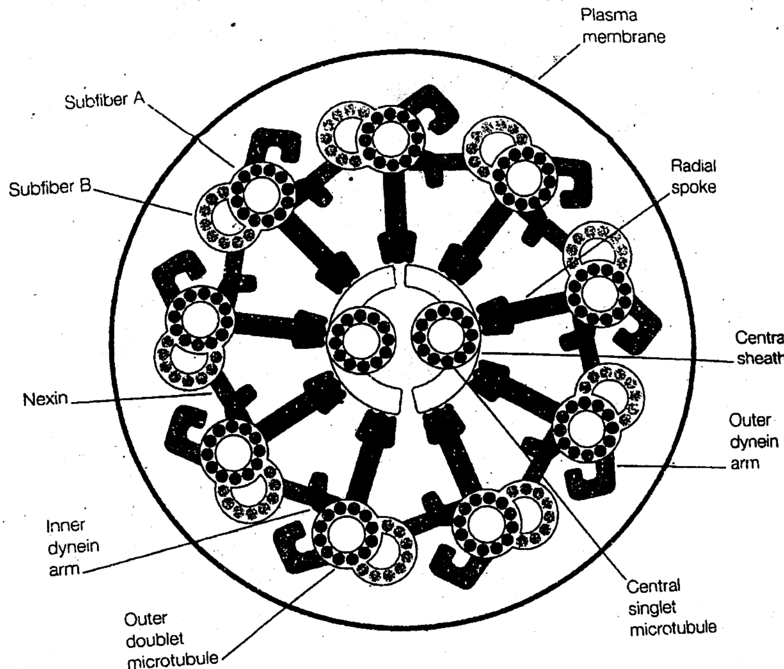


Fig 4.10 : Cross-sectional diagram of a cilium.

Basic structure of cilia and flagella

Flagella are of longer length compared to cilia. In eukaryotic cells, the cilia and flagella are of similar internal structure. Cilia and flagella contain microtubules. The microtubules are arranged as a bundle of nine doublets around the periphery with a pair of single microtubules running within them. This structure is called *axoneme.* In an axoneme, the 9+12 array has specific arrangement (Fig 4.10).

Each of the nine outer doubtlets appears like the figure eight. The smallar circle of the figure is termed subfiber A. The larger circle, subfiber B.

Subfiber A is joined to a central sheath by radial spokes. The neighbouring doubtlets are held together by nexin links. Two dynein arms emerge from each subfiber A with all the arms in a molecular cilium pointing in the same direction.

Mechanism of movement

The dynein in a large protein (MW 1000-2000 kDa). It consists of one, two or three heads depending on the source. The heads of dynein form cross bridges with the subfibres B and its has ATP binding site as well as ATPase activity (Fig. 4.11)

Binding of ATP to dynein causes it to dissociate from B subfiber. The ATPase activity of dynein splits ATP to ADP and Pi. On hydrolysis of ATP, dynein again binds with subfiber B with subsequeat release of ADP and Pi. This ATPase cycle leads to the movement of the cilium as the outer doublets of the axoneme slide past each other. Dynein is involved in converting the energy released from ATP hydrolysis into mechanical energy for movement. Movement is produced by the interaction of

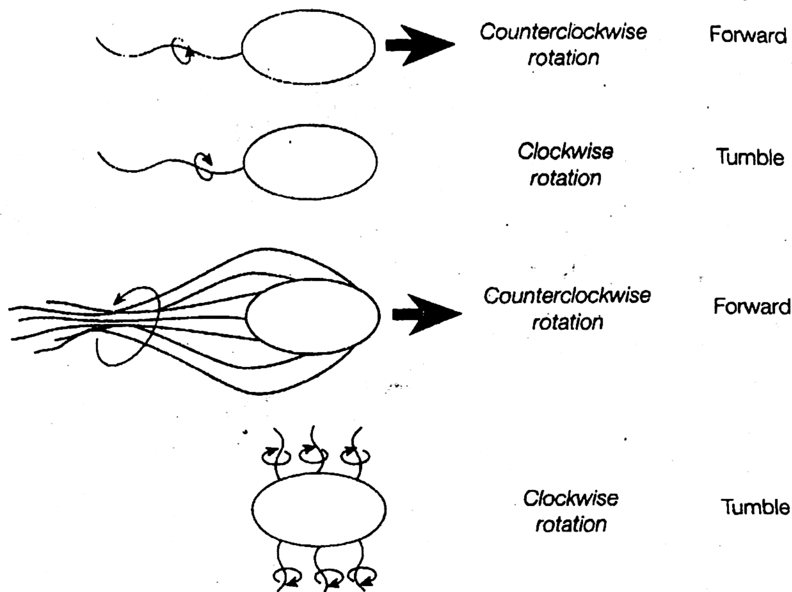


Fig 4.11 : Effects of flagellar rotation on prokaryote movement.

the dynein arms with one of the microtubules of adjacent doublets. The force between adjacent doublets is generated by the dynein cross bridges. Thus the dynein arms on subfibre A of one doublet walk along subfiber B of the adjacent doublet. The radial spokes prevent sliding motion as happens in the muscle, and so the motion is converted to a local bending. The highly flexible protein, nexin, keeps adjacent doublets together during this process. Bending of the flagellum occurs when the extending dynein arms attached to the neighbouring B tubule, inducing active sliding movements out the expense of ATP.

Movements and its importance

1. A flagellum, like the tail of a sperm, beats with a symmetrical undulation that is propagaled as a wave along the flagellum. A cilium beats asymmetrically with a fast or dash like stroke in all direction followed by a slower recovery motion in which a bending cilium returns to its original position.
2. The number of cilia or flagella present in cell show much variation. A paramoecium may have several thousand cilia, cliliated cell in the respiratory passage may have 250 cilia. Few cilia are present in the epithelial lining of fallopian lube. Cilia in the respiratory passage help in the removal of mucous by mucous cell of the respiratory lining, along with trapped particles toward the nasal opening. Ciliary movement in the reproductive tract help in propalsion of ovum from the ovary to the uterine tube as this is necessary for fertilization and implantation of fertilized ovum.

   A flagellalled cell carries usually one or a few flagella. The sperm of a vast number of animals swim by means of flagella. Flagella exhibit typically sinusoidal motion in propelling fluid/water parrallel to their axis. The undulating action of the flagellum either propels water away from the surface of the cell body or draws water towards the cell body. The cilia exhibit an oar like motion, propelling water parallel to the cell surface.
3. Cilia and flagella are found in many protozoans and mainly related to locomotion, cilia are found in all animal phyla. Modified ciliary structure are present in insect eyes as well as in the majority of other sense organs.
4. Small animals use cilia and flagella for locomotion and muscles are used for the purpose in large animals.

Bacterial flagella : (Chemotaxis) by rotating their flagella. The flagella of bacteria are quite different. They are thinner (about 0.2 μm in diameter, against 0.25 μm for true flagella and cilia), short and relatively rigid. They are related by forces at the base where the are attached to the cell. They differ for eukaryotic cilia and flagella in two ways— (i) each bacterial flagellum is made up of if flagellin (53 kDa sub unit) as opposed to tubulin as (ii) it rotates rather than kinds. The rotary motion of the flagellum is driven by the basal body which acts like a motor. The direction of

flagellar rotation determines the type of movement. Prokaryotes with a single flagellum move forward during counter clockwise rotation and tumble where the flagellum rotates clockwise. Where there are more than one flagellum they behave as a single bundle during counter clockwise rotation and thus move forward, however, during clockwise rotation the flagella act independently and the organisms tumbles. Bacteria can move through the extra cellular medium towards attractants and away from repellents.

4.3 Specialized organs (eg : electric organs and tissues)

Fishes are unique in the animal kingdom in being capable of producing electric current. These are produced from an organ in the tail region and called electric organ. About 250 species of fishes, both chondrichthyes and osteichthyes are reported to possess electric organs. These species have evolved the electric organs independent of each other.

The following are the most important fishes that are known to possess the electric organ.

Elasmobranchs : (i) Electric Rays; (ii) Skates.

Teleosts : (i) Mormyridae; (ii) Gymnotidae, (iii) Siluridae.

It has been found that some fishes produce strong current, some produce weak current and there are some which can only sense electricity but cannot produce electricity. On this basis, electric fishes have been divided into three main categories :

A. Strongly electric fish :

i) electric eel.

ii) electric catfish.

iii) electric rays.

B. Weakly electric fish :

i) Knife fishes.

ii) elephant nose.

C. Fishes that can only sense electricity :

i) Sharks.

ii) Rays.

iii) Skate.

iv) Catfish.

v) Paddle fish.

Structure of electric organ

The shape and position of the electric organs differ greatly in different species of fishes but all of them have more or less a similar microscopic structure. It is made up of disc like cells called electroplates or electrocytes which are modifed muscle fibers.

These are embedded in a jelly like material and are bound together by connective tissue into an elongated compartment. One face of each electroplate is supplied by nerve fibers and the jelly receive blood capillaries. Each electroplate is a multinucleated cell with nearly transparent cytoplasm. Hence, the elecetric organ looks like a clear gelatinous mass as compared to the muscles.

Stimulation of electric organ

Normally a resting potential exists across both innervated and noninnervated surfaces. In the *Electrophorus* it is about 90 mv. the inside being negative. It is due to difference in the distribution of electrolyte concentration within and outside its cell. At the peak of the discharge, the membrance potential across the nervous face of the electroplate is reversed (60 mv) but the potential across the non nervous face remains unchanged. As a result a potential difference of 150 mv (60 + 90) develops

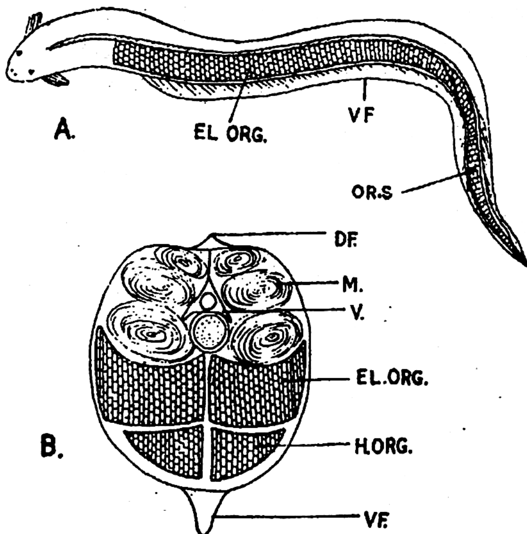


Fig 4.12 : (A) *Electrophorus electricus*, dissected to show electric organs. (B) T.S. *Electrophorus*. DF., Dorsal fin ; EL. ORG., Electric organs ; H. ORG., Hunter's organ ; M., Myotome ; OR. S., Organ of Sach's ; V., Vertebra ; VF., Ventral fin.

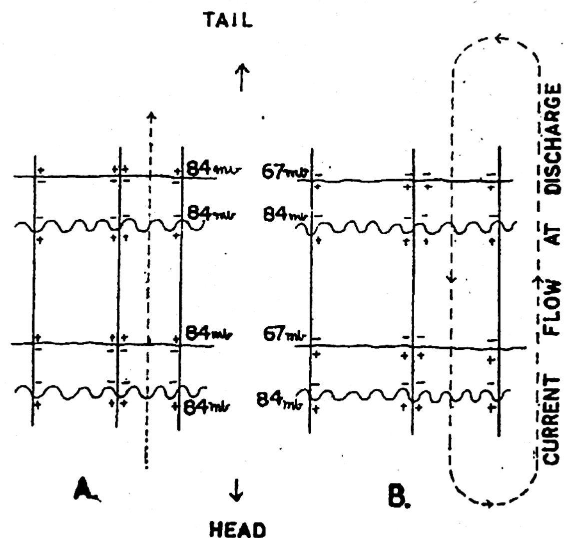


Fig 4.13 : Diagramatic representation of current flow in an electric fish. (A) at rest ; (B) at discharge.

between the two surface of a cell. The voltages of successive electroplates are added up in series and a strong current flows. According to ionic hypothesis, at first sodium ion and them potassium ion enter into the cell due to alteration of membrane permeability and thus results in potential difference. (inside positive and outside negative) (Fig 4.12, 4.13).

These electrocytes or electroplates receive simultaneous command signals from the brain to 'fire'. At the moment of 'firing' the electrocytes are asymmetrically polarized acting as serially connected batteries. The simultaneous firing of electrocytes results in the electric organ discharges (EODs) which are emitted in the surrounding water. In strongly electric fishes, such as electric eel, electric catfish and electric rays, the electric organ is huge containing numerous electrocytes and so their discharge voltage can reach as high as 600 volts. However, in weekly electric fishes, it is small and often less than a volt.

The electric discharge of the electric organs are of two types. In all strongly electric fishes and some weakly electric fishes the discharge is of pulse type. They discharge short electrical pulses intermittently. However, some weakly electric fishes produce wave-type discharge, they produce wave like continuous AC electricity. Some fishes like *Electrophorus* produce both strong and weak current according to need. Most of the electric fishes can produce electricity as well as can sense electricity. However, there are fishes which can only sense electricity but cannot produce it.

Strength of current generated :

The values of electric current produced by some electric fishes are given below :

Skate :		4 volts.
Electric rays :	*Torpedo* :	40 Volts.
	Narcine :	37 Volts.
Electric eel :	*Electrophorus* :	370 – 550 Volts
Electric Cat fish :	*Malapterurus* :	350 – 450 Volts.
Star-gazar :	*Astroscopus* :	50 Volts.

Functions of electric organ

Electrolocation :

The ability to locate an object with the help of electricity is called electrolocation. Fishes have a very sensitive sensory organ which can receive electricity. These are called electroreceptors which remain embedded in the skin.

There are two types of electroreceptors. An **ampullary receptor** contains

supportive cells that lie at the bottom of a narrow channel filled with gelatinous mucopolysaccharide. Afferent nerves embrace the receptor cells.

The tuberous receptor lies buried under the skin in an invagination beneath a loose layer of epithelial cells. This loose layer may differentiate into covering cells that cover the sensory cells are a superficial set of plug cells. These receptor respond to higher frequency than the ampullary receptors. It also can sense the electrical discharge of the fishes own electric organ.

Electroreceptors are used to detect a slight change of electric field caused by nearby objects. Hence, they can see objects electrically. The electrolocation may be active or passive. When the source of electricity used for electrolocation is their own electric organ it is called active electrolocation. The fishes, which cannot produce electricity but can sense it, can sense very weak electricity produced by prey animals. For example, a shark can find a small fish buried in sand by the weak electricity given off by the prey. This type of electrolocation is passive electrolocation because here the electricity is produced by the prey and not the predator which help in locating the object.

Self protection :

Electric fishes are well protected against their own current discharge and against the discharge of each other. This is achieved possibly by developing high insulation around nervous system. As in *Electrophorus*, the spinal cord and the swimming muscles are embedded in a thick layer of fat. Their nerves and muscles may also develop unusually high threshold of excitation.

Importance of electric organs

The form, position are strength of the electric organs show much variation and this is related to there functions.

Fishes with powerful electric organs use these both offensively in their hunt for food and defensively against their enemy. The primary function of the electric organs in all fishes is possibly defensive. Both *Torpedo* and *Electrophorus* have been observed to paralyse small fishes before eating them. Fishes with weak electric discharge create an electric field around themselves and if any object is close to it the electric field breaks up and thus the fish become aware of the presence of the object. Hence it may act as an warning device and it is of considerable value in muddy water where vision is not possible.

Such weak discharge also helps the fish to find direction in the dark water.

Electric organ discharge also helps in maintaining the territoriality by individual fish. It may also be useful for speices or even sex recognition.

Fish can use electricity to feel the environment and also can communicate with each other using electrical singal.

Jamming avoidance response

The wave type electric fish normally discharge at a fixed frequency and each individual has its own frequency. When two individuals having similar frequency meet, however, their EODs interfere with each other causing problem in electrolocation. This is called jamming. To avoid jamming the two fishes shift their frequencies until there frequencies differ in such a way so that operation of electrolocation becomes possible.

4.4 Suggested questions

1. Describe the basic structure of an electric organ and mention its importance.
2. What is jamming avoidance response.
3. Write briefly on electrolocation.
4. Describe the basic structure of cilia and flagella.
5. Write a note on the mechanism of movement of cilia and flagella.
6. Write briefly on the mechanism of ameoboid movement.
7. What is titin ? How it helps in muscle contraction ?
8. How the muscle shortens during muscular contraction ?
9. Describe the sequence of events in muscular contraction with a diagram.
10. Comment on length tension relationship in muscle contraction.
11. How collaping of hollow viscera is related to contraction of smooth muscle cells.
12. Comment a differential distribution of skeletal muscle fiber ?
13. What is caldesmon ? How it helps in muscle contraction ?

Unit 5 ❑ Adaptation

Structure

5.1 Introduction
5.2 Levels of adaptation
5.3 Mechanism of adaptation
5.4 Significance of body size
5.5 Suggested questions

5.1 Introduction

There are two basic concepts in natural selection. These are fitness and environment. Environment means the kind of habitat in which the living organism or animal lives. Whereas fitness is strongly linked with adaptation. Environments are largely variable in relation to stress that they impose on their inhabitants. This stress may be both abiotic and biotic.

Whenever there is a change in the environment, compensatory changes occur in the living organisms to cope with the environmental modification. Short term compensatory changes in respense to environmental disturbance is called acclimatization or acclimation whereas long-term compensatory changes in known as adaptation. Adaptation is defined as a process; the means by which natural selection adjusts frequency of genes that codes for traits affecting fitness. For example, increasing haemoglobin concentration ; *in taxa* might be seen as an adaptation to potentially low oxygen environment. Adaptation in this sense, is a process that normally occurs very slowly, over hundred or thousand of generations and is usually not reversible. However, in extreme environment or where selective pressure from human interference are strong it can sometimes occurs very quickly. Adaptation is often used as a term for the characters or traits observed in animals that are the results of selection. For example presence of hemoglobin can be said to be an adaptation to increase the oxygen carriage in the blood. The processes of adaptation is usually a slow one that occurs over generations and is rarely reversible. Acclimatization on the other hand, is more rapid phenomenon whereby a biochemical or physiological change occur within the life of an individual animal resulting from exposure to a new condition in the environment. Thus an ascent to high altitude (mountain) may lead to acclimatization to low oxygen and low pressure; movement from arctic areas to southwards will lead to acclimatization to warmer temperature. When similar processes are allowed to occur in the laboratory it is called acclimation. A polar bear is said to be adapted to

polar temperature. The shape of the chest of sherpas are adapted for respiration at low oxygen pressure at high altitude. The human polar explorer or mountaneer get acclimatized to the environment. But they revert back gradually to normal when the environmental condition is changed. Mouse forcefully subjected to cold exposure in the laboratory, get acclimatized to cold. It reverts back to normal when the laboratory temperature is reverted to normal.

The avoiders have or develop mechanisms for getting away from or to avoid an environmental problem either in spece or in time. Avoidance in space is brought about by behavioural change for a small animal it may involve a search for an appropriate habitat using phototactic or chemotactic responses. They look for less stressful microhabitat in crevices or burrows. Larger migratory species try for large scale migration with the help of physiological adjustments. e.g. accumulation of food reserves. Avoidance in time require more complex responses at all levels. An animal entering into torpor accumulate food, construct or find a refuge; huddle in a ball to reduce its surface area for preventing heat loss in a cold environment; it may reduce its core temperature and metabolic rate, it may acquire a thick insulating layer to cope with temperature extrem; it may mobilize or generate new form of enzymes and new forms of membrane components.

The conformers change their internal states, similar to changes in the external environment. They do not try to maintain a homeostatic condition. This involves biochemical and physiological modifications. If the internal environment varies in terms of osmoticity, salinity or temperature, the cells must have a biochemical-physiological system that can function at diverse conditions especially enzymes and membrane stabilization. Their status is such that the animal is kept functioning at extreme conditions avoiding damaging effects of freezing on hypoxia etc. However in general the physiological and biochemical changes are small and cheap, hence are more economic.

The regulators on the other hand maintain their internal environment almost constant irrespective of changes in the external environment. This involves substantial and expensive biochemical and physiological adjustments. For example, to maintain temperature homeostasis, even in the best endothermic mammals, behavioral changes like basking, burrowing, wallowing, huddling, erecting or concealing appendages etc are adapted as a first line of defence. Along with this substantial physiological and biochemical adjustments, occur to have optimal effect. These include changes of blood flow or respiratory rate or nonshivering modification of thermogenesis and thermolysis or the production of heat-shock proteins or anti-freeze molecules.

5.2 Levels of adaptation

When an animal is confronted with changes in its environment it normally exhibit three types of responses - avoidance, conformity or regulation. These responses are the final outcome of adaptations at four levels. Biochemical, Physiological, Morphological and Behavioral. Adapted responses may appear at different spatial levels. Some responses are essentially subcellular, some affect the morphology or activity of whole cells; others manifest as effects on entire tissue or organs like changes in muscle size heart volume or arrangements of vascularisation.

However, there is a limit of conforming and regulating. For example, the osmoconformers show some regulation in extreme low salinity to avoid cell damage due to excessive swelling. Similarly the osmoregulators become unable to regulate at lower salinities and turn to conformers, homeothermic animal allow their extremities to become poikilothermic at extreme cold.

These strategies adapted to counteract enviromental changes are associated with different costs and benefits thus the 'avoidance' by shutting down is cheap but this causes the animal to remain out of the 'race' for some time without any growth and reproductive output. In case of avoidance by way of migration may be expensive but it allows the animal to continue with growth in another environment. Avoidance in poor physical environment by way of shut down or migration give additional benefits like avoiding predation or competition.

Conformity at the extremes of temperature, salinity or hypoxia may allow a minimal lifestyle but over a broad range it can maintain a reasonably productive life style at a cheaper cost. Regulation is rather definitely expensive; osmoregulation takes about substantial amount of energy but thermal regulation draws up of total energy budget. However, it can gather food all through and the conformers and avoiders become prey. With extra food they can grow and reproduce faster. Despite high cost they become dominant in many ecosystems.

Adaptation at different spatial levels

Adaptive responses may occur fandamentally at the molecular level, but they are manifested at different spatial levels in the whole animal. Animals are made up of several distinct compartments. Each of them may show different adaptive responses that leads to a change in the animal as a whole. The individual cells contain intracellular fluid (ICF) which is its own fluid environment. These cells are directly bathed in tissue fluid or extracellular fluid (ECF). In may species ECF is distinct from blood (ECF enclosed in specialized channel known as blood vessels) or lymph (ECF present in lymph vessels) or hemolymph. These fluids may be different in composition from the classical ECF. The relation between fluids of these three compartments and their

homeostasis is highly complex. Adaptation to environment may require modification at all these levels. For many invertebrate marine animals, the ECF and blood are identical with sea water and so adaptation occurs at cell–ECF level and within cells i.e. across intracellutar compartments. But in case of nonmarine animals there occur extensive regulation at the boundary between external environment and blood i.e. skin, as well as at the cellular lavel.

In terms of the whole animal, adaptations occur at different spatial levels or site.

1. To maintain difference between the outside world and the circulating blood, adaptation occurs at the outside surface e.g. skin. This surface (skin) may be made up of relatively unspecialized epidermal cells or a complete multilayered structure with chitinous, keratinous or lipid containing elements. Sometimes, variability in structure is confined to a particular area of the skin such as gill surface and other areas of skin are relatively inert or impermeable.

2. Adaptations can occur at the boundary between ECF and the circulating fluid. This mainly occurs in vertebrates when some constituents of blood pass out from blood capillaries into ECF and vice versa; some constituents of ECF goes back to blood via lymphatic system.

3. Adaptation at the boundary between ECF & ICF. It involves the cell membrane itself that control exchanges between ECF and ICF. The exact make up of ICF is very different from that of ECF. Total concentration of the fluid of the two compartments, however, should be similar to prevent osmotic swelling or shrinkage of the cells.

4. Adaptation within the cell : Cells themselves are strongly compartmentalized and the organelle menbranes are involved in regulating the exchanges between the cytoplasm and the nucleus, mitochondria or endoplasmic reticulum.

As the environmental condition is changed the animals show adaptation at different functional and spatial levels to cope with the environment which is essential for its survival.

5.3 Machanism of adaptation

Structural and functional modification of living organism occur as the environment undergoes changes. This is essential for survival of the living objects. Temporary changes occur to counteract the environmental changes and it reverts back to the previous form when the environmental changes are over. This type of change is called acclimatizion e.g. cardiospiratory changes in a mountaineer during ascent to high altitude.

But sometimes permanent changes occur to cope with the environmental changes involving genes (actually in DNA). This is called adaptation. The specific shape of the chest of sherpas living at high altitude is an adaptive respiratory change.

Proteins are basically involved in cellular processes. Cell division is the result of DNA duplicatian. Protiens are the products of genetic code of DNA. Alteration of DNA will alter protein structure, hence will alter cellular functions and thus will lead to phenotypic changes at the organism level. Adaptation occurs when any change in the DNA level becomes expressed via protein changes, as a trait that is beneficial in a particular environment and so persists due to selective advantage, eventually spreading through population. Hence anything that controls proteins is at the core of adaptation.

A change in the shape of the protein is associated with many cell-functions like switching effects via kinase-phosphetase system (e.g. phosphorylation of glycogen synthase makes it inactive — glycogen synthesis is inhibited, dephosphorylation of the enzyme makes it active – glycogen synthesis is stimulated. Former is caused by glucagon and the latter by insulin ; motor effects like ATP driven shape change in myosin causing its movement along actin during muscle contraction; change in shape of protein pump causing chanelling of ions (Na^+–K^+ pump) and other molecules across membranes and between cell compertments. These functions are again subjected to extremely complex regulation and amplification to produce controlled effects on whole organism.

DNA, RNA and protein synthesis

Most of the chromosomal DNA in any animal cell does not code for RNA or for protein. Only 1% of DNA sequence is transcribed into functional RNA sequence. DNA is duplicated during cell division. The transposons (Short pieces of DNA consisting of a few hundred to 10,000 nucleotide pairs) present in DNA organisation are involved in mutation. There is some evidence that they undergo long periods of quescence in the genome and then exhibit sudden bursts of activity (transposition bursts) being triggered by environmental change. These bursts form a link between environment and adaptation. They cause increased biological diversity because transpositions will potentially bring together two or more new traits which (of little value alone) become very useful by working together. Thus these transposition bursts can produce randomely modified progeny. This happens at times of environmental stress. Hence transposons act as useful symbiotic factors, generating diversity just when it is most needed.

A change in DNA is the fundamental mechanism of evolution. DNA is *insulted* in a normal course by various factors like thermal degradation. UV radiation metabolite action as oxygen radical. DNA repair enzymes like DNA repair nudclease. DNA polymerase & DNA ligase are induced by DNA - damage. The DNA is repaired and normal function is left undisturbed. This is a safety mechanism of the cell. Such repair is crucial in genetic recombination in meiosis where chromosomes undergo crossing-over. It has been found that minor mis-matches are corrected by such repair

but where there is a particularly poor match between the recombining strands, the pairing is usually aborted and no recombination occurs; excessive scrambling of the genome which might be detrimental to physiological functions is thus averted.

DNA forms RNA by transcription. The RNAs undergoes posttranscriptional modification and active RNAs are formed. There are three types of RNA – mRNA, rRNA and tRNA. mRNA with the help of rRNA, tRNA join aminoacids through peptide bond and form polypeptides by the process called translation.

The polypeptides formed by translation process undergo posttranslational modification and are converted to active polypetides. These undergo proper folding with the help of chaperans and functional proteins are produced.

The proteins that can be formed theoretically are many. But the proteins that are selected for in evolution are those that can reliably and repeatedly folded up into stable forms which can be subjected to conformational changes by controlling mechanisms.

Protein effect is a balance between protein synthesis and protein breakdown. Degradation of abnormal proteins is mediated by intracellular proteosome-ubiquitin mechanism. However, in case of degradation of normal protein with specific halflife is dependent on aminoacid sequence on the N-terminal side of the peptide. Certain amino acids offer protection while other such as Arg, Asp and Glu promote proteolytic attack. Thus the aminoacid sequence of this region can be selected over evolutionary time to give protein with appropriate half life.

The protein synthesis at genomic level is regulated by regulating proteins at different levels—

(i) Transcrptional control; (ii) RNA processing control; (iii) RNA transport control; (iv) translational control, mRNA degradation control; Protein activity control.

The control at the transcriptional level involves the actions of repressor protein (inducible enzyme); corepresser - aporepressor complex (repressible enzyme); transcription activators and transcription repressor; transcription factors operating at the enhancer site and repressor site causing enhancement and suppresion of transcription respectively.

Adaptation is achieved by way of formation new structure and development of new functions for better survival in the existing environment. Environmental factors act as stimulus. They in turn modulate intracellular mechanisms by way of genetic alteration and gene expression that ultimately leads to phenotypic changes.

Permanent changes in protein structure can be brought about by mutations and recombinational changes in the DNA sequence of a cell. This can produce subtle changes in enzyme activity, signalling activity and subcellular morpthology, as well as expression of other proteins. All these in turn can lead to permanent heritable

change in the development of organisms. These are seen at a series of levels. At biochemical levels, there may be increased thermal tolerance of an enzyme or expression of a more pH stable allozyme; morphologically there may be developmental changes of gene expression leading to altered positioning of muscle cells, nerves, blood vessels, even completely modified appendages; Physiologically there may be increase in heart rate under the influence of Ca^{++} and cAMP signalling regime on Na^{+} pumping; changes in the rate of Na^{+} pumping as more or different channel proteins are synthesized; at the behavioral level there may be an increase in the speed of attack, greater sensitivity to a sexhormone or a concious change in response. All of these proteins induced changes the raw material for adaptation. Environmental stimuli modulate intracellular system through neurotransmitter and hormones. In relation to adaptive changes the hormones are the major controllers of genetic expression and thus of protein synthesis.

5.4 Significance of body size

An unconditional generalisation cannot be made about adaptive responses like avoidance, conforming and regulating across animal kingdom. It is dependent on body design and habitat.

1. Smaller and soft bodied animals are more likely to be avoider and conformers. They can use microhabitat more effectively. They enter into protected crevices, burrows or rest on and in other organisms. Since these animals have a high surface area to volume ratio (small animal) rapid fluxes will occur (water, ion, air, thermal energy) and so restoration to normalcy is expensive. They also have little inbuilt protection from swelling and shrinkage and lack complex outer layer which can provide same insulation or impermeability. In these animals in estuarine habitats, a switch over to cyclic avoidance is common an conforming is also seen. Those terrestrial habitats where there is both continuous high environmental stress and high fluctuation, conforming may not be an option, rather exceptional strategies for avoidance are common (torpor, estivation, encystment etc.).

2. Animals with hard outer layers (exosceleton) may have better options for some regulations are a greater independance of their environments Some animals like arthropods likely develop partial regulation of osmotic concentration. Because of exoskeleton, the outer surface can have reduced permeability and may be partly thermally insulated by cuticular hairs. So fluxes are slowed. But in these animals, behavioral avoidance, aided by efficient limbs (and sometimes wings) that can be built from an exoskeleton becomes a major part to cope with environmental change especially in the more rapidly changing terrestrial habitats.

3. Large animals are much more likely to be regulating in all environments with

important exception of the relatively equable and unchanging open ocean. Larger animals operate in a larger scale environment where rapid changes are relatively unimportant. Because of larger surface (lower surface area–volume ratio) more time is available for regulatory mechanism to operate. They may have better opportunities for energy storage. They may have room for complex neurohormonal control mechanism. In terrestrial habitats, where environmental changes are faster, all these factors work together and the only option left to large animals is regulation.

Thus the smaller animals adapt to avoidence and conforming specially if they have soft body . In case of small and medium sized animals with hard exoskeleton, some regulation and behavioral avoidance means are adapted to cope with the environment. The large animals cope with the environment by adapting regulation.

Anatomical and functional changes occur in a predictable way with increasing body size. The study of the size-related effect is known as scaling.

4. Heart rate, O_2 consumption, metabolic rate, all are higher in small animals compared to large animals. This is partly related to the disproportional increase of tissue of low metabolic rate like skeleton, fat and connective tissues in large animals. The activity of oxidizing enzymes is higher in corresponding tissues of small animals than larger ones. Cytochrome oxidase and malic dehydrogenase have been shown to be more in small than large mammals. The muscles of small animals consume much more energy during steady running than do the muscles of large ones which is due to higher metabolic rate in small animals. (It is known that large divers remain submerged for longer period of time than small divers. This is because the large animals have less O_2 consumption and less metabolic rate.) Small mammals have relatively larger surface area and so heat dissipates more readily from them. In case of fish, the energy cost for swimming per unit mass of body unit declines with increased size.

For an evolutionary and ecological reason, there is an optimum body size for a taxon. Scaling and size dependency are crucial factors in all comparative physiology and patterns in animal adaptation. The size of a particular species of animal is determined by several factors like —

(i) **Phylogenetic inheritance.** e.g. insects are small and vertebrates are large.

(ii) **Basic physiological design.** e.g. animals with open circulation are larger than those with no circulatory system, and those with close circulation may be larger again.

(iii) **Basic mechanical design.** e.g. animals with hydrostatic skeletons are usually relatively small as are those with exoskeleton; while those with tubular endoskeletons are relatively large.

(iv) **Habitat.** e.g. any given design may be larger in aquatic habitat giving internal support than on land where self-weight is a problem.

Scaling effect have been observed in migration. In case of avoidence as a measure to cope with stress birds usually migrate; while terrestrial animals, specially smaller ones, are inclined to escape by hibernation and torpor. The smallest migrating mammals have a body mass of about 20 Kg (some African antilope) ; where as very small birds and even monarch butterfly having one gram body mass are regular migrators. Thus different sizes and locomotory modes affect the ability to migrate effectively. For any given migration time, flying animals will be able to complete a much longer distance than walkers or swimmes of similar size or alternatively, to achieve the same distance in the same time, walkers or swimmers would have to be much larger. Global, terestrial or marine migrations are therefore slow and prolonged often taking many months (as in salmonid fish). Whereas aerial migration by birds can be completed in a matter of days and weeks. Adaptation of the capability to store food is an important factor in migration. Few animals undertake nonstop migration relying on food stores; many animals accumulate fat stores of 25 – 50% of body weight before migrations. Since fishes are generally ectothermic with 10 fold lower metabolic rate than similar sized birds and mammals, they can migrate much further without feeding and have the greatest migratory capability.

Several experimental and other observations indicate that adaptation is related to body size in respect of cellular adaptation as well as in organism level.

Because of small size, the desert rodents evade the heat by retreating to their underground burrows during the day. However ground squirrel, although of small size is similarly handicapped in hot weather but they adapt in a different way. When they are outside their burrows on a hot day they get heated very rapidly. They cannot tolerate very high temperature and die if heated to 43^0 C. However a temperature of $42{\cdot}4^0$ C is tolerable by them without apparent ill effects. When it is heated it goes to a relatively cold burrow as get cooled rapidly due to large surface area and the exposure to hot climate is tolerated.

5.5 Suggested questions

1. Explain acclimatization, acclimatian and adaptation. Write briefly on the levels of adaptation.
2. Give brief accounts of mechanism of adaptation.
3. Name the adaptive responses seen during environmental change. How are they related to body size ?
4. Write briefly on scaling with examples. Comment on or name the different factors that determines the size of an animal.

Unit 6 ❑ Physiological adaptation to different environments

Structure

6.1 Marine adaptations

6.2 Adaptations for life in an estuary

6.3 Fresh water adaptations

6.4 Extreme aquatic environments

6.5 Parasitic habitats

6.1 Marine adaptations

There are thousands of species of marine life, from tiny zooplankton to enormous whales. Each is adapted to the specific habitat it occupies. Throughout the oceans, marine organisms must deal with several things that terrestrial life do not

- regulating salt intake
- obtaining oxygen
- adapting to water pressure
- dealing with wind, waves and changing temperatures
- getting enough light

Salt regulation : Fish can drink salt water, and eliminate the salt through their gills. Seabirds also drink salt water, and the excess salt is eliminated via the nasal, or "salt glands" into the nasal cavity, and then is shaken, or sneezed out by the bird. Whales don't drink salt water, instead get the water they need from the organisms they eat.

Oxygen : Kish and other organisms that live underwater can take their oxygen from the water, either through their gills or their skin.

Marine mammals need to come to the water surface to breathe, which is why the deep-diving whales have blowholes on top of their heads, so they can surface to breathe while keeping most of their body underwater.

Whales can stay underwater without breathing for an hour or more because they make very efficient use of their lungs, exchanging up to 90% of their lung volume with each breath, and also store unusually high amounts of oxygen in their blood and muscles when diving.

Temperatures : Many ocean animals arc cold-blooded (ectothermic) and their internal body temperature is the same as their surrounding environment.

Marine mammals, however, have special considerations because they arc warm-

blooded (endothermic), meaning they need to keep their internal body temperature constant no matter the water temperature. Marine mammals have an insulating layer of blubber (made up of tat and connective tissue) under their skin. This blubber layer allows them to keep their internal body temperature about the same as ours, even in the cold ocean. The bowhead whale, an arctic species, has a blubber layer that is 2 feet thick.

Water pressure : In the oceans, water pressure increases 15 pounds per square inch for every 33 feet of water. While some ocean animals do not change water depths very often, far-ranging animals such as whales sometimes travel from shallow waters to great depths several times in a single day.

Whales can dive deeply. The sperm whale is thought to be able to dive over $1^1/_2$ miles below the ocean surface, and they can do that successfully because their lungs and rib cages collapse when diving to deep depths.

Wind and waves : Animals in the intertidal zone do not have to deal with high water pressure, but need to withstand the high pressure of wind and waves. Many marine invertebrates and plants in this habitat have the ability to cling on to rocks or other substrates so they are not washed away, and have hard shells for protection.

Light : Organisms that need light, such as tropical coral reefs and their associated algae, are found in shallow, clear waters that can be easily penetrated by sunlight. Since underwater visibility and light levels can change, whales do not rely on sight to find their food. Instead, they locate prey using echolocation and their hearing.

In the depths of the ocean abyss, some fish have lost their eyes or pigmentation because they are just not necessary. Other organisms are bioluminescent, using light-giving bacteria or their own light-producing organs to attract prey or mates.

6.2 Adaptations for life in an estuary

The term estuary comes from Latin *aestus,* meaning tide. The adjective *aestuarium* means tidal. Thus estuary is defined as 'the tidal mouth of a great river, where the tide meets the current'.

There are a number of vertical and horizontal attributes to estuarine ecosystems. The intertidal zone is alternatively flooded and exposed. There may be salt marsh or mangrove wetlands, algal beds, sand or mud flats, reefs of oysters, mussels or calms in this region. Organisms that live in this region have developed special adaptations.

Like other aquatic ecosystems the vertical gradient of light is a limiting condition for photosynthetic activity. At euphotic zone, where light reaches the bottom plants can live attached to the bottom. Estuarine water clarity tends to be much greater near the ocean, so both rooted and planktonic plants generally photosynthesize in greater

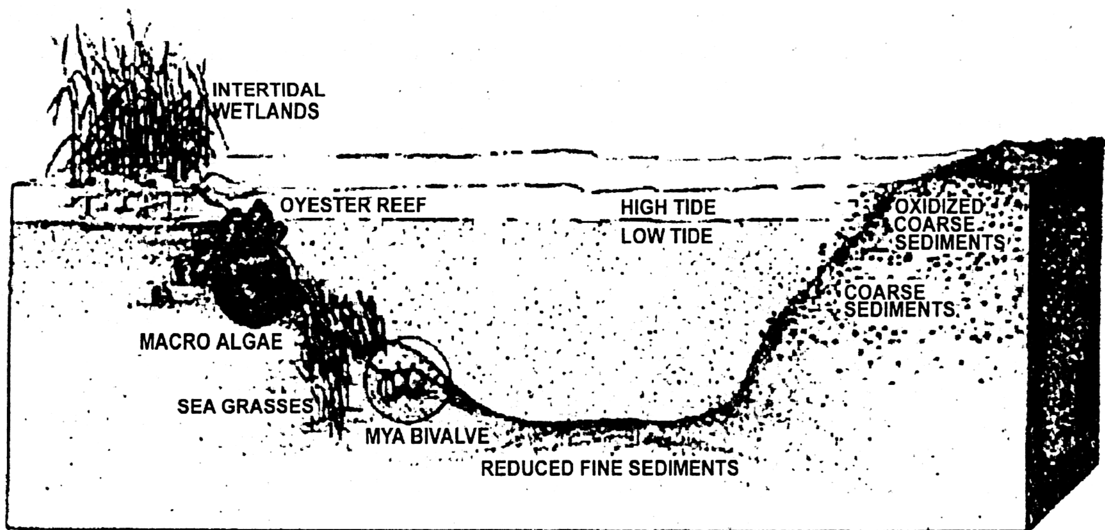


Fig. 5.1 : Vertical zonation of estuarine habitat

depths than in low salinity regions. Animals of the aphotic zone are dependent on transported food from somewhere else. Estuaries are productive environments for aquatic life. They are rich in nutrients compared with rivers and oceans, and they are also good animal refuges, offering protection from storms, competitors and parasites. However, estuaries do experience sudden and often widespread changes in salinity, temperature and dissolved oxygen levels, so aquatic organisms must find ways to cope with these changes.

Dissolved oxygen content is another important parameter for estuarine life. There exists a gradient from oxidizing (aerobic) to reducing conditions (anaerobic or anoxic) in estuaries for biological and chemical processes.

Salinity

Salinity is perhaps the most important factor affecting aquatic species. Most aquatic animals are adapted to life in sea water. These animals vary in terms of the degree to which they are able to tolerate the lower salinities of the estuary. A smaller number of animals are adapted to life in fresh water, and few of these species tolerate salinities in excess of 2 ppt (parts per thousand).

Ocean salinity is 35 ppt. The salinity level of the estuary varies along its length, with depth and with the seasons. There are extreme changes in salinity from almost freshwater conditions in winter to saline (or almost hypersaline) conditions in summer. This change in salinity causes a change in the concentration of dissolved gases (fresh water contains more oxygen than sea water at the same temperature) and in the density and viscosity of the water.

Adaptations to changing salinities

Estuarine organisms possess adaptations or behaviours that enable them to cope with changing salinities. Few organisms remain in an estuary for the whole of their life cycle. Some fish are truly estuarine, spending their whole lives in the estuary. Some use it as a nursery habitat only and others are marine visitors, coming in when the salinity suits them. Mobile animals like fish and crabs can swim away from unfavourable conditions. Prawns and crabs move out of the estuary in winter when waters are less saline. However, less mobile (sedentary) animals such as barnacles and worms have to either seal themselves inside their shells or adapt to the conditions. Many sedentary animals die when conditions are unfavourable and must recolonise when conditions change. Many algae and seagrasses die off during winter periods when salinity levels become too low. Some organisms are able to tolerate extreme conditions for a short time only. A sudden change, such as an unusual heavy summer fall of rain which produces a freshwater flow into the estuary, or extreme conditions of salinity, will produce a variety of responses. For example, worms, molluscs and fish produce slime or mucus to cover and protect their sensitive body surfaces. Some polychaete worms and crabs retreat into holes or burrows, plugging them. Other animals withdraw their sensitive body parts, or close their shells. If an organism cannot escape or reduce contact with the water during times of abnormal salinity it must use a physiological response. The marine species generally reside near the mouth of the estuary, the freshwater species in the low salinity areas and the estuarine species somewhere in the middle. Many estuarine species are osmoregulators, meaning they can maintain a constant salt balance, no matter what the salinity of the water is. Alternatively, an animal may modify its metabolic rate or change its patterns of activity. The physiological response of an animal to salinity changes takes time to complete, so it is often supplemented by a behavioral response that enables it to either delay or moderate exposure to unfavourable conditions, completely avoid them, or slowly adapt their body to the new saline environment. For example, some bivalves close their shell valves when sea water suddenly becomes diluted. After a while they become used to these conditions. Organisms that are capable of dealing with varying salinities are euryhaline, and organisms that can only deal with small changes in salinity are stenohaline. Stenohaline animals rely on coping methods such as moving out of the area, burrows in the sand, excreting excess salts or closing their shells, and worms, molluscs and fish can produce mucus or slime to cover sensitive body parts. The mussel, *Xenostrobus securis,* deals with salinity of 2g/L by closing up their valves for up to many months. When an organism is unable to move or reduce contact with the unfavorable salinity, it then relies on physiological responses such as osmosis (become iso-osmotic with estuarine water) or changes its activities or its metabolic rate. Generally mature organisms are better able to handle the stress of

salinity changes than reproducing adults or newly hatched eggs. Many sessile organisms die if conditions become too harsh and have to re-colonize when conditions are once again suitable.

Euryhaline crustaceans respond to changes in salinity by molecular processes. Vertebrates respond by regulated blood osmotic concentrations and controlling ion fluxes and organic osmolytes. Invertebrates can regulate blood osmotic concentration, cell volume or both.

Sodium chloride is very important in regulating blood osmolarity in euryhaline crabs, and regulating fluxes and permeability's of these ions is how the crabs deal with the salinity.. Green shore crabs respond quickly to salinity variations and within six hours, their blood Na^+ level reaches a steady state. It is thought that the crabs possess a sensory organ on their legs known as "hair peg" which is what responds to the salinity variations and sets the wheels in motion for response. Gill structure is also helpful in dealing with changes in salinity as the gills contain two different sets of epithelial cells, which are believed to be crucial to ion and gas exchange.

Adaptations to changing temperatures

Temperatures are more variable in the estuary than they are in the ocean. In winter the estuary is colder than the ocean, and in summer it is warmer. Daily temperature fluctuations can also be extreme, especially in the shallows. Also, the solubility of oxygen depends on temperature. (More oxygen dissolves in cold water than in warm water.) Adverse temperatures may cause responses of avoidance and escape. One of the most common mechanisms that certain organisms use to cope with conditions in the cold winter months is to transform into a resting stage. Another habit is to burrow into the mud or sand on the bottom of the estuary. Fortunately in the Swan-Canning system temperatures are rarely extreme, never reaching freezing in the winter or going above 40°C in the summer. Organisms that are able to withstand varying temperatures are eurythermal. Those that can't must use other responses such as moving, burying themselves, or to transform to their resting stage. Temperature can fluctuate over seasonal cycles in temperate estuaries. Bacteria have adapted to this by reorganizing their biochemical pathways and adjusting protein and DNA synthesis rates.

Adaptations to changing oxygen levels

In water containing low amounts of oxygen, organisms have physiological and behavioral mechanisms to survive. In sessile animals that cannot move to a more oxygen rich environment, they must lower their energy demand, maintain their metabolism or use a method of creating energy without oxygen. Many animals do this by increasing their heart rate and the flow of water past respiratory surfaces.

Some organisms have respiratory pigments, which allow them to maximize the oxygen they do get. Others are very good regulators of oxygen uptake and maintain constant rates of uptake until the oxygen falls to a 'critical pressure'. They then switch to anaerobic metabolism. This can only be maintained for a short time. Some organisms, such as oyster larvae, have been shown to lower there aerobic metabolism in response to hypoxia.

Nektonic adaptations to estuarine environment

General adaptation for true nektons include the existence and position of swimming organs (e.g. fins), a smooth streamlined body, a specific gravity close to water, the ability to extract oxygen at a relatively rapid rate from the surrounding water in order to support the large energy requirements of swimming. Additionally, well developed sensory organs are necessary for most organism with active nektonic existence. Fishes mostly achieved these requirements. They have streamline body and are able to control their depth through varying their specific gravity by changing the amount of gas in the swim bladder. Fishes have gills well adapted for rapid oxygen exchange, thus meet an active and sustained movement. Their specialized sensory organ, the lateral line, is very sensitive to sound waves and to changes in water density. The chin barbells in some fishes help to locate food.

There are specific adaptations of nekton that allow them to flourish in estuaries. This is interesting since the estuarine environment is apparently hostile or stressful. The rapidly changing physical and chemical environment imposes great energy demand of fish so that most species cannot survive there. Estuarine species may be divided according to tolerance depending on temperature and salinity. Some species require high salinities and are not found in the riverine reaches of estuaries. Some other species possess a wide range of salinity tolerance. There are only a few species which are adapted to withstand extreme variation in both temperature and salinity.

6.3 Fresh water adaptations

Aquatic animals develop various types of adaptations according to changes in aquatic environment. This is essential for the maintenance of life in aquatic medium.

Majority of herbivores are algal grazers. Animals show variation as they live in ripples and send bottom areas. But most animals are seen in these two areas.

First fautra of the hill stream of India are very rich and varied. *Botia dayi, Botia dario, Nemacheilus aureus, Nemacheilus beavani, Nemacheilus montanus, N. rupicola* are bottom living fishes usually found under stones and boulders. Because living at the bottom, they have broad head, dorso-ventral flattening of the anterior part of the body, presence of thorasiç adhesive apparatus, horizontal alignment of paired fins. Many fishes at the bottom stay under stones and boulders and thus avoid water

currents. The paired fins are fanlike and horizontally placed. The ventral surface is smooth, without scales, and flattened.

Those, which are inhibitant of fast flowing water, need to move without the favour of water current, by means of mascular effect. *M. armatus* has an elongated cylindrical and cel like body and the posterior part is highly compressed laterally. The snout is produced into a long beak-like process to reduce the pressure exerted by the water current. Some fishes like *N. botia* are inhibitants of slow running water and usually abundant in ports. These fishes assemble just below the surface of the water and move through the weed-beds for feeding and returns to the pools. In fishes which feed on insects, mainly larva and nymphs, the mouth is ventral and suited to the bottom feeding habit. They make adjustments to withstand the current. Many nymphs have efficient adaptations, which enable themto tolerate current strength upto 300 cm/sec. There animals cling to the surface of the rock keeping their heads towards the current and when pressed firmly against the substratum. The water current exerts a downward pressure which helps to held the animal in position. Body flattering is thus one of the adaptations to enable organisms to take shelter beneath stones and avoid the force of the current. The inches, which usually live under the stones have flattened streamlined bodies.

Streamlining of the body helps to offer the least resistance to water and is also found among many stream-invertebrates. The tapering bodies of the nymphs of the mayfly illustrate this very well.

Development of suckers helps the animal to attach if itself to the substrate. The larva of the blackfly, *Simulum* are found in large numbers, attached to stones in stream-bed. They are one of the mostly adapted animals to the life in fast-flowing water. Simulum possess large salivary glands which secrete a pad of sticky silk on the substratum to which it attaches itself by means of a pair of modified prolegs at the rear end of the abdomen. At the posterior end of the semicrect body is a circle of rows of outwardly directed hooks, which when the muscles of the disc are relaxed, move outward and cling on to a silk web previously placed there by the larvae. The anterior end of the body then swings freely in the current. There is a fan like food gathering organ on each side of the mouth which traps food particles from the water current.

The development of hooks is also an adaptation to current. Caddies fly larvae occur most abundantly in streams with moderate to swift currents. They usually construct cases made of leaves, twigs, sand grains and stones cemented together with silk secreted by the animal itself. In swift water, the cases are stout, cylindrical, tappered posteriourly and are usually swollen and more solidly so constructed of sand, pebbles or rock fragments.

Hill stream fishes have also become highly adpated to this dynamic environment.

The chief factors affecting the life cycle of these organisms are : (A) Strength of the current, (B) Hill streams are shallow and clear, so they have to bear intense light during day-time, (C) Sufficient food is available but in the form of algae covering stones and rocks.

Fishes have to develop special adaptations to live in the environment. The head and the body of most hill stream fishes are greatly flattened and in the highly specialized form the body is leaf like. The ventral profile is straight while the dorsal profile is only slightly arched. The head is usually small and semicircular. The size is generally small so that they can easily take shelter any rock and beneath them, and can conveniently live in shallow water protected from direct sunlight. The scales of these fishes undergo reduction or are very minute or embedded in the skin as in *Nemacheilus*. This is of special significance to adhesions to rocks and stones so that although scales are present in the dorsal in the dorsal and lateral aspects, they are absent in the region of the chest. The paired fins specially the pectorals are modified for adhesion and the number of inner rays is increased. The fins are shifted that it can act as hydroplanes and adhesion to rocks and stones is facilitated. In most species, the lower lobe of caudal fin is longer than the upper one. Some species possess (eg. *Nemacheilus,* and *Glyptosternum*) develop a band shaped caudal peduncle which appears to be an adaptationto life in fast flowing waters.

The position of the mouth is shifted from the anterior and of the snout to the ventral side towards the tip of the snout. Instead of a transverse the mouth is generally horny covering which help to scrape the algal material from the stone-surface, for feeding.

The mouth is surrounded by sensitive barbules beneath the head. These help in testing the substratum. In some species (eg. *Nemacheilus*) the lips are divided in the middle and are swollen so that they form a saucer, when pulled outwards. In *Glyptosternum,* the lips are reflected and spread round the mouth to form a bread sucker for attachment. The eyes are generally small in size and pushed towards the upper surface where they come to lie close together.

Besides formation of additive sucker for the lips, the skin is thrown into grooves and ridges on the ventral side of the body, specially between the pectroal fins of the body. Such straited structures serve as friction plates for attachment to stones.

The gill openings are restricted to sides only and do not extend beyond the pectoral fin. Any effect on respiration is copresneted by the well acrated water of the stream. The bottom lining forms required negative buoyancy ; the air bladder is considerably reduced or degenerate and becomes completely a useless hydrostatic organ. This helps the bottom living organisms to aquire negative buoyaney. Most fishes expend much energy in fighting the current, hence they spend long periods in resting.

Amplibians found in torrent waters generally develops small lungs, presumbly to reduce buoyamoy. Size of the appendages and body are reduced or a streamline shape is achieved to reduce the surface areas exposed to the full impact of current.

Most animal species, though have developed devices for clinging to the bottom, they are more abundant at the underside of the rocks in riffles, than they are on the upperside. Some species, however, such as rotifers, water mites, protozoans find shelter within the mass of algae that may cover the top of the rock. Even swift water fishes take all the advantages of whatever protection available.

Inspite of various mechanisms for maintaining position against the current, a continuous drift animals occurs downstream. The driff is more prominent at night than daytime. A continuous drift of downstream is compensated by the adult insects to lay their eggs in shallow water. The adult insects emerging downstream commonly fly upstream for reproduction. Some fishes move upstream for spawning.

Lake is a stretch of water surronded on all sides by land. Different types of lakes are there like glacial lakes of high altitude, tectonic lakes formed due to movement of deeper parts of earth's crust, lakes formed due to volcanic activity, lakes formed by wind action or land slide.

In the lakes, there is a gradual rise of temperature as one moves from the depth towards the surface.

Amount of O_2 present in a lake or pond depends on extent of contact between water and air, oı. the circulation of water and on amount produced and consumed by the lake community. Animals and microgamisms present in water, use O_2 and produce CO_2.

According to habitat characteristics, the pond and lakes exhibit 3 zones— littoral, limnotic and profundal zones. The shallow water which has light for the surface to the bottom is called litteral zone. The bottom part that does not receive day light is called profundal zone.

Light serves as the initiator or driving force of the ecosystem. It controls the development of pigmentation in animals, and stimulates the development of various adaptations. The development of vision depends on availability of light in the media. Fossorial forms have generally reduced vision and so do deep sea forms. In some organisms locomotory activity depends on light intensties. This is called photokenesis.

In cave dwelling and fossorial or borrowing animals, the size of the eyes are generally reduced due to absence of light or is found embedded in the integument. Eyes are absent in cave dwelling fishes and cave amphibians. In burrowing amphibious such as the Apoda, the eyes are covered by a fold of the integument. In abyssal depth, where there is absence of light, the organisms either have reduced vision or the eyes are highly developed to preceive low light, which spread the occurrance of biofuminiscente at those depths.

Light controls locomotor activity of many animals by a direct action on locomotion. This is called photokinesis. The rate of swimming in mussel crab has been shown to be upregulated where the intensity of light is increased in experimental condition.

Temperature

Heat is an ecological factor which has an wide ranging influence directly on the biota as well as indirectly in combination with other factors. It is a direct regulator of the climatic conditions of a place as well as modulator of other factors such as density and salinity. Organisms have adjusted themselves, and have developed various adaptations to meet the temperature ranges. Solar radiation falling on earth supplies energy to the living organisms in the form of heat, the activity of the organisms depend on transfer of heat from the media in which they live. In their adaptations, organisms have specialized themselves to live whether narrow limits in small scale environment (microhabitat) that are significantly different from the larger environments (habitats) of which they form a part.

In case of poikilothermic animals, the physiological activities are adapted to function in spite of the temperature strees. Poikilotherms are ectotherm and the body temperature is dependent on the amount of heat they take up from water (environment).

In case of homeothermic animals, the heat generated in the body is retained. Hence, it has high body temperature. They have a high metabolic rate compared to poikilothermic animals. They are endothermic animals.

Homeothermy is necessary for organisms to inhibit the terrestrial environment, because air and land experience violent fluctuations of temperature and climatic conditions.

1. Animals produce eggs that can survive thermal extremes.

2. In arctic regions animals have their freezing point of plasma lowered for –80°C to –1.47°C, in response to seasonal changes in temperature. They produce anti-freeze, which reduces their & risk from freezing.

3. Amphibians in land lose water very fast by evaporation through skin. Their risk of dessication is partially compensated by a reuction in urine flow, update of water for urinary bladder and absorption through skin, all promoted by vasotocin. Their survival is further aided by a retread to underground burrows, where the temperature is much lower than on the surface.

Reptiles have impermeable skin, but they also lose temperature byinceased evaporation, although the rate is lower than amphibia. However, some reptiles can control the body temperature to some extent. In *Varanus*, as the temperature of environment rises, powerful pumping movement of the mouth and neck occur. This is the functional equivalent of ponting in mammals. It can also increase its body temperature by increasing its metabolic rate.

In general most of the poikilotherms undergo hibernation and estivation to meet temperature extremes, while homeotherms are able to regulate their body temperature through special adjustments. Hibernation occurs to tide over extremes of cold. The term is used to describe conditions or instances in which metabolism is reduced, animal puts itself in a state of rest with reduced metabolic rate. There occur a marked drop in body temperature. It is of wide occurrance in poikilotherms specially at higher latitudes.

Terrestrial reptiles avoid overwintering by retreating into rocks, crevices, or borrows as do the amphibians. Many aquatic turtles spend the winter buried in mud beneath ponds and stream borders. Among mammals hibernation is reported for the order Monotremata, Marsupialia, Insectivora, Chiroptera, and Reptilia. At least one bird is known to hibernate. During awakening from hibernation a sequence of events occur— (i) rapid rise of body temperature, (ii) warming of heart promotes circulation of blood which carries heat in a restricted way such that the heat passes largely to the respiratory system and the brain during the early stages of awakening.

Aestivation

Dormancy during summer months, when temperature is high, or excessive dryness and shortage of food products is called onset of dry season, burry in the mud of a swamp or river bed and forms mucous lined cell in which it aestivates. This may last for as long as seven months, with the return of rains and subsequent rise of water level, the fish energes from its aestivation chamber.

In many insects, dormancy take the form of diapause (during this time growth and development are suspended or retarded). In homeothermic animals, body temperature is kept constant in spite of changes of environmental temperature. These animals are curythermal and are capable of regulating their body temperature by thermostatic regulation through physiological adjustments.

Amphibia move from direct sun to sheddy place. Insects move from the sun to the stones and beneath leaves. Some insects freeze at night and thaw out in the day in cold climate. O_2 content of water is less compared to air. Thus, to ensure O_2 supply, respiratory adaptations occur. These include (i) increase in respiratory surface, (ii) use of higher affinity oxygen storing pigments, (iii) modulation of ventillatory or circulatory rates.

In fresh water vertebrates, gills are the most common adaptations to ensure O_2 supply. Diferent forms of gills develop eg. serial by repeated filamentous gills, tentucular crown gills, enclosed lamellate gills are seen in molluses and annelids, tracheal gills, spiracle gills, rectal gills in insects. Freshwater vertebrates use cutaneous or gill based respiration. Skin breathing is quite common in fresh water fish larvae and many eels. Cat fish rely on it most extensively to sustain their metabolic rate. Many species depend largely on skin respiration. eg. Salamander, frogs. Although

most fishes, and juvenile or neotenic amphibions use gills and nearly all adult amphibians, reptiles, birds and mammals (being secondarily aquatic) **rely** on air breathing through lungs.

The haemoglobin saturation curve shows a 'left shift', when the O_2 demand is increased than occur increased rate of ventillation in fresh water animals which may arise from hypoxia of the environment, or increased metabolic activity. In small animals like sponges and rotifiers, ventillation is mainly done by cilia and flagella, their activity increasesas needed.

In cray fish, hyperventillation is seen initially, but as hypoxia sets in, the animal shows brady cardia and the circulatory flow pattern alter to give increased flow anteriorly over the brain.

Fresh-water vertebrates show sophisticated ventilatory response to hypoxia. In fish, hypoxia is sensed by oxygen receptors located in the brain and aorta. Both rate and stroke volume are increased in hypoxia, by changing the buccal and opercular pumping pattern.

In freshwater vertebrates, when O_2 demand is increased due to hypoxia in environment or increased metabolic activity, ventillation is increased, by changing the buccal and opercular pumping pattern. In snakes and turtles, ventilation is intermilttent at rest, but becomes continuous during steady swimming. Green turtle shows a seven fold increase in mean ventillation frequency and increase in both pulmonary and aortic blood flow.

In bimodal breathers, besides aquatic breathing, air breathing devices are present. For example, in arthropods, air bubbles and plastrons are used as air supply systems. Special diving adpatations are found in fresh water reptiles, birds and mammals.

Many essentially freshwater animals are able to use both aerial and aquatic oxygen and may switch over to air breathing when their aquatic habitat begins to dry up or overheat and becomes hypoxic, or it becomes too rich in H_2S, due to decomposition.

Custaccans, fishes and amphibians are most notable as bimodal breathers. Many bimodal crabs have reduced gill-area, typical of air breathers.

Fresh water habitat give rise to problems related to reproductive strategies. There is a general tendency for the fresh water invertebrates to have very short life cycles. Commonly there is a very high reproductive output in freshwater invertebrates. In *Drussena* (Zebra mussel) one female may release 1 million oocytes per year. Reproduction in fresh water vertebrates also show a similar pattern. However, secondary freshwater vertebrates that are essentially land animals, usually resort to a terrestrial site for reproduction. Turtles lay their eggs in the upper shore.

Fresh water is rarely very deep, the largest lakes being only 1000-1500 meter, so that freshwater animals do not normally have to cope with great pressure. However,

buoyancy is more difficult in fresh water due to reduced specific gravity of the medium giving very little lift. Swim-bladder is present in fish and it is 6-9% of body volume. Swim bladder gives the buoyant effet.

Suckers and hooks for adhesion develop specially in case of bottom dwellers. Certain fish have ventral sucker and blackfly have hooks to hold on to rocks.

The four eyes fish Anableps can focus simultaneously on terrestrial and aquatic image having practically two eyes with a pear shaped lens and two sets of pupils above and below the water miniscus. Fresh water beatles, bugs, and fishs such as pike have eyes with large pupil. This is also seen in surface dwelling betles and bugs. Chemoreception is also an important device in many freshwater animals for prey location, predator avoidance and the location of hosts and mates in river. Many aquatic insects have water pressure receptors ; flow receptors are also found. In crustaceans and fishes they are located in cephatic appendoges like antennae or vibrissae. Electro reception occurs in a range of fresh water fishes with a very weak and strong field producers. Echolocation is seen in freshwater dolphins, but it is largely lost in freshwater animals.

Most of the freshwater zooplankton are filter feeders. Hydra takes small prey that brush against and trigger the shinging cells in its tentacles. Aecolerated cutrophication is an well recognised process in both rivers and lakes. The reduced O_2 level leads to submerged plants disappear as light is cut off and animal casualty follow. The submerged animals change eg. *Tubifex* worms replace crustaccans and then surface dwellers decline.

Fresh water can be polluted in different ways. Contaminated natural freshwater is the single biggest source of human disease of the world today. Some of the diseases is due to parasites and pathogens naturally present in freshwater. But a huge additional hazard is added by pathogens derived from sewage and animal wastes.

Global warming leading to acid rain makes the water acidic. This causes— (i) increased mortality of fresh water animals, (ii) It solubilizes minerals. Alluminium sloubility causes aluminium poisoning of many fishes, (iii) Cuticle formation in insects and crustaceams is affected, thus leads to defective osmoregulation. (iv) Hatching of some salmonid fishes is decreased.

Thus, abudance of many species decline, particularly snails, amphibians, small crustaccans, leading to decrease in zooplankton. Other fishes such as eel, insects and rotifers are also destroyed it the pH stays below 5 for long time.

6.4 Extreme aquatic environments

The deepsea physical environment has special characteristics. To cope with this environment, the animals living there undergo suitable adaptation.

Four remarkable characteristics prevail in this area. These are :

(1) ***Absence of sunlight.*** Beyond 200 fathoms their is no sunlight.

(2) ***Quiescence :*** Because of depth the movement of water is almost absent.

(3) ***Cold environment :*** In the deepsea the temperature is nearing the freezing point and the temperature remains constant.

(4) ***Pressure :*** The pressure is high as it increases with depth.

(5) ***Lack of green vegetation :*** Total absence of light is the reason for total absence of green vegetation in the deep sea environment.

For survival in the deepsea environment, the animals have to develop adaptive changes to cope with adverse physical condition present there. The general characterstics of deepsea animals are as follows :

(i) The deepsea animals are weak and delicate.

(ii) The body is generally simplified.

(iii) They are either totally blind or they possess powerful telescopic eyes to catch maximum amount of light.

(iv) They develop long feelers to act as tactile organs.

(v) Almost all the deepsea animals are luminescent.

(vi) Most of the deepsea fishes live on the exudes of decaying matters and so the animals lose the masticatory power. There are other animals which possess powerful jaws.

(vii) Most of these develop wonderful devices for caring the youngs. other produces large number of youngs to overcome the hostile environment.

(viii) Small size is an important charasteristic of deep sea living.

Structural modifications in deep sea animals.

Almost all the phyla have representatives who lead deep sea life. The modification of the invertebrates are diverse compared to vertebrates.

Modification of the vertebrates of the deep sea have been found to be as follows. Amongst the elasmobranchs the true sharks do not exhibit deep-sea charasteristics excepting the luminous sharks. The silver shark, however, show deep-sea characteristics in having huge eyes and long alternated body and tail.

Amongst the teleosts, the typical deep sea form is *Cetomimus*. It has a long mouth, small teeth, very small eyes and scaleless body. However, in *Ipnops* there are no eyes and only two large luminous organs are found on the head. Scaleless body and well developed luminescent organs are the characteristic features in Stomiatidae. In *Gastrostomus*, the body is long, slender with rows of luminous organs on the lateral sides of the body and the mouth is bounded by very large jaws. The Gadiformes

(cod like forms) have reduced mouth and dentition; the eyes are extremely large, the trunk is reduced and has a filamentous tapering tail.

The anglers show typical deep sea characteristics. The paired fins are adapted for crawling on the bottom of the sea and the anterior finrays of the dorsal fin function as a lure. In *Linophryne*, the finrays are provided with luminous organs to attract the prey. The *Oneirodes*, however, is blind but has luminous organs to compensate for the loss of eyes. In another deep sea fish, *Protostomias*, specialised light producing organs are present in rows on the lateral side of this body.

Another important feature of deep sea fishes is flatness of the body to adjust with high pressure. The body in these fishes becomes flattened and the mouth is shifted to the lateral side of the body.

These structural modifications in deep sea forms are due to peculiar physical condition of the deep sea environment. The deep sea forms are geologically very recent in origin. These forms were originally the inhabitants of the pelagic or littoral regions which migrated to the deep sea and become adapted.

6.5 Parasitic habitats

Parasites depend fully on their host for their living. Hence, they adapt themselves accordingly. Adaptation to specific environments is a dynamic feature of all living organisms. Pararitism starts with an accidental meeting of few animals. Gradually one, the guest, starts to lead a more dependent life upon its host for food and shelter. This change from a free living life to a life in which food and shelter become available without any effort bring about profound modifications in the make up of parasites.

The helminths are modified morphologically and physiologically to live in their particular environment. The modification depend on the degree of parsitism.

Morphological adaptations

Every part of the body of a helminth parasite exhibits twist due to parasitic mode of life. The strutural modifications involves two aspects — degeneration and attainment of new organs.

Degeneration : There occur loss or simplification of unused organs or parts. In helminthes the loss or degeneration involves particularly the digestive and locomotory organs.

(a) ***Digestive / Alimentation.*** As the parasites begin to live on digested or semidigested food of the host, there occur reduction in their alimentation and digestive glands.

In the adult trematodes, the digestive tube is a blind gut. In the larvae of

trematodes, the gut is either very simple or completely eliminated. In adult tape worm the alimentation is completely absent. As the animal lives amidst digested and semidigested food, the food is absorbed directly through the general surface of the body. *Trichinella* and cystacercus larvae in vertebrate muscles occur in such location that they are constantly surrounded by rich nutritious food. The food is absorbed through the outer layer of the body.

(b) ***Locomotor organs :*** Thes locomotory organs are not necessary for the parasites because they live their entire life in the body of the host. Hence, locomotor organs are totally reduced. However, in the free living larval stage of the parasites, such as miracidian and hexacanth the ectoderm is ciliated.

(c) ***Sense organs :*** These sense organs in helminthes are also simple structures. This happens because they lead a sedentary life in a sheltered habitat. This correlation is especially seen in the endoparasites.

2. Attainment of new structures/organs

Parasitic existance is made possible due to modification of old structures and formation of new structures. These are necessary and helpful in food absorption, protection, attachment and vast reproduction.

(a) ***Integumet :*** The outer integument or cuticle of helminth parasite becomes highly modified and is so adapted as to resist against the digestive juices, passage of food and for adhesion.

Cuticle become thin to absorb food in those parsites which live in rich nutritious environments e.g. adult liver fluke in bile, blood flukes in blood, tapeworms in intestine. *Trichinella* and cysticereus in vertebrate muscle, several larval forms developing in lymph spaces and in blood stream.

In case of some gut parasites e.g. tapeworms, nemotodes etc. which remain attached to the wall of the gut the cuticle is suitably modified— it becomes thick impregnated with chitin like substance and enzyme resistant, so that it is not digested by digestive enzymes but remain permeable to water.

In most trematodes the outer integument contains spines, spinnules or scales of various kinds. These cuticular modifications protect the outer surface of the worms against the abrasive action of food and roughage flowing around them. In the chinese liver fluke *Cloriorchis sinesis*, the larval stage has a spinous cuticle, this suggests that possibly it was a gut parasite and subsequently converted to a parasite of the bile passage.

(b) ***Musculature :*** The muscles are well developed in tapeworm (e.g. *Taenia*). This enables *Taenia* to spread and elongate their bodies along the length of the intestine of their host. Similarly, power of locomotion enables the round worm (e.g. *Ascaris*) to counteract gut peristalsis and thus maintain their position in the intestine. In this way the parasites becomes capable to obtain predigested nutrients of the host.

(c) ***Organs for attachment :*** All parasites develop suitable devices for attachent to their hosts, either to the exterior or to the interior of host cavities. Helminthes are variously modified for adhesion to the body of their hosts. In all adult parasitic flatworm acetabula or suckers develop. The liver fluke (e.g. *Fasciola*) has two suckers on the ventral side of the body one anteriorly and another posteriorly. In the tape worms the scolex bears either four sucking cups (e.g. *Taenia solium*) or accessory suckers (e.g. *Myzoplyllo bothrium*) or lateral sucking grooves or bothria (e.g. Diplyllo bothrium).

Some cestodes and nematodes also develop hook-like structures near the cephalic ends that further help in attachment. In some, a basal circlet of hooks (*T. solium*) or rows of hooks develop (*Dipylidium carninum*). A buccal armature bearing toothlike structure has been found in *Macracanth orhynchus*.

In some helminths e.g. miracidium and cerceria, unicallular secretary glands develop which help during penetration into the host. The secretion contain a lytic enzyme that digests the host's time make passage through which the worms move. In larval trematodes cystogenous glands develop whose secretion help in cyst formation. These glads degenerate after their functions are over. Hook worm contain buccal glands which pour secretion that are anticoagulant and also has histolytic action.

(d) ***Vast reproduction :*** Parasitic adaptation involves a significant development of reproductive organs with much increased capability of reproduction. In both flat worms and round worms, the interior of the body is mostly occupied by the genital organs. The chances for survival is increased by astronomical production of eggs. Self fertilization is more common than cross fertilization. The life history usually includes several larval stages for multiplication and for easy and sure transfer from one host to anohter. The nervous system in all parasitic helminthes and excretory system, paticularly in trematodes show little deviation or adaptation to perticular mode of life.

Physiological adaptation

Apart from structural modification physiological or functional adaptation are also seen in parasitic adaptation.

1. ***Intracellular digestion :*** The parasites develop intracellular digestion because they feed on tissue elements and inflammatory excudates. This has been observed in flukes.

2. ***Osmoregulation :*** The osmotic pressure of the interior of parasitic worms remains less than or same as that of their hosts and thus there occur no difficulty in the exchange of water. It has been found that cestodes have well developed osmoregulatory system and their pH tolerance is also high.

3. ***Anaerobic respiration :*** The parasites adapt to decrease their oxygen demand

because they live in less or no oxygen environment. The intestinal parasites live in an environment completely devoid of free oxygen. They develop a very low metabolic rate which requires a minimum amont of oxygen. In the absence of free oxygen, energy is obtained by the fermentation of glycogen which is broken by glycolysis carbon di-oxide and fatty acid. The glycogen and lipid contents in than body tissues have been found to be high whereas the protein content is less.

4. ***Antienzyme :*** One important parasitic adaptation is to develop antienzymes so that they are not attacked on destroyed by the host enzymes. Most of the helminth parasites, particularly intestinal parasites, secrete antienzymes in order to protect themselves from the gastric juices and digestive enzymes of the host. A dead worm cannot secrete these enzymes and so they are digested by the host enzymes. Medicines are used to destroy this anti-enzyme action and thus the parasites are subsequently destroyed by the host.

Group A(II)

Biochemistry

Unit 1 ❐ Glucose Metabolism *via* EMP and HMP Pathway, TCA Cycle

Structure

1.1 Introduction

1.2 Glucose metabolism *via* EMP and HMP pathway, TCA cycle

1.3 Oxidation of fatty acid

1.4 Phenylalanine

1.5 Catabolism of purine

1.6 Terminal Questions

1.1 Introduction

The major function of carbohydrate in metabolism is as a fuel to be oxidized and provide energy for other metabolic processes. In this role, carbohydrate is utilized by cells mainly in the form of glucose. The three principle monosaccharides resulting from the digestive process are glucose, fructose and galactose. Fructose may assume considerable quantitative importance if there is a large intake of sucrose. Galactose is of major quantitative significance only when lactose is the principal carbohydrate of the diet. Both fructose and galactose are readily converter to glucose by the liver. Pentose sugars as xylose, arabinose, and ribose may be present in the diet, but their fate after absorption is obscure. D-Ribose is synthesized in the tissues for incorporation into nucleotides.

1.2 Glucose metabolism *via* EMP and HMP pathway, TCA cycle

1.2.1 Glycolysis through EMP pathway

Glucose enters into the glycloytic pathway by phosphorylation to glucose-6-phosphate. It is completed by the enzyme hexokinase (here glucokinase) in liver parenchymal cells. ATP is required as phosphate donor and Mg^{++} as cofactor. Glucose-6-phosphate is the junction of several metabolic pathways (glycolysis, gluconeogenesis, hexose monophosphate shunt pathways, glyconeogenesis, glycogenolisis). Glycogen is hydrolyzed to glucose-6-phosphate *via* glucose-1-phosphate by the enzyme phosphorylase. In glycolysis it is converted to fructose-6-phosphate by phosphohexose isomerase. This fructose-6-phosphate in presence of the enzyme phosphofructokinase coverted to fructose-1, 6-bisphosphate. It requires ATP and Mg^{++}. Fructose-1, 6-

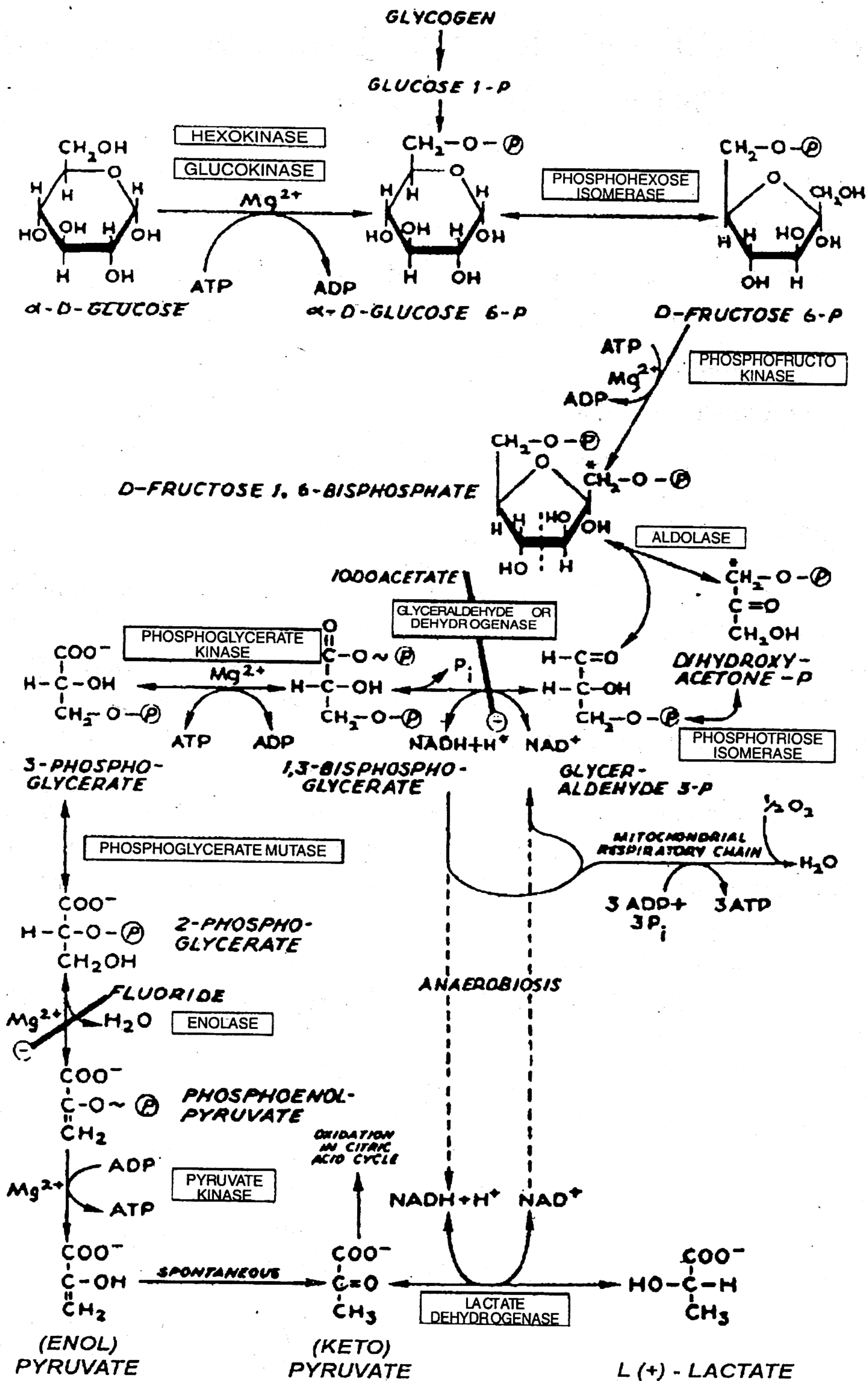


Figure 1.1 : Embden-Meyerhof pathway of lycolysis. (Ⓟ– PO_3^{3-}; Pi, $HOPO_3^{3-}$; ⊖, inhibition)

bisphosphate splits by aldolase into two triosephosphate (glyceraldehyde-3-phosphate and dihydroxyacetone phosphate). Glyceraldehyde-3-phosphate and dihydroxyacetone phosphate are inter converted by the enzyme phosphotriose isomerase. Glyceraldehyde-3-phosphate by oxidation in presence of NAD is converted to 1, 3-bisphosphoglycerate. 1, 3-bisphosphoglycerate is converted to 3-phosphoglycerate in presence of the enzyme phosphoglycerate kinase. It is subsequently converted phosphoenol pyruvate by the enzyme enolase. Phosphoenol pyruvate is converted to pyruvate (Keto) and the spontaneously to Keto pyruvate for the activation of the enzyme ATP and Mg^{++} is very much essentia. Keto form of pyruvate converted to lactate by lactate dehydrogenase. NADH + H^{++} generated for the oxidation of glyceradehyde-3-phosphate is utilized for the activation of the enzyme lactate dehydrogenase.

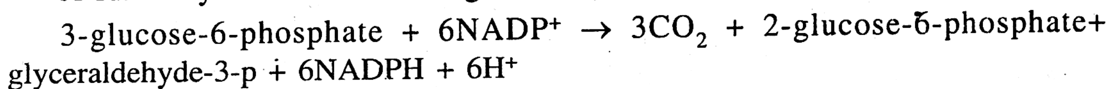
1.2.2 Hexose monophosphate shunt/pentosephosphate pathway

Major functions of the hexose monophosphate shunt pathway are to provide NADPH for reductive syntheses outside the mitochondria and to provide ribose for nucleotide and nucleic acid, synthesis. This pathway for the oxidation of glucose occurs in certain tissues. It is active notably in liver, lactating mammary gland, adernal cortex, thyroid gland, erythrocytes, testis in addition to Embden Meyerthof pathway of glycolysis. It is clear that this pathway is markedly different from the EMP of glycolysis. Oxidation occurs in the first reactions and CO_2, which is not produced at all in the EMP. Three molecules of glucose-6-phosphate give rise to three molecules of CO_2 and three 5-carbon residues. The latter are rearranged to generate two molecules of glucose-6-phosphate and one molecule of glyceraldehye-3-phosphate. In shunt pathway NADP and not NAD^+ used as a hydrogen acceptor. The enzymes of this shunt pathwasys are found in the extramitochondrial soluble portion of the cells.

In the first phase glucose-6-phosphate undergoes dehydrogenation and decarboxylation to give pentose, ribose-5-phosphate, *via* ribulose-5-phosphate. In the second phase ribulose-5-phosphate derived from second molecules of glucose-6-phosphate is converted to xylulose-5-phosphate and then by series of reaction forms fructose-6-phosphate and erythrose-4-phosphate. Third molecules of glucose-6-phosphate converted to xylulose-5-phosphate. This xylulose-5-phosphate and erythrose-4-phosphate form fructose-6-phosphate & glyceraldehyde-3-phosphate.

Three molecules of glucose-6-phosphate finally form two molecules of fructose-6-phosphate and one molecule of glyceraldehyde-3-phosphate. So it can be considered that three molecules of glucose-6-phosphate finally forms 2½ molecules of glucose-6-phosphate.

A summary of the reaction is given below :



3-glucose-6-phosphate + $6NADP^+ \rightarrow 3CO_2$ + 2-glucose-6-phosphate+ glyceraldehyde-3-p + 6NADPH + $6H^+$

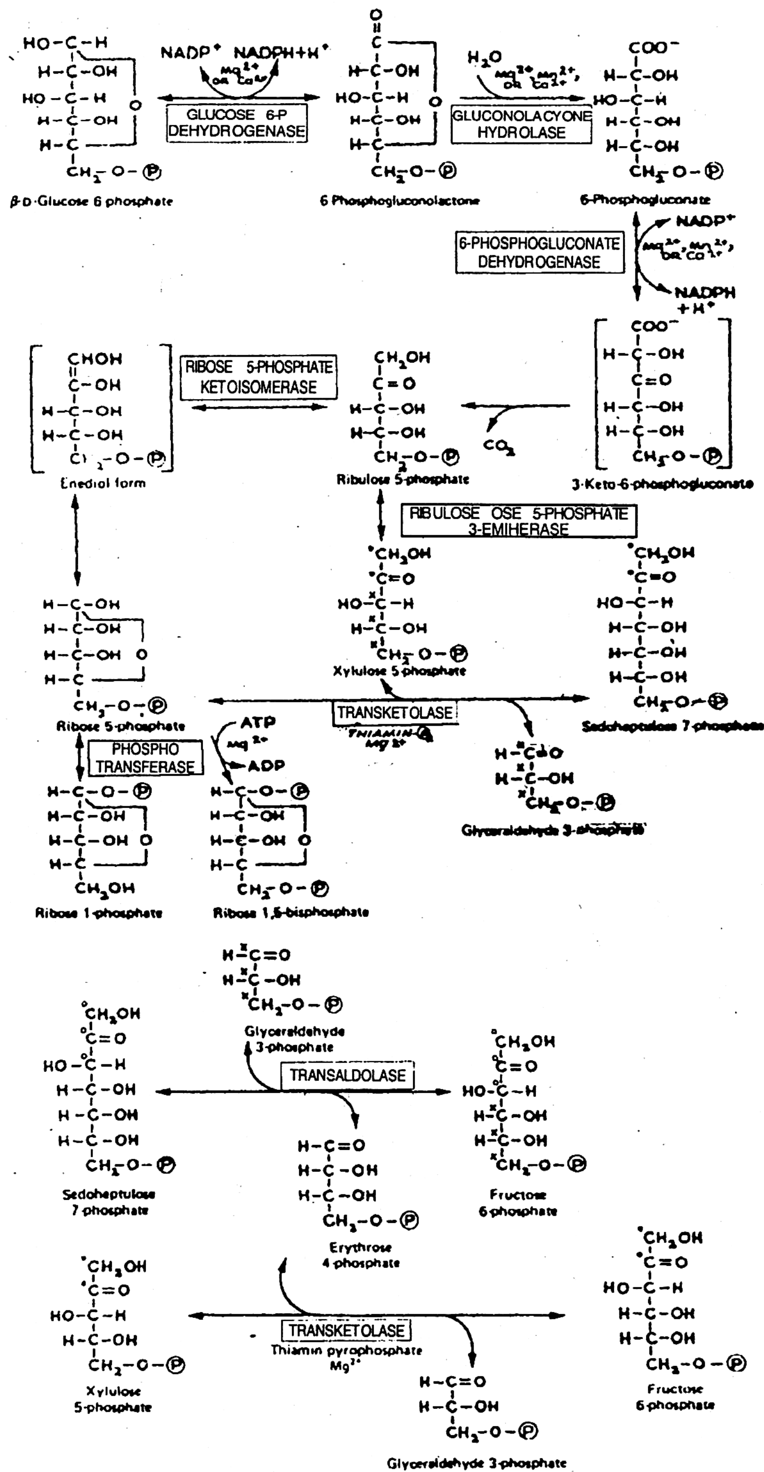


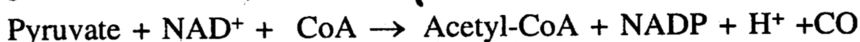
Figure 1.2 : The hexose monophosphate shunt (pentose phosphate pathway) (Ⓟ – PO_3^{3-})

Metabolic significance :

It is active in liver dipose, adrenal cortex, thyroid peritrocytes, testis, lactating mammary and, NADPH of the shunt pathway help in the synthesis of fatty acids and steroid. This NADPH also responsible for the synthesis of the amino acid via glutamet dehydrogenase. In erythrocytes oxidised glthatione is reduced to reduced gluthatione by this NADPH, catalised by gluthatione reductase. Reduced gluthatione remove H_2O_2 from the erythrocyte whih is catalyzed by glutathione peroxidese. This reaction is important. The accumulation of H_2O_2 may decrease the life span of erythoracyte by increasing the oxidation of haemoglobin to methemoglobin. The hexose monophosphate shunt pathway provides pentoses for nucleotide and nucleic acid synthesis.

1.2.3 Oxidation of pyruvate of acetyl-CoA

Before pyruvate can enter the citric acid cycle, it must be transported into the mitochondria via a special pyruvate transporter that aids it passage across the inner mitochondiral membrane. Within the mitochondria, pyruvate is oxidatively decarboxylated to accetyl-CoA. This reaction is catalyzed a mltienzyme complex and designated as pyruvate dehydrogenase complex.



Pyruvate + NAD^+ + CoA → Acetyl-CoA + NADP + H^+ +CO

1.2.4 The Citric Acid cycle (Krebs cycle, tricarboxylic acid cycle)

At the beginning of the cycle Acetyl-CoA combines with 4-carbon carboxylic acid oxaloacetate, resulting in the formation of a 6-carbon tricarboxylic acid citrate. A small quantity of oxalocacetate is needed to facilitate the conversion of a large quantity of acetyl units to CO_2, oxalocetate may be considered to play a catalytic role.

The major fnction of the cycle is to act as the first common pathway for the oxidation of carbohydrae. protein and lipid or glucose, fatty acid & amino acid. Further the citric acid cycle liberated a good amount of energy during the oxidation of carbohydrate, lipid & protein. During the course of oxidation of Acetyl-CoA in the cycle reducing equivalents in the form of hydrogen or of electrons are formed. These reducing equivalent then enter the respiratory chain, where large amont of ATP generated in the process of oxidative phosphorylation. the enzymes of the citric acid cycle are located in the mitochondrial matrix, either free or attached to the inner surface of the mitochondrial membrane. It facilitates the transfer reducing equivalent to the adjacent enzymes of the repiratory chain. It is of further significance that citric acid cycle has a dual or amphibolic role. Through this cycle both synthesis and catabolism of glucose, fatty acid and amino acid take place.

In citric acid cycle at the beginning the oxaloacetate combines with acetyl CoA in presence of citrate synthatase to form citrate. Citrate is then converted to isocitrate

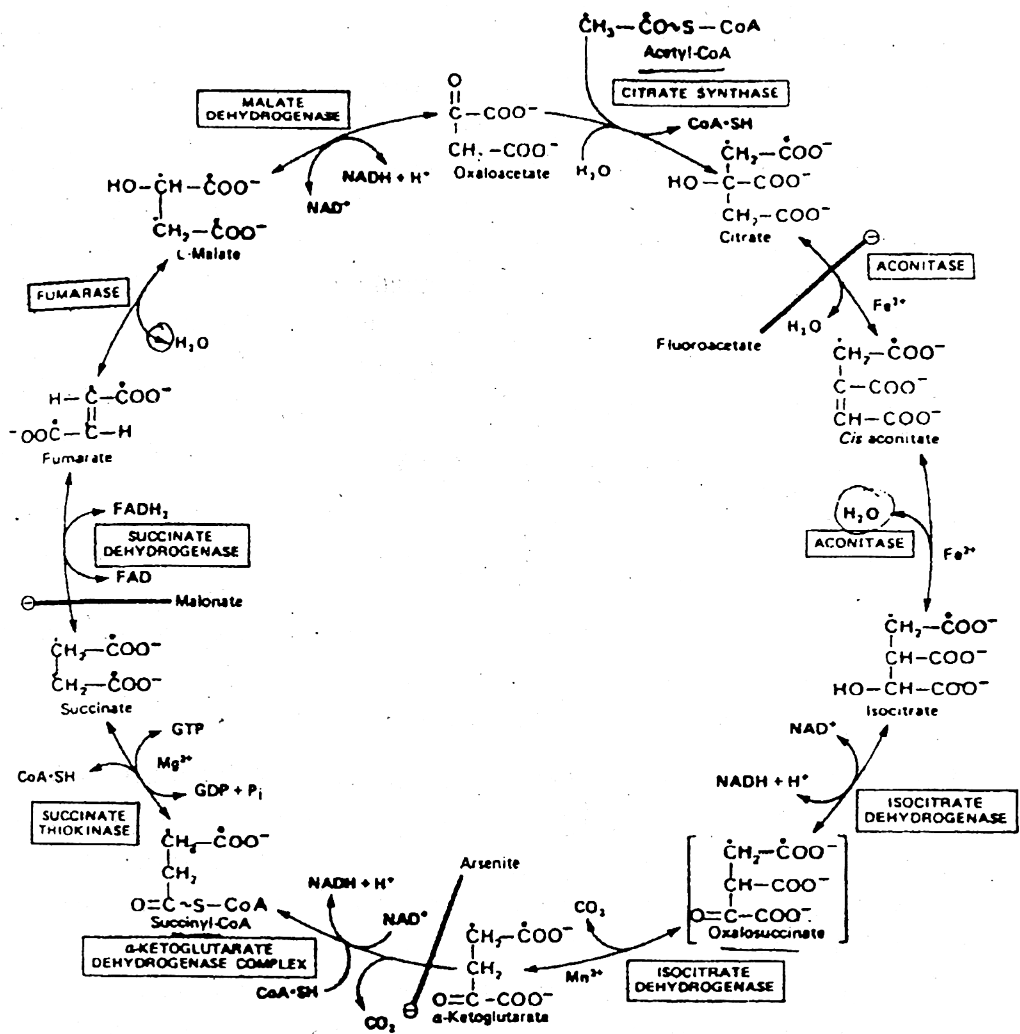


Figure : 1.3 The citric acid (Krebs) cycle. Oxidation of NADH and $FADH_2$ in the respiratory chain leads to the generation of ATP via oxidative phosphorylation. In order to follow the passage of acetyl-CoA through the cycle, the 2 carbon atoms of the acetyl radical are shown labeled on the carboxyl carbon (using the designation [•]) and on the methyl carbon (using the designation [•]). Although 2 carbon atoms are lost as CO_2 in one revolution of the cycle, these atoms are not derived from the acetyl-CoA that has immediately entered the cycle but from that portion of the citrate molecule which derived from oxaloacetate. However, on completion of a single turn of the cycle, the oxaloacetate that is regenerated is now labeled, Which leads to labeled CO_2 being evolved during the second turn of the cycle. Because succinate is a symmetric compound and because succinate dehydrogenase does not differentiate between its 2 carboxyl groups, "randomization" of label occurs at this step such that all 4 carbon atoms of oxaloacetate appear to be labeled after one turn of the cycle. During gluconeogenesis, some of the label in oxaloacetate is incorporated into glucose and glycogen. In this process, oxaloacetate is decarboxylated by release of the carboxyl group adjacent to the CH_2 group. As a result of recombination of the resulting 3-carbon residues in a process that is essentially reversal of glycolysis, the eventual location of label from acetate in glucose (or glycogen) is distributed in a characteristic manner. Thus, if oxaloacetate leaves the citric acid cycle after only one turn from the entry of labeled acetyl-CoA (acetate), label from the carboxyl carbon of acetate is found in carbon atoms 3 and 4 of glucose, whereas lebel from the methyl carbon of acetate is found in carbon atoms 1, 2, 5 and 6. For a discussion of the stereochemical aspects of the citric acid cycle, see Greville (1968). The sites of inhibition (⊖) by fluoroacetate, malonate, and arsenite are indicated.

via cisaconitate in presence of Aconitase. Isocitrate inpresence of NAD^+ and isocitrate dehydrogenase converted oxalosuccinate which is decarbonylated to α-ketaglutarate by the enzyme, Mg^{++} -dependent isocitrate dehydrogenase. α-ketaglutarate by the utilization of NAD^+ in presence of α-ketaglutarate dehtdrogenase complex oxidized to form succinyl CoA which is oxidized to succinate by sccinate thiokinase with the help of GDP. Sccinate in presence of succinate dehydrogenase & FAD forms fumarate. The enzyme fmarase form malate from fumarate which is ultimately oxidized to oxaloacetate by enzyme malate dehydrogenase and NAD^+. Again the reaction sarts and it is repeated in cycle order.

Table 1. Generation of high-energy bounds in the catabolism of glucose

Pathway	Reaction Catalyzed By	Method of~Ⓟ Production	Number of ~Ⓟ Formed per Mole of Glucose
Glycilysis	Glyceraldehyde 3 phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH	6*
	Phosphoglycerate kinase	Osidation at substrate level	2
	Pyruvate kinase	Osidation at substrate level	2
			10
Allow for consumption of ATP by reactions catalyzed by hexokinase and phosphofructokinase			−2
			Net 8
Citric acid cycle	Pyruvate dehydrogenase	Respiration chain oxidation of 2 NADH	6
	Isocitrate dehydrogenase	Respiration chain oxidation of 2 NADH	6
	α-ketaglutarate dehydrogenase	Respiration chain oxidation of 2 NADH	6
	Succinate thiokinase	Oxiadation at substrate level	2
	Succinate dehydrogenase	Respiratory chain oxidation of 2 $NADH_2$	4
	Malate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
			Net 30
Total per mole glucose under aerobic conditions			38
Total per mole glucose under anaerobic conditions			2

*It is assumed that NADH formed in glycolysis transported into mitochondria via the malete shuttle. If the glycerophosphate shuttle is used. only 2 ~ □ would be formed per mole of NADH, the total net production being 36 instead of 38. The calculation ignores the small loss of ATP due to a transport of H^+ into the mitichondrion with Pyruvate and a similar transport of H^+ inthe operation of the malate shuffle, totaling about 1 mol of ATP.

1.3 Oxidation of fatty acids

β-oxidation of fatty acids

Several enzymes, known cllectively as "fatty acid oxidase" are found in ,otechondral matrix.adjacent to the respiratory chain (in inner membrane). These catalyze the oxidation of acyl CoA to acetyl-CoA, the system being coupled with the phosphorylation of ADP to ATP.

After formation of acyl CoA and its penetration through the mitochondrial membreane via the carnitine transporter system, there follows the removal of 2 hydrogen atom from the 2 (α) and 3 (β) carbon atoms, catalyzed by acyl-CoA

dehydrogenase. The results in the formation of α, β unsaturated or Δ^3 unsaturated acyl CoA. The coenzyme for the dehydrogenase is a falvoportein, contaning FAD as prosthetic group, whose reoxidation by the repiratory chain requires the mediation of another flavoprotein termed electron transferring falvoprotein.

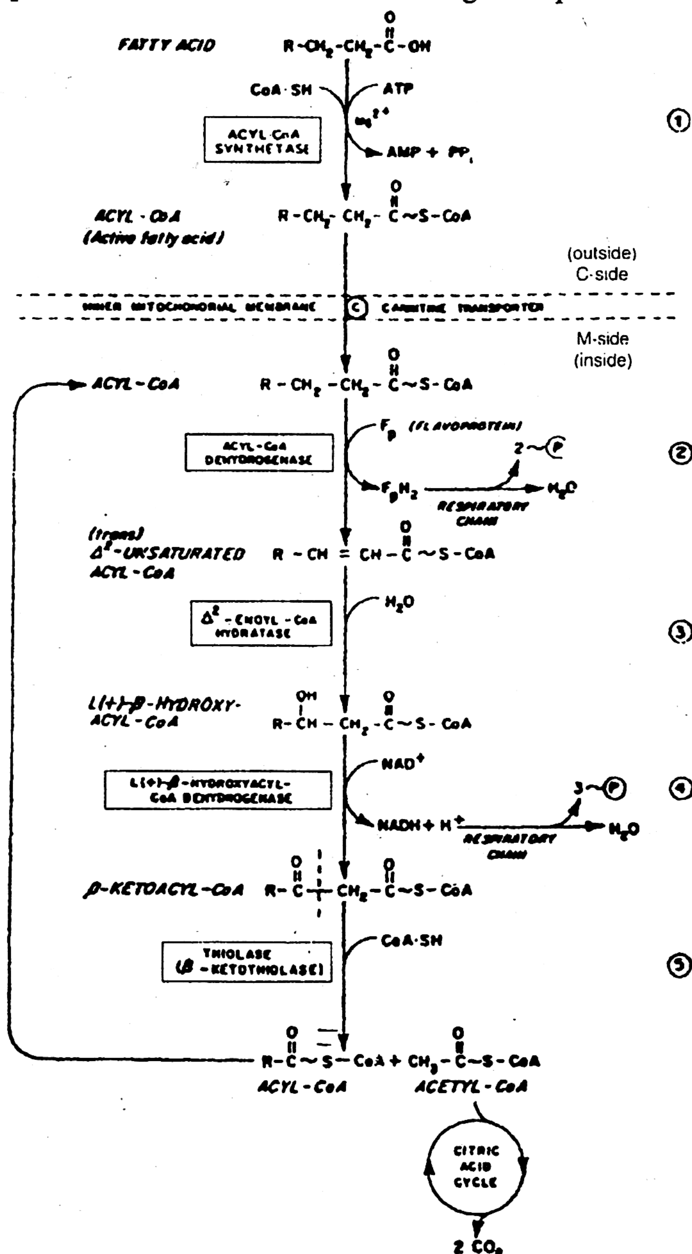


Figure 1.4 : β-oxidation of fatty acids. Long-chain acyl-CoA is cycled through reactions ②– ⑤ acetyl-CoA being split off each cycle by thiolase (reaction ⑤). When the acyl radical is only 4 carbon atoms in length, 2 acetyl-CoA molecules are formed in reaction⑤

Water is added to saturate the double bond and form β-hydrxyacyl-CoA catalyze by the enzyme crotonase. By further dehydrogenation β-hydroxy derivative [β-carbon (β-hydroxylacyl CoA dehydrogenase)] form the corresponding β-ketol CoA compound NAD acts as coenzyme. β-keto acyl CoA splits at β-position by thiolase involving another molecle of Coa and forms acetyl CoA and acyl CoA contaning 2 carbon less that original acyl CoA. In this way a long chain fatty acid may completely degraded to acetyl CoA and this can be completely oxidized to CO_2 and water *via* citric acid cycle.

Fatty acids with an odd number of carbon atom are oxidized by this pathway.

Peroxisomal fatty acid oxidation :

This is the modified form of β-oxidation of long chain fatty acid by the peroxidomal enzyme shortens the CoA thioester of very long fatty acid to octanoyl CoA.

α and ω oxidation of fatty acid :

α-oxidation is the removal of one carbon at a time from the carboxyl end of molecule. It doesn't require CoA intermediate and does not generate high energy phosphate.

ω-oxidation is brought about by hdroxylase enzymes involving cytochorme P-450 in microsomes. The CH_3 group is converted to a CH_2OH group that sbsequently is oxidized to COOH, thus forming a dicatboxylic acid.

Oxidation of unsaturated fatty acid :

The CoA ester of unsaturated fatty acid are degraded normally by the enzyme responsible for β-oxidation until either a Δ^3-cis-acyl-CoA compound or a Δ^2-cis-acyl-CoA compound is formed, depending upon the position of the double bond. The former compound is isomerized to the corresponding Δ^2-trans-CoA state, which in turn is hydrated by Δ^2-enoyl-CoA hydratase to L (+) β-hydrozyacyl-CoA. The Δ^2-cis-acyl-CoA compound is first hydrated by Δ^2-enoyl-CoA hydratase to the D(–) β-hydroxyacyl-CoA derivative. This ndergoes epimerization [D(–)-β-hydroxyacyl-CoA epimerase] to give the normal L (+)-β-hydroxyacyl-CoA state is β-oxidation.

Microsomal Peroxidation of Polyunsatured fatty acids :

Polyunsaturated fatty acids in membrane initially destroyed by lipid peroxidation a hydrogen atom is removed, leaving a lipid free radical. After rearragement of the double bond, a lipid hydroperoxide or endoperoxide is formed by the addition of moleclar oxygen. NADPH dependent peroxidation of unsaturated fatty acids is catalyzed by microsomal enzymes.

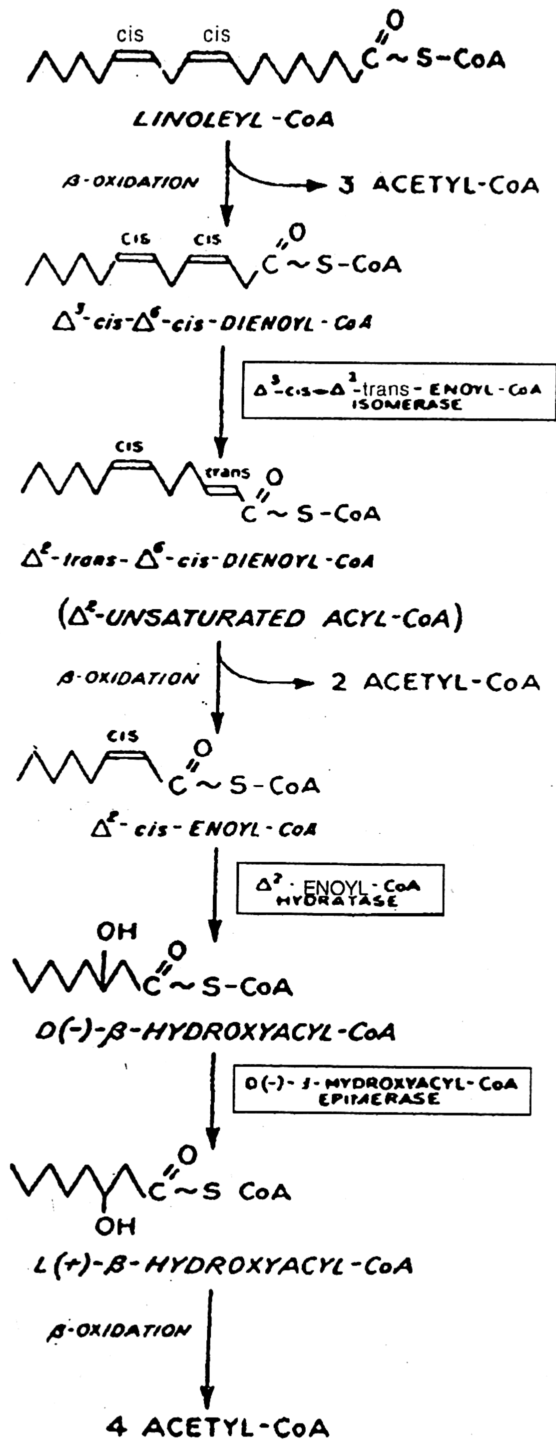


Figure 1.5 : Sequence or reactions in the oxidation of unsaturated fatty acids, e.g. Inoleic acid.

1.4 Phenylalanine

Phenylalanine is a glycogenic and ketogenic amino acid, through tyrosine & it forms (a) catecholamines, (b) melamins, (c) thyroid hormones, (d) fumerate and acetoacetate.

(a) The metabolism of catecholamines

The pathway for the formation of catecholamines are give below :

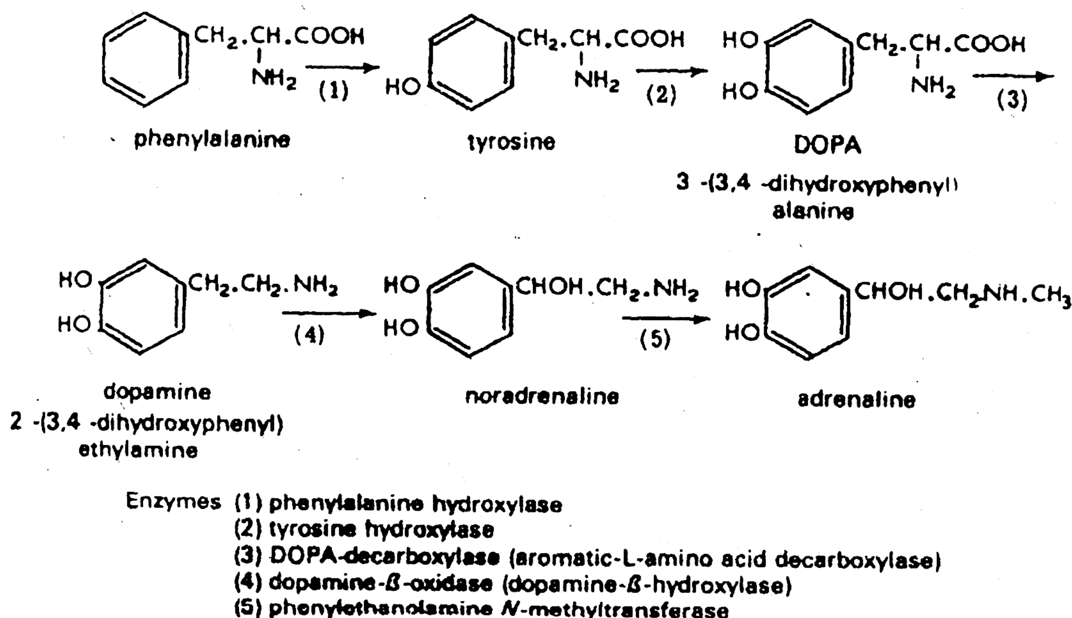


Figure 1.6 : The principal biosynthetic pathway for dopamine, noradrenaline and adrenaline

Catecholamines are speedily inactivated by methylation at eh orthohydroxyl group by catecholamine-O-methyltransaminase (COMT) & adenosylmethionin and oxidative deaminetion by monoamine oxidase (MAO) and aldehyde dehydrogenase. Conjugated and unconjugated end products such as metnoradrenatine, metadrenatine, vanilic acid are excreted through urine.

(b) Melanin :

This is protien-bound polymeric pigment present in skin melanocytes, hair, iris, choroids, retinal pigmented cell and substantia nigra.

noradrenaline
adrenaline
N-methyladrenaline
3,4-dihydroxy-mandelic aldehyde
(3,4-dihydroxy-phenyl)-ethanediol
3,4-dihydroxy-mandelic acid
3-methoxy-4-hydroxy-mandelic acid (MOMA, HMMA or VMA)
N-methylmetadrenaline
normetadrenaline (normetanephrine)
3-methoxy-4-hydroxy-mandelic aldehyde
metadrenaline (metanephrine)
(3-methoxy-4-hydroxy-phenyl) ethanediol
vanillic acid
Urine (in free and conjugated forms)

MAO = monoamine oxidase
COMT = catechol-O-methyl transferase
AO = aldehyde oxidase
AD = alcohol dehydrogenase
Dotted arrows indicate minor pathways

Figure 1.7 : The breakdown of adrenaline and noradrenaline after Axelrod (1963, 1965), Nodified from Crossland, 1967, by kind permission of Messrs Butterworths.

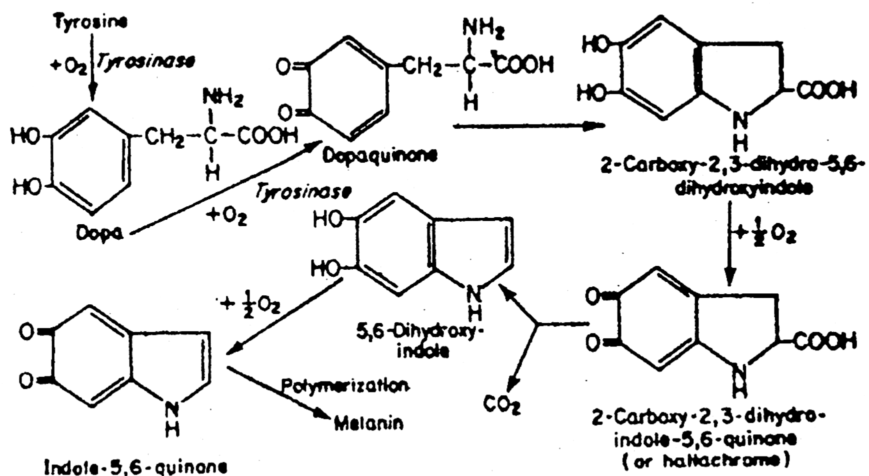


Figure 1.8 : Possible pathway for melanin synthesis

(c) Thyroid hormones :

Phenylalanine by phenylalanine hydroxylase are converted to tyrosine. By iodination at 3 position forms monoiodotyrosine (MIT) and then by further iodination at the 5th position it forms diiodotirosine (DIT). Then by coupling of one mono-and one-di-or by coupling of 2 diiodotyrosine formation of triiodtyrosine (T_3) or tetraiodo thyronime (T_4) formation take place. The enzyme responsible for the T_3 and T_4 formation are peroxidase.

The thyroid hormones themselves are deiodinated in the liver, salivary glands and kiney. The iodine can be used again. the remainder of the molecules are disposed of as conjugated in the urine.

(d) Phenylalanine and tyrosine form acetoacetate and fumerae. Then through TCA cycle they are completely degrated or through this cycle they synthesize, or lipid or protein.

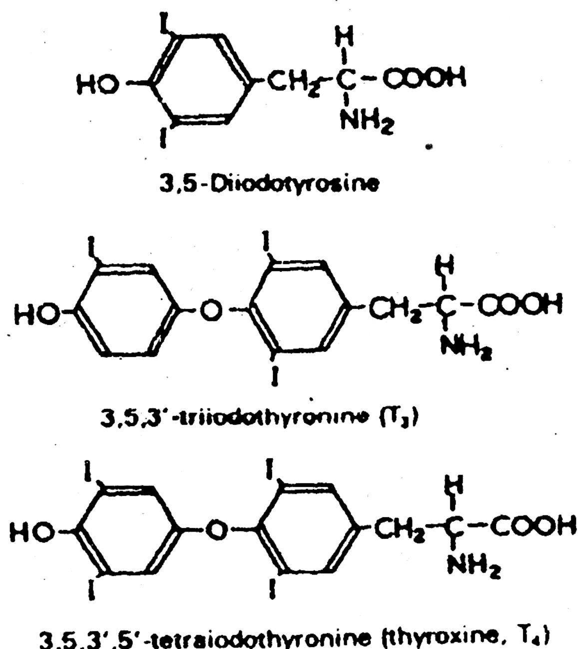


Figure 1.9 : Iodinated derivatives of tyrosine

1.5 Catabolism of purine

Primates, dalmation dogs and uricotelic animals, catabolize purine to uric acid. Human eliminates 600-800mg. of uric acid daily; 80 to 90% through urine and rest through bile. Catabolism of purinase to uric are given in figure 1.12.

Figure 1.10 : Catabolism of purines to uric acid

1. Transamination

Transamination transfer the amino group of one amino acid to a ketoacid, changing the later into a new amino acid and original amino acid into a new keto acid.

Alamine + α-ketogutarate = Pyruvate + glutamate

Except threonine, lysine, proline and hydroxyprolinem all α-amino acid can participate in transaminations and change into respective keto acid by donating α-amino groups. Transaminations are double displacement type of bisubstrate reations. The two substance (i.e. amino acid and keto acid) bind separately and successively

with the prosthetic group of the enzyme. L-glutamic acid first combines with the enzyme bound pyridoxal phosphate and forms enzyme bound Schiff base with the liberation of water. It is hydrolysed to release glutamic acid as a product leaving enzyme bound pyridoxamine phosphate (PMP). The pyruvic acid (second substrate) binds with the PMP to form a new schiff base. After intermolecular rearrangement, dissociates with the help of water forms alamine and enzyme bound pyridoxal phosphate.

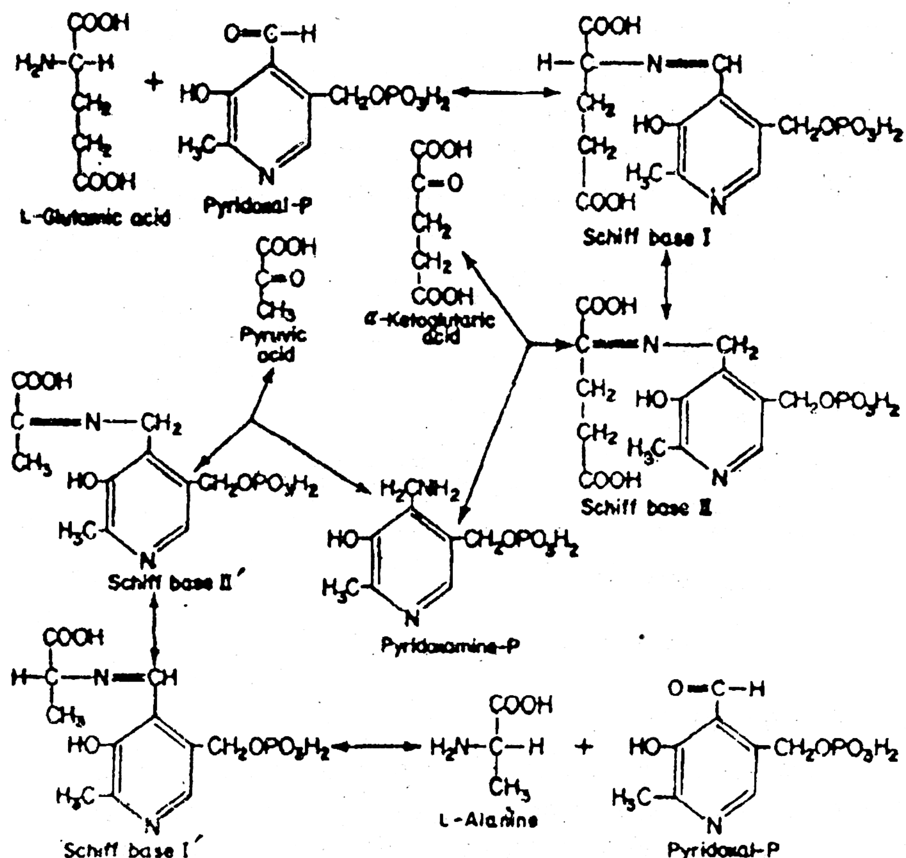


Figure 1.11 : Mechanism of transamination by glutamate pyruvate aminotransferase

The reaction may be summarized as follows :

α-Amino acid, + enzyme PLP = a-keto acid, + Enzyme PMP.

α-keto acid 2 + enzyme PMP = α-amino acid 2 + enzyme PLP.

Each ransaminase specific for each amino acid as substrate for the first stage of transmination.

Cliunically two imporant transaminases are–

Serum-glutamate oxaloaretate ransaminase (SGOT)

Serum-gltamate pyruvate transminase (SGPT).

2. Transdeamination

In mammulian tissue particlarly the liver amino acids are deaminated by transamination with the help of aminotransferases (transaminase) followed by oxidative deamination of the resulting L-glutamate in the mitochondria by a polymeric and allosteric enzyme. L-glutamate dehydrogenase, present in liver mitochondria requires NAD^+ or $NADP^+$ by the action of L-glytamate dehydrogenase.

Dehydrogenase is reversible. It may also synthesize glutamate by the reductive animation of α-ketoglutarae. The better is then spontaneously hedrolyzed into α-ketoglutamate and ammonia by cytoplasmic glucomate dehydrogenase with has low Km ammonia. It mainly catalyzed the biosynthesis of glutamate sing NADPH, NADH, ATP and GTP. It is allosterically activated by NAD^+, $NADP^+$, ADP, GDP and AMP.

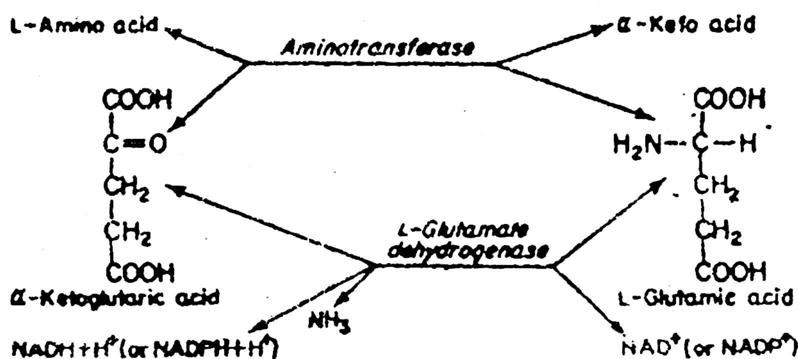


Figure 1.12 : Transdeamination of L-amino acid

3. Oxidative deamination

Oxidative deamination is the direct removal of the amino group as ammonia by flavoprotein containing enzyme amino acid oxidase. It is found in peroxisomes of mammalion liver and kidney cells. It ocurs in mamalian renal mitochondria and microsome and too poor inmammalian livercell.

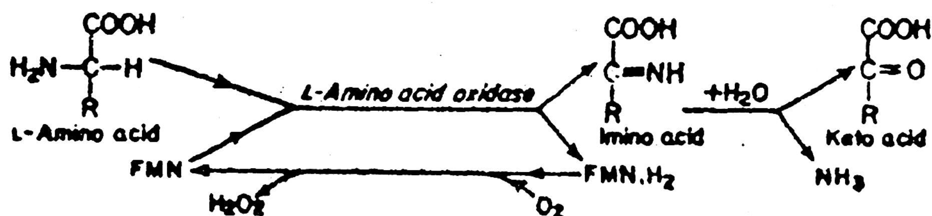


Figure 1.13 : Oxidative deamination by L-amino acid oxidase

It oxidize corresponding imino acid by transferring reducing equivalent from the amino acid to the flavin mucleotide in its prosthetic group. The amino acid reacts spontaneously with water to give an α-keto acid and ammonia. The reduced nucleotide of the prosthetic group is reoxidised directly by molecular O_3, producing H_3O_3.

1.6 Terminal questions

1. Write the significance of the citric acid cycle.
2. State schematically the generation of high energy bonds in the catabolism of glucose.
3. Discuss about the glycolysis.
4. Write about the glycogenesis.
5. Discuss critically about the hexose monophosphate shunt pathway.
6. Write about the metabolic significance of hexose monophosphate shunt pathway.
7. Discuss about the role of phenylalanine for the generation of biologically active molecules.
8. Discuss about the sybthesis & function of neutrotransmitters / T_3 & T_4 or thyroid hormones/melanin from phenylalanine.
9. Write about the catabolism of purine.
10. Discuss about the β-oxidation of fatty acid.

Unit 2 ❐ Biological Oxidations

Structure

2.1 Introduction

Objectives

2.2 Oxidoreductases

2.3 Redox potential

2.4 Mitochondrial respiratory chain

2.5 Oxidative phosphorylation

2.6 Summary

2.7 Terminal questions

2.8 Answers

2.1 Introduction

You will read about biological oxidations in this unit. Oxidation and reductions in tissues consist of transfers of *reducing equivalence* (viz., electrons and H^+) from one substrate to another; the electron-donor substrate is thereby oxidized while the electron-acceptor substrate gets simultaneously reduced. In this way, electrons may flow between successive substrates of one or more pairs, depending mainly on the relative electron-affinities of the electron-donor and the electron-acceptor substrates each time. In *aerobic metabolic pathways,* molecular O_2 is required to serve as the final electron-acceptor during such electron-flow from intermediate to intermediate of the pathway. But in *anaerobic metabolism,* no O_2 is required to act an electron acceptor. Glycolysis and TCA cycle are examples of respectively anaerobic and aerobic oxidative pathways. Both anaerobic and aerobic oxidations can generate high amounts of free energy which may be trapped by forming high-energy bonds such as those of ATP and GTP. Thus, electrons removed from substrates of aerobic oxidations are transported by successive electron-carriers of the *mitochondrial respiratory chain,* ultimately to be accepted by molecular O_2; the energy released during such electron transports to O_2 is harnessed to phosphorylate ADP to ATP by *oxidative phopshorylation.*

Objectives

Reading of this unit should enable you to :

- Understand the nature and actions of different classes of oxidoreductases,

- Know the natures of redox potentials and electron-transfer potentials and their roles in electron transfers between substrates.
- Describe different components of the mitochondrial electron transport chain and their respective roles in electron transport.
- Explain the free energy changes during electron flow along different respiratory chain complexs of the electron transport chain.
- Understand the difference between substrate-level and oxidative phosphorylations,
- Describe the structural organization of ATP synthase of inner mitochondrial membrane particles and its mode of action in oxidative phosphorylation of ATP.
- Explain the coupling of the mitochondrial oxidation and ATP synthesis in terms of the chemiosmotic theory.
- Describe the redox loop mechanism and the proton pump mechanism to explain the formation of a transmembrane proton gradient with the energy released by mitochondrial electron transport.
- Discuss the regulation of oxidative phosphorylation.

2.2 Oxidoreductases

Oxidation of a substrate consists of either the removal of reducing equivalent (viz, electrons and H^+) or the addition of O_2 to it. Reduction is the reverse process of addition of reducing equivalents to a substrate. Oxidation and reductions are mostly catalyzed in biological systems by specific enzymes called *oxidoredctases*. You will know the natures and modes of action of different classes of oxidoredctases from their brief classification given below.

2.2.1 Oxygenases

They oxidize their respective substrates by incorporating oxygen into the molecules of the latter. They belong to two main subclasses.

(a) Dioxygenases :

These incorporate both oxygen atoms of O_2 into each substrate molecule, often breaking the C-C bond between the oxygen-accepting Carbons in the latter; e.g., tryptophan 2, 3-dioxygenase and homogentisate, 1, 2-dioxygenase of liver oxidizing respectively tryptophan and homogentisate.

(b) Monooxygenases or hyodroxylases :

These catalyze the hydroxylation of their substrate by incorporating it in one of the oxygen atoms from O_2 and similtaneously reduce the other oxygen atom to H_2O

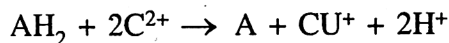
by reducing equivalents from specific coenzymes/cofactors such as cytochrome b_5 and tetrahydrobiopterin; e.g. phenylalanine hydroxylase of liver and dopamine β-hydroxylase of adrenal medulla, hydroxylating their substrates respectively using tetrahydrobiopterin and L-ascorbate.

2.2.2 Oxidases

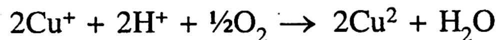
The catalyze the oxidation of their respective substance by transferring reducing equivalents (electrons and H^+) from the substrate to molecular O_2 alone, producing H_2O in most cases.

(a) Copper-containing aerobic oxidases :

These are copper-protein complexes. The Cu^{2+} ion of the enzyme receives the electron from the substrate (AH_2) to from Cu^+ which then transfers the electron to molecular O_2 directly; the corresponding proton (H^+) from the substrate follows the electron to join the O_2 to form H_2O.



$$AH_2 + 2C^{2+} \rightarrow A + CU^+ + 2H^+$$

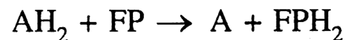


$$2Cu^+ + 2H^+ + \tfrac{1}{2}O_2 \rightarrow 2Cu^2 + H_2O$$

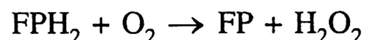
E.g., cytochrome oxidase, a Cu^{2+} –heme-protein complex of inner mitochondrial membrane, oxidized reduced cytochrome C.

(b) Flavoprotein oxidases :

These are flavoproteins (EP) with FMN or FAD as prosthetic group. They oxidize their substrates (AH_2) by transferring reducing equivalents (e and H^+) from the latter to their own prosthetic groups, reducing them to $FMNH_2$ or $FADH_2$. $FMNH_2$ of $FADH_2$ is reoxidized by the transfer of reducing equivalents directly to molecular O_2, producing H_2O_2; eg. L- and D-amino acid oxidases of respectively kidney and liver, bearing FMN and FAD respectively; xanthine oxidase of liver, containing Fe^{3+}, Mo^{6+} and FAD. Flavoprotein oxidases can also transfer the reducing equivalents to methylene blue, reducing the latter.



$$AH_2 + FP \rightarrow A + FPH_2$$

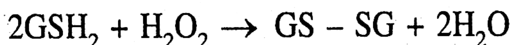


$$FPH_2 + O_2 \rightarrow FP + H_2O_2$$

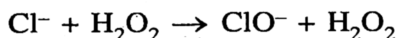
2.2.3 Hydroperoxidases

These hemoproteins of hepatic and renal peroxisomes, thyroid cells, granulocytes and erythrocytes serve to reduce H_2O_2 with reducing equivalents from specific substrates such as glutathione, halides (Cl^- or I^-) and cytochrome C, which are thereby oxidized; eg., glutathione peroxidase (RBC) reduces H_2O_2 while oxidizing

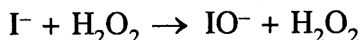
glutathione (GSH) to GS-SG, myeloperoxidase (granulocytes) oxidized Cl^- to hypochlorite (Cl^-) for reducing H_2O_2, and iodine peroxidase (thyroid) oxidized I^- to hypoiodite (IO^-) for reducing H_2O_2.



$$2GSH_2 + H_2O_2 \rightarrow GS - SG + 2H_2O$$

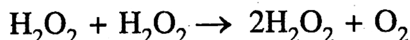


$$Cl^- + H_2O_2 \rightarrow ClO^- + H_2O_2$$



$$I^- + H_2O_2 \rightarrow IO^- + H_2O_2$$

Catalases of peroxisomes, in addition, can reduce H_2O_2 to H_2O with electrons from another H_2O_2 molecule :



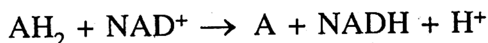
$$H_2O_2 + H_2O_2 \rightarrow 2H_2O_2 + O_2$$

2.2.4 Dehydrogenases

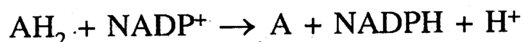
They transfer reducing equivalents from their substrates to specific electron-aceptors other than molecular O_2 |——| never to the latter. The electron-acceptor thus reduced gets reoxidized by transferring the reducing equivalents in turn to some other electro-acceptors. A chain of such electron-transfers may be carried out by the serial actions of several dehydrogenases (an *electron transport chain*) until an oxidase transfers the electrons from the preceding electro-acceptor to molecular O_2 directly.

(a) Pyridine-linked dehydrogenases :

While oxidizing their substrates, these enzyme transfer two electrons and a proton |——| as a hydride ion (H^-) ion |——| from the substrate to a pyridine nucleotide (either NAD^+ or $NADP^+$) serving as the electron-acceptor coenzyme; the other proton from the substrate (AH_2) is released in the medium while the coenzyme is reduced to BADH or NADPH.

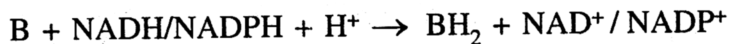


$$AH_2 + NAD^+ \rightarrow A + NADH + H^+$$



$$AH_2 + NADP^+ \rightarrow A + NADPH + H^+$$

The reduced coenzyme is next released from the dehydrogenase, joins some other dehydrogenase as the electron-donor coenzyme of the latter, and gets reoxidized by donating its hydride ion to substrate (B) of that enzyme and reducing it.



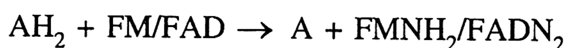
$$B + NADH/NADPH + H^+ \rightarrow BH_2 + NAD^+/NADP^+$$

In most cases, NADH gets reoxidized by donating its H^- ion to an electron-acceptor of the *mitochondrial respiratory chain* (see 2.4). But NADPH is mainly reoxidized by donating its H^- ion to specific substrates/intermediates of the *reductive synthesis* pathways such as those for palmitic acid and cholesterol synthesis.

NAD^+ *–dependent dehydrogenases* include cytoplasmic L-lactate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase for glycolysis, mitochondrial pyruvate dehydrogenase, α-ketoglutarate dehydeogenase and malate dehydrogenase for aerobic oxidative metabolism of pyruvate, and mitochondrial 3-hydroxycyl-CoA dehydrogenas for beta-oxidation, *$NADP^+$ – dependent dehydrogenases* include cytoplasmic glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of HMP shunt.

(b) Flavin-linked dehydrogenases :

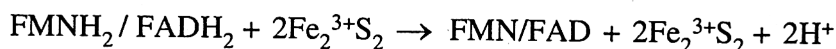
Their prosthetic groups consists of riboflavin-derivatives such as FMN and FAD. While oxidizing their substrates (AH_2), these enzymes transfer two elections and two protons from the substrate to their flavin prosthetic grops, reducing the latter to $FMNH_2$ and $FADH_2$ respectively.



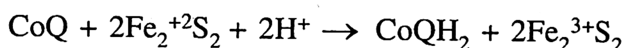
$$AH_2 + FM/FAD \rightarrow A + FMNH_2/FADN_2$$

FMN-dependent dehydrogenases include NADH dehydrogenase of mitochondrial respiratory chain. *FAD-dependent dehydrogenases* include mitochondrial succinate dehydrogenase of the TCA cycle and acyl –CoA dehydrogenase of beta-oxidation. Dihydrovatate dehydrogenase, a mitochondrial enzyme for pyrimidine synthesis bears both FMN and FAD as its prosthetic groups, along with Fe^{3+}.

Most flavin-dependent dehydrogenases get their reduced prosthetic groups ($FMNH_2$) oxidized be transferring their reducing equivalents through mitochondrial iron-sulfur proteins to coenzyme Q of the respiratory chain.



$$FMNH_2 / FADH_2 + 2Fe_2{}^{3+}S_2 \rightarrow FMN/FAD + 2Fe_2{}^{3+}S_2 + 2H^+$$



$$CoQ + 2Fe_2{}^{+2}S_2 + 2H^+ \rightarrow CoQH_2 + 2Fe_2{}^{3+}S_2$$

(c) Iron-sulfur proteins :

These are electron-transporting components of the mitochondrial respiratory chain (see 2.4). They occur in the inner mitochondrial membrane mainly as protein-bound $2Fe_2S_2$ and Fe_4S_4 clusters, in which respectively 2 and 4 nonheme iron atoms are linked respectively to as many acid-labile inorganic sulfides and are also coordinately bound to the side chain SH groups of specific cystaine residues of apoproteins. Iron-sulfur proteins transport electrons from the reduced flavin prosthetic groups, such as $FMNH_2$ of NADH dehydrogenase and $FADH_2$ of succinate dehydrogenase, to coenzyme Q of the mitochondrial chain. During such process, the Fe^{3+} ions-sulfur proteins get first reduced to Fe^{2+} ions on accepting electrons from the reduced flavins and next oxidized back to Fe^{3+} ions by donating those electrons to CoQ.

(d) Cytochromes :

These are hemoproteins containing Fe^{3+} in their oxidized ferricytochrome ($CyFe^{3+}$) forms and Fe^{2+} in their reduced ferrocytochrome ($CyFe^{2+}$) forms. In aerobic cells such as hepatocytes, myocytes, adipocytes and cardiac myocytes, cytochromes b_H, b_L, b_{560}, C_1, C, a and a_3 occur as components of the mitochondrial respiratory chain; they differ from each other in their apoproteins (apocytochromes) and/or in their heme prosthetic groups (eg. *heme a, heme b* and *heme c*). Ferricytochrome ($CyFe^{3+}$) gets reduced to ferrocytochrome ($CyFe^{2+}$) receiving electron from an iron-sulfur cluster or another ferrocytochrome, and gets reoxidized $CyFe^{3+}$ by donating that electron to CoQ or iron-sulfur cluster or another ferricytochrome or molecular O_2. Of the cytochromes of the inner mitochondrial membrane, cytochromes a and a_3 together constitute an oxidase, *cytochrome oxidase* (Cy a-a_3), that transfers the electron directly to molecular O_2; but the others function as dehydrogenases, not donating electrons directly to molecular O_2 and instead, carrying electrons in a specific serial order to reach the electrons to Cy a-a_3 for the final flow of those electrons to molecular O_2 : $CoQ \rightarrow Cyb_L \rightarrow Cyb_H \rightarrow Cyc_1 \rightarrow Cyc \rightarrow Cya \rightarrow Cya_3 \rightarrow O_2$.

2.3 Redox potential

Each substance occurs in any system in two forms : its oxidized or oxident form can accept electrons from another substance, thereby oxidizing the latter and itself getting reduced while its reduced or *reductant* form can donate electrons to some other substance, thereby reducing the latter and itself getting oxidized to change into the oxidant form. The oxidant and reductant forms of any substance remain in equilibrium with one another and constitute a *conjugate redoxpair or redox couple;* e.g., pyruvate (oxidant)/lactate (reductant), FAD/$FADH_2$, FMN/$FMNH_2$, NAD^+/NADH, $NADP^+$/NADPH, $Fe_4^{3+}S_4$, oxaloacetate/malate, fumarate/succinate, CoQ/$CoQH_2$, ferricytochrome a/ferrocytochrome a.

Redox potential or oxidation-reduction (OR) potential of any substance is a measure of its electron-affinity. As the electron-affinity is determinant for the donation or acceptance of electrons by a substance, its redox potential gives an estimate of its tendency to accept or donate electrons.

Redox potential of substance is estimated by placing an electrode in an equimolar solution of its oxidant and reductant forms and measuring its potential against a standard hydrogen electrode. For measuring the *standard redox potential* (*Eo*) of a substance at pH o.o, a *standard reference half-cell* is constituted by dipping a hydrogen electrode in a 1 molar solution of H^+ at 25°C and bubbling H_2 gas at 1 atmosphere pressure through hat solution (Fig. 2.1) A *sample half-cell* is also constituted by

immersing another electrode in an equimolar solution of the oxidant (Ox) and reductant (Red) forms of the substance, each of a concentration of 1M. The electrodes in the two half-cells are connected to a voltmeter while the electrical continuity is maintained by connecting the solutions of the half-cells by an agar or salt bridge. The voltmeter reading gives the E_0 of the substance under in vestigation. The *standard redox potential of biological system* (E'_0) is, however, determined using a standard reference half-cell containing 10^{-7} M solution (pH 7.0) of H^+ ions.

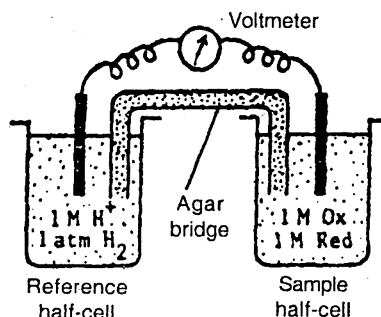


Figure 2.1 : Set-up for measuring the Eo of a redox couple
(From D. Das, Biochemistry, Academic Publishers, 2002)

For a substance with a *higher electrons-affinity* than H_2, electrons donated by H_2 of the reference half-cell will flow from there to the sample half-cell to join the oxidant member (O_x) of that substance; this will make the sample half-cell electrode positive to the reference electrode and the E'_0 and from the voltmeter will be positive. Thus, a higher, i.e., more positive or less negative, redox potential indicates a *stronger electron—affinity,* and consequently a *stronger oxidizing capacity* of the oxidant (O_x) member of the relevant redox couple. For a substance with a *lower electron-affinity* than H_2, electrons donated by its reductant form will flow from the sample half-cell to the reference half-cell to join H_2 ions there; this will make the sample half-cell electrode electronegative to the reference electrode and the E'_0 given will become negative. Thus, a lower, i.e., more negative, redox potential indicates a *lower electron-affinity* of the reductant. It follows, therefore, that :

(*i*) the higher or more positive the E'_0 of a redox pair the stronger is its oxidant member (e.g., ferricytochrome c) as an oxidizing agent and the weaker is its reductant member (e.g., ferrocytochrome c) as a reducing agent;

(*ii*) the lower or more negative the E'_0 of a redox pair, the weaker is its oxidant member (e.g. $NADP^+$) as an oxidizing agent and the stronger is its reductant (e.g. NADPH) as a reducing agent;

(*iii*) in any oxidation-reduction reaction, electrons will flow form reductant member of a redox couple with a lower E'_0 to oxidant member of another redox couple with

a lower E'_0 to the oxidant member of another redox couple with a higher E'_0 |—| this would oxidize the reductant of the first redox couple to its oxidant form and would reduce the oxidant of the second redox couple to its reductant form.

To cite an example, isocitrate gets oxidized to α-Ketoglutarate by donating electrons to NAD^+ and reducing the latter to NADH, because the E'_0 (–0.38 volt) of E'_0 the α-Ketoglutarate / isocitrate redox couple for lower than the (–0.35 volt) of the NAD^+/NADH redox couple.

However, the redox potentials of two redox couples would be the sole determinant of electron-flows between them, only when the oxidant and reductant members of each couple occur in-equimolar concentrations, i.e., in the so-called standard conditions. Whenever the oxidant and reductant members of either couple are not present in the system in equimolar concentrations, electron-flow between the two redox couples will depend on the resultant effect of their respective E'_0 values and the ratio of oxidant and reductant concentrations of each couple, and is consequently determined by the determined by the difference between the *electron transfer potentials (E)* of the two couples. For each couple. E is given by the following *Nernst equation.* Where R is the molar constant (8.314 joules/degree/mol). T is one absolute temperature (assumed here as 298 K or 25°C), F is the Faraday constant (96487 coulombs/mol) and n is the number of electrons transferred.

$$E = E_0' + \frac{RT}{nF} \text{In} \frac{[\text{Ox}]}{[\text{Red}]}; \text{of}, E = E_0' + \frac{0.059}{n} \log \frac{[\text{Ox}]}{[\text{Red}]}$$

Thus, a change in the relative concentrations of the oxidant and reductant members of one or both the redox couples may after the difference (ΔE) between their electron transfer potentials so as to reverse the direction of electron-flow between those couples. For example, during anaerobic glycolysis, pyruvate is reduced to lactate by the reducing equivalents (e and H^+) from NADH, because (*i*) the pyruvate / lactate redox couple has a higher E'_0 of –0.185 volt than the E'_0 (–0.315 volt) of the NAD^+ / NADH couple and (*ii*) the [OX]/[Red] ration of the first couple is increased while that of the second one is lowered by the rise in cytoplasmic concentrations of respectively pyruvate and NADH of glycolysis, thus lowering the electron transfer potential of the second couple much below that of the first one. In the aerobic condition after glycolysis, on the contrary, (*i*) the difference ($\Delta E'o$) between the $E'o$ values of the two redox couples remains as before, but (*ii*) the [Ox]/[Red] ratio of the pyruvate/lactate couple is lowered by the accumulated lactate while that of the NAD^+/NADH couple is raised by the reoxidation of the accumulated NADH; these result in a rise in the electron transfer potential (E) of the latter couple for above that of the former, bringing about electron flow from lactate to NAD^+ and redoxidation of lactate to pyruvate.

2.4 Mitochondrial respiratory chain

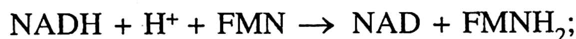
Mitochondrial *respiratory or electron transport chain* consists of a specifically arranged set of electron transporters in the inner mitochondrial membrane for carrying out aerobic oxidations of NADH and reduced flavins ($FMNH_2$ and $FADH_2$) by transporting reducing equivalents (*e* and H^+) from them to molecular O_2. Each electron transporter of this chain acts as a *redox couple* |——| you have read about redox couples in the preceding section 2.3 on redox potential. Such electron carriers are located in proper and steric relation to each other in order of progressively rising redox potentials. This enables electrons to flow from the reduction member of each such redox couple of the chain to the oxidant member of the next one having a higher E_0 unit they are finally donated to molecular O_2. The mitochondrial ET chain they constitutes the *final electron-flow* path from tissue substrates to molecular O_2. Two components of the ET chain, viz., cytochrome c and co-enzyme Q, are not intergral proteins of the membrane |——| cytochrome c is a peripheral protein on the cytoplasmic (outer) surface of the inner membrane while coenzyme Q (ubiquinone) is nonpolar, lipid-soluble benzoquinone derivative, not bound to any protein and diffusing freely in that membrane. Other components of the ET chain, viz, NADH dehydrogenase, succinate dehydrogenase, iron-sulfur clusters and cytochromes b_{560}, b_{11}, b_1, c_1, a and a_3, remain bound to specific integral proteins of the inner membrane; each such set of intergral protein-bound components |——| except cytochrome C and CoQ |——| is organized into one or other of four *respiratory chain complexs* which together constitute a *respiratory chain assembly.* Thousands of such respiratory chain assemblies may occur per cubic micrometer of the inner membranae of an aerobic cell.

2.4.1 Respiratory chain complexes

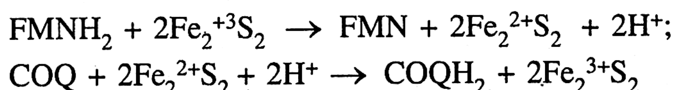
The integral protein-bound components of each ET chain are organised into the following four respiratory chain complexes in the inner membrane. Components of each such complex are oriented asymmetrically in the membrane and arranged in specific steric relations to each other and in an ascending order of their redox potentials.

a. Complex I or NADH-Q reductase :

This large transmembrane oligomeric integral protein (MW 850 Kdal) has FMN and several FE_2S_2 and FE_4S_4 iron-sulfur clusters as its prosthetic groups. It functions as NADH dehydrogenase transferring reducing equivalents (*e* and H^+) from NADH through FMN and iron-sulfur c lusters of its prosthetic groups to coenzyme Q, thereby oxidizing NADH to NAD^+ and reducing CoQ (ubiquinone) to $CoQH_2$ (ubiquinone).



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



$$FMNH_2 + 2Fe_2{}^{+3}S_2 \rightarrow FMN + 2Fe_2{}^{2+}S_2 + 2H^+;$$
$$COQ + 2Fe_2{}^{2+}S_2 + 2H^+ \rightarrow COQH_2 + 2Fe_2{}^{3+}S_2$$

You have already read that *COQ* is a lipid-soluble benzo-quinone derivative, not bound to any protein and not belonging to any of the respiratory chain complexes. Diffusing freely in the lipid bilayer of the inner membrane, CoQ can make alternate contacts with other electron transporters such as iron-sulfur clusters and cytochromes of different respiratory chain complexes and transports electrons between them. In this way, it functions as a mobile electron transporter between either complexes I and III or complexes II and III. Mammalian CoQ is called Q_{10} because of then C_5 isoprenoid units forming its long nonpolar sidechain.

b. Complex II or succinate-Q reductase :

This oligomeric nonspanning integral protein (MW 127 Kdal) consists of a dimeric flavoprotein called *succinate dehydrogenase* with FAD prosthetic group, a few Fe_4S_4 ion-sulfur clusters, and cytochrome b_{560} with a heme to prosthetic group. Succinate dehydrogenase oxidized succinate to fumarate using FAD as the electron acceptor. The $FADH_2$ is reoxidized by the transfer of its reducing equivalents through cytochrome b_{560} and iron-sulfur clusters to CoQ, reducing the latter to QH_2. You should bear in mind that electrons cannot pass from succinate to COQ through complex I because succinate has a higher *E'o* (+ 0.30 V) than *E'o* (– 0.315) of the NAD^+ of that complex.

c. Complex III for QH_2-cytochrome c reductase :

This large oligomerie integral protein (MW 280 Kdal) consists of a single transmembrane *apocytochrome b* bearing two *heme b* prosthetic groups at two different sites, one *cytochrome* c_1 having a *heme c* prosthetic group, and nonspanning and nonheme *Rieske iron-sulfur protein* with a Fe_2S_2 cluster. Because of their locations at different sites on apocytochrome b molecule, the two heme b molecules, differ in their electron-affinities and consequently function as cytochromes b_L (*E'o* = –0.03 V) and b_H (*E'o* = + 0.03V) respectively.

Complex III reoxidized QH_2, formed by the electron-transport by either complex I or complex II, to COQ by transferring its electrons through a postulated *Q cycle* (see 2.5.5) to ferricytochrome c_1 (Cye_1 Fe^{3+}) thereby reducing the latter ferrocytochrome c_1 (Cyc_1 Fe^{3+}).

Ferrocytochrome c_1 next gets reoxidized by donating its electron to ferricytochrome c (Cye Fe^{3+}) and reducing the latter to ferrocytochrome c (Cye Fe^{2+}). You should realize that *cytochrome c,* though not a component of any respiratory chain complex, serves as an electron-transferring link between complexes III and IV by receiving electrons from cytochrome c_1 or complex III and donating them to cytochrome a of complex IV.

d. Complex IV or cytochrome oxidase :

This large oligomeric transmembrane protein (MW 200 Kdal) carries two *heme* a molecules (heme a and a_3) bound to two different sites on its peptic subunit I, and two copper ions (Cu_a and Cu_{a3}) bound respectively to its subunits II and I. Because of the bindings of heme groups and copper ions to separate sites on the apoprotein, the heme a-Cu_a combination has a lower electron affinity (lower *E'o*) than that of the heme a_3-Cu_{a3} combination. The meme a-Cu_a combination forms the *cytochrome c-binding site* and acts as the *anaerobic oxidizing unit* (cytochrome a) of complex IV|——| it accepts electrons from ferrocytochrome c to reoxidize it to ferricytochrome c and subsequently donates them to the heme a_3—Cu_{a3} combination; the latter, having a higher *E'o* forms the *O_2-binding site* and acts as the *aerobic reduction unit* (cytochrome a) donating the electrons, received from heme a-Cu_a unit, to molecular O_2. Both the heme iron and the copper ion of each of the two units alternate between respectively ferric $\rightleftarrows$ ferrous and cupric $\rightleftarrows$ cuprous forms while transporting electrons. Complex IV thus functions as a *copper-containing aerobic oxidase or cytochrome a–a_3* complex (see 2.2.2).

2.4.2 Electron-flow paths

You will read below now the electrons from different metabolites, being oxidized, follow different routes along the mitochondrial ET chain to reach molecular O_2.

(*a*) Some metabolites such as pyruvate from glycolysis, α-ketoglutarate and isocitrate of the TCA cycle, and 3-hydroxy cacyl-CoA of beta-oxidation posses electron transfer potentials lower than that of NAD^+ of their respective dehydrogenases at their respective normal [Ox] / [Red] ratios in mitochondria. So, these substrates donate their electrons to NAD^+ producing NADH during their oxidations. Again, because of a higher *E'o* of the FMN of NADH-Q reductase than that of NADH, the latter can transfer the electrons so that FMN, thus reaching the electrons to complex I. Electrons from the $FMNH_2$ of complex I then flow through the successive components of

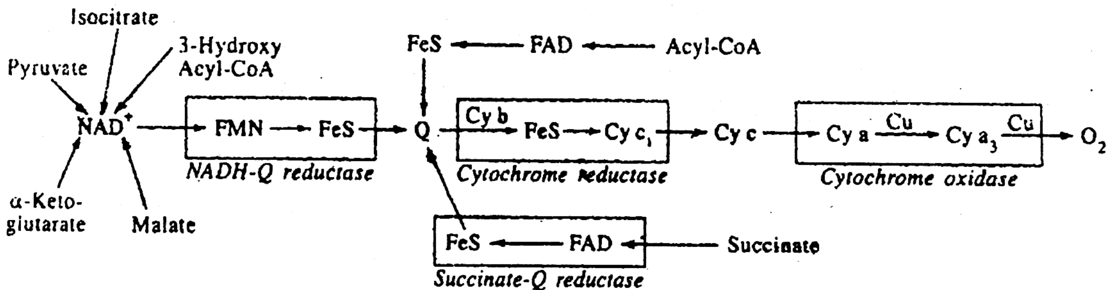


Figure 2.2 : Electron-flow paths through the respiratory chain.
(From D. Das, Biochemistry, Academic Publishers, 2002)

complex I to CoQ along their progressively rising $E'o$ and E values, reducing CoQ to QH_2 (Fig. 2.2)

(*b*) Succinate from the TCA cycle is oxidized by succinate dehygenase which transfers its reducing equivalents to FAD. Similarly, acyl-CoA dehydrogenase oxidizes acyl-CoA in betaoxidation, using FAD as the electron-acceptor. From the $FADH_2$ thus produced, electrons can pass through iron-sulfur relatively higher $E'o$ and E values of $FADH_2$ (see 2.4.1).

(*c*) Electrons from QH_2, whether coming from complex I or complex II, pass successively through the components of complex III, cytochrome C and complex IV. Because of their progressively rising $E'o$ and Eo values, and finally reach molecular O_2.

2.4.3 Free energy changes during electron flow

For any oxidation-reaction, the *negative free-energy change* ($\Delta G^{o\prime}$) due to electron-flow from the reductand of one redox couple to the oxidant of another redox couple with a higher $E'o$ is given by the following equation depending on the difference in redox potential ($\Delta E'o$) between the two redox couples, the calorie equivalent (F) of the Faraday constant (F = 23.063 Kcal), and the number (n) of electrons passing from one redox couple to the other.

$$\Delta G^{o'} = - nF\Delta E'o.$$

The $\Delta G^{o'}$ of the elections transfer between two successive redox couples at any step of mitochondrial electron transport must-exceed the $\Delta G^{o'}$ (– 8 Kdal) of hydrolysis of a high-energy ATP bond it any such bond has to be formed with the energy released at that step of mitochondrial electron transport. You will understand from the following computation that when two electrons (n = 2) flow over a $\Delta E'o$ + 0.22, $\Delta G^{o'}$ amounts to –10.15 Kcal and may be almost the minimum for generating an ATP bond ($\Delta G^{o\prime}$ –8 Kcal) :

$$\Delta G^{o'} = - nF\ \Delta E'o = - 2 \times 23.063 \times 0.22$$
$$= - 10.15 \text{ Kcal}$$

Negative free energy changes (– $\Delta G^{o\prime}$) along the rising $\Delta E'o$ at different sites of mitochondrial electron transport are brought about by the *exergonic transfer of electrons* between successive components of the respiratory chain complexes. If the negative $\Delta G^{o'}$ resulting at a particular site of such electron transfer is sufficiently higher in magnitude than the positive $\Delta G^{o'}$ of the endergonic phosphorylation of ADP to ATP the exergonic electron transport at that site may by coupled with the endergonic synthesis of ATP to drive the latter reaction.

(a) *Complex I* (NADH-Q reductase) transports two electrons over a $\Delta E'o$ of more than +0.36V from NADH ($E'o$ = 0.315 V) to CoQ ($E'o$ = + 0.045V). So, using the aformentioned equation, the $\Delta G^{o'}$ per electron-pair transported by complex I amounts to about – 16.61 Kcal/mol which suffices for the formation of one ATP bon (Table 2.1).

$$\Delta G^{o'} = nF\Delta E'o = -2 \times 23.063 \times 0.36 = -16{,}61 \text{ Kcal.}$$

(b) *Complex II* (succinate-Q reductase) transports two electrons over the $\Delta E'o$ of +0.015V from succinate ($E'o$ = +0.030V) to CoQ ($E'o$ = +0.045), thus generating the $\Delta G^{O'}$ of – 0.69 Kcal/mol. This poor $\Delta G^{o'}o$ does not suffice for forming any hing energy bond of ATP.

$$\Delta G^{o'} = nF\Delta E'o = -2 \times 23.063 \times 0.015 = -0.69 \text{ Kcal.}$$

(c) *Complex III* (QH_2 –cytochrome C reductase) transports two electrons over $\Delta E'o$ of + 0.19 V from QH_2 ($E'o$ = + 0.045V) to cytochrome C ($E'o$ = + 0.235 V), thus generating the $\Delta G^{o'}$ of – 8.76Kcal/mol. |——| this suffices for forming a high-energy ATP bond.

$$\Delta G^{o'} = nF\Delta E'o = -2 \times 23.063 \times 0.19 = -8.76 \text{ Kcal.}$$

(d) *Complex IV* (cytochrome oxidase) transports two electrons over the $\Delta E'o$ of + 0.58 IV from ferrocytochrome C ($E'o$ = + 0.235V) to molecular O_2 ($E'o$ = + 0.816 V), thus generating the $\Delta G^{o'}$ of – 26.80 Kcal |——| this suffer for forming another ATP bond.

$$\Delta G^{o'} = nF\Delta E'o = -2 \times 23.063 \times 0.581 = -26.80 \text{ Kcal.}$$

Table 2.1 ET Chain sites for forming high-energy bonds.

Complex	Electron transfer	$\Delta E'o$	$\Delta G^{o'}$	~P bonds formed
I	NADH → CoQ	+ 0.360 V	–16.61 Kcal	I
II	Succinat → CoQ	+ 0.015 V	–0.69 Kcal	0
III	QH_2 → Cyc Fe^{3+}	+ 0.190 V	–8.76 Kcal	I
IV	Cyc Fe^{2+} → O_2	+ 0.51 V	–26.80 Kcal	I

Thus, the $\Delta G^{o'}$ resulting from the transport of an electron-pair from pyruvate, α-ketoglutarate, malate, isocitrate or 3-hydroxyacyl-CoA to molecular O_2 by complexes I, III and IV successively, can be utilized to from one high-energy bond of ATP at each of those sites, making a total of *three high-energy bonds* during the aerobic oxidation of those substrates. But the transport of an electron-pair from succinate of acyl-CoA bypasses complex I and instead passes through complex II, III and IV,

resulting in a total yield of *two high energy bonds.,* because the $\Delta G^{o'}$ of electron-transport but complex II does not suffice in forming any ATP.

2.5 Oxidative phosphorylation

Phosphorylation of ADP to ATP involves the endergonic formation of a high-energy phosphorisc anhydride bond, having a high positive $\Delta G^{o'}$. This endergonic reaction has to be driven by being coupled with and exergonic reaction with a negative $\Delta G^{o'}$ of higher magnitude. This can be accomplished by either substrate-level or oxidative phosphorylations.

2.5.1 Substrate-level and and oxidative phosphorylations

These are two important processes of ATP formation by the phosphorylation of ADP.

(*a*) *Substrate-level phosphorylation :*

These can take place in both anaerobic and aerobic metabolisms. A high-energy metabolic intermediate is first formed as an intermediate in a metabolic pathway; e.g., 1, 3-bisphosphoglycerate and phosphoenolpyruvate, each carrying a high-energy phosphate bond, are formed in glycolysis while succinyl-CoA having a high-energy thioester bond is formed in the TCA cycle. Next, the exergonic cleavage of the high-energy bond in the metabolic intermediate, with a high negative $\Delta G^{o'}$, is coupled with the endergonic phosphorylation of ADP or GDP respectively to ATP and GTP directly utilizing the bond energy of the metabolic intermediate. Such phosphorylations of ADP or GDP do not involve aerobic respiration, use of molecular O_2, and mitochondrial electron transport.

(*b*) *Oxidative phosphorylation :*

These can take place during aerobic metabolisms only and involve the transport of electrons from substrates, being oxidized, to molecular O_2 by the components of the mitochondrial ET chain. Yo may recall that such mitochondrial electron transport is *exergonic* because it takes place along the progressively rising redox potentials of the successive components of respiratory chain complexes; at specific sites of the chain, the exergonic electron transport has negative $\Delta G^{o'}$ values of higher magnitudes than the positive $\Delta G^{o'}$ of the *endergonic* phophosylation of ATP to ADP. So, the exergonic mitochondrial electron transport can be used for driving the endergonic phosphorylation of ADP by the ATP *synthesis* of inner membrane particles. This is known as oxidative phosphorylation.

2.5.2 P : O ratio

It is ratio between the number of phosphate groups incorporated into ATP by esterification to form high-energy phosphate bonds, and the number of oxygen atoms reduced by electron-pairs transferred to them by mitochondrial electron transport to accomplish that esterification.

$$P : O = \frac{\text{number of phsohate groups esterified}}{\text{number of oxygen atoms reduced}}.$$

The P : O ratio serves as an index of oxidative phosphorylation, indicating the number of high-energy phosphate bonds formed by the transfer of electron-pairs from specific substrates to one oxygen atom. For example, oxidations of substrates such as pyruvate and malate by NAD^+_dependent dehydrogenases produce a P : O ratio of 3, because each electron-pair from NADH is transported of one oxygen atom successively by repiratory chain complexes I, III and IV, generating three ATP bonds |——| one at each of those three sites of the respiratory chain. On the contrary. FAD-dependent oxidations of substrates such as acyl-CoA and succinate produce a P : O ratio of 2, because each electron-pair from $FADH_2$ would reduce an oxygen atom by being transported only by complexes II, III and IV, by-passing complex I and thus generating only two ATP bonds |——| one at each of the sites in complexes III and IV. However, these prevalent P : O ratios are now thought actually to amount to 2.5 and 1.5 respectively because of factors such as mitochondrial proton leakages and presence of AMP in mitochondria in addition to ADP and ATP.

2.5.3 ATP synthesis

An inner membrane protein, called the *proton-translocating ATP synthesis,* catalyzes the endergonic phosphorylation of ADP to ATP in the mitochondrial matrix the negative $\Delta G^{o'}$ of the exergonic electron transport along the mitochondrial ET chain. This enzyme occurs in the headpiece of each of the numerous dumpbell-shaped *inner membrane particles* projecting into the mitochondrial matrix from the

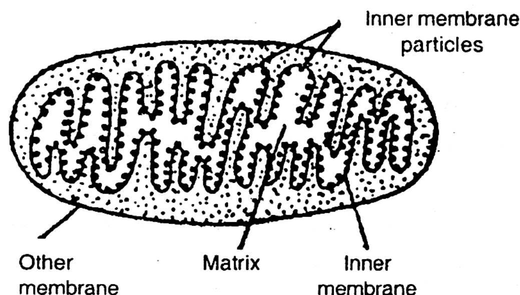


Figure 2.3 : Sectional view of a mitochondrion
(From D. Das Biochemistry, Academic Publishers, 2000)

matrix surface of the membrane and its cristase (Fig. 2.3). Each such particle (MW 450 Kdal) consists of the following three main parts.

(*i*) A cylindrical *basepiece,* 22 nm in diameter, is lodged in the inner membrane as nonpolar transmembrane oligomeric integral protein (*Fo unit*). A circular ring of a *DCCD-binding proteolipid* (MW 8 Kdal) eneireles a polar *proton channel* running across the membrane from its cytoplasmic surface to its matrix surface (Fix. 2.4).

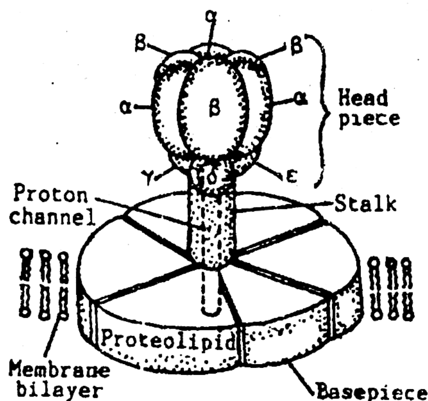


Figure 2.4 : Schematic diagram of an inner manbrane Particle
(From D. Das, Biochemistry, Academic Publishers, 2000)

(*ii*) *An oligomycin-sensitivity conferring protein* (MW 23 Kdal) and a *coupling F_6 protein* (8 Kdal) form a narrow 5-mm long *stalk* joining the F_O unit of basepiece to the head-piece of the particle.

(*iii*) A 370-KD polar, oligomeric, peripheral membrane protein (*F_l unit*) constitutes the spheroidal *head piece* of the particle, and held by its stalk, projects into the mitochondrial matrix. The F_1 unit 10nm × 8nm in size, is made of three α, three β, and one each of γ, δ and ε peptide subunits. The α and β subunits are arranged alternately in a circle in the F_1 unit, in the form of three αβ pairs; the α and β subunits of each such pair partly differ from those of the other two pairs in their conformations as also in their special relations with the single γ subunit. The β subunit of each αβ pair bears a catalytic site of the *ATP synthase.* The δ subunit of F_1 is coupled to the matrix and of the proton channel running through the F_0 unit and thus constitutes functionally a gate of that channel leading to F_1 unit.

So long as the transmembrane proton channel remains open in the inner membrane particle and an inward proton gradient is maintained across the inner membrane, the energy released by the exergonic down-gradient inward translocation of protons through the proton channel drives the endergonic phosphorylation of ADP to ATP by the ATP synthase of the F_1–F_0 particles. But if (*i*) the proton channels are closed, eg., by the action of DCCD (*ii*) the trans-membrane proton gradient is abolished by the

action of proton-translocating ionophores such as dinitrophenol or (*iii*) the F_1 unit is isolated from the F_o unit by the action of urea, then the F_1 unit nolonger acts as the ATP synthesis and functions instead as *ATP* to hydrolyze ATP to ADP and P_1.

Boyer's binding-change model, in its present modified from, proposers that in every F_1 unit, the catalytic site on each β subunit acts cyclically due to conformation changes of the F_1 unit brought about by a transmembrane inward translocation of protons down a proton gradient resulting from electron transport. This model is briefly described below.

The β subunits of three αβ pairs of each F_1 unit have different conformations at any time : (*i*) the catalytic site on the β subunit of one αβ pairs exists as an *inactive O site* with an open conformation and possesses no affinity to bind to the substrates. ADP and Pi; (*ii*) the catalytic site on the β subunit of the second αβ a pair occurs as catalytically inactive *L site* binding only loosely to the substrates ; (*iii*) the catalytic site of the β subunit of the third αβ pair exists as the tightly binding and active *Tsite* (Fig. 2.5). The down-gradient proton inflow through the proton channel in the inner membrane particle produces the following changes cylically in the β subunited of each αβ pair of the F_1 unit.

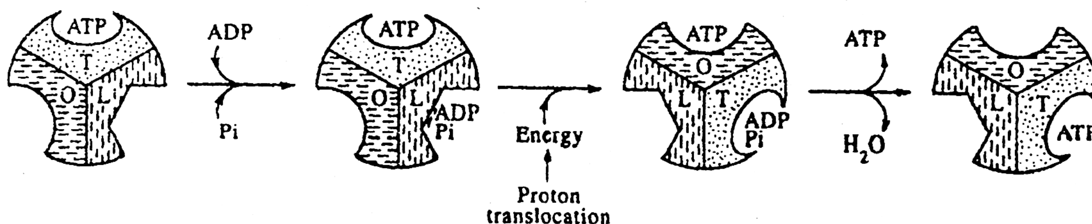


Figure 2.5 : Boyer's binding change model for ATP synthese action (Atter R. L. Coss, Annu Rev Biochemistry, 50 : 687, 1981)

(*a*) The L site holds ADP and Pi initially loosely, the T site holds and ATP tightly, and the O site holds no substrate.

(*b*) The down-gradient proton-inflow through the proton channel of the F_1 unit releases energy which causes the rotation of its γ subunit; this changes (*i*) the conformation of the *T site to an O site,* releasing the ATP from it, (*ii*) that of the *L site to a T site* which now holds on the loosely-bound ADP and Pi moretightly, catalyzes the formation of ATP from them and then continues to hold that ATP tightly, and (iii) the conformation of the *pre-existing O site to a new L site* which binds loosely to ADP and Pi now.

These changes are cyclically repeated to make the β subunit of each αβ pair pass successively through O → L → T conformational and associated functional changes, bringing about the successive steps of ATP synthase activity.

2.5.4 Chemiosmotic theory

There is no direct physical association between the respiratory chain complexes of the inner membrane and the ATP synthase in the headpiece of the inner membrane particle; so, the energy released during mitochondrial electron transport has to be conserved by its conversion to a form that can be subsequently utilized by ATP synthease in ATP formation. This process is known as the *energy transduction or coupling between mitochondrial oxidation and ATP synthesis* Peter Mitchel proposed nis *chemiosmotic theory* to explain this coupling.

According to this theory, the free energy released by electron transport along the mitochondrial ET chain is immediately harnessed in actively translocating protons from the mitochondrial matrix across the inner membrane to the outer compartment of mitochondrion. This active outward trans-membrane proton-translocation results in a higher proton (H^+) concentration outside that membrane. This in turn leads to (*i*) a *pH gradient* (ΔpH) with the outside pH 1.4 units below that in the mitochondrial matrix, and (*ii*) a *transmembrane potential* ($\Delta\Psi$) with the inner side of the membrane electron-negative to the extent of 0.14 volt relative to its outer side. The resultant of these two is an *electrochemical potential* (ΔP) with a consequent inward transmembrane proton (H^+) gradient. Where R and F are respectively the molar gas constant (8.314J/K/mol) and the Faraday constant (96-187C/mol. and T is the temperature of 298K.)

$$\Delta P = \Delta\Psi - 2.303\frac{RT}{F}\Delta pH = 0.14 - 2.303\times\frac{8.314\times 298}{96487}\times(-1.4)$$

$$= 0.14 - 0.059\ (-\ 1.4) = 0.224\ \text{V}.$$

As the protons, thus pumped out, flow inward down the consequent proton gradient through the proton channel of the inner membrane particle, the energy released by their down-gradient exergonic inflow is used by ATP synthase of F_1 unit in catalyzing the endergonic phosphorylation of ADP to ATP.

At least two protons must be actively translocated outward across the inner membrane to suffice for the formation of own ATP bond; Mitchell proposed in his *redox loop model* (See 2.5.5) that two protons can be so translocated during the transport of each electron-paid by each of the repiratory chain complexes I, III and IV. So, three ATP bonds should be expected to be formed in consequence of the transport of an electron-paid from NADH to molecular O_2 through complexes I, III and IV. However, *in vivo* nearly three protons may have to be translocated to provide for each ATP bond, because some translocated protons may diffuse back across the inner membrane, lowering the electrochemical gradient ΔP below what is theoretically anticipated.

2.5.5 Redox loop mechanism

According to this model proposed by Mitchell, the mitochondrial ET chain is organized into *three redox loops* (Fig. 2.6) Each loop includes a respiratory chain complex and transports electrons first from the inner (matrix) surface to the outer (cytoplasmic) surface of inner membrane and then, back again to the inner surface. During such trnasport of each electron-pair, each loop simultaneously translocatres two protons outward and releases them into the cytoplasm from the outer surface of the membrane. For functioning in this manner, the respiratory chain complex are oriented a symmetrically in the inner membrane.

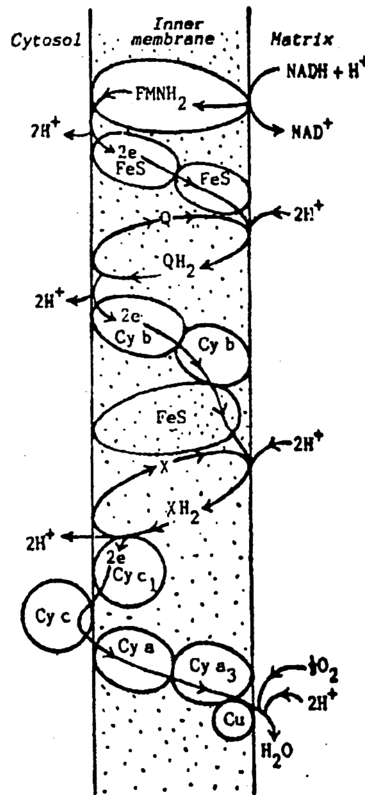


Figure 2.6 : Mitechell's redox loop model
(From D. Das, Biochemistry, Academic Publishers, 2000)

(*a*) *First redox loop :*

This is constituted by an integral membrane protein which spans the entire thickness of the membrane and functions as *NADH-Q reductase* or respiratory chain *complex 1.* Its. FMN prosthetic group receives a pair of electrons along with protons from NADH of the matrix and gets reduced to $FMNH_2$ The latter donates the lecrons

to the iron-sulfur clusters of complex I near the outer surface of the membrane and simultaneously release two protons from that surface to the cytoplasm. The iron-sulfur clusters then transport the electrons back to the inner surface of the membrane and transfers them to CoQ there; the latter is reduced to QH_2 (ubiquinol) by taking up two protons simultaneously from the matrix.

(*b*) *Second redox loop :*

This consists of another transmembrane complex which functions as *complex II* or *cytochrome c reductase.* QH_2 (see above) diffuses through the membrane lipid bilayer to its outer surface where it gets reoxidized to CoQ by donating its electrons to cytochrome b_L (b_{560}) of complex III and releasing its two protons into the cytoplam. These electrons are then transferred to cytochrome b_H(b_{562}) and iron-sulfur cluster of complex III. It was initially supposed that a hypothetical mobile electron-carrier (X) would receive the electrons from the iron-sulfur cluster of complex III near the inner surface of the membrane and two protons from the adjacent matrix to become reduced to XH_2 which would then carry the electrons to the next redox loop by diffusing through the membrane lipid bilayer.

(*c*) *Third redox loop :*

This loop, Mitchell proposed, consists of *cytochrome C* and *complex IV* or *cytochrome oxidase.* The reduced hypothetical carrier (XH_2) was supposed to diffuse across the inner membrane to its outer surface where it would get reoxidized by donating its electrons to cytochrome c_1 and simultaneously releasing its protons into the adjacent cytoplasm. Electrons received by cytochrome c_1 would then be carried successively by cytochromes c, a and a_3 to the inner surface of the membrane where the electrons would finally be transferred to molecular O_2 in the matrix.

Mitchell tried to explain in this way how three pairs of protons were translocated from the matrix to the cytoplasm during the transport of one electron-pair successively by complexes I. III and IV. But the hypothical electron-carrier (X) supposes to link the second and third loops could never be found.

Q cycle :

This cycle of events has been proposed to avoid assuming a hypothetical electron-carrier (X) for linking complexes III and IV, and to suggest as alternative way of proton translocation other than the direct involvement of complex IV as a third redox loop. The Q cycle proposes the translocation of *four protons* by the second redox loop itself during its role in electron-transfer, evading the need to assumed the translocation of two protons by each of second and third loops. Its proposed steps are summarized below (Fig. 2.7).

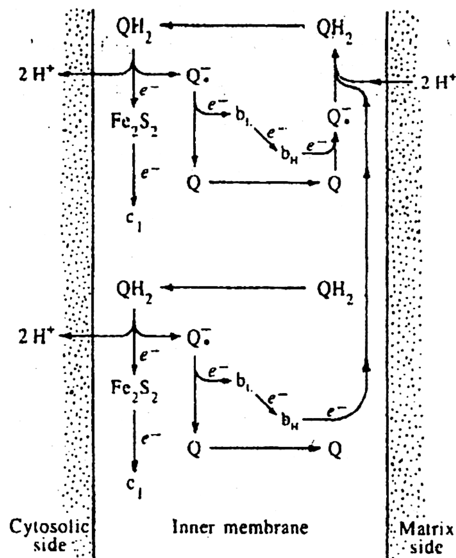


Figure 2.7 : The Q cycle (From D. Das, Biochemistry, Academic Publishers, 2005)

(*i*) QH_2, formed by the transfer of electrons from complex I of the first redox loop, diffuses from the inner (matrix) side to the outer (cytosolic) side of the inner membrane where it donates one electron to the Rieske iron-sulfur protein of complex III, releases two protons in the cytosol and forms a semiquinone anion (Q^-). The latter is reoxidized to CoQ by donating its other electron to ferricytochrome b_L, reducing it to ferrocytochrome b_L.

(*ii*) The Rieske iron-sulfur protein gets reoxidized by giving the received electron to ferricytochrome c_1 of complex III, to reduce it to ferrocytochrome c_1.

(*iii*) Two ferrocytochrome b_L molecules are reoxidized by donating their respective electrons to two cytochrome b_H (b_{562}) molecles reducing them to ferrocytochrome b_H.

(*iv*) Two ferrocytochrome b_H molecules get reoxidized by donating their electrons to a CoQ molecule near the inner (matrix) surface of the membrane; the CoQ molecule simltaneously accepts two protons from the matrix to be reduced to QH_2. The latter diffuses back to the outer (cytosolic) surface of the membrane to restart the cycle from step (i).

Thus each Q cycle translocates a total of four protons outward across the inner membrane during the transport of each electron-pair by the complex III itself.

2.5.6 Proton pump mechanism

This is an alternative model proposed to explain the outward proton translocation across the inner membrane during mitochondrial electron transport. According to this

model, some proton-translocating proteins occur in the inner membrane with two separate conformations in their oxidized and reduced forms, repectively. The oxidized form of such a proton-translocating protein has specific amino acid side-chains of its proton-binding site exposed on the inner (matrix) surface of the inner membrane; in this conformation, those amino acid side chains behave as weak acidic groups with high acid ionization exponent (pk) values and consequently possess high proton-affinities. So, protons form the matrix bind easily to such weak by acidic groups of the proton-binding site (Fig. 2.8). *Cytochrome oxidase* of the ET chain may act as such a proton-translocating protein, but there may also be other such proteins that are not direct components of the ET chain.

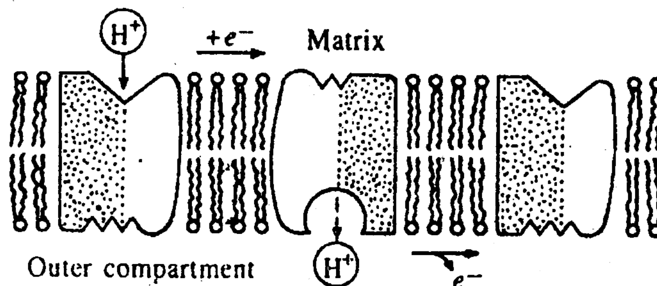


Figure 2.8 : Changes in a portion - translocator during a proton pump cycle (From D. Das, Biochemistry, Academic Publishers, 2005)

As the protonated proton-translocator gets reduced by accepting electrons from a mitochondrial respiratory chain complex, it undergoes changes in conformation and proton-affinity. As a result, the amino acid side chains at the proton-binding site of the reduced and protonated translocator get exposed now on the outer (cytoplasmic) surface of the membrane, behave as stronger acid groups with low pK values and weaker proton-affinites, and consequently release their protons into the cytosol. The subsequent reoxidation of the translocator by the transfer of its electrons to the next-electron-acceptor changes its conformation to its original deprotonated form so that its proton-acceptor site gets exposed again on the inner surface of the membrane, regains its original weak acid groups and higher proton-affinity (higher pK) and awaits fresh protonation to star the next cycle.

2.5.7 Regulation of oxidative phosphosrylation

The rate of oxidative phosphorylation depends on the mitochondrial ATP concentration. In other words, the ATP *mass action ratio* in the mitochondrical matrix determines the rate of oxidative phosphorylation—this ratio is given by [ATP] / [ADP][Pi] which is the ratio of the molar concentration of ATP and the product of molar concentrations of ADP and Pi in the matrix. A fall in this ratio or a rise in the

ratio of mitochondrial concentrations of the reductant and oxidant forms of the NAD^+/ NADH redox couple, viz., [NADH]/ [NAD^+], enhances the mitochondrial concentration of ferrocytochrome c ($CycFe^{2+}$); the latter being the substrate of *cytochrome oxidase,* the activity of the enzyme is consequently enhanced, leading to a rise in the rate of mitochondrial oxidation as also of the oxidative phosphorylation coupled with it. Stated in a different way, mitochondrial oxidation and oxidative phosphorylation are controlled largely by the mitochondrial ADP concentration (*respiratory or acceptor control*).

To maintain the rate of oxidative phosphorylation in an active tissue, the ATP mass action ratio is normally kept adequately low in the mitochondrion by the action of a homodimeric intergral protein. *ATP-ADP translocator,* of the inner mitochondrial membrane. This 60-KD protein carries out and *ATP-ADP antiport* across the inner membrane. Thus, the ATP-ADP translocator, driven by the membrane potential, transports ATP molecules outward and ADP molecules inward across the inner membrane. This normally keeps the matrix [ADP]/[ATP] ratio at about tenfold of the cytoplasmic [ADP]/[ATP] ratio. In this way, the ATP-ADP translocator functions as a *rate-limiting factor* in maintaining an adequately low ATP mass action ratio in the mitochondrion. Its inhibition by the mould anitibiotic bongkrekic acid or the glycoside atractyloside decreases the transmembrane ATP-ADP transport, there raises the mitochondrial ATP mass action ratio, and consequently brings about the inhibition of mitochondrial oxidation as also of oxidative phosphorylation. You may read further details of this translocator action in 3.4.5.

In a resting tissue, relatively less ATP needs to be to be hydrolyzed for energy production and the ATP mass action ratio remains high. This keeps the ferrocytochrome c concentrations low in the mitochondrion with a consequent decline in cytochrome oxidase activity. So rates of mitochondrial electron transport and ATP formation remain low. On the contrary, in a highly active tissue, ATP is hydrolyzed in high amounts to ADP and Pi for meeting the enhanced energy requirement, and ATP-ADP antiport is heigthened. The mitochondrial ATP mass action ratio consequently falls and ferrocytochrome c concentration rises. This leads to a rise in cytochrome oxidase activity, with resultant rises in mitochondrial oxidation and oxidative phosphorylation.

Uncoupling of oxidation and phosphorylation :

Some lipid-soluble substance such as penta-chlorophenol and dinitrophenol possess strong proton-affinities because of their weakly acid nature. So, they can bind to protons on the outer side of the inner membrane to change into neutral protonated forms which diffuse across the membrane and release the protons into the mitochondral

matrix. Thus, acting as *monile, protontranslocating ionophores,* they lower or abolish the transmembrane proton gradient, resulting in (i) a decline in proton inflow through proton channels of F_1-F_0-particles and a consequent lowering of the ATP synthase action of F_1 unit, and (*ii*) an enhanced ATP ase action of F_1 unit because of the rise of proton concentration in the matrix. The resultants of these two effects consist of an enhanced mitochondrial oxidation due to the rise in matrix ADP concentration, and decline in oxidative phosphorylation. So, the energy generated by enhanced mitochondrial oxidation is given of as heat. In this way, these lipid-soluble proton-translocators bring about an *uncoupling* of oxidation and phosphorylation. The *thermogenic* (temperatue-raising) *affect* of thyroid hormones may result partly from the uncoupling of mitochondrial oxidation and oxidative phosphorylation.

In hibernating mammals and new-born furless mammals, adrenaline secreted on cold exposure brings about an uncoupling of mitochondrial oxidation and oxidative phosphorylation. The secreted adrenaline activatives the lipolysis of brown fat in adipocytes into fatty acids which open up inner membrane proton channels made of an integral protein called *thermogenin.* This causes inward proton translocation across that membrane through the thermogenin channels and nullifies the transmembrane proton gradient, uncoupling oxidation and phosphorylation. Thus the energy from mitochondrial oxidation of fatty acids from brown fat is dissipated as heat instead of being utilized for ATP synthesis.

2.6 Summary

Biological oxidations and reductions are mostly catalyzed by oxidoreductases of four main classes, viz, oxygenases which incorporate oxygen into their substance, oxidases which oxidize their substrates by transferring electrons from the latter to molecular O_2 directly, hydroperoxidases that transfer electrons from substrates of H_2O_2, and dehydrogenases which transfer electrons from their substrates to electron-acceptors other than molecular O_2. Of the dehydrogenases, pyridine-linked dehydrogenases use either NAD^+ or $NADP^+$ as electron-acceptor coenzymes, flavin-linked dehydrogenases posses either FMN or FAD as the prosthetic group for accepting electrons from the substrates, iron-sulfur proteins have nonheme iron-sulfur clusters which accept electrons from the substrates, while cytochromes are hemoproteins with their heme-iron functioning as electron-acceptors.

Every substance exists in an oxidant form which may accept electrond from other substance, oxidizing the latter and itself being reduced to a reductant form; the latter, in turn, may donate the electrons to other electron-acceptor substances, reducing the latter and itself being reoxidized to the oxidant form. Electrons can flow

in this way from the reductant member of a substance having lower electron-affinity to the oxidant member of another substance having higher electron-affinity. Redox potential is a measure of the electron-affinity of a substance. So, it is an estimate of the tendencies for accepting and donating electrons by a substance. Electrons would flow from the reductant form of a substance having a lower or more negative redox potential, i.e., a lower electron-affinity, to the oxidant form of another substance having a higher or more positive redox potential, i.e., a higher electron-affinity. Besides, the direction of electron flow between two substances also depends on the ratios molar concentrations, of their oxidant and reductant forms in a system.

A number of redox (oxidant-reductant) couples, mostly integral proteins of the inner mitochondrial membrane, constitute a mitochondrial electron-transport or respiratory chain, transporting electrons from various substrate to molecular O_2 ultimately, in accordance to the order of their progressively ascending redox potentials. Each such ET chain consists of four respiratory chain complexes, viz., complex I transporting electrons from NADH to coenzyme Q, a mobile nonprotein nonpolar ubi-quinone of the inner membrane, complex II transporting electrons from succinate of CoQ, complex III carrying electrons from CoQ. H_2 to a peripheral protein cytochrome c, and complex IV receiving electrons from reduced cytochrome C and donating them to molecular O_2. Except cytochrome oxidase, an oxidase of complex IV, all other integral proteins of the ET chain function as dehydrogenases. The exergonic transport of each electron-pair along the rising redox potentials of complexes I, III and IV release sufficient free energy for forming three high-energy ATP bonds.

Oxidative phosphorylation is the endergonic phosphorylation of ADP to ATP, harnessing the energy released by the exergonic electronic-transport along the respiratory chain. This phosphorylation is catalyzed ATP synthase activity of the F_1 protein of the headpiece of inner membrane particles. According to Peter Mitchell, for this oxidative phosphorylation the ATP synthase activity is coupled with the mitochondrial electron-transpost by a transmembrane proton gradient created across the inner membrane by the outward transfer of protons through the latter during the election transport. Mitchell proposed a redox loop mechanism, and subsequently the Q cycle in modifying that mechanism, to explain how mitochondrial electron transport may lead to the inward transmembrane proton gradient. An alternative proton pump mechanism has also been proposed to explain the coupling of mitochondrial oxidation and ATP synthase action, assuming changes in the proton-affinities and conformations of some proton-translocator proteins of the inner membrane during mitochondrial electron transport. In his binding-change model of ATP synthase action. Boyer has proposed cyclic changes in that action of the beta-subunits of each of three $\alpha\beta$-subunit-pairs of the F_1 protein during electron transport.

Oxidative phosphorlation is mainly regulated by the ATP mass action in the mitochondrial matrixm, viz., [ATP]/[ADP][Pi], which is normally kept adequately low in the matrix by a transmembrane ATP-ADP anitport, carried out by an inner-membrane ATP-ADP translocator. Uncoupling of mitochondrial oxidation and oxidation and oxidative phosphorylation enhances mitochondrial. Oxidation and decrease oxidative phosphorylation, leading to the release of the energy from aerobic oxidation as heat.

2.7 Terminal questions

1. (a) Discuss the differences between substrate-level phosphorylations and oxidative phosphorylations, citing examples.
   (b) Describe the components of the inner membrane particle and its F_1 unit housing the ATP synthaes, using a suitable diagram.
   (c) Discuss with a suitable diagram Boyer's binding-change model for the mitochondrial ATP synthase action.
2. (a) Describe the chemiosmotic theroy about the coupling of mitochondrial oxidation and ATP synthesis.
   (b) Describe the proton-pump mechanism for proton translocation across the inner membrane during mitochondrial electron transport.
   (c) Discuss the roles of ATP mass action ratio and ATP-ADP translocator in regulating oxidative phosphorylation.
3. (a) Give an outline of electron transports by pyridine-linked dehydrogenases, flavin-linked dehydrogenases and iron-sulfur proteins.
   (b) Give brief accounts of oxygenases and oxidases, with examples of the subclasses of both.
   (c) Discuss how to mitochondrial cytochromes belong to two different classes of oxidoreductases, giving examples.
4. (a) Explain what you understand by redox potential and standard redox potentials. Discuss the significance of redox potential in the electron flow between electron-donors and electron-acceptors.
   (b) Describe the relation between electron transfer potential and redox potential, quoting the relevant Nernst equation.
   (c) Explain how electrons flow from NADH to pyruvate during anaerobic glycolysis, but from lactate to NAD^+ in the post-glycolysis aerobic condition.

5. (a) Describe the components and functions of the repiratory chain comlexes constituing each mitochondrial respiratory chain assembly.
   (b) Describe using a suitable flowcahrt the different paths followed by electrons along the respiratory chain complexes during their flow from different metabolites to molecular O_2.
   (c) Discuss, quoting the relevant equation, the free energy changes during electron flow along different repiratory chain complexes, and identify there from the sites of the repiratory chain where high-energy bonds may be formed.
6. (a) Described the oxidation-phosphorylation coupling using Mitchell's chemiosmotic theory.
   (b) With a suitabl. .iagram, describe the redox loop mechanism of electron transport to explain proton translocation during mitochondrial repiration.
7. (a) Describe the Q cycle with a sitable flowchart.
   (b) Discuss the regulation of mitochondrial oxidative phosphorylation.
   (c) How can mitochondrial respiration and phosphorylation be uncoupled? Describe such uncoupling in hibernating mammals.
8. Write notes on the followin :
   (a) Redox couples.
   (b) Hydroperoxidases.
   (c) ATP-ADP translocator.
   (d) ATP-ADP mass action ratio.
   (e) Proton-translocating ATP synthase.
   (f) P : O ratio

2.8 Answers

1. (a) See Section 2.5.1.
   (b) See Section 2.5.3.
   (c) See Section 2.5.3.
2. (a) See Section 2.5.4.
   (b) See Section 2.5.6.
   (c) See Section 2.5.7.
3. (a) See paragraphs (a), (b) and (c) Section 2.2.4.

(b) See Sections 2.2.1 and 2.2.2.

(c) See paragraph (d) of Section 2.2.4.

4. (a) See first to fifth paragraphs Section 2.3.

(b) See sixth paragraph of Section 2.3.

(c) See seventh (last) paragraph of Section 2.3.

5. (a) See Section 2.4.1.

(b) See Section 2.4.2.

(c) See Section 2.4.3.

6. (a) See Section 2.5.4.

(b) See Section 2.5.5.

7. (a) See Section 2.5.5.

(b) See Section 2.5.7.

(c) See fourth and fifth paragraph of Section 2.5.7.

8. (a) See first, fourth, fifth and sixth paragraphs Section 2.3.

(b) See Section 2.2.3.

(c) See second and third paragraphs of Section 2.5.7.

(d) See second paragraph of Section 2.5.7.

(e) See Section 2.5.3.

(f) See Section 2.5.2.

Unit 3 ❑ Membrane Transport, Biosyntheses and Muscle Contraction

Structure

3.1 Introduction

Objectives.

3.2 Membrane transport

3.3 Active transport

3.4 Biosynthesis of Urea

3.5 Glycogenesis

3.6 Gluconeogenesis

3.7 Glutathione biosynthesis

3.8 Norepinephrine and Epinephrine biosyntheses

3.9 Serotonin and melatonin biosynthesis

3.10 Saturated Fatty Acid biosynthesis

3.11 Prostaglandin bisynthesis

3.12 Muscle contraction

3.13 Summary

3.14 Terminal questions

3.15 Answers

3.1 Introduction

You will learn in this unit about the characteristics, kinetics and significances of active transports across membranes and some mechanisms of such transports.

You will then proceed to read about the biosynthesis of several substances of biological importance such as norepinephine, epinephrine, glutathione, serotonin, melatonin, glycogen, saturated fatty acids, prostaglandins and urea.

Finally, major muscle proteins will be briefly descibed, sliding filament model of muscle contraction and the molecular mechanism of muscle contraction will be presented to you.

Objectives

- After reading this unit, you should be able to :
- Differentiate between nonmediated and carrier-mediated transports,
- Distinguish between different stoichiometric categories of active transports,
- Describe the characteristics, significance and kinetics of actine transports,
- Discuss the mecahnism of ATP-driven active transports such as the sodium-potassium pump.
- Understand and describe the mechanisms of ion gradient-driven active transports such as the ATP-ADP antiport,
- Describe the pathway, energetics and significance of urea biosynthesis and explain its significance,
- Give an account of how glucose is converted to glycogen for storage in tissues,
- Discuss how glucose can be formed from non-carbohydrates,
- Describe how amino acids are used in synthesizing specialized products such as glutathione, norepinephrine, epinephrine, serotonin and melatonin,
- Give an account of synthesis of the saturated fatty acid, palmitic acid, and of its elongation into longer saturated fatty acids,
- Explain how polyunsaturated fatty acids can be used in synthesizing prostaglandins by the cyclo-oxygenase pathway.
- Develop a basic idea about the structural and molecular characteristics of major muscle proteins,
- Understand the concept of the sliding filament model of muscle contraction,
- Understand and explain the molecular mechanism of muscle contraction, based on actine-myosin interaction in striated muscles,
- Describe the myosin-based contraction of smooth muscles.

3.2 Membrane Transport

There are two major processes for the transport or flow of solutes across in **intact membrane.**

(a) Nonmediated transport or diffusion :

It is a simple physical process in which the substrate flows down its electrochemicl gradient across the membrane, requiring neither any energy expenditure nor any transporter or carrier protein to ferry it from one side to the other, continuing only

so long as the transmembrane gradient exists, and getting affected by the Gibbs-Donnan effects of nondiffisuble ions.

(b) Carrier-mediated transports :

They are far speedier processes with specific transporters, translocases or carrier proteins transferring specific molecules or ions across the membrane. Such mediated transports may again be of two main types, according as cellular work is or is not involved in the process.

(i) Passive mediated transport or facilitated diffusion :

Here, specific carriers transfer specific substrates for more rapidly than diffusion and down their respective concentration or electrical gradients across the membrane so long as such a gradient exists, but requiring no energy expenditure and not being affected by cold, lack of O_2 or metabolic inhibitors; e.g., transports of fructose and mannose across the intestinal cell membrane.

(iii) Active transport : This type of transport is even more rapid and is carried out by carrier proteins having high specificities for their substrates; but active transport transfers substrate molecules against their electrochemical gradients, consequently involves the expenditure of cellular energy and so, gets depressed by cold, metabolic inhibitors and hypoxia; e.g., transmembrane transports of Na^+ and K^+ and Na^+K^+ pump.

3.3 Active Transport

Active transports are *endergonic, carrier-mediated transports* of specific solute molecules or ions by specific carriers in a specific direction across a membrane and even against the transmembrane electrochemical gradient of the transported substrate. Like facilitated diffusions, active transports also belong to three categories *stoichiometrically;* viz., *(i) uniport* or the transmembrane transport of a single substrage, e.g., extrusion of cytosolic Ca^{2+} to the extracellular fluid; *(ii) symport or* the simultaneous transport of two different substrates in the same direction across a membrane, e.g., simultaneous active transport of boty N^{a+} and glucose from the intestinal lumen to the enterocyte across its membrane; (iii) *antiport* or *counterport* which is the simultaneous transport of two different substrates in opposite directions across a membrane, such as the active Na^+ efflux and K^+ influx by $Na^+ - K^+$ pump across the plasma membrane.

3.3.1 Characteristics of active transports :

(a) Active transprot is *far more rapid* than both nonmediated transport and passive mediated transport.

(b) The carrier or transporter for any active transport possesses very high *substrate-specificity,* transferring only a specific substrate or a specific pair of substrates across the membrane.

(c) Active transport is *unidirectional*–unlike diffusion and facilitated diffusion which can take place in either direction across a membrane depending on the direction of the electrochemical gradient of the substrate, active transport of a substrate always takes place in a single specific direction across the membrane. This can be explained by the *asymmetric disposition of the transporter* for any active transport in the membrane. When not bound to its substrate, this asymmetric disposition keeps its substrate-binding site on a particular surface of the membrane and binding to the substrate only on that surface; this changes the conformation of the carrier either to place the carrier-bound substate on the other surface of the membrane or to constitute a polar channel across the membrane to its other surface. This enables the carrier to release the substrate from that surface. The carrier does not itself diffuse through the membrane during the process and regains its original conformation after the substrate has been released from it.

(d) The darrier always transports the substrate in a *single specific direction* across the membrane, *even against the electrochemical gradient* of the substrate. This enables the active transport to absorb the entire amount of a substrate from the lumen of intestine or renal tubule. It also produces a steep electrochemical gradient of the substrate across a membrane.

(e) Active ion transport is *electrogenic,* producing a potential difference between the two sides of the membrane when it segregates positive and negative ions on its opposite sides. But if it is either a symport of two counterions or an antiport of two like-chanrged ions, active ion transport is *electroneutralI* with no trans-membrane potential difference resulting from it.

(f) As active transport is carried out against the electrochemical gradient of the substrate, it is an *endergonic process* with positive free-energy change ($\Delta G^{o'}$). Where R is the molar gas constant (8.314J/K/mol) and T is the absolute temperature (298K ≡ 25°C), the ΔG^{01} for the active transport of n moles of solute from its lower molar concentration (C_1) to its higher molar concentration (C_2) across a membrane, and the work done (Wcal) by the cell in that transport are given by :

$$\Delta G^{o'} = RT\, l\,n \frac{C_2}{C_1} = 2.303\ RT \log \frac{C_2}{C_1}\ ;$$

$$W = 2.303\ nRT \log \frac{C_2}{C_1}.$$

(g) Because active transports are engergonic, they have to be driven by being *coupled with exergonic reactions* with high negative $\Delta G^{0'}$ such as the hydrolysis of high-energy bonds of ATP (*ATP-driven active transports*) and the electrochemical potential gradients created by transmembrane ion gradients (*ion gradient-driven active transports*). [See 3.3.4 and 3.3.5 below]

(h) Because transportable substrate molecules for outnumber the limited number of specific carrier molecules, progressive rise in substrate concentration [S] leady to a gradual decline in the rate of increase in the transmembrane flow or *flux (J)* of the substrate and ultimately reaches a maximum flux (*J*max) with a saturating substrate concentration—the *J*max cannot be exceeded by raising the [S] further. This is reflected in the *rectangular hyperbolic graph* obtained by plotting *J* values against the respective [S] values (Fig. 3.1). Thus the flux of active transport is a *rectangular hyperbolic*

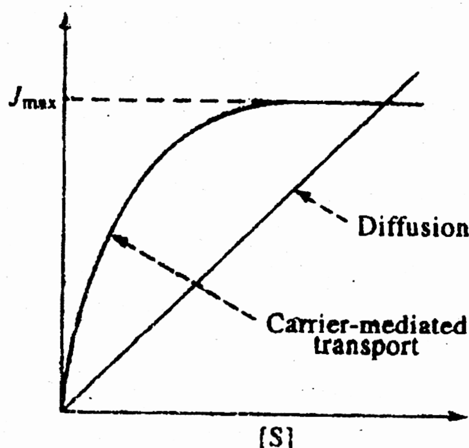


Figure 3.1 : Hyperbolic Kinetics of active transports, in contrast to the kinetics of diffusion. [From D. Das, *Biochemistry*, Academic Publishers, 2005]

function of the molar concentration of substrate, and follows the equation for the *Michaelis-Menten hyperbolic Kinetics;* where K_m is the molar concentration of sybstrate for attaining $^1/_2$ Jmax, $J = \frac{J\max[S]}{K_m + [S]}$

(i) Some cases of simultaneous active transports of more than one substrate molecule, however, follow the *sigmoid saturation Kinetics* owing to a *positive cooperatively* between the substrate molecules.

(j) Some active transports are *competitively inhibited* by suitable substrate analogues competing with the substrate for occupying the substrate-binding sites of the carrier. This indicates the existence of only *a limited number of sites* available in the carrier for such mediated transports.

3.3.2 Significances of active transports

By transporting specific solute molecules or ions in a particular direction across a membrane, active transports help to accomplish the following :

(i) Absorb or reabsorb the specific solutes from intestinal or renal tubular lumens very rapidly, independent of their transmembrane electrochemical gradients and consequently, even totally from those lumens,

(ii) Create and maintain ion gradients in specific directions across a membrane.

(iii) Keep the resting membrane polarized, a repolarize it after its depolarization,

(iv) Create the resting membrane potentials of neuromuscular tissues and also participate in the course of action potentials during their excitation and conduction of impulses, and

(v) Help in the distribution of water in different body compartments due to the obligatory osmotic transfer of water across the membranes along with the actively transported solutes.

3.3.3 Mechanisms of active transports

It may be recalled that being *endergonic* in nature, active transports have to be conducted by being *coupled with exergonic reactions* which in most cases consist of either ATPase catalyzed hydrolysis of high energy phosphate bonds of ATP, or dissipation of ion-gradients across the membrane to be crossed by the transported substrates. These two mechanisms are described below with examples. (See 3.3.4 and 3.3.5.)

3.3.4 ATP-driven active transports

In these cases, endergonic active transports are conducted by being coupled with the exergonic hydrolysis of ATP so that the free energy released by the latter chemical reaction can be harnessed for the endergonic physical process of active transmembrane transport. Four types of ATPases, catalyzing ATP hydrolysis for active ion-transports, occur in animal membranes, viz., *P-type ATPases* of plasma membrane and endoplasmic reticulum membrane, *V-type ATPases* of lysosomal membrane, *F-type ATPases* of inner mitochondrial membrane particles—all these three types are associated with active transports of specific cations such as Na^+, K^+, Ca^{2+}, and H^+, and A-type ATPases for active transports of anions such as Cl^- ions. Two such ATP-driven active transports are described below.

(a) $Na^+ - K^+$ pump :

This integral protein of plasma membrane is also known as *$Na^+ - K^+$ ATPase* and carries out the active antiport of three Na^+ ions outward and two K^+ ions inward across the membrane against their respective concentration gradients, hydrolyzing

one ATP molecule into ADP and Pi : $3Na^+_{in} + 2K^+_{out} + ATP + H_2O \rightarrow 3N_a^+{}_{out} + 2K^+_{in} + ADP + Pi$. This antiport helps to maintain the extracellular and intracellular Na^+ concentrations respectively at about 150 mM and 15 nm, and the respective K^+ concentrations at about 5mM and 100mM. This ATPase, a tetrapeptide consisting of two nonglycosylated α and two glycosylated β peptidesubunits, is a *P-type ATPase* —like other P-type ATPases, this enzyme also occurs in the plasma membrane, is inhibited by vanadate, and gets phosphorylated by ATP during its action. The enzyme exists in two conformation,s viz., E_1 and E_2 (see below).

The Na^+ – K^+ ATPase is activated **in vivo** by any rise in intracellular Na^+ concentration or in extracellular K^+ concentration. Then, it carries out the Na^+ – K^+ antiport across the plasma membrane in the following way to maintain the normal inward concentration gradient of Na^+ and the normal outward gradient of K^+ (Fig. 3.2).

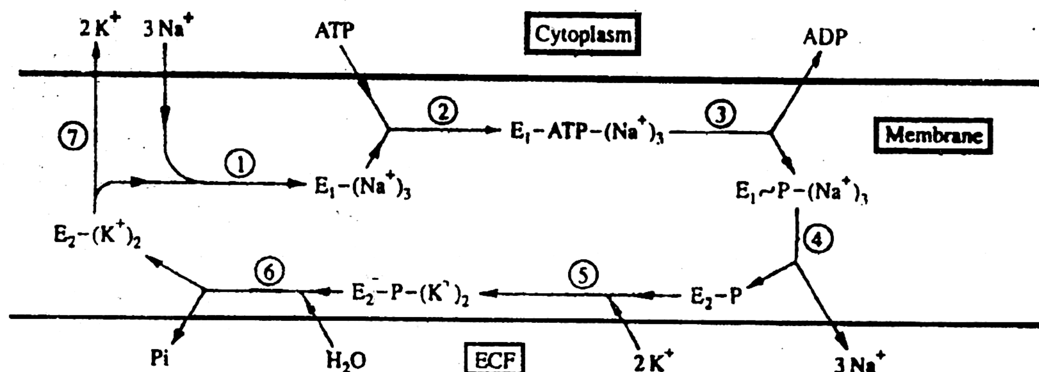


Figure 3.2 : Active antiport of Na^+ and K^+ by Na^+–K^+ [From D. Das, *Biochemistry*, Academic Publishers, 2005]

(i) The cation-binding site of the E_1 form of Na^+ – K^+ ATPase has a high Na^+ affinity, is situated on the cytoplasmic surface of the membrane, and binds to three Na^+ ions from the cytosol, forming E_1 – $(Na^+)_3$ complex; the latter then binds to a cytosolic ATP molecule to form an E_1 – ATP-$(Na^+)_3$ complex.

(ii) The E_1 – ATP $(Na^+)_3$ complex changes into E_1 ~ P – $(Na^+)_3$, a high-energy intermediate, by using the bound ATP to phosphorylate a specific aspartate residue of the enzyme into a phospho-aspartate residue, and releases ADP into the cytosol.

(iii) The high-energy E_1 ~ P – $(Na^+)_3$, intermediate next changes into a low-energy E_2–P – $(Na^+)_3$, form; this change in the conformation of the enzyme translocates its cation-binding site to the extracellular surface, lowers the Na^+ – affinity of that site, consequently release the Na^+ ions into the extracellular fluid, and changes into E_2 – P intermediate with high K^+ – affinity.

(iv) The cation-binding site of the E_2 – P intermediate now binds to two extracellular K^+ ions to form an E_2–P–$(K^+)_2$ intermediate whose phosphoaspartate residue releases P_1 into the ECF; this gives rise to an E_2–$(K^+)_2$ intermediate.

(v) This changes the conformation of the latter into E_1 – $(K^+)_2$ with its cation-binding site translocated on the cytosolic surface, lowering its K^+ – affinity; the two K^+ ions are consequently released in the cytosol and the enzyme resumes its original E_1 conformation.

Functions :

By maintaining transmembrane inward and outward concentratiion gradients of Na^+ and K^+ respectively, the Na^+ – K^+ ATPase principally helps in **(i)** active intestinal and renal tubular absorptions of Na^+ ions from their lumens, **(ii)** maintenance of the resting polarized state and the post-excitation repolarization of neuromuscular membranes, and **(ii)** maintenance of intracellular osmolarity and fluid content.

(b) Ca^{2+} – ATPases :

These are also *p-type ATPases* occurring as integral proteins in plasma membrane, endoplasmic reticulum membrans and muscles sarcoplasmic reticulum menbranes. Acting in a manner similar to the Na^+ – K^+ ATPase, Ca^{2+} – ATPases carry out the extrusion of each pair of Ca^{2+} ions from the cytosol to either the ECF or the ER/SR lumens, against the electrochemieal gradient of Ca^{2+}, utilizing the energy from one ATP bond hydrolyzed in the process. $2Ca^{2+}{}_{in} + ATP + H_2O \rightarrow 2Ca^{2+}{}_{out} + ADP + Pi$. Thus, Ca^{2+} – ATPases help to maintain **(i)** a much higher Ca^{2+} concentration in the ECF in the cytosol, and **(ii)** Ca^{2+} pools in the lumens of endoplasmic and sarcoplasmic reticulums.

3.3.5 Ion gradient-driven active transports

In these cases, endergonic active transports are conducted by coupling them with the exergonic dissipation of transmembranes ion gradients established by processes like mitochondrial electron transport and photosynthetic reactions. You will find below how two such ion gradient-driven active transports are carried out.

(a) ATP-ADP translocator :

This homodimeric transmembrane integral protein of the inner mitochondrial membrane carries out an *electrogenic antiport* by transporting one ATP^{4-} outward and in exhcnage, one ADP^{3-} inward across that membrane. The translocator molecule has a single binding site for an adenine nucleotide (ATP or ADP) at the area of contact between its two peptide subunits—ATP and ADP have to complete with one another for binding to that site. The translocator exists in two alternativc conformations, viz., one with its binding site exposed to the outer mitochondrial compartment adjoining the outer surface of the inner membrane, and the other having its binding site exposed to the mitochondrial matrix. Binding of either ATP or ADP to the binding site from

any side of the membrane changes the conformation of the translocator, placing its binding site on the other surface of the membrane. Such altermating conformational changes on binding to the adenine nucleotides enable the translocator to carry out their antiport. This antiport is *electrogenic* because of the overall extrusion of one negative charge in the direction of ATP^{4-} movement, and has to be driven by the difference in membrane potential resulting from the inward proton gradient across the inner membrane. The ATP-ADP antiport creates the respective transmembrane gradients of ATP and ADP. It is inhibited by *bongkrekic acid,* a mould antibiotic, and by *atyractyloside,* a plant glycoside, acting respectively from the inner and outer sides of the inner membrane. (See also 2.5.7 for the role of the translocator in regulating oxidative phosphorylation.)

(b) **Na^+ – glucose symport :**

The symport of dietary Na^+ and gloucose, driven by the Na^+ gradient across the luminal membrane of brush-bordered intestinal epithelial cells, helps in their intestinal absorptions from food. The mechanism depends upon three membrane transport systems of those cells.

(i) The **Na^+ – K^+ ATPase** (Na^+ – K^+ pump) of the basal membrane of the cell hydrolyzes a high-energy bond of an ATP molecule and utilizes that energy to bring about an active Na^+ – K^+ antiport transporting three Na^+ ions outward and two K^+ ions inward across the basal plasma membrane (Fig. 3.3). This keeps the intracellular

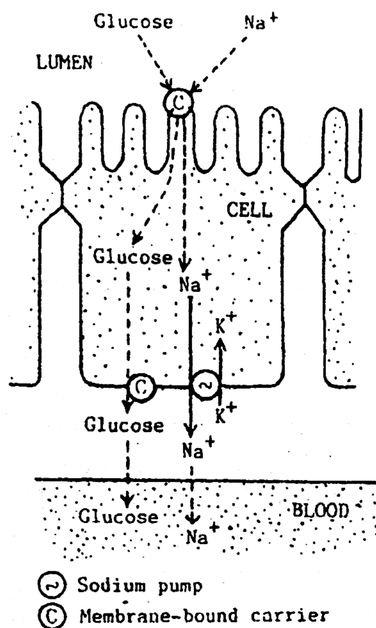


Figure 3.3 : Intestinal Na^+–glucose sumport. [From D. Das, *Biochemistry*, Academic Publishers, 2000]

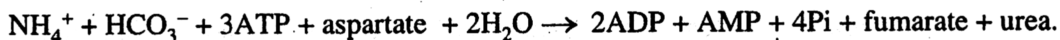
Na^+ concentration lower than the Na^+ concentration in the intestinal lumen to provide an inward Na^+ gradient from the lumen to the cell interior.

(ii) **A Na^+ glucose cotransporter** of the luminal plasma membrane of the cell utilizes this Na^+ gradient for concentrating both Na^+ and glucose from the lumen on that membrane; for this, luminal Na^+ first binds to the Na^+-binding site of the cotransporter to enhance its glucose-affinity, folowed by the subsequent binding of luminal glucose to the glucose-binding site of the cotransporter. The successive bindings of Na^+ and glucose changes the conformation of the cotransporter; exposing both the binding sites on the cytoplasmic side of the membrane, lowering their affinities for the respective ligands and releasing them in the cytoplasm. The cotransporter thereby regains its original conformation and the substrate-affinities of its binding sites which are again exposed now on the luminal surface of the membrane. *Phlorizin* inhibits this Na^+ -dependent cotransporter by binding to its domains on the extracellular surface of the membrane.

(iii) **A passive mediated glucose transport system** of the basal plasma membrane finally transpoprts glucose across that membrane passively—down the outward concentration gradient of glucose—to release it into the ECF (**facilitated diffusion**).

3.4 Biosynthesis of Urea

Urea is the principal nitrogenous end product of amino acid catabolism in *ureotelic animals* such as mammals, elasmo-branches, terrestrial amphibia and aquatic reptiles. It is the main uninary nonprotein nitrogenous (NPN) waste product in those animals. Most of the urea is synthesized in the liver of ureotelese by the *arginine-urea pathway* which was first outlined by Krebs and Henseleit and has since been considerably elaborated and modified. In this process, ammonia from catabolized amino acids and the amino group of aspartate serve as the sources of the nitrogens of urea while HCO^-_3 (or CO_2) acts as the source of its carbon. Ornithine, another amino acid, participates, in the pathway, but is ultimately obtained back after passing through the successive intermediates, citrulline and arginine, which are also amino acids. The overall reaction of the pathway is summarized below :

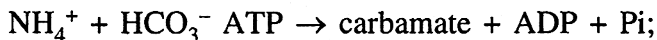


$$NH_4^+ + HCO_3^- + 3ATP + \text{aspartate} + 2H_2O \rightarrow 2ADP + AMP + 4Pi + \text{fumarate} + \text{urea}.$$

The initial reactions of the pathway take place in hepatic *mitochondria* where ammonia, bicarbonate, ornithine and two ATP molecules interact to produce citrulline. The latter is then translocated to the hepatic *cytosol* where aspartate interacts with it to give rise to arginine. Arginine is finally hydrolyzed to ornithine and urea in the cytosol. These steps of the arginine-urea cycle are elaborated below (Fig. 3.4).

Figure 3.3 : The arninine-urea pathway [From D. Das, *Biochemistry*, Academic Publishers, 2000]

(i) Mitochondrial *carbomoyl phosphate synthase I* condenses free ammonia obtained from amino acid catabolism, with HCO_3^- to form carbomoyl phosphate at the expense of two high-energy bonds of ATP.

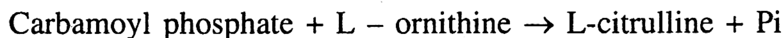


$NH_4^+ + HCO_3^-$ ATP $\rightarrow$ carbamate + ADP + Pi;

ATP + carbamate $\rightarrow$ carbamoyl phosphate + ADP.

This enzyme is allosterically activated by N-acetyl-glutamate, which enhances its affinity for ATP molecules largely.

(ii) *Ornithine transcarbamoylase,* closely associated with the carbomoyl phosphate synthase I in mitochondria, next transfers the carbamoyl group from carbamoly phosphate to L-ornithine, changing the latter to L-citrulline.

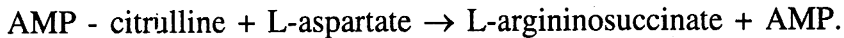


Carbamoyl phosphate + L – ornithine $\rightarrow$ L-citrulline + Pi

(iii) A *citrulline-ornithine transporter* of the inner mitochondrial membrane then carries but a *passive mediated antiport* of citrulline and ornithine, transferring citrulline outward to the cytosol across that membrane and in exchange transferring ornithine inwards from the cytosol to the mitochondrial matrix.

(iv) In the cytosol, *argininososuccinate synthase* catalyzes the stepwise reaction between L-citrulline and L-aspartate to from L-argininosuccinate at the expense of both high-energy phosphate bonds of an ATP.

ATP + L-citrulline → AMP - citrulline + PPi;


AMP - citrulline + L-aspartate → L-argininosuccinate + AMP.

Thus, amino groups of different amino acids may be transferred by transamination to oxalocetate, changing it to aspartate; the latter then forms argininosuccinate in the arginine-urea pathway.

(v) Another cytosolic enzyme, *argininosuccinate lyase*, next splits L-argininosuccinate into L-arginine and fumarate. The latter may be changed in the TCA cycle to oxaloacetate which may be transminated again to aspartate for use in the arginine urea pathway.

(vi) Finally, *arginase* of hepatic cytosol hydrolyzes L-arginine into ornithine and urea. Ornithine is next translocated back into the mitochondrion by the citrulline-ornithine transporter (see above) and is used again in the arginine-urea pathway.

Energy expenditure :

A total of *four high-energy bonds* of three ATP molecules are spent as follows in synthesizing one urea molecule. (i) Two high-energy bonds, one from each of two ATP molecules are spent in synthesizing carbamoyl phosphate by the action of *carbamoyl phosphate synthase I.* (ii) Two more high-energy bonds of a third ATP molecule are spent in the synthesis of L-argininosuccinate by *argininosuccinate synthase.*

Significnace of urea synthesis :

(i) Highly toxic ammonia, formed by amino acid catabolism, is *detoxicated* in ureoteles by being converted to less toxic urea before the latter can be transported by blood to the kidneys for urinary eliminations. Genetic disorders affecting the arginine-urea pathway produces abnormal rise in blood ammonia and sympotoms of ammonia toxicity.

(ii) Arginine, formed as an intermediate in urea synthesis, constitutes a major nondietary source of this amino acid in ureotelic organisms. Uricoteles such as birds and lizards as well as ammonoteles such as fishes and salamanders do not run the arginine urea pathway and eesentially require the dietary supply of this amino acid.

Regulation of urea synthesis :

(i) The rate of urea synthesis os largely regulated by the *allosteric modulation* of its rate limiting enzyme, *carbamoyl phosphate synthase I.* N-Acetylglutamate binds to the positive allosteric site of the enzyme and enhances its substrate-affinity for ATP to enhance the urea synthesis rate.

(ii) The rate of urea synthesis is decreased by the *feed-back inhibitions* of carbamoyl phosphate synthase I ornithine transcarbamoylase and argininosuccinate systhase by the respective products of their actions, viz., carbamoyl phosphate, citrulline and argininosuccinate.

3.5 Glycogenesis

Glycogenesis is the biosynthesis of glycogen, a homoglycan polysaccharide, from glucose in the *cytoplasm* of many tissue cells. It is carried out most extensively in liver and muscles and to much smaller extents in other tissue cells, except brain cells, erythrocytes and renal cells.

(a) Glucose is firs tphosphorylated to glucose 6-phospahte by either *glucokinase* or *hexokinases* I, II and III, spending a high-energy phosphate bond of ATP (Fig. 3.5). Of these four isozymes, glucokinase predominates in the liver over the other three, and has a higher substrate-specificity but a lower substrate-affinity for glucose. Hexokinases predominate in extrahepatic tissues such as muscles and intestinal mucosa, possess less substrate-specificities but higher substrate-affinities for glucose, compared to glucokinase.

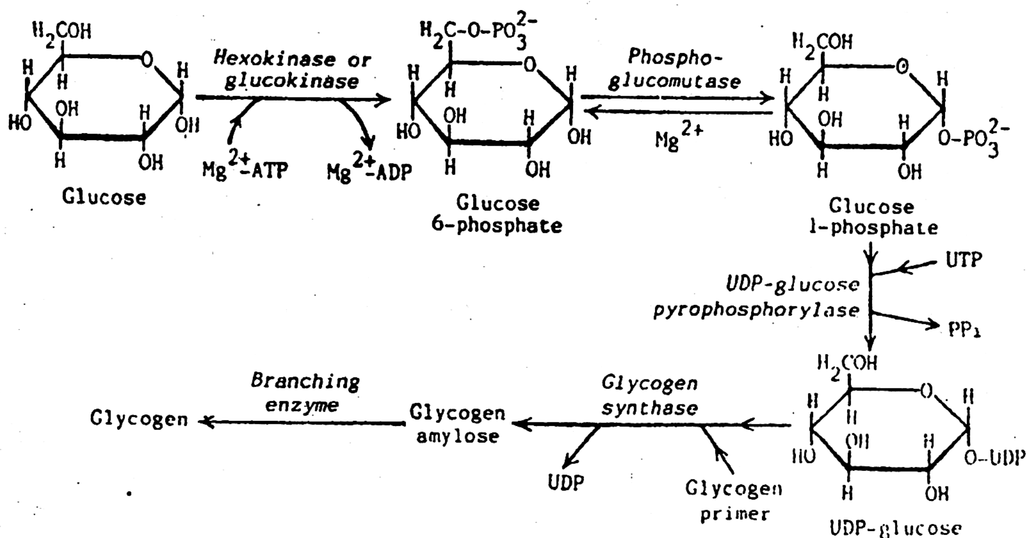


Figure 3.5 : Pathway for glycogenesis. [From D. Das, *Biochemistry*, Academic Publishers, 2000]

(b) *Phosphoglucomutase* next catalyzes the isomerization of glucose 6-phosphate to glucose 1-phosphate through an enzyme-bound glucose, 1, 6-bisphosphate intermediate. The latter is formed by the transfer of a phosphate group from a phosphoserine residue of the enzyme to glucose 6-phosphate, and is subsequently changed to glucose 1-phosphate by donating its C^6–phospahte group to the smae serine residue of the enzyme.

Enz-phosphoserine + glucose 6-phosphate → Enz-serine + glucose 1, 6-bisphosphate;

Enz-serine + glucose 1, 6-biphosphate → Enz-phosphoserine + glucose 1-phosphate

(c) *UDP-glucose pyrophosphorylase* next replaces the two terminal phosphoryl groups of UTP by glucose 1-phosphate, relasing PPi and forming UDP-glucose (Fig.3.5.).

(d) *Glycogen synthase* then transfers the glucose residue of UDP-glucose to the nonreducing end of a pre-existing polyglucose chain (*glycogen primer).* The C^1 of each newly added glucose molecule gets bound by an 1, 4-*glycosidic bond* to the C^4– OH of the terminal glucose residue of the primer molecule. Each such addition extends the polyglucose chain by one glucose residue. Because a poly-glucose chain is thus extended by the successive additions of glucose molecules by identical 1, 4–glycoside bonds only, the product becomes a *glycogen amylose* molecule with unbranched chains.

(e) After ten or more molecules of glucose have been so added to an unbranched polyglucose chain of glycogenamylose, *amylo–1, 4 → 1, 6-transglycosylase* (branching enzyme) transfers the terminal oligosaccharide chain of five or more glucose units from its nonreducing end to the nonreducing end of another such polyglucose chain of the molecule, joining the transferred chain by an 1, 6-glycoside bond to the C^6 – OH of the terminal glucose, of that new chain, resulting in the branching of the latter, as occurs regularly in a glycogen chain (Fig. 3.6).

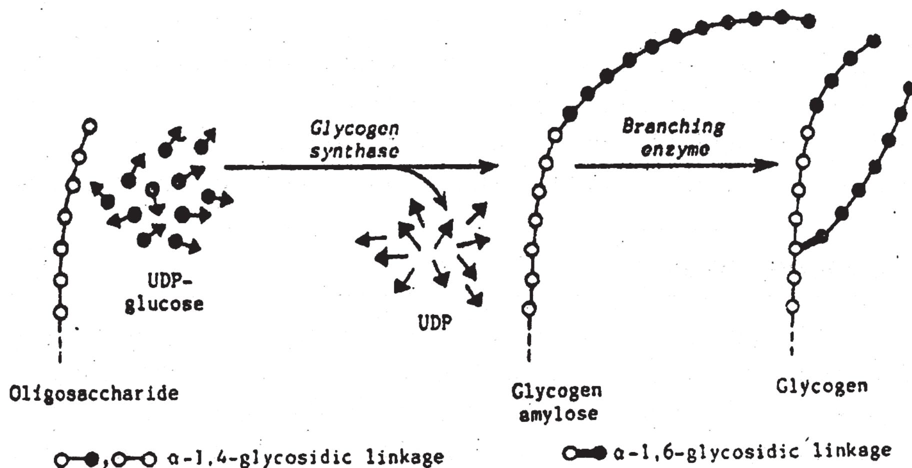


Figure 3.6 : Actions of glycogenasynthase and branchinagenzyme. [From D. Das, *Biochemistry*, Academic Publishers, 2005]

Such successive actions of glycogen synthase and branching enzyme are repeated to form branched glycogen molecules by the polymerization of glucose.

Energetics :

Two high-energy phosphate bonds are spend in adding each glucose residue to the glycogen amylose molecule in the following reactions. **(i)** One ATP bond is hydrolyzed for phosphorylating glucose to glucose 6-phosphate by hexokinase or glucokinase. **(ii)** A second ATP bond is spent in rephosphorylating UDP, released by the action of glycogen synthase from UDP-glucose, to UTP by the action of *nucleoside diphosphokinase.* Thus *two high-energy phosphate bonds* of ATP are spent for extending the polyglucose chain of glycogen amylose by one glucose unit.

Significances of glycogenesis :

(i) Blood glucose that is in excess of the immediate need for energy production is stored in liver and muscles as glycogen for future utilization in energy production and in maintaining the blood sugar.

(ii) Polymerization of glucose to glycogen decreases the bulk of stored glycogen.

(iii) Glycogenesis enables, the cellular storage of large amounts of insoluble glycogen granules without any significant rise in the intracellular osmolarity.

Regulation of glycogenesis :

(i) Insulin *induces* the synthesis of hepatic *glycogen synthase,* the rate-limiting enzyme of glycogenesis, and *glucokinase,* leading to enhanced glycogenesis. This is a long-term regulation of glycogenesis.

(ii) Its short-term regulation depends on *reversible covalent modifications* of *glycogen synthase* by phosphorylation and dephosphorylation. Phosphorylation of specific serine residues of the enzyme to phosphoserine residues by an ATP-dependent *protein Kinase* decreases the activity of the synthase and causes a decline in glycogenesis; dephosphorylation of those phosphoserine residues by a protein phosphatase increase the activity of the synthase with a consequent rise in glycogenesis.

3.6 Gluconeogenesis

Gluconeogenesis is the process of biosynthesis of glucose from noncarbohydrate sources. Gluconeogenesis from metabolites of glycolysis such as pyruvate and lactate takes place mainly in the liver. Moreover, particularly during starvation, many amino acids such as alanine and serine are used in gluconeogenesis in both liver and kidneys. Gluconeogenesis is carried out partly in mitochondria and partly in the cytoplasm.

3.6.1 Significances of gluconeogensis

(i) Lactate and other products of glycolysis, released by contracting muscles and other extrahepatic tissues into the blood, are converted by the liver into glucose which is transported to those tissues by blood for energy production and for replenishing their depleted glycogen stores (*Cori cycle*).

(ii) During fasting or on a poor carbohydrate intake, gluconeogenesis from the catabolites of amino acids from tissue proteins helps to prevent hypoglycemia and to maintain the supply of blood glucose to extrahepatic tissues such as the brain, muscles, heart and erythrocyts.

(iii) Gluconeogenesis serves for the final metabolism of the carbon-skeletons of glycogenic amino acids after their nitrogenous parts have been removed and catabolized.

3.6.2 Gluconeogenesis from lactate

(a) Lactate is first reoxidized to pyruvate by the NAD^+ -dependent *lactate dehydrogenase* in the hepatocyte cytosol.

(b) Pyruvate is next transported into the mitochondrial matrix by a *pyruvate-H^+ symport* carried out by a *Pyravata transporter* of the inner mitochondrial membrane.

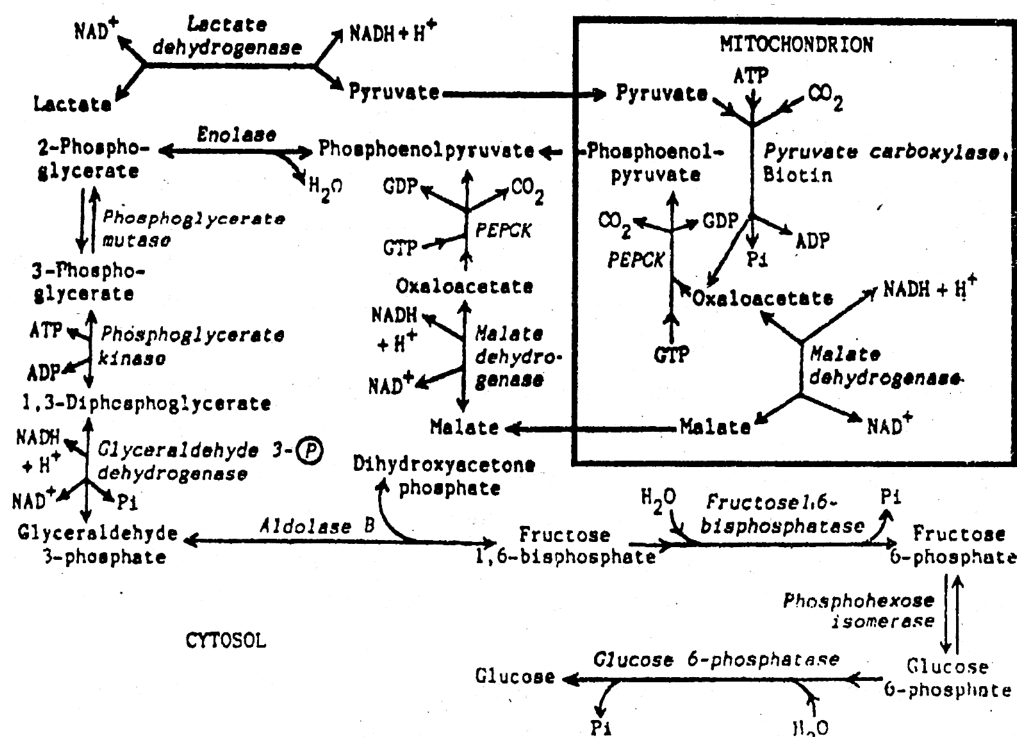


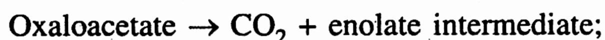
Figure 3.7 : Gluconeogenesis from lactate, [From D. Das, *Biochemistry*, Academic Publishers, 2000]

(c) Biotin-dependent *pyruvate carboxylase* of mitochondrial matrix then carboxylates pyruvate to oxaloacetate, using its biotin prosthetic group, CO_2 and the energy from a high energy bond of ATP (Fig. 3.7).

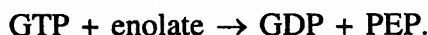
Enz-biotin + ATP + CO_2 → Enz-carboxybiotin + ADP + Pi,

Enz-carboxybiotin + pyruvate → Enz-Biotin + oxaloacetate

(d) In some animals such as lagomorphs and pigeons, mitochondrial *phosphoemolpyruvate carboxykinase* (PEPCK) first decarboxylates oxalocetate to an enolate intermediate and then phosphorylates the latter into phosphoenolpyruvate (PEP) using a high-phosphate group from GTP; PEP is then transfered to the cytosol by an inner membrane transporter.



Oxaloacetate → CO_2 + enolate intermediate;



GTP + enolate → GDP + PEP.

In rodents, on the contrary, oxaloacetate is reduced by mitochondrial *malate dehydrogenase* and NADH to malate which is translocated to the cytosol by a *malate-*HPO_4^{2-} *antiport* brought about a *dicarboxylate transporter* of the inner membrane. In the cytosol, malate is next *reoxidized to oxalocetate by cytoplasmic malate dehydrogenase and* NAD^+*; oxaloacetate is then* converted to PEP by the cytoplasmic *PEPCK,* using GTP.

(e) PEP is next converted to fructose 1, 6-bisphosphate in the cytosol by the successive actions of several glycolytic enzymes (Fig. 3.7) ; **(i)** *enolase* hydrates PEP to 2-phosphoglycerate, **(ii)** the latter is isomerized by *phosphoglycerate mutase* to 3-phosphoglycerate which **(iii)** is phosphorylated by ATP and *phosphoglycerate kinase* to 1, 3-bisphosphoglycerate, **(iv)** the latter is dephosphorylated and reduced by NADH and *glyceraldehyde 3-phosphate dehydrogenase* to give Pi and glyceraldehyde 3-phosphate, and **(v)** the latter is condensed with dihydroxyacetone phosphate by *aldolase* B, giving fructose1, 6-bisphosphate.

(f) Fructose 1, 6-bisphosphate is hydrolyzed into fructose 6-phosphate and Pi by *fructose 1, 6-bisphosphatase,* a specific cytosolic enzyme for gluconeogenesis.

(g) Fructose 6-phosphate is next isomerized by a glycolytic enzyme, *phosphohexose isomerase,* into glucose 6-phosphate.

(h) Finally, microsomal *glucose 6-phosphatase,* another specific enzyme for glucoeogenesis, hydrolyzes glucose 6-phosphate to glucose which may then pass into the blood.

For gluconeogenesis from each lactate (C_3) moleule, **(i)** one high-energy ATP bond is spent during pyruvate carboxylase action, (ii) one high-energy bond of GTP is spent in PECK action, and **(ii)** one high-energy bond of GTP is spent in PEPCK

action, and (iii) a second ATP bond is spent during the action of phosphoglycerate kinase. As two lactate molecules are used in forming each glucose (C_6) molecule, a total of *six-energy phosphate bonds* are spent in the gluconeogenesis of one glucose molecule from lactate.

3.6.3 Gluconeogenesis from amino acids

Catabolism of *glycogenic amino acids* such as glycine, alanine, serine, aspartate, glutamate, methionine, valine, cysteine, histidine, arginine, proline and hydroxyproline, and of *glycogenic-ketogenic amino acids* such as phynylalanine, tyrosine, tryptophan, threonine and isoleucine, yield gluconeogenic intermediates like pyruvate, fumarate, α-Ketoglutarate, succinate and oxaloacetate, which are changed to glucose by successive actions of enzymes of carbohydrate metabolism including the glycolytic and specific gluconeogenic enzymes described above (See 3.6.2 and Fig. 3.7). But Ketogenic amino acids, viz., leucine and lysine, do not yield any glucose by gluconeogenesis.

3.7 Glutathione biosynthesis

You may recall that glutathione is a tripeptide, made of glycine, L-glutamate and L-cysteine, and functions as a reducing agent, acts in detoxications and participates in the biosynthesis of pheomelanin pigments of skin. However, unlike the body peptides in general, glutathione cannot be translated by polysomes because its N-terminal peptide bond between glutamate and cysteine is a γ-*peptide bond,* and an α-

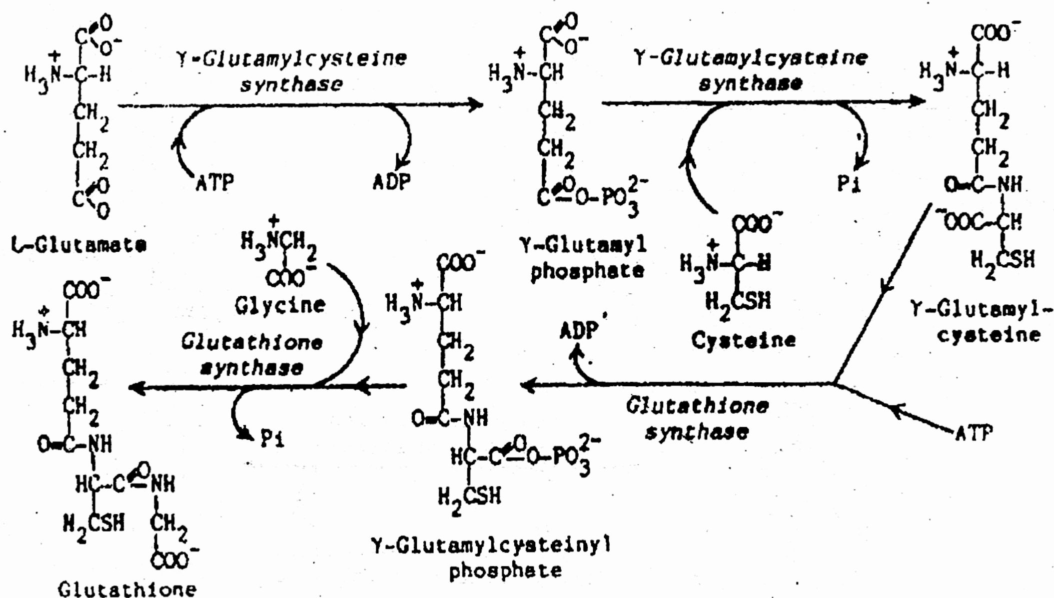


Figure 3.8 : Biosynthesis of glutathione. [From D. Das, *Biochemistry*, Academic Publishers, 2000]

peptide bond. Consequently, glutathione is synthesized by the successive actions of the following cytoplasmic enzymes without the participation of ribosomes, tRNA and mRNA. Each molecule of glutathione is synthesized spending *two high-energy bonds* of ATP.

(i) γ-*Glutamylcysteine synthase* first transfers a phosphate group from ATP to the sidechain γ-carboxyl group of l-glutamate of form γ-glutomyl phosphate, and then replaces that phosphate group by transferring a cysteine molecule in its place, releasing Pi and γ-glutamylcysteine (Fig. 3.8)

(ii) *Gluathione synthase* next phosphorylates the carboxyl group of the cysteine residue of γ-glutamylcysteine using ATP, and then replaces that phosphate group by glycine, releasing Pi and glutathione.

3.8 Norepinephrine and Epinephrine biosynthesis

You are aware that norepinephrine and epinephrine (also called *noradrenaline* and *adrenaline)* are catechdamines released as neurotransmitters at sympathetic axon terminals and secreted from adrenal medulla as its hormones. These two *biogenic amines* are synthesized from L-tyrosine in adrenergic neurons and adrenal chromaffin cells (Fig. 3.9).

(i) *Tyrosme hydroxylase* uses molecular O_2 and the electron-donor *terahydrobioterin* to hydroxylate L-tyrosine to dihydroxyphenylalanine (dopa).

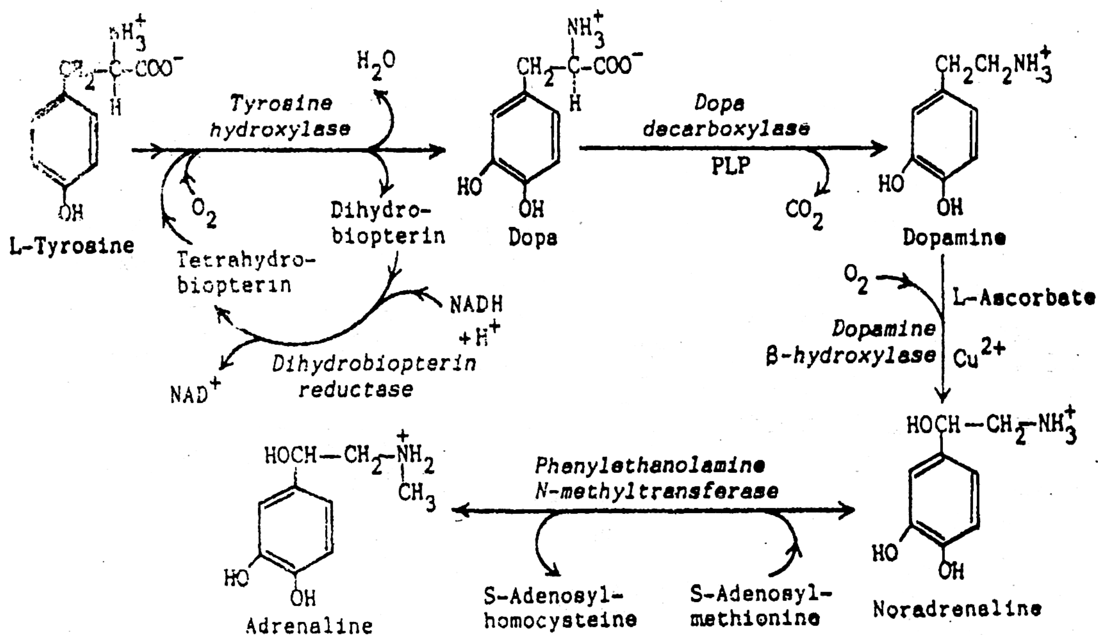


Figure 3.9 : Biosynthesis of catecholamines. [From D. Das, *Biochemistry*, Academic Publishers, 2000]

(ii) *Aromatic L-aminoacid decarboxylase* (dopa decarboxylase), an enzyme with pyridoxal phosphate (PLP) as its prosthetic group, release the carboxyl group of dopa as CO_2 and thus forms dopamine.

(iii) *Dopamine β-hydroxylase,* bearing Cu^+ ion at its active site and using vitamin C as an electron donor cofactor, hydroxylates dopamine to norepinephrine (noradrenaline).

(iv) Finally, *phenylethanolamine N-methyl ferase* transfers the labile methyl group of S-adenosylmethionine ("active" methionine) to the sidechain-NH_2 group of norepinephrine, changing the latter to epinephrine (adrenaline) and the "active" methionine to S-adenosylhomocysteine.

3.9 Serotonin and Melatonin biosynthesis

Serotonin is synthesized from the amino acid L-tryptophan in serotonergic neurons of the hypothalamus, brain-stem, basal ganglia and analgesic neural pathways of the central nervous system and released from their axon terminals. Melatonin is synthesized from serotonin by the pineal gland cells.

(i) *Tryptophan hydroxylase* uses molecular O_2 and the electron-donor *tetrahydrobiopterin* to hydroxylate L-tryptophan to 5-hydroxytryptophan (Fig. 3.10); the sidechain of the latter is next decarboxylated by *aromatic L-amino acid decarboxylase* (5-hydroxytryptophan decarboxylase), an enzyme bearing PLP as its prosthetic group, thus giving rise to serotonin.

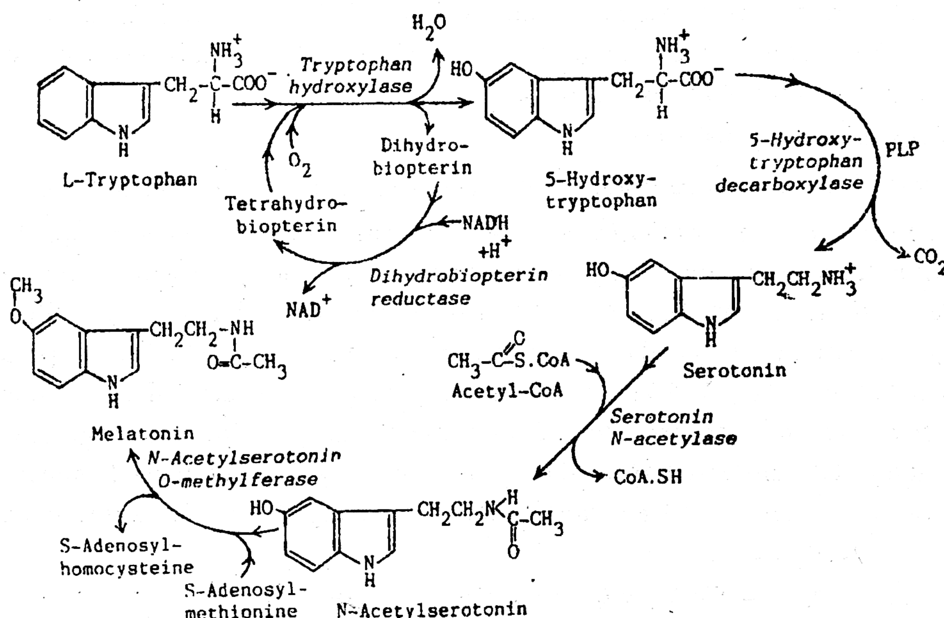


Figure 3.10 : Serotonin and metatonin biuosynthesis [From D. Das, *Biochemistry*, Academic Publishers, 2000]

(ii) *Serotonin N-acetylase* of pineal gland cells transfers the acetyl group of acetyl-CoA to the sidechain—NH_2 group of serotonin to convert the latter to N-acetylserotonin; next, *N-acetylserotonin O-methylferase* transfers the labile methyl group of S-adenosylmethionine ("active" methionine) to C^5 –OH group of N-acetylserotonin, changing them respectively to S-adenosyl homocysteine and melatonin. Melatonin synthesis is photo-regulated.

3.10 Saturated Fatty Acid biosynthesis

In animals such as mammals and birds, tissues like the liver, mammary glands and adipose tissues mostly synthesize palmitic acid (C_{16}) as the basic saturated fatty acid, with the help of two cytosolic multienzyme systems, viz., *fatty acid synthase* and *acetyl-CoA carboxylase.* Longer saturated fatty acids ($>C_{16}$) are subsequently formed by the stepwise addition of C_2 units to palmitate with the help of mainly a *fatty acid elongase* system of smooth ER membranes, while shorter fatty acids ($<C_{16}$) are produced by the stepwise removal of C_2 units from palmitate and other long-chain fatty acids by the mitochondrial β-oxidation enzymes. However, some fatty acid synthase systems of lactating mammary glands are endowed with the ability to turn out short chan fatty acids like butyric and hexanoic acids for secretion in the milk.

3.10.1 Cytoplasmic synthesis of palmitate

The C_2 units (acetyl groups) of acetyl-CoA and the reducing equivalents (H^+ and electron) from the electron-donating coenzyme NADPH constitute the ultimate source materials for synthesizing palmitic acid.

(a) Sources of acetyl-CoA :

Acetyl-CoA used in palmitate synthesis comes from two sources in the cytoplasm.

(i) In *nonruminant animals,* acetyl-CoA formed in the mitochondrion from the oxidative decarboxylation of glycolytic pyruvate, is condensed with oxaloacetate by *citrate synthase* of the TCA cycle to form citrate (Fig. 3.11). When citrate is in excess of the immediate need for its oxidatiion in the TCA cycle for energy production, it is transferred to the cytoplasm by a *citrate-malate antiport* across the inner membrane by a *tricarboxylate transporter* of that membrane and in exchange, malate is simultaneously transferred from the cytoplasm to the mitochondrial matrix. In the cytoplasm, *ATP-citrate lyase* cleaves citrate into acetyl-CoA and oxaloacetate, using ATP and coenzyme A.

Citrate + ATP + CoA → Citryl – CoA + ADP + Pi

Citryl – CoA → oxaloacetate + acetyle – CoA

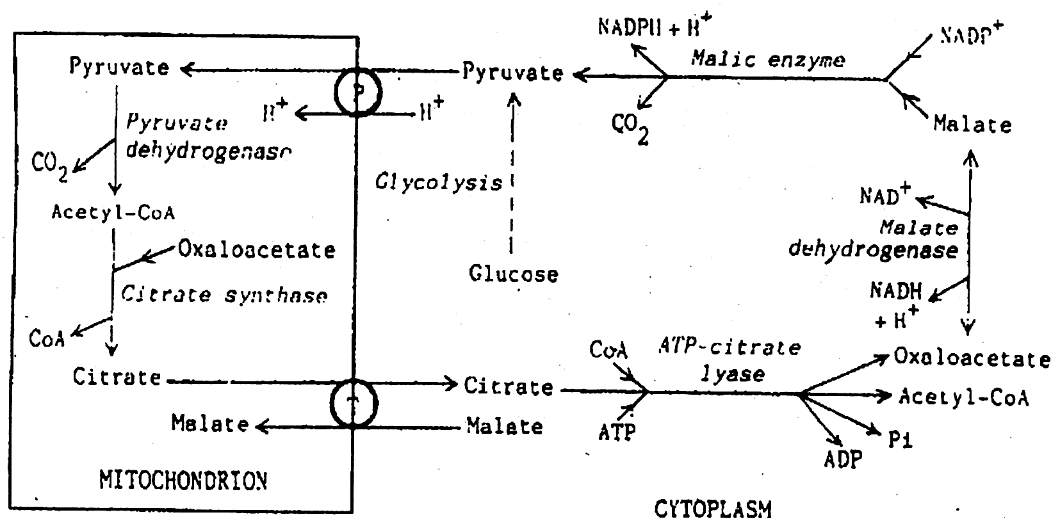


Figure 3.11 : Provision for acetyl-CoA and NADPH in cytoplasm for palmitate synthesis in non ruminants. P : pyruvate transporter. T : tricarboxylate transporter. [From D. Das, *Biochemistry*, Academic Publishers, 2000]

(ii) In *ruminants,* acetic acid absorbed from the rumen as a product of cellulose fermentation is thioesterified with CoA by cytoplasmic *acetyl-CoA synthase (acetatethiokinase)* to form acetyl-CoA at the cost of two high-energy bonds of ATP.

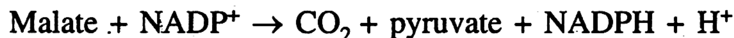
Acetate + ATP + CoA → acetyl –CoA + AMP + PPi

(b) **Sources of NADPH :**

NADPH is generated in the cytoplasm in more than one way for acting as an electorn-donor in palmitate synthesis.

(i) Both in *ruminants and nonruminants,* $NADP^+$ is reduced to NADPH by the actions of *glucose 6-phosphate dehydrogenase* and *6-phosphogluconate dehydrogenase* in the pentose phosphate pathway. This is why that pathway takes place at high rates in lipgenic tissues like adipose tissues.

(ii) In *nonruminant animals,* oxaloacetate obtained by ATP-citrate lyase action on citrate (see above) is reduced by NADH and cytoplasmic *malate dehydrogenase* to form malate; the latter is next decarboxylated and converted to pyruvate by cytoplasmic *malic enzyme* ($NADP^+$ – malate dehydrogenase), with the simultaneous reduction of $NADP^+$ to NADPH (Fig. 3.11).



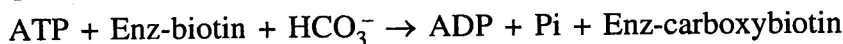
Malate + $NADP^+$ → CO_2 + pyruvate + NADPH + H^+

(iii) In *ruminants,* citrate is isomerized to isocitrate in the cytoplasm by *aconitase;* isocitrate is next oxidized by $NADP^+$—dependent cytoplasmic *isocitrate dehydrogenase* and then decarboxylated to α-ketoglutarate, simultaneously reducing $NADP^+$ to NADPH.

Isocitrate + $NADP^+$ → CO_2 + NADPH + H^+ + α-Ketoglutarate.

(c) Acetyl-CoA carboxylase action :

Acetyl-CoA carboxylase, a cytoplasmic *multienzyme protein* carboxylates acetyl-CoA to malomyl-CoA, using HCO_3^- and spending an ATP bond (Fig. 3.12). This enzyme system bears a *biotin prosthetic group* on one of its peptide subunits and active sites for *biotin carboxylase* and *transcarboxylase* on two other subunits. The *biotin carboxylase* uses a high-energy ATP bond to carboxylate the biotin prosthetic group into carboxybiotin.



$$\text{ATP} + \text{Enz-biotin} + HCO_3^- \rightarrow \text{ADP} + \text{Pi} + \text{Enz-carboxybiotin}$$

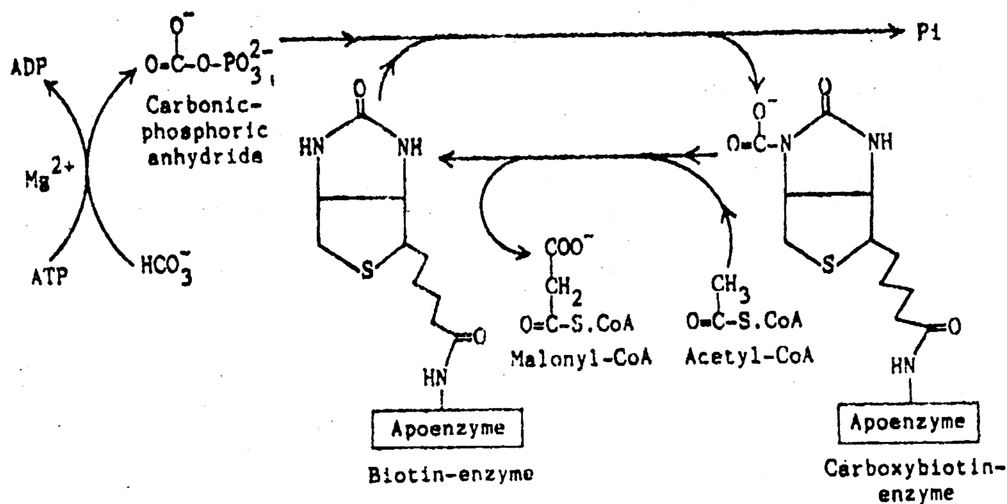
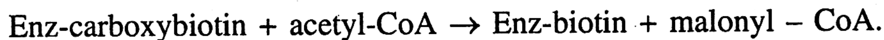


Figure 3.12 : Acetyl-CoA carboxylase action. [From D. Das, *Biochemistry*, Academic Publishers, 2000]

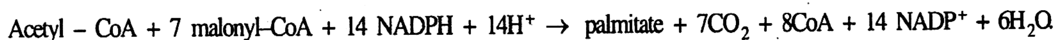
Transcarboxylase then carboxylates acetyl-CoA to malonyl-CoA by the transfer of the carboxylate ion to the substrate from the carboxybiotin prosthetic group.



$$\text{Enz-carboxybiotin} + \text{acetyl-CoA} \rightarrow \text{Enz-biotin} + \text{malonyl} - \text{CoA}.$$

(d) Falty acid synthase :

Sevel malonyl-CoA molecule and are acetyl-CoA molecule are next used by fatty acid synthase, another cytoplasmic *multienzyme protein,* in synthesizing one palmitic acid molecule through seven cyles of reactions, in mammals and birds. Overall :



$$\text{Acetyl} - \text{CoA} + 7\ \text{malonyl-CoA} + 14\ \text{NADPH} + 14H^+ \rightarrow \text{palmitate} + 7CO_2 + 8\text{CoA} + 14\ \text{NADP}^+ + 6H_2O$$

In these animals, this multienzyme protein exists as an ellipsoid homodimer, its two monomers being noncovalently bound to each other in an antiparallel manner with the N-terminal end of each facing the C-terminal end of the other. Each minomer

consists of N-terminal *domain-I (condensation unit);* a middle *domain-2 (reduction unit),* a C-terminal *domain-3 (palminate release unit),* and these domains face respectively the domains 3, 2 and 1 of the other monomer. The domains of each monomer carry the following active sites : **(i)** domain 1: 3-ketoacyl synthase, malonyl transacylase and acetyltransacylase; **(ii)** domain-2 : 3-hydroxylacyl dehydratase, enoyl reductase, 3-Ketoacyl reductase and acyl-carrier protein (ACP); **(iii)** domain 3 : thioesterase. The 3-ketoacyl synthase active-site carries of cysteine residue with a sidechian SH (Cys-SH) group, the ACP has a 4'-phosphopantetheine prosthetic group with another SH group (Pan-SH), and the Cys-SH of one monomer faces the Pan-SH of the other and vice versa. The two monomers, as long as held together as the homodimer, act as the multienzyme synthase, synthesizing two palmitate molecules simultaneously from the two ends of the dimer; with the coordinated participation of the Cys-SH of each monomer and the Pan-SH of the other. In this process, the enzymes of domain-1 of each monomer and those of domains-2 and 3 of the opposite monomer function together as one unit.

In contrast, bacteria, **euglena** and potato tuber cells carry separate and individual enzymes and the ACP molecule each occurring singly to participate in fatty acid synthesis, instead of existing and acting as a dimeric multienzyme system.

(e) Reactions catalyzed by the multienzyme synthase :

(a) Acetyl transacylase and **malonyl transacylase** of domain-1 of each monomer of the multienzyme complex transfers the acetyl group from an acetyl-CoA and the malonyl group from a malonyl-CoA respectively to the Cys-SH of the same monomer and to the Pan-SH of the other, forming an acetyl-malonyl-enzyme (acyl-malonyl-Enz) complex (Fig.3.13).

(b) 3-Ketoacylsynthase of domain-1 of the first monomer transfers the acetyl group from its Cys-SH to the methylene –C (C^2) of the malonyl group held by the domain-2 Pan-SH of the other monomer, releasing the carboxyl-C of that malonyl group as CO_2 and forming a Pan-S-bound 3-Ketoacyl group on the second monomer.

(c) **3-Ketoacyl reductase** of domain-2 of the second monomer next used NADPH to reduce the Pan-S-bound 3-ketoacyl group to D(–)-3-hydroxyacyl group.

(d) **3-Hydroxyacyl dehydratase** of the same domain-2 then changes the Pan-S-bound D(–)-3-hydroxyacyl group to Δ^2-*trans* enoyl group by releasing H_2O from it.

(e) **Enyl reductase** of the same domain-2 next reduces the Δ^2-double bond in the Pan-S-held enoyl group using NADPH and giving rise to a new Pan-S-held C_4-acyl group at the end of this first cycle of reactions.

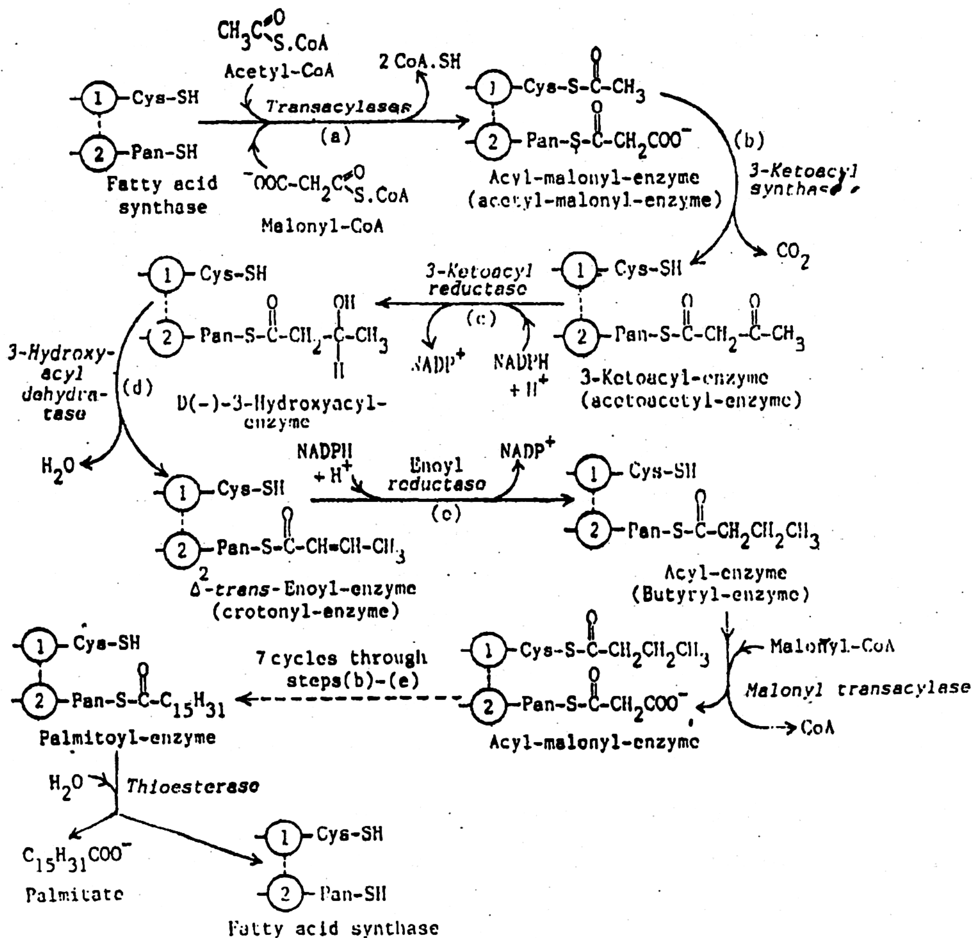


Figure 3.13 : Palmitate synthesis by fatty acid synthesis. [From D. Das, *Biochemistry*, Academic Publishers, 2000]

A second cycle then starts with the transfer of a malonyl group from a fresh malonyl-CoA molecule by *malony transacylase* of domain-1 of the first monomer to the domain-2 Pan-SH group of the second monomer while the Pan-S-held C_4-acyl group gets transferred to the domain-1 Cys-SH of the first monomer. A new acyl-malonyl enzyme complex is thus formed and the second cycle follows through the reactions described in (b) to (e) above.

After seven such cycles, a C_{16}-acyl (palmitoyl) group is formed on the Pan-SH. Now, *thioesterase* of domain-3 of the second monomer hydrolyzes the thioester bond holding the palmitoyl group and releases the latter as palmitic acid. However, lactating mammocytes posses fatty acid synthases having thioesterase action capable of releasing different short and medium-chain fatty acids, instead of palmitic acid, from the Pan-SH. This is how such lower fatty acids occur in the milk.

(f) **Energetics of palmitate synthesis :**

In synthesizing each palmitate molecule, **(i)** a total of 8 high-energy bonds of ATP are spent by ATP-citrate lyase in cleaving 8 citrate molecules to 8 acetyl-CoA molecules, and **(ii)** 7 more high-energy ATP bonds are spent by acetyl-CoA carboxylase in carboxylating 7 acetyl-CoA molecules to 7 malonyl-CoA molecules. Thus a total of *15 high-energy phosphate bonds* are spent in forming each palmitate molecule.

(g) **Regulation of fatty acid synthesis :**

Short-term regulation of palmitate synthesis is carried out mainly by regulating *acetyl CoA earboxylase,* the rate-limiting enzyme of the process. (i) *Citrate,* produced by high rates of carbohydrate metabolism when on high-carbohydrate diet, allosterically activates acetyl-CoA carboxylase to raise its Vmax and consequently enhances the availability of malonyl-CoA for palmitate synthesis, thus increasing the rate of the latter. (ii) *Palmitoyl-CoA* and other long-chain acyl-CoA *antagonize the allosteric effect of citrate* on acetyl-CoA carboxylase to decrease palmitate synthesis, when on a high-fat diet. (iii) *Reversible cocalent activation* of acetyl-CoA carboxylase by its *phosphorylation* increases its activity, leading to enhanced palmitate synthesis; *dephosphorylation* produces the opposite effect.

Long-term regulation depends mainly on the *inducing action of insulin* on the synthesis of acetyl-CoA carboxylase, fatty acid synthase, ATP-citrate lyase, and NADPH-generating enzymes such as malic enzyme and the dehydrogenases of the pentose phosphate pathway.

3.10.2 Microsomal elongation of fatty acids

Longer saturated fatty acids ($>C_{16}$) are mostly formed from palmitate by its stepwise elongatin catalyzed by separate individual enzymes of the *microsomal elongase system* of the smooth ER membranes, using C_2 units from malonyl-CoA molecules. You may recall that malonyl-CoA is formed in the cytoplasm by the carboxylation of acetyl-CoA by acetyl-CoA carboxylase; such malonyl-CoA can be used for both the synthesis of palmitate and its elongation to longer fatty acids. However, during their elongation, fatty acids are not carried by any acylcarrier protein nor do the individual enzymes of the elongase system occur as a multienzyme protein. In non-neural tissues, the microsomal elongase system enzymes almost solely catalyze the elongation of palmitic acid (C_{16}) to stearic acid (C_{18}) through the following reactions; but such enzymes of brain microsomes catalyze the elongation of palmitate to much longer ($C_{18} - C_{24}$) fatty acids for brain lipids. Overall reaction in elongating palmitate to sterate is quoted below :

Palmitoyl-CoA + malonyl – CoA + 2NADPH + $2H^+$ → stearoyl-CoA + $2NADP^+$ + H_2O

(a) Microsomal *3-Ketoacyl-CoA synthase* transfers the palmitoyl group from palmitoyl-CoA to the methylene-C (C^2) of malonyl-CoA, relasing the CoA from palmitoyl-CoA and the carboxyl-C (C^3) of malonyl-CoA as CO_2 (Fig. 3.14). This results in the formation of 3-ketostearoyl-CoA (a 3-ketoacyl-Coa).

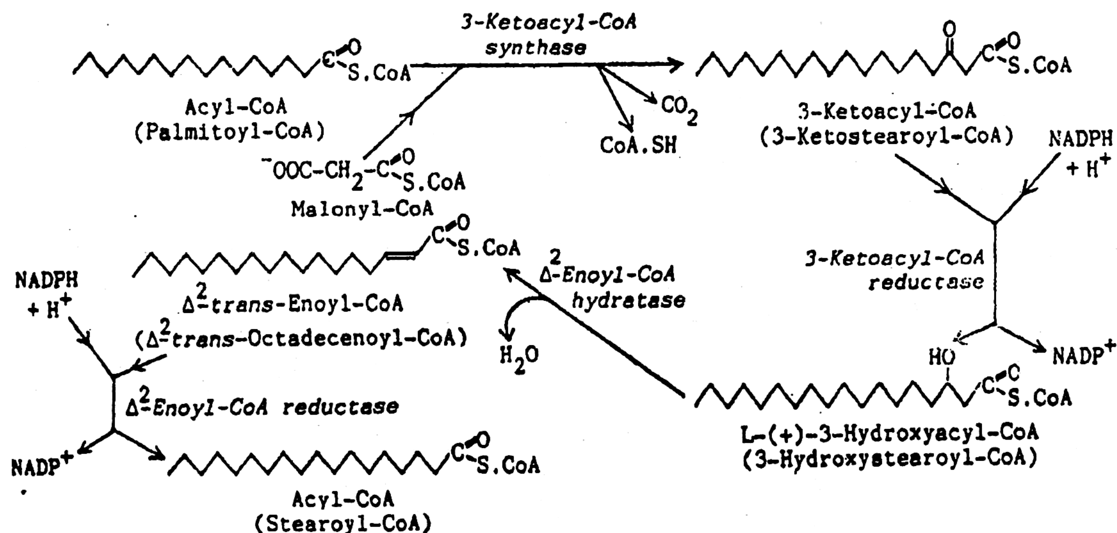


Figure 3.14 : Elongation of polmitoyl-CoA to stearotyl-CoA [From D. Das, *Biochemistry*, Academic Publishers, 2000]

(b) *3-Ketoacyl-CoA reductase* next used NADPH to reduce 3-ketosteraoyl-CoA to L(+)-3-hydroxystearoyl CoA (a 3-hydroxyacyl-CoA).

(c) Δ^2-*Enoyl-CoA hydratase* releases H_2O from L(+)-3-hydroxystearoyl-CoA to give Δ^2-*trans*-octadecenoyl-CoA (a Δ^2-*trans*-enoyl-CoA).

(d) Δ^2-*Enoul-CoA rductase* finally reduces the product of reaction (c) using NADPH, to form a C_2-higher saturated acyl-CoA which, in the present example, is stearoyl-CoA.

3.11 Prostaglandin biosynthesis

Prostaglandins (PG) are monoenoic or polyenoic, hydroxy-substituted and Keto-substituted, cyclic C_{20}-fatty acids. The molecule consists of an unsaturated liner carbon-chain with a saturated C_5-cyclopentase ring in the middle of that chian. Prostaglandins are classified into PG_1, PG_2 and PG_3 series with the subscripts indicating the number of double-bonds in their linear chains. Prostaglandins of each series are sub-classified into PGD, PGE, PGF, etc., according to the positions and types of the substituent groups on their cyclopentane rings (Fig.3.15).

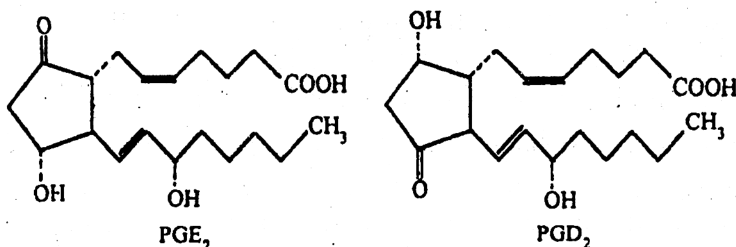


Figure 3.15 : Two prostaglandins.

3.11.1 Cyclo-oxygenase pathway of PG synthesis

Prostaglandins are synthesized by this pathway by enzymes of smooth ER membranes from different C_{20}-polyenoic (polyunsaturated) fatty acids in diverse tissues such as lungs, kidneys, seminal vesicles, vascular endothelia, liver, uterus, brain gastrointestinal tract, thymus, heart, adipocytes and platelets. Prostaglandins of PG_1, PG_2 and PG_3 series, containing respectively 1, 2 and 3 double-bonds in their linear molecular chains, are biosynthesized respectively from dihomo-γ-linotenic acid, arachidonic acid and timnodonic acid, released from membrane phospholipids into the cytoplasm by the action of Ca^{2+} dependent specific *phospholiphases* such as phospholipases A_1, A_2 and C. These three fatty acids released from membranes are C_{20} polyenoic fatty acids bearing respectively 3, 4 and 5 double-bonds. The phospholipase-catalyzed release of these C_{20}-polyenoic acids is the *rate-limiting reaction* of PG biosynthesis—while angiotensin, bradykinin and hypoxia activate the phospolipase to enhance PG synthesis, mepacrine and corticosteroids inhibit the phospholipases and depress PG synthesis. The released C_{20}-polyenoic acids are converted to prostaglandins by the *cyclo-oxygenase pathway* summarized below (Fig. 3.16). The principal enzyme system of this pathway is a microsomal heme-containing multienzymes system, *prostaglandin endoperoxide synthase.*

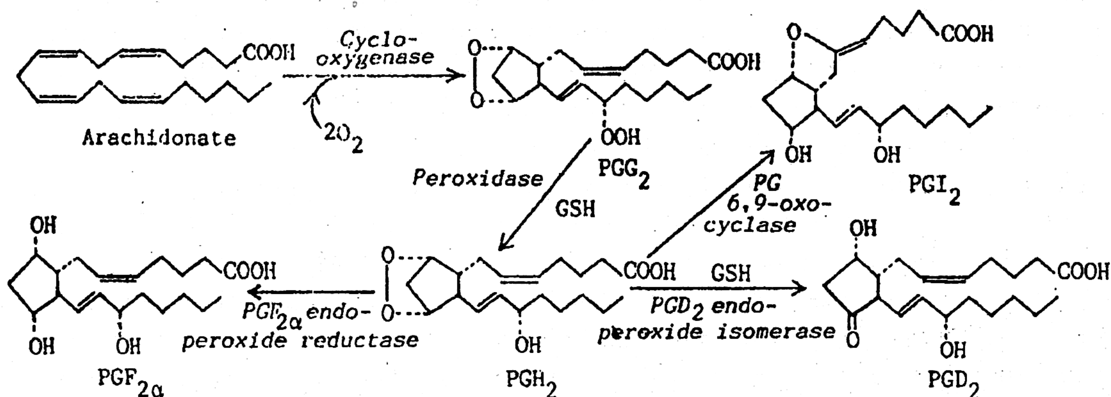


Figure 3.16 : Cyclo-oxygenase pathway for PG_2 series. [From D. Das, *Biochemistry*, Academic Publishers, 2000]

(a) The *fatty acid cyclo-oxygenase* component of the PG endoperoxide synthase system oxidizes and cyclizes the C_{20}-polyenoic acids (e.g., arachidonic acid) into the respective unstable cyclic endoperoxides (e.g. PGG_2 from arachidnate).

(b) The *Peroxidase* component of the PG endoperoxide synthase system changes the endoperoxides (e.g. PGG_2) into the other respective unstable cyclic endoperoxides (e.g., PGH_2) using glutathione as a reducing cofactor and H_2O_2 as an electorn-acceptor.

(c) Microsomal PG *endoperoxide isomerases* may then use glutathione as a cofactor in isomerizing the unstable PG endoperoxides into prostaglandins like PGD_2 and PGE_2.

(d) *Prostaglandin endoperoxide reductases* may then reduce prostaglandins like PGH_2 and PGE_2 into still other prostaglandins, like PGF_{2a} from PGH_2 or PGF_2.

Catecholamines, vasopressin and angiotension enhance PG biosynthesis by increasing PG endoperoxide synthase activity; acetylsalicylate, indomethacin and nonsteroid antiinflammatory agents inactivate the fatty acid cyclo-oxygenase and thereby decrease PG biosynthesis.

3.12 Muscle contraction

There are significant differences between the molecular mechanisms of contrcaction of striated and smooth muscles. These basiclly arise from differences between their muscle proteins. Here, you will read first about muscle proteins of striated muscles and their roles in the mechanism of contraction. You will next read about how the differences in muscle proteins between striated and smooth muscle fibres result in a distinctive mechanism of smooth muscle contraction.

3.12.1 Major muscle proteins

Dark anisotropic and electron-dense A bands of each myofibril of a striated muscle fibre contain *thick protein myofilaments,* made of parallel and staggered molecules of the muscle protein **myosin.** Each hexameric myosin molecule is made of two heavy and four light L peptide chains (Fig. 3.1.7). The α-helical C-terminal segments of the two H chains are entwined into a left-handed coil forming a double-stranded fibrous rod-like domain, while the N-terminal end of each H chain bears a pear-shaped globular S_1 *domain* with *myosin-ATPase* activity—an *essential L chian* (ELC) and a *regulatory L chiain* (RLC) remain associated with the S_1 domain. Trypsin hydrolyzes myosin into a *heavy meromyosin* (HMM) molecule consisting of two S_1 domains connected to a C-terminal rod-like tail of healically coiled fractions of two H chains, and a double-stranded rod-like *light meronyosin* (LMM) molecule consisting of the helically entwined C-terminal ends of the two H chains. Hydrolysis of HMM by papain yields two *S_1 Subfragments* with their associated L chains, and a double-helical rod-like *S_2 subfragment* (Fig. 3.17).

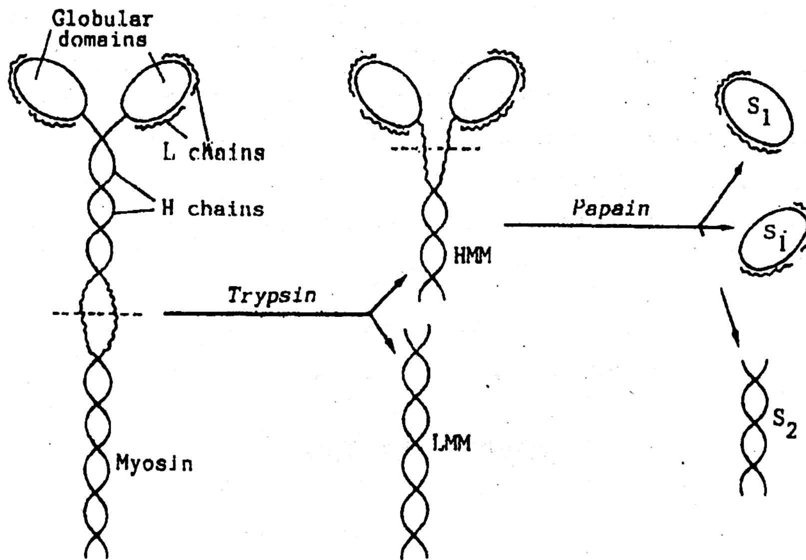


Figure 3.17 : A myolecule and its cleavage frogments. [From D. Das, *Biochemistry*, Academic Publishers, 2000]

In each thick myofilament, many parallel myosin filaments are arranged in two bundles with their N-terminal S_1 domains oriented towards opposite poles at the two margins of the A band and the C-terminal ends of both bundles meeting end-to-end at the less dense H zone in the middle of the A band. Moreover, myosin molecules are staggered both longitudinally and helically along the thick myofilament so that their S_1 domains protrude from the two outer segments of the filament at regular intervals along their and at staggered angles from the filament (Fig. 3.19).

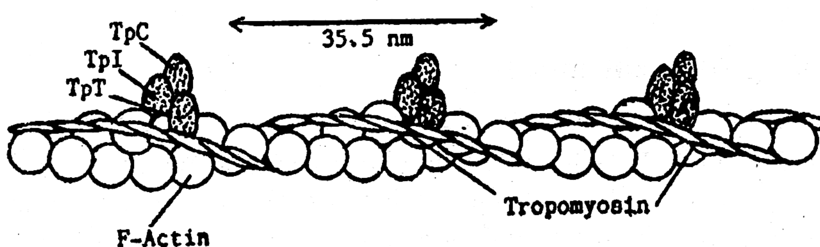


Figure 3.18 : A thin myofilament. [From D. Das, *Biochemistry*, Academic Publishers, 2000]

Light, isotropic I bands consist mainly of parallel, double-stranded, *thin protein myofilaments,* each made of two helically entwined, long and beaded strands of *F-actin polymers* (Fig.3.18). F-actin is formed by the ATP and Mg^{2+} dependent polymerization of soluble *G-actin* molecules at isotonic ionic concentration. Double stranded cables of the protein *tropomyosin* course along the helical central groove of

F-actin double-strands which also remain associated at intervals with three inter-connected globular peptide subunits of *troponin.* The F-actin and tropomyosin double-strands and the troponin subunits together constitute a thin myofilament (Fig. 3.18). F-actin filaments extend from the central Z line of an I band upto some distance into the neighbouring A band and interdigitate with the thick myosin filaments of the latter (Fig. 3.19). F-actin strands acquire an arrow-head pattern along their lengths due to their binding to myosin S_1 heads, giving a polarity to their filaments.

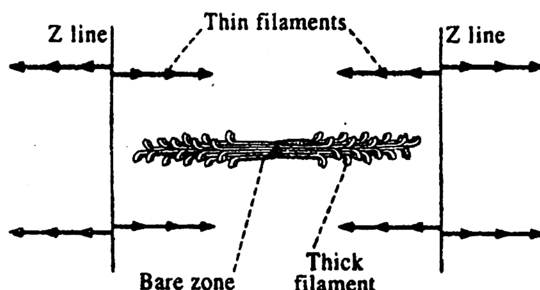


Figure 3.19 : Polarities of thick and thin filaments. [From D. Das, *Biochemistry*, Academic Publishers, 2000]

Peptide subunits of each *troponin system* associated with the F-actin double strand consist of **(i)** *troponin* C (TpC) having Ca^{2+} binding sites, **(ii)** *TpT* binding to tropomyosin and (iii) *Tpl* binding to F-actin and TpC and inhibiting the actin myosin interaction (Fig. 3.18).

Besides the major muscle proteins described abvoe, several minor muscle proteins such as α-actinin, desmin, vimentin, C-protein and M-protein also help in assembling myofilaments in myofibrils.

3.12.2 Sliding filament model

According to this model of muscle contraction, myofilments themselves do not change in length during the contraction or relaxation of the muscle; instead, the muscle fibres are shortened due to the shortening of their *sarcomeres.* Sarcomeres are successive segments of each myofibril and function as the functional units of the latter—each sarcomere extends along the myofibril from one Z line to the next (Fig.3.19). According to this model, thin actin filaments slide during muscle contraction towards the centre of the A band, penetrating deeper between the thick myosin filaments interdigitating with them within that band. Such sliding of actin filaments in between the myosin filaments of the A band draws the Z lines at the two ends of the sarcomere closer to each other, shortening the length of each sarcomere from ~2300 nm in a relaxed myofibril to ~1500nm in its contracted condition and thereby shortening the muscle

fibre. Shortening of the sarcomeres during muscle contraction increases the overlap between the thin actin filaments and the thick myosin filaments in each A band and greatly shortens the lengths of I bands, but does not affect the length of any A band because its length depends solely on the lengths of its myosin filaments slide out from between the interdigitating thick myosin filaments so that the overlap between the two type of filaments decreaes. This enhances the length of each I band considerably and elongates each sarcomere from ~1500 nm in the contracted myofibril to ~2300 nm in the relaxed one, but again leaves the length of the A band unaltered.

3.12.3 Molecular mechanism of muscle contraction

In striated muscles, contraction and relaxation result from an actin-myosin interaction cycle. Contraction of nonstriated muscles, on the contrary, is principally myosin based.

Actin-myosin interaction :

The modern concept of the contraction of a muscle due to the actin-myosin-ATP interaction, and of the ATP-related dissociation of actomyosin (actin-myosin complex) during its relaxation, is based primarily on *Szent-Gyorgi's findings* that **(i)** addition of an action solution to a myosin solutin resulted in an increased viscosity of the latter due to actomyosin formation by actin-myosin interaction, **(ii)** addition of ATP to that mixed solution decreased its viscosity again, indicating the dissociation of actomyosin into actin and myosin, **(iii)** actomyosin threads were found to contract on being immersed in a solution containing Mg^{2+}, K^+ and ATP, and **(iv)** myosin threads did not contract when dipped into a similar solution.

According to the modern concept of the striated muscles contraction, the attachment of the globular S_1 domains of myosin-heads with the adjacent F-actin strands gives rise to cross-bridges between the myosin and actin filaments, leading to the sliding-in of the thin actin filaments deeper between the thick myosin filaments. This *endergonic* sliding-in of the filaments is accomplished by coupling it with the *exergonic* hydrolysis of ATP by *myosin-ATPase* of the S_1 domains of myosin-heads—the activity of the myosin-ATPase is manifold heightened by the binding of F-actin to myosin. During relaxation, on the contrary, the detachment of the S_1 domains of myosin-heads from F-actin cleaves the cross-bridges between the acting and myosin filaments, leading to the sliding-out of thin actin filaments from between the thick myosin filaments, leading to the sliding-out of thin actin filaments from between the thick myosin filaments. The sequence of reactions in this actin-myosin interaction cycle is described below (Fig.3.20).

(a) During relaxation, *Ca^{2+} –ATPase* of the sarcoplasmic reticulum membrane actively transports Ca^{2+} ions from the sarcoplasm to the SR cisternae where the protein *calsequestrin* binds to the Ca^{2+} ions and sequesters them. This maintains the sarcoplasmic

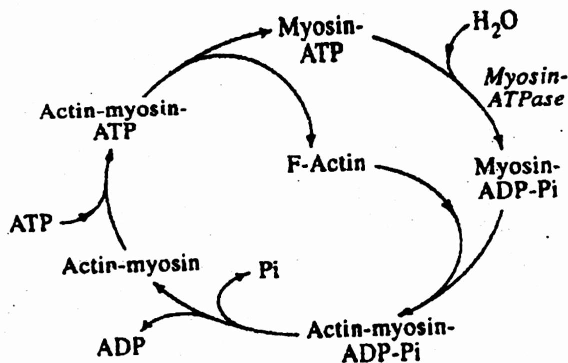


Figure 3.11 : Actin-myosin interaction cycle during contraction and relaxation of striated muscle. [From D. Das, *Biochemistry*, Academic Publishers, 2005]

Ca^{2+}concentration below 10^{-6} M and thus keeps the *trponin* C (TpC) of thin filaments almost free from Ca^{2+} and consequently incapable of promoting any actin-myosin interaction. In other words, this lack of TpC activity permits the *troponin I* (TpI) to continue inhibiting actin-myosin interaction, probably by blocking the S_1-binding sites of F-actin and thereby keeping the latter detached from the myosin S_1 domains. Myosin still binds to ATP which is hydrolyzed by the *myosin-ATPase* of its S_1 domains to ADP and Pi; but in the absence of attachments between F-actin and myosin, most of the ADP and Pi fail to be released from myosin and continue to remain as the high-energy myosin-ADP-Pi complex, retaining the free energy of ATP hydrolysis. In this state, the S_1 headpieces of myosin make an angle of ~90° with the axis of the thick myosin filament and neither actin-myosin interaction nor filament sliding can take place. This continues to prevent the shortening of myofibrils in the relaxed muscle.

(b) When the muscle fibre is stimulated, the depolarization of membranes of its T-tubular system leads to the opening of *ligand-gated Ca^{2+} –release channels* of the SR membrane; the consequent release of Ca^{2+} ions through these opened Ca^{2+} channels into the sarcoplasm from the SR cisternae, where they were so long sequestered by calsequestrin, increases the sarcoplasmic Ca^{2+} concentration above 10^{-5} M, causing the binding of Ca^{2+} to the *troponin C* (TpC) component of F-actin-bound TpC from active TpC-$(Ca^{2+})_4$ comples. The latter allosterically rolls the troponin T-bound *tropomyosin double-stranded cables,* coursing through the central groove of the F-actin filament, to move them deeper into that groove. This makes the S_1-binding sites of F-actin more accessible and enables their electrostatic bonding with the myosin S_1 heads. This counteracts the inhibitory effect of TpI on actin-myosin interaction.

(c) A weak initial binding of F-actin of S_1 headpieces releases Pi from the high-energy myosin-ADP-Pi complex and increases the actin-affinity of myosin S_1 heads. This causes a stronger binding of the two. It leads to a change in the high-energy

conformation of the actin-bound myosin to a low-energy conformation bringing about a rotation of the myosin heads from an angle of 90°–to a 45°–angle with the myosin filament axis (Fig.3.21), releasing ADP from the myosin head and resulting in the formation of actin-myosin complex. The rotation of myosing heads acts as the *power stroke* utilizing the energy released by the conformational change of the actin-bound myosin, and pulls the thin actin filaments to slide them by >7 nm towards the A band centre and between the interdigitating thick myosin filaments.

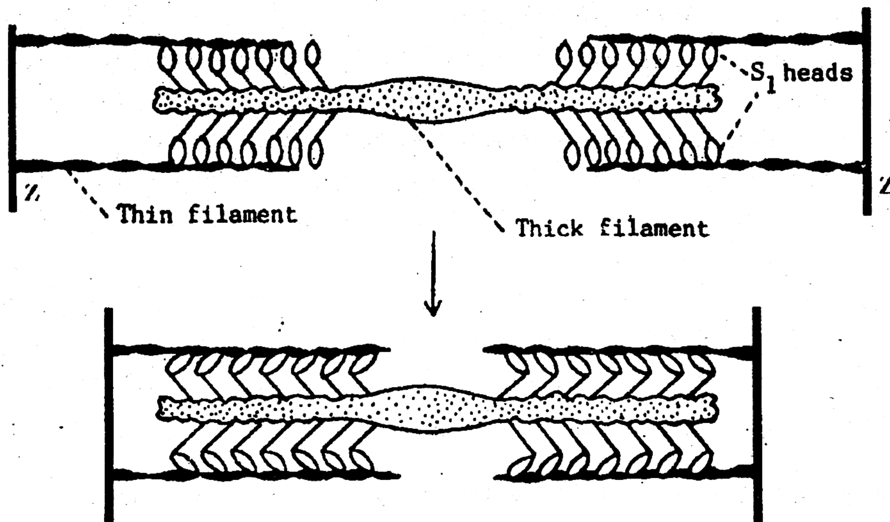


Figure 3.21 : Mechanism of sliding of myofilaments during muscle contraction. [From D. Das, *Biochemistry*, Academic Publishers, 2000]

(d) Next, a fresh ATP molecule binds to the myosin S_1 domain to form an actin-myosin-ATP complex. This weakens the actin-affinity of myosin, detaches the myosin S_1 heads from F-actin, thereby cleaves the cross-bridges between actin and myosin filaments, and make them slide away from each other. This lengthens the myofibril in the relaxed muscle, while myosin is left behind as a myosin-ATP complex.

(e) Myosin-ATPase hydrolyzes the myosin-bound ATP to ADP and Pi, and the resulting myosin-ADP-Pi complex awaits the next cycle of actin-myosin interaction (Fig. 3.20).

3.12.4 Myosin-based contraction

Smooth muscle fibres also depend for their contraction on the interaction between longitudinally oriented thin and thick myofilaments made principally of F-actin and myosin molecules respectively. But smooth muscle fibres differe in many ways from striated muscle fibres with respect to microanatomical structure, chemical constituents

including muscle proteins, and regulatory mechanisms. These make the events in smooth muscle contractions considerably more dependent on myosin.

In smooth muscle cells, myofilaments are not organized into myofibrils; sarcoplasmic reticuluns and T-tubules are rather rudimentary, sarcomeres are not aligned to give a striated appearance, and no troponin system is associated with the F-actin strands; instead of troponin, a protein *caldesmon* remains bound to F-action in the relaxing muscle, inhibiting the actin-myosin interaction—this interaction is promoted in the contracting muscle by ht erelease of caldesmon from actin. Moreover, smooth muscle myosin possesses characteristic light peptide chains, called *p-light chains* or *pL-myosin,* different from the L chains of striated muscle myosin; this pL-myosin exists in an inactive phosphorulated from and an active dephospho form, and plays the role of an inhibitor of actin-myosin interaction in place of troponin I. The sarcoplasm also contains **(i)** a *myosin p-light-chain kinase* (ML CK) which catalyzes the ATP-driven phosphorylation and consequent inhibition of pL-myosn, **(ii)** a Ca^{2+} –binding protein *calmodulin* (CaM) whose Ca^{2+}– bound form (CaM.4Ca^{2+}) participates in activating MLCK, and **(iii)** a Ca^{2+}– independent *myosin light-chain phosphatase* which can catalyze the dephosphorylation of phospho-pL-myosin into active dephospho-pL-myosin.

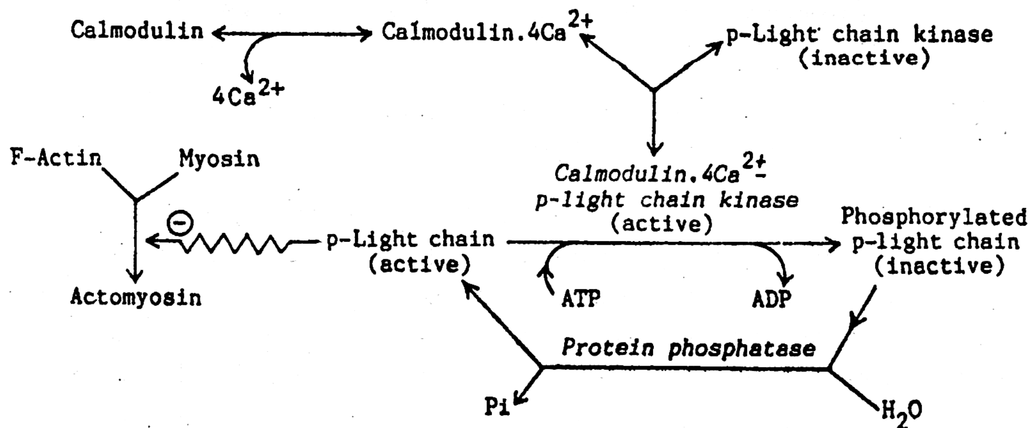


Figure 3.22 : Regulation of smooth muscle contraction by Ca^{2+} ions. [From D. Das, *Biochemistry,* Academic Publishers, 2000]

(a) In the relaxing smooth muscle with a sarcoplasmic Ca^{2+} concentration lower than 10^{-7}M, actin-myosin interaction remains inhibited by the active *dephospho-pL-myosin* as well as the acting-bound *caldesmon.* Antonomic nerve impurses, reaching the smooth muscle cell, depolarizes its plasma membrane; this enhances the Ca^{2+} influx across the latter from the ECF to the sarcoplasm, where *calmodulin* binds to

the inflowing Ca^{2+} to form the $CaM.4Ca^{2+}$ complex (Fig. 3.22). When the sarcoplasmic Ca^{2+} concentration exceeds about 10^{-5} M in this way, optimum amounts of $CaM.4Ca^{2+}$ –molecules bind to the dephospho form of MLCK to activate the latter. The active $CaM.4Ca^{2+}$ -MLCK now catalyzes an ATP-driven phosphorylation of active dephospho-pL-myosin into inactive phosphorylated pL-myosin which can not longer inhibit the actin-myosin interaction. Simultaneously, the $CaM.4Ca^{2+}$ complex may bind to the actin-bound caldesmon and dislodge it from actin. Thus actin-myosin interaction is now freed from the inhibitory effects of both pL-myosin and caldesmon. This leads to the attachment of myosin heads to actin filaments and the sliding of myosin and actin filaments towards each other for muscle contraction.

(b) With the onset relaxation, membrane *C^{2+} –ATPase* actively extrudes Ca^{2+} from the sarcoplasm to lower the sarcoplasmic Ca^{2+} concentration below 10^{-7} M. This releases Ca^{2+} from sarcoplasmic $CaM.4Ca^{2+}$ complex, changing it to calmodulin. **(i)** The lowering of sarcoplasmic $CaM.4Ca^{2+}$ concentration allows the actin-inhibiting protein caldesmon to bind again to actin, thereby inhibiting the actin-myosin interaction. **(ii)** Having lost its Ca^{2+}, calmodulin is released from its binding with MLCK which is thereby inactivated and unable to phosphorylate and to inactive pL-myosin. **(iii)** The Ca^{2+} independent *myosin light chain phosphatase* dephosphorylates and activates the remaining phospho-pL-myosin. **(iv)** The rise in active phospho-pL-myosin due to **(ii)** and **(iii)** enhances its inhibitory action on actin-myosin interaction. All these events lead to the detachment and sliding of actin and myosin filaments away from each other.

3.13 Summary

Active transports are transmembrane transfers fo specific molecules or ions by some membrane proteins, acting as specific transporters or carriers against the electorchemical gradients of the transppported substrates across the membrane. Such transports are endergonic and must be carried out by being coupled with exergonic reactions such as either the exergonic hydrolysis of high energy ATP-bonds by $Na^+ - k^+$ ATPase or the exergonic dissipation of transmembrane ion-gradients as in cases of mitochondrial ATP-ADP translocase and intestinal Na^+-glucose symport. Active transports may be uniports, symports or antiports, according respectively to whether a single substrate, or two substrates in the same direction or two substrates in opposite directions are transported against their respective electrochemical gradients. Active transport is unidirectional in a specific direction, sigmoid kinetics in some cases of symport and antiport.

In ureofelic animals, the nitrogenous waste-products of amino acid catabolism are converted to urea as the ultimate urinary NPN end product in the hepatic arginine-urea cycle spending four ATP bonds per urea molecule formed.

Glucose absorbed from dietary carbohydrates in the intestine is converted by glycogenesis in liver, muscle and many other tissues to glycogen for storage, spending two high energy ATP bonds per glucose molecule incorporated in glycogen.

Noncarbohydrates, particularly many amino acids and products of glycolysis such as lactate and pyruvate may be converted back to glucose by gluconeogensis in liver and kidneys, Gluconeogenesis from pyruvate or lactate requires the spending of six high-energy phosphate bonds per momecule of glucose synthesized.

Gluathione, a tripeptide acting as an important reducing agent, is synthesized from glutamate, cysteine and glycine at the cost of two ATP bonds in the cytoplasm, but without the participation of polysomes because one of its peptide bonds is a γ-peptide bond instead of an α-peptide bond. Norepinephrine and epinephrine are synthesized in adrenergic neurons and adrenal medulla from the amino acid tyrosine. Tryptophan is used in synthesizing serotonin in serotonergic neurons and melatonin in pineal gland cells.

Saturated fatty acids, plamitic acid in particular, are synthesized in liver, adipocytes and mammocytes by two cytoplasmic multienzyme proteins, viz., acetyl-CoA carboxylase and fatty acid synthase, using acetyl-CoA and NADPH. Such synthesis of a molecule of palmitate involves the expenditure of 15 high energy phosphate bonds. Usually, longer saturated fatty acids are synthesized from palmitate by the stepwise additions of C_2 units with the help of microsomal fatty acid elongase system of enzymes. C_{20}-polyenoic fatty acids, released by phospholipase action on membrane phospholipids, are used in synthesizing prostaglandins by the cyclo-oxygenase pathway with the participation of a midrosomal multienzyme system called the prostaglandin endoperoxide synthase.

According to the sliding filament model, muscle contraction results from the sliding-in of thin actin filaments between thick myosin filaments interdigitating with each other in myofibrils. In striated muscle fibres, the molecular mechanism of muscle contractiion involves and elaborate actin-myosin interaction. In monstriated muscles, contraction is more myosin-based. These mechanisms have been discussed in some detail.

3.11 Terminal questions

1. (a) Give an account of the elongation of palmitate to stearate by smooth ER enzymes.

   (b) Describe how the following specialized products are synthesized from amino acids : (i) glutathione, (ii) epinephrine, (iii) melatonin.

2. (a) Differentiate between active and passive carrier-medialted transports.

   (b) Discuss the characteristics of active transports, including their kinetics.

   (c) Describe the significances of active transports.

3. (a) Describe the synthesis of glucose from lactate, using a suitable flow chart.

   (b) State the significances of gluconeogensis.

   (c) Using a suitable diagram, describe the enzymatic reactions of the principal steps of the cyclo-oxygenase pathway for synthesizing PGD_2- and $PGF_{2\alpha}$ from C_{20}-polyenic acids of membrane.

4. (a) Describe how acetyl-CoA and NADPH are made availabel for fatty acid biosynthesis in the cytoplasm of ruminant na dnonruminant animals.

   (b) Describe a suitable diagram the action of acetyl-AoA carboxylase for palmitate synthesis.

   (c) Give an account of the domains of multienzyme fatty acid synthase of mammals and the enzymatic and nonenzymatic sites in thsoe domains.

   (d) Discuss the short-term and long-term regulations of fatty acid synthesis.

5. (a) What are the significnaces of urea biosynthesis in animals?

   (b) Describe the arginine urea pathway of urea biosynthesis, using a suitable flowchart and mentioning the energetics of the process.

   (c) Discuss the regulation of urea biosynthesis.

6. (a) Describe different active sites in the domains of multienzyme fatty acid synthase.

   (b) With a suitable flow chart discuss the reactions of palmitate synthesis by the enzymic components of fatty acid synthase.

   (c) Mention the energetics of palmitate synthesis.

7. (a) Discuss how glycogenesis is regulated.

   (b) Describe using suitable diagrams the process of glycogenesis, mentioning its energetics.

   (c) How is glycogenesis regulated?

8. (a) Mention different types of ATPases involved in ATP-driven active transports.
   (b) Describe with a suitable flow-chart the action of the $Na^+ - K^+$ ATPase as an ATP-driven active antiport.
   (c) Discuss the role of ATP-ADP translocator in ion gradient-driven active transport.
9. (a) Describe Na^+ -glucose symport with a diagram, as an example of ion gradient driven active transport.
   (b) Describe the sliding filament model of muscle contraction.
10. Discuss how actin-myosin interaction results in the contraction and relaxation of striated muscle fibres.
11. (a) Describe with a flowchart the myosin-based contractions of smooth muscles, regulated by Ca^{2+} ions.
   (b) Write briefly about the following two muscle proteins : (i) troponin system; (ii) myosin.

3.15 Answers

1. (a) See Section 3.10.2.
   (b) See Sections 3.7-3.9.
2. (a) See part (b) of Section 3.2.
   (b) See Section 3.3.1.
   (c) See 3.3.2.
3. (a) Section 3.6.2.
   (b) See Section 3.6.1.
   (c) See Section 3.11.1.
4. (a) See parts (a) and (b) of Section 3.10.1.
   (b) See part (c) of Section 3.10.1.
   (c) See part (d) of Section 3.10.1.
   (d) See part (g) of Section 3.10.1.
5. (a) See relevant part of Section 3.4.
   (b) See relevant parts of Section 3.4.
   (c) See relevant part of Section 3.4.

6. (a) See part (d) of Section 3.10.1.
   (b) See part (e) of Section 3.10.1.
   (c) See part (f) of Section 3.10.1.
7. (a) See relevant part of Section 3.5.
   (b) See relevant parts of Section 3.5.
   (c) See relevant part of Section 3.5.
8. (a) See first paragraph of Section 3.3.4.
   (b) See part (a) of Section 3.3.4.
   (c) See paragraph (a) of Section 3.3.5.
9. (a) See part (b) of Section 3.3.5.
   (b) See Section 3.12.2.
10. (a) See Section 3.12.3.
   (b) See first, second and third paragraph of Section 3.12.1.

Unit 4 ❐ Enzymes

Structure

4.1 Introduction

Objectives.

4.2 Enzyme - Substrate interaction

4.3 Specificities of enzymes

4.4 Michaelis - Menten kinetics

4.5 Covalent Modifications of enzymes

4.6 Allosteric Modulations of enzymes

4.7 Isozymes

4.8 Ribozymes

4.9 Rate-limiting enzymes

4.10 Summary

4.11 Terminal questions

4.12 Answers

4.1 Introduction

You are aware that in living organisms, chemical reactions are generally catalyzed by enzymes which are proteins in nature. In this unit, however, you will know about some RNA molecules also, that function as enzymes. This unit will describe how highly reactive but transient complexes are formed by enzyme-substrate interactions for the catalysis. Each encyme can bind to and change only one or a few substrates; different enzymes may possess different types of such substrate specificity. You will learn here about the frequent existence of more than one protein for catalyzing the same reaction.

Therefore of enzyme-catalyzed reactions may be either rectangular hyperbolic functions or sigmoid functions of the substrate concentration, according as they follow the Michaelis-Menten hyperbolic kinetics or the Hill equation for sigmoid kinetics. These will be elaborated in this unit.

Activities of some enzymes may be regulated by reversible addition and removal of specific groups to/from them through the formation/cleavage of covalent bonds. Some inactive proenzymes are irreversible changed to active enzymes by the hydrolytic removal of a part their paptide chain. This unit will also describe the activation or

inhibition of some enzymes by substances called allosteric modulators, binding to specific sites of the enzyme molecule, distinct from the substrate-binding site. Often a metabolic pathway may be augmented or impeded by modulating in various ways one or more rate-limiting enzymes of theat pathway.

Objectives

On reading this unit, you should be able to :

- Understand the transition state theory of enzyme-sybstrate interaction,
- Describe the models of enzyme-substrate interactions,
- Know about the bonds involved in forming enzyme-sybstrate complexes,
- Explain different types of substrate specificities of enzymes,
- Understand the Michaelis-Menten substrate saturation Kinetics,
- Explain the significance and narrate the characteristics of K_m,
- Describe reversible covalent modifications of enzyme,
- Narrate how some proenzymes undergo irreversible convalent activation,
- Learn about ribozymes and isozymes,
- Define rate-limiting enzymes and their characteristics,
- Describe the characteristics of allosteric modulations,
- Understand the sigmoid saturation kinetics and its changes during allosteric modulations,
- Narrate different models of allosterism.

4.2 Enzyme-Substrate Interaction

Like inorganic catalysts, enzymes bind to their substrates to form transient and highly reactive enzyme-substrate complexes (ES Complexes).

4.2.1 ES Complex formation

The action of an enzyme (E) on its substrate (S) is always initiated by the binding of specific groups of the substrate molecule to the sidechains of specific amino acid residues at the *active site* of the enzyme molecule to form the activated but unstable ES complex. The latter would soon dissociate into the unchanged enzyme and the product (P) formed by the catalyzed change of the substrate. That such ES complexes are formed during enzyme actions, has been supported by (i) the *isolation* of some ES complex of glyceraldehyde 3-phosphate dehydrogenase, (ii) the *electron microscopic demonstration* of some ES complexes like those of nucleic acid

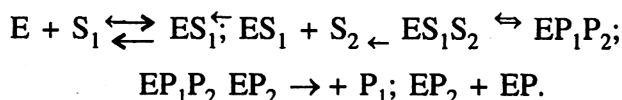
polymerases, (iii) the *X-ray crystallography* of some ES complexes such as that of carboxypeptidase A, and (iv) the *spectroscopic detection* of some ES complexes either by nuclear or electorn magnetic resonance (NMR or EMR) spectroscopy or by fluorescence spectroscopy, e.g., the ES complex of prokaryotic tryptophan synthase.

Formation and fate of ES complexes follow different routes in different types of enzymatic reactions. For example :

(a) In *single-substrate reactions* such as those catalyzed by isomerases in isomerizing a single substrate,

$$E + S \rightleftarrows ES \rightarrow E + P$$

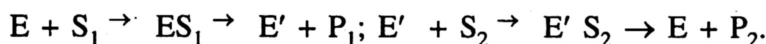
(b) In *bisubstrate reactions* between two substrates (S_1 and S_2), there are the following two alternative routes : (i) In *single-displacement bisubstrate reactions* such as those catalyzed by phosphotransferases (e.g., glucokinase) or by purine-dependent dehydrogenases (e.g., lactate dehydrogenase), S_1 and S_2 bind successively to E to form a ternary ES_1S_2 complex of the enzyme with both substrates; the products (P_1 and P_2) produced by the enzyme action are then released successively :



$$E + S_1 \rightleftarrows ES_1; ES_1 + S_2 \leftarrow ES_1S_2 \leftrightarrows EP_1P_2;$$

$$EP_1P_2\ EP_2 \rightarrow + P_1;\ EP_2 + EP.$$

But in (ii) *double-displacement bisubstrate reactions* such as those catalyzed by transaminases, one substrate (say, S_1) binds to the enzyme singly to form the first ES complex (ES_1) which dissociates to release the first product (P_1) and a modified E′ to form the second ES complex ($E'S_2$) which subsequently releases the second product (P_2) and the original form of E :



$$E + S_1 \rightarrow ES_1 \rightarrow E' + P_1;\ E' + S_2 \rightarrow E'S_2 \rightarrow E + P_2.$$

Bonds involves in the formation of ES complexed may be of several types :

(a) *Noncovalent bonds : (i) hydrogen bonds* between polar groups of the substrate and the enzyme, such as that between a uracil residue of RNA and sidechain OH groups of specific serine and threonine residues of pancreatic RNase; (ii) *ionic bonds* between counterionic polar groups of the substrate and the enzyme, such as that between the sidechain NH_4^+ group of a lysine residue of a histone (substrate) and a sidechain COO^- group of the enzyme trypsin; (iii) *hydrophobic interactions* holding the nonpolar groups of the substrate and the enzyme together, (iv) *van der Waals forces of attraction* between both polar and nonpolar groups, acting as dipoles in the substrate and the enzyme.

(b) *Covalent bonds* such as (i) *thioester or thiothemiacetal bonds* between sidechain SH group at the active site of the enzyme and a carboxyl or an aldehyde group of its substrate, as in the ES complex of glyceraldehyde 3-phosphate and glyceraldehyde 3-phosphate dehydrogenase, and (ii) *Schiff bases* between the sidechain NH_2 group of a basic amino acid residue (e.g., lysine) at the active site of the enzyme and a ketonyl group of the substrate, as in the ES complex of fructose 6-phosphate and transaldolase.

Role of enzyme-substrate complexes :

Formation of the ES complex may help in catalysis in various ways.

(i) The ES complex formation may *lower the energy barrier* for the reaction to be catalyzed, by providing alternative reaction paths requiring much less activation energy (vide 4.2.4).

(ii) It may bring the bond to be changed in the substrate very close to and *in proper alignment* with such groups of the enzyme as would participate in the catalysis.

(iii) It may strain the bond to be changed in the substrate to help in its change.

(iv) It may provide functional acidic, basic or other groups like NH_3^+, COO^-, thiol and phenolic OH groups for accepting or donating specific ions or groups during catalysis.

4.2.2 Active sites of enzymes

Several amino acid residues of the enzyme molecule function together to constitute its *active site*. Such amino acid residues may be situated in the peptide chain of the enzyme at distances considrably apart from each other, but have been brought in close proximity and proper steric relations to each other by the coils and folds of the three-dimensional secondary and tertiary structures of the enzyme molecule. Such higher orders of its molecular structure may also form a nonpolar cleft or crevice amidst the peptide chain coils, which is accessible to substrates possessing specific sizes and three dimensional shapes and contains specific amino acid residues of the enzyme with sidechains capable of binding to specific groups of the substrate; this constitutes the *substrate-binding site* in the active site of the enzyme. Specific amino acid sidechains of the binding site and/or of a nearby *catalytic site,* similarly constituted by the three-dimensional form of the enzyme, may catalyze changes of the relevant covalent bond in the enzyme-bound substrate.

Specific amino acid residues must occur at the active site of the enzyme for its action. Thus, *thiol enzymes* (e.g., papain, glyceraldehyde 3-phosphate dehydrogenase and HMG-CoA reductase), *lysine enzymes* (e.g., transaldolase), and *serine enzymes* (e.g., trypsin, chymotrypsin and acetylcholinesterase) must have respectively a cysteine, a lysine and a serine-residue, each at a specific position on the peptide chain and with a free sidechain, at their active sites.

Evidently, the substrate-affinity and the rate of enzyme action may be augmented or diminished if any change in the higher orders of its molecular structure affect the three-dimensional form of its active site. Moreover, the binding of specific nonsubstrate molecules to such a goup of the active, site of an enzyme, as is essentially required to remain free for the enzyme action, inactivates the enzyme; e.g., the binding of iodoacetate to the cystainyl-SH group at the active site any thiol enzymes inhibits the latter.

4.2.3 Models for enzyme-substrate interaction

Two models have been proposed for enzyme-substrate interactions.

(a) ***Fisher's template or lock and key model :***

Fisher proposed that irrespective of the presence or absence of the substrate, the active site of an enzyme exists in the three-dimensional form fully suitable for catalytic activity, and needs no change in that conformation for binding to and changing the substrate. He thus considered that the active site, even when existing by itself free from any substrate, occurs as a *rigid, pre-shaped template* with such three-dimensiional molecular form, size and groups as would readily fit with those of its substrate, therefore, the latter would bind to the active site wihtout any change in the pre-existing three-dimensional form of that site (Fig. 4.1) |——| the binding of the substrate to the active site could be compared with the fitting of a key into the pre-shaped keyhole of a lock (*lock-and-key model*). This would be followed by the enzyme-catalyzed change of the substrate into the product which is released leaving behind the active site in the same unchanged rigid template-like conformation. This model explained the sterospecificity of enzymes, proposing that only one specific stereoisomer of the substrate, but not its other stereoisomers, can fit into and bind to the pre-existing rigid template-like active

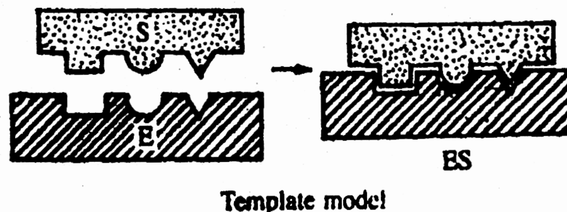


Figure 4.1 : Template model for enzyme-substrate interaction
[From D. Das, Biochemistry, Academic Publishers, 2000]

site. The coenzyme and the substrate would need to bind to the active site in a successive sequence, because the first of such ligands would bind to the rigid template-like active site, providing therby additional groups or binding sites for the binding of the next ligand. Fisher's template model met with no problems in case of the simple form of hyperbolic substrate-saturation kinetics, but could not explain how enzyme-activity changes on the binding of allosteric modulators at sites other than the substrate-binding site if the active site was rigid with no flexibility.

(b) ***Koshaland's induced fit model :***

Koshland proposed that the active site of an enzyme is not a rigid template with its full catalytic activity in its pre-shaped three-dimensional form in which it exists in the absence of the substrate. According to his model, the active site possesses significant *flexibility* and changes its three-dimensional conformation as the substrate molecule comes close to or binds loosely to it to form an initial superficial complex (Fig. 4.2). This initial interaction with substrate-alters the conformation of the active site to a final and much more active form which now binds the substrate more tightly and exerts the full catalytic action on it. This concept of flexibility and substrate-induced change of the active site has been strengthened by the conformational changes detected in many enzymes such as carbomoyl phosphate synthase I (mitochondria) and hexokinase (cytoplasm) on their initial binding to their repective substrates. Moreover, the allosteric modulation of enzymes can be explained by the idea of such conformational changes of the active site when a ligand binds to the allosteric site of such enzymes (vide 4.6.2 and 4.6.3).

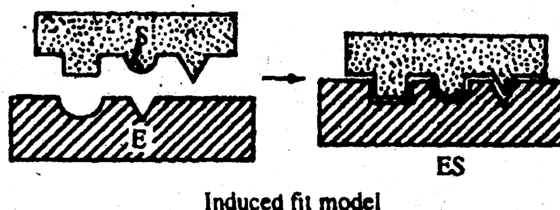


Figure 4.2 : Induced fit model for enzyme-substrate interaction
[From D. Das, Biochemistry, Academic Publishers, 2000]

4.2.4 Transition state theory

For any reaction, the reactants must collide or approach each other with a minimum Kinetic energy called the *activation energy* to overcome the *energy barrier* for forming or cleaving the bonds (*Kinetic theory of reactions)*. In any mass of reactants, however, the molecules possess different Kinetic energies, some having the free energy higher than the required activation energy and others falling short of it. A rise of temperature increases the reaction rate by enhancing the kinetic energies of reactant molecules so that a higher number of reactant molecules now have kinetic energies exceeding or equalling the activation energy and can thus overcome the energy barrier for the reaction. According to the *transition state theory* (activated complex theory), the *activation energy* necessary for crossing the energy barrier of a reaction is to be provided by the difference between the free energy of the reactants (A, B) and that of a transient activated intermediate (AB*) formed as a *transition complex* by the initial binding together of the reactants (Fig. 4.3). This transition complex soon dissociates into the products (C, D) if its free energy equals or exceeds the activation energy of the reaction.

$A + B \rightarrow AB^* \rightarrow C + D$

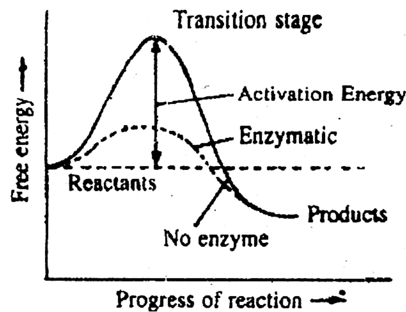


Figure 4.3 : Activation energy of a reaction in presence and absence of an enzyme [From D. Das, Biochemistry, Academic Publishers, 2000]

The transition state theory further proposes that the enzyme catalyzing a reaction binds to the substrate to lower the energy barrier for the reaction probably by providing alternative reaction paths so that much lower activation energy is required. Thus, even at the relatively low temperature in living organisms, the Kinetic energy of a larger number of reactant molecules enables them to form such transition complexes as have free energies equalling or exceeding the lowered activation energy requirement in present of the enzyme, and consequently yield the products at a higher rate.

4.3 Specificities of Enzymes

Each enzyme possesses two types of specificaties, viz., reaction specificity and substrate specificity.

4.3.1 Reaction specificity

Each enzyme catalyzes only a specific type of reaction of its substrate or substraes. For example, creatine phosphokinase, a phosphotransferase, would catalyze only the transfer of a phosphate group from phosphocreatine to ADP or from ATP to creating and no other reaction. Similarly, phosphothexose isomerase, an aldose-ketose isomerase, would carry out only an isomerization reaction between glucose 6-phosphate and fructose 6-phosphate. Because of such reaction specificities, enzymes have been categorized into classes like oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases, according to the specific type of reactions they catalyze.

4.3.2 Substrate specificity

Each enzyme can act upon either a single substrate as a small number of closely related substrates, because the catalytic action of the enzyme requires the substrate to bind to and interact with specific amino acid sidechains and groups at the three-dimensional active site of the enzyme and therefore, to possess specific groups and

three-dimensional form befitting the active structural specifications cannot bind to the active site and consequently cannot be changed by the enzyme. Several types of substrate specificities are described below.

(a) Group specificity :

An enzyme can often act only on a specific type of covalent bond associated or connected with only specific groups or residues in the substrate molecule. For example, gastric pepsin can hydrolyze only such peptide bond which connects the α-COOH group of an aromatic amino acid (Phe, Tyr or Trp) with the α-NH_2 group of another aromatic or dicarboxylic (Glu or Asp) amino acid; intestinal sucrase can hydrolyze such a β-2, 1-glycosidic bond as binds the α-anomeric OH group of C^1 of a glucopyranose the the β-anomeric OH group of C_2 of a fructopyranose. This type of specificity is called the group specificity.]

(b) Chain-length specificity :

Some enzymes can act only on substrates having specific lengths of carbon chains in their molecule. For example, fatty acid thiokinases possess such chain-length specificities for fatty acids; long-chain thiokinases thioesterify only long-chain fatty acids such as palmitic (C_{16}) and stearic (C_{18}) acids, medium-chain thiokinases act on C_4–C_{14} fatty acids, and acetate thiokinase acts similarly on only acetic (C_2) and propionic (C_3) acids.

(c) Geometric or cis-trans sterospecificity :

Some enzymes can act on substrates with double-bonds in their molecular chains, only if the double-bond has a specific steric configuration, either *cis* or *trans*. Such specificity of an enzyme for either *cis* or the *trans* isomer of the substrate, not for both, is called geometric or *cis-trans* stereospecificity. For example, fumarase can act only on the *cis*-isomer, fumaric acid, but not on its *trans*-isomer maleic acid.

(d) D-L stereospecificity

Some enzymes cna act only on the D-stereoisomers of their substrates and not on their L-steroisomers. For example, hexokinases phosphorylate several D-hexoses such ad D-glucose and D-glucosamine, but not their L-stereoisomers. Then, there are other enzymes which act specifically on L-stereoisomers of their substrates, but not on their D-stereoisomers. For example, L-glutamate, but not D-gluamate. Such *D-L stereospecificity* of enzymes indicates that the enzyme and its substrate bind to each other by at least a *three-point binding* so that the enzyme binds to one of the stereoisomers of the substrate; but being a mirror image of that isomer, the other stereoisomer of the latter cannot bind to the active site of the enzyme.

(e) D-l optical specificity :

Enzymes having D-L steresopecificity act also on only one specific optical isomer of the substrate, either its dextrorotatory *(d)* isomer or its levorotary *(l)* isomer, but not on both the optical isomers. This is because the D and L stereoisomers of a substance are also the *d* and *l* optical isomers of each other. For example, L(+)-3-hydroxyacyl-CoA dehydrogenase acts specifically on the dextrorotatory isimers of 3-hydroxacyl-CoA molecules, but not on their levorotatory isomers.

4.4 Michaelis-Menten kinetics

The rate of an enzyme-catalyzed reaction is usually measured and expressed as its *initial velocity* (V_0). The latter is defined as the rate of an enzymatic reaction when very little substrate has yet been converted into the product so that the resultant decline in the molar concentration [S] of the subhstrate has not alterted yet the rate of reaction significantly.

for many enzyme-catalyzed reactions, the initial velocity (V_0) has been found to be a *rectangular hyperbolic function* of the molar concentrations [S] of the substrate or substrates. In other words if the V_0, measured using different molar concentration [S] of a substrate, are plotted against the respective [S] values, the plotted points are found to be distributed along a rectangular hyperbolic curve (Fig. 4.4). This relation between sybstrate concentrations and initial velocities is known as the *Michaelis-Menten rectangular hyperbolic substrate-saturation kinetics* of enzymes. Many of the enzymes catalyzing single-sybstrate, bisubstrate and multisubstrate reactions obey this hyperbolic kinetics. We are, however, restricting ourselves here to single-substrate reactions only, such as those catalyzed by isomerases. A single-substrate reaction, you know, results from the interaction of the enzyme (E) and a single substrate (S).

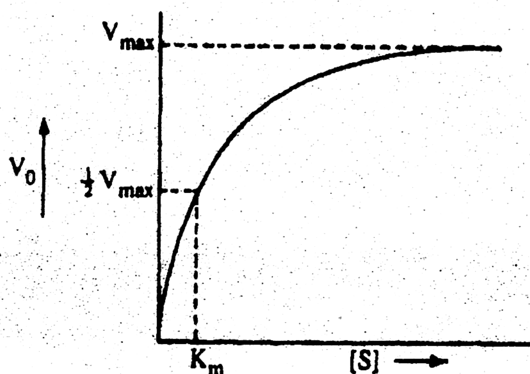


Figure 4.4 : Hyperbolic substrate saturation sainetics
[From D. Das, Biochemistry, Academic Publishers, 2000]

The hyperbolic substrate-saturation plot of a single-substrate reaction shows an *almost linear rise* in the rate of V_0 with the rise in [S] when [S] is low, because at low substrate concentrations, the rises proportionately with the rise in [S]. But at higher substrate concentrations, the *rate of rise in V_o declines progressively* to produce a curved *hyperbolic plot;* this indicates that the enzyme is the *limiting factor* in the enzyme substrate reaction because of a far lower number of enzyme milecules than thsoe of the substrate |——| this leaves less and less numbers of free enzyme molecules available for binding to fresh substrate molecules, with the rise in substrate concentration. Ultimately, at a still higher substrate concentration, the plot reaches a *plateau-like flat summit* because all enzyme molecules are now already saturated with substrate and interact with the substrate at their maximum capacity. The V_o now reaches the maximum level called the *maximum velocity* (V_{max}) which cannot be surpassed by increasing the [S] any further. This hyperbolic kinetics obeys the *Michaelis-Menten equation* which for a single-substrate reaction, may be expressed as follows :

$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

Where, K_m is the *Michaelis constant* serving as an index of the substrate-affinity, and V_{max} is the maximum rate of enzyme action at saturating substrate concentrations as also an index for the concentration of active enzyme in a tissue.

4.4.1 Michaelis constant

Michaelis constant (K_m) is regularly measured for enzymes to estimate their substrate-affinities, kinetics and their changes due to inhibitions and modulations.

Characteristics of Michaelis constant :

(a) K_m is the molar concentration of the substrate at which the initial velocity (V_o) amounts to half the maximum velocity, i.e., $^1/_2\, V_{max}$. Thus, where $V_o = {}^1/_2\, V_{max}$.

$$V_o = \frac{V_{max}[S]}{K_m + [S]}, \text{ or } K_m = [S]\left(\frac{V_{max}}{V_o} - 1\right)$$

$$\text{or, } K_m = [S]\left(\frac{V_{max}}{\tfrac{1}{2}V_{max}} - 1\right) = [S]$$

K_m is expressed in moles per litre. It is a constant for each particular enzyme-substrate concentration so long as other conditions like pH, temperature and ionic strengths remain unaltered.

(b) At very low substrate concentrations far below the k_m,

$$[S] \langle\langle K_m, \therefore K_m + [S] \cong K_m$$

$$\therefore V_o = \frac{V_{max}[S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m} = K[S].$$

Where k is a new constant, being the ratio of V_{max} and k_M for the given enzyme-substrate combination. This indicates that at very low substrate concentrations, V_o of a single-substrate reaction depends only on the molar concentration of the substrate *(a first-order reaction)* and rised lineraly with [S] in the initial part of the hyperbolic plot (Fig. 4.4).

(c) At high substrate concentrations far exceeding the k_m,

$$[S] \rangle\rangle K_m, \therefore K_m + [S] \cong [S]$$

$$\therefore V_o = \frac{V_{max}[S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m} = V_{max}.$$

Thus, when [S] far surpasses the k_m, V_o reaches the V_{max} and is no longer proportional to reactant (substrate) concentration (a **zero-order reaction**). This forms the plateau-like summit of the hyperbolic curve (Fig. 4.4). It follows that with saturating substrate concentrations, usually when $[S] \geq 10^2\ k_m$, V_{max} depends on the prevailing *molar concentration of the active -enzyme.*

(d) K_m is a measure of the *substrate-affinity* of the enzyme and ranges from about 10^{-7} to about 10^{-1} M. The higher is the K_m, the lower the substrate-affinity; the lower is the k_m, the higher the substrate affinity. This is because the higher is the affinity of enzyme for a substrate, the lower is the substrate concentration required to attain the V_{max} as well as the $\frac{1}{2}V_{max}$.

(e) The substrate-affinity and so, the k_m would vary from substrate to substrate for an enzyme. For example, pyruvate carboxylase, catalyzing a reaction between ATP, pyruvate and HCO_3^- to form oxaloacetate, has progressively higher k_m values for those substrates, viz., 6×10^{-5} M, 4×10^{-4} M and 1×10^{-3} M respectively for them. So, this enzyme possesses its highest affinity for ATP and the lowest affinity for bicarbonate.

(f) K_m as well as substrate-affinity may be changed by any condition that either changes the higher orders of structure of the enzyme protein or alters the ionization of its active site. Thus, K_m may change with changes in temperature, pH and ionic concentration.

(g) K_m of an enzyme is increased by the competitive inhibition of the enzyme, because the competitive or substrate-analogue inhibitor competes with the substrate for binding to the substrate-binding site of the enzyme, decreasing the availability of free substrate-binding sites to the substrate and consequently, diminishing the substrate-

affinity. But K_m remains unchanged in noncompetitive inhibitiion because the noncompetitive inhibitor, not being a substrate-analogue, does not bind to the substrate-binding site of the enzyme and consequently does not affect the substrate-affinity of the latter.

(h) Different proteins, catalyzing the same reaction of identical substrates, are called *isozymes* or *isoenzymes* and differ between themselves in their tissue distributions. Such isozymes of an enzyme differ from each other in their substrate-affinities and so, possess different K_m values for the same substrate. For example, the hexokinase isozyme (glucokinase) of hepatocytes has a hundred-fold higher K_m for glucose than the other hexokinase isozymes of muscles, intestinal cells and brain. Such differences in K_m between isozymes play an important role in conducting the metabolism of a substrate differentially in different tissues.

(i) Allosteric enzymes of the *K-series*, such as phosphofructo-kinase-1, suffer changes of their K_m on the binding of specific ligands (*allosteric modulators*) to their allosteric sites. For example, the binding of some ligands (*activators*) to such sites increases their substrate affinities and consequently decreases the K_m; but the binding of some other ligands (*inhibitors*) decreases the substrate affinity and raises the K_m. On the contrary, the substrate affinity as well as the K_m remains unaltered in M series of allosteric enzymes, such as acetyl-CoA carboxylase, on the binding of allosteric modulators to them.

4.4.2 Linear transformations of Michaelis-Menten equation

For regular measurements of K_m and V_{max} values of enzymes in estimating their substrate-affinities, active enzyme concentrations, and their changes, it is far more convenient to use the following linear forms of the Michaelis-Menten equation than its heperobolic form itself.

Linewaver-Burk double-reciprocal plot :

The Lineweaver-Burk equation is derived from the Michaelis-Menten equation (MME) in the following way :

$$V_o = \frac{V_{max}[S]}{K_m + [S]} \quad or, \quad \frac{1}{V_o} = \frac{K_m + [S]}{V_{max}[S]}$$

$$Or, \quad \frac{1}{V_o} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

On scaling $\frac{1}{V_o}$ and $\frac{1}{[S]}$ along the ordinate and the abscissa, respectively, and plotting each experimentally measured $\frac{1}{V_o}$ against the corresponding $\frac{1}{[S]}$, a linear

double-reciprocal plot is obtained (Fig. 4.5). They y-intercept, the negative x-intercept and the slope of the plotted straight line give respectively the $\frac{1}{V_{max}}$, $-\frac{1}{K_m}$ and $\frac{K_m}{V_{max}}$.

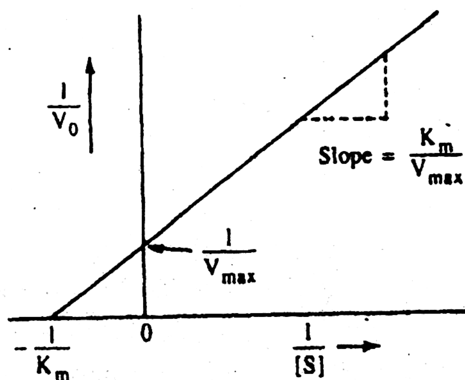


Figure 4.5 : Double reciprocal plot
[From D. Das, Biochemistry, Academic Publishers, 2000]

Eadie-Hofstee plot :

This linear form of the MME is obtained as follows :

$$V_o = \frac{V_{max}[S]}{K_m[S]}, \quad \text{or,} \quad \frac{V_o}{[S]}(K_m + [S]) = V_{max},$$

$$\frac{V_o}{[S]}K_m + V_o = V_{max}, \therefore \; V_o = V_{max} - \frac{V_o}{[S]}K_m$$

On scaling V_o and $\frac{V_o}{[S]}$ along the ordinate and the abscissa, respectively, and plotting each measure V_o value against the corresponding $\frac{V_o}{[S]}$, a linear *Eadie-*

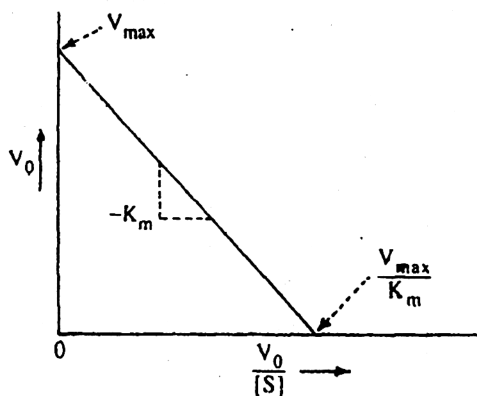


Figure 4.6 : Eadic-Hofstee plot [From D. Das, Biochemistry, Academic Publishers, 2000]

Hofstec plot is obtained (Fig. 4.6). In this plot, they y-intercept, the x-intercept, and the slope give respectively the values of V_{max}, $\frac{V_{max}}{K_m}$ and –Km.

Wolf-Hanes plot :

The linear Wolf-Hanes equation results on multiplying both sides of the Lineweaver Burk equation by [S] :

$$\frac{[S]}{V_o} = \frac{K_m}{V_{max}} + [S] \times \frac{1}{V_{max}}$$

A linear *Wolf-Hames plot* is drawn by scaling $\frac{[S]}{V_o}$ and [S] along the ordinate and the abscissa, respectively, and plotting each measured $\frac{[S]}{V_o}$ against the corresponding [S] value (Fig. 4.7). They intercept, the negative x-intercept and the slope of this linear plot gives respectively the $\frac{K_m}{V_{max}}$, the -K_m and the $\frac{1}{V_{max}}$ values.

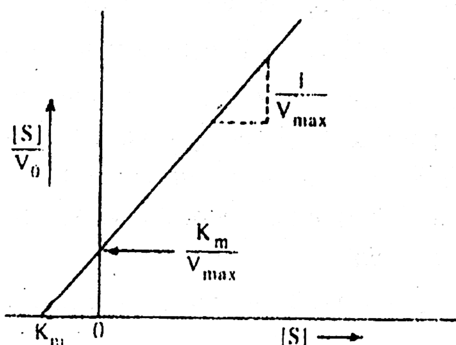


Figure 4.7 : Wolf-Hanes plot [From D. Das, Biochemistry, Academic Publishers, 2000]

4.5 Covalent modifications of enzymes

Covalent modifications of enzymes consist of either addition or removal of specific groups to/from the enzyme molecule by respectively the formation and cleavage of a covalent bond, leading to either an increase or a decrease in the enzyme activity. Covalent modifications may be either irreversible or reversible. They are important mechanisms of *in vivo* regulations of enzyme activities.

4.5.1 Irreversible covalent activation

Stronge protein-hydrolyzing enzymes are in many cases synthesized and secreated as inactive precursor molecules called *proenzymes,* so as to prevent their unintended

action on body-tissue components. These inactive proenzymes reach their intended sites of action in the tissues where specific peptide bonds of their molecules get hydrolyzed under appropriate conditions like pH changes or by the actions of other specific proteases. That results in the removal and release of specific segments of the peptide chain of the proenzyme as one or more inactive peptides called *unmasking substances;* the remaining part of the proenzyme molecule becomes an active enzyme due to consequent donformational changes in its molecule and exposure of specific substrate-binding or catalytic groups at its active site. This change is *irreversible* |——| the activated enzyme, once formed, cannot bind again with the released inactive peptide to form back the original proenzyme. Two examples are cited below :

(a) The gastric protease, pepsin, is secreted in the gastric juice of many vertebrates, including mammals, as an inactive proenzyme called *pepsinogen.* In the gastric lumen, a specific peptide bond of pepsinogen is hydrolyzed either spontaneously by the strong acidic gastric pH, or by the autocatalytic action of already activated pepsin molecules at a pH of ~4.6. This releases an inactive polypeptide of 44 amino acid residues from pepsinogen, changing the latter to pepsin.

Pepsinogen + H_2O → Pepsin + inactive polypeptide.

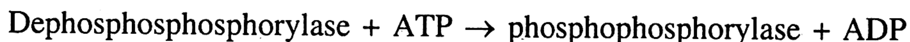
(b) Similarly, the pancreatic inactive protease *trypsinogen* has to reach the intestinal lument where a specific peptide bond is hydrolyzed either by enteropeptidase of intestinal juice at a pH of 5.2 to 6.0, or by already activated trypsin molecules at a pH near 7.9; this releases an inactive hexapeptide from trypsinogen to change the rest of its molecule to active *trypsin.*

Trypsinogen + H_2O → trypsin + inactive hexapeptide.

4.5.2 Reversible covalent modification

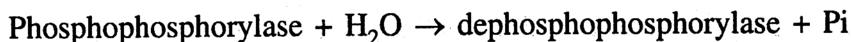
Some enzymes get activated or inhibited by enzyme-catalyzed addition of specific groups such as phosphate and adenylate groups, through the formation of covalent bonds between the target enzyme molecules and the group added. Moreover such a process is reversible |——| the opposite change of the same covalent bond, catalyzed by some other enzyme, results in the opposite effect on the target enzyme activity. Two examples of such reversible covalent modifications are cited below.

(a) *Glycogen phosphorylase* of hepatocytes is activated and inhibited by reversible covalent modifications through phosphorylatiion and dephosphorylation, respectively. *Phosphorylase Kinase* used a high-energy phosphate group of ATP to phosphorylate a specific serine residue of the inactive phosphorylase *(dephosphophosphorylase),* changing it to the active phosphorylase *(phosphophosphorylase).*



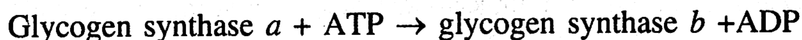
Dephosphophosphorylase + ATP → phosphophosphorylase + ADP

On the contrary, *protein phosphatase-I* hydrolyzes the phosphoester bond in phosphophosphorylase to release Pi and inactive dephosphophosphorylase.



Phosphophosphorylase + H_2O $\rightarrow$ dephosphophosphorylase + Pi

(b) Active *glycogen synthase* a also undergoes reversible covalent modification; however, phosphorylation inhibits it and dephosphorylation activates it. Active glycogen synthase *a* is inactivated to *glycogen synthase b* by phosphorylation catalyzed by *protein Kinase a* using an ATP.



Glycogen synthase *a* + ATP $\rightarrow$ glycogen synthase *b* +ADP

On the contrary, *protein phosphatase-I* hydrolyzes the phosphoester bond in glycogen synthase *b* to dephosphory late it to active glycogen synthase *a,* releasing the phosphate group as Pi.

4.6 Allosteric modulations of enzymes

In addition to the substrate binding site present in the enzymes, some enzymes possess also one or more other specific sites called *allosteric sites* which play important roles in the regulation of such enzymes. Binding of specific low-MW ligands to such allosteric sites either enhances of decreases the activity of these enzymes. Such low-MW ligands, binding with allosteric sites to regulate these enzymes, are known as *allosteric modulators* and their regulatory actions on the enzymes are called *allosteric modulations.* According as such modulators enhance or decrease the enzyme activity, they are respectively called positive allosteric modulators (*allosteric activators*) and negative allosteric modulators *(allosteric inhibitors).*

4.6.1 Characteristics of allosteric modulations

(a) The allosteric site and the active or substrate-binding site *(isosteric site)* are located on different peptide subunits of an oligomeric allosteric enzyme.

(b) Changes in pH and temperature, high ionic concentrations, mercurials, radiations, mutarotations and peptidase actions frequently affect or destroy the allosteric property of an enzyme without affecting its catalytic activity. This indicates that the allosteric and the substrate binding sites of an enzyme are not identical.

(c) Denaturation of the active site of an enzyme is sometimes prevented by the presence of the allosteric modulator, but not by the presence of the substrate itself; this also indicates that the allosteric and the isosteric sites of an ênzyme are distinct from each other.

(*d*) Only specific ligands can bind to the allosteric site to about the allosteric modulation of the enzyme (*modulator-specificity* of allosteric sites).

(*e*) Some enzymes possess allosteric capable to binding to only one type of modulators, either activators or inhibitors, and do not possess sites for binding to the opposite type of modulators. But some others bear separate allosteric sites, one binding to activators and the other to inhibitors. For example, cytoplasmic carbamoyl-phosphate synthase II possesses both positive and negative allosteric sites which respectively bind to PP-ribose-P and UMP; but mitochondrial carbamoylphosphate synthase I bears only a positive allosteric site where N-acetylglutamate binds to activate the enzyme allosterically, while allosteric inhibition of this enzyme has not been demonstrated.

(*f*) In some cases, either the substrate or an intermediate of an earlier step of a pathway binds to the positive allosteric site of an enzyme for a subsequent step to activate the latter allosterically (*feed-forward allosteric activation*). An example is the allosteric activation of pyruvate Kinase of glycolysis by fructose 1,6-bisphosphate produced by an earlier step of the same pathway.

(*g*) Sometimes, a metabolic product of a pathway binds to the negative allosteric site of an enzyme catalyzing one of its earlier steps and inhibit that enzyme allosterically (*product feedback allosteric inhibition*). For example, an end product UMP of the pyrimidine synthesis pathway allosterically inhibits carbamoyl-phosphate synthase II, an initial enzyme of that pathway.

(*h*) Allosterism results from different types of cooperativities between the ligands binding to an allosteric enzyme. Allosteric inhibitors have a *negative cooperativity* with the substrate of the enzyme; in other words, on binding to the allosteric site, an allosteric inhibitor decreases either the binding of the substrate to the substrate-binding site or the catalytic action of the enzyme on the substrate. On the contrary, on binding to the allosteric site, allosteric activators show a *positive cooperatively* with the substrate, enhancing the enzyme-substrate interaction and catalysis. As the allosteric ligand is different from the substrate, such cooperativity between the two is called *heterotropic allosteric effect.*

(*i*) According to the nature of change in the allosteric enzyme Kinetics by the heterotropic allosteric effects of modulators, allosteric enzymes are categorized into a K-series (e.g. PFK-1) and a *V or M series* (e.g. acetyl-CoA carboxylase). Allosteric ligands change the substrate-affinities (and so, the K_m values) of K-series of enzymes, but alter the V_{max} of V or M series of enzymes.

(*j*) The peptide subunits of an allosteric enzyme bear more than one substrate binding site and so, several substrate molecules may successively bind to the substrate-binding sites of an enzyme. This produces the *homotropic effect* of substrates of an

allosteric enzymes due to a *positive cooperativity* between these substrate molecules |——| the binding of one substrate molecule to a substrate-binding site of the enzyme brings about a change in three-dimensional conformation of the latter, promoting the binding of a second substrate molecule to the next substrate-binding site of the same enzyme molecule, with similar repetitions for successive substrate molecules.

(k) Allosteric enzymes obey the *sigmoid substrate-saturation Kinetics,* instead of the Michaelis-Menten hyperbolic Kinetics of nonallosteric enzymes (vide 4.6.2.).

4.6.2 Sigmoid Kenitics of allosteric enzymes

Because of the homotropic effect of substrate molecules of an allosteric enzyme, resulting from the positive cooperativity between them, the initial velocity (V_0) for such an enzyme action is a *sigmoid function* of the molar concentration [S] of the substrate. This *sigmoid substrate saturation Kinetics* conforms to the *Hill equation* given below for a single-substrate reaction :

$$V_o = \frac{V_{max}[S]^n}{K' + [S]^n}$$

where V_0 is the initial velocity with a substrate concentration [S], V_{max} is the maximum velocity at the saturating substrate concentration K' is a constant which is distinct from K_m and n is the *Hill coefficient,* serving as a measure of positive cooperativity between the substrate molecules, and dependent on the number of substrate-binding sites of each enzyme molecule as well as the nature and strength of interaction between those sites. On plotting the V_0 against the corresponding substrate concentrations, a *sigmoid curve* is produced (Fig. 4.8). If n exceeds 1, a positive cooperativity between the ligands, viz., substrate-substrate or substrate-activator cooperativity, promotes the binding and interaction of the enzyme with more substrate molecules; when n is lower than 1, a negative substrate-inhibitor cooperativity decreases the binding and interaction of substrate molecules with the enzyme; in the absence of any cooperativity between the ligands, n equals 1 and the Kinetics is hyperbolic instead of sigmoid.

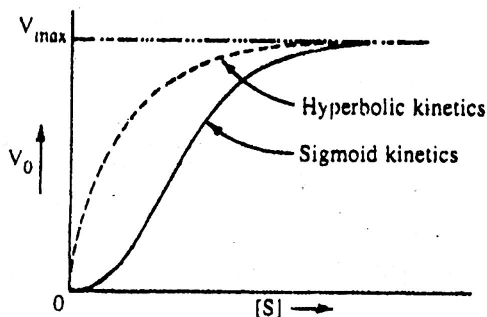


Figure 4.8 : Sigmoid and hyperbolic kinetics [From D. Das, Biochemistry, Academic Publishers, 2000]

The sigmoid plot indicates that Vo rises at a low rate with the rise of [S] at low substrate concentrations; with further rise of [S], V_0 rises more steeply with a higher slope due to positive cooperativity between substrate molecules; as [S] approaches the saturating concentration, the curve reaches its flat top summit (V_{max}) because of the nonavailability of free enzyme molecules for interaction with more fresh substrate molecules.

Changes during allosteric modulations :

(a) In *K-series* of enzymes such as PFK-1, binding of an allosteric activator to the positive allosteric site of the enzyme shifts the sigmoid curve to the left making it steeper, and *lowers the* K_m (Fig. 4.9), but leaves the summit and the V_{max} *unchanged.* Binding of an allosteric inhibitor to the negative allosteric site shifts the sigmoid curve to the right making it flatter, and *raises the* K_m, but leaves its summit and V_{max} *unchanged.*

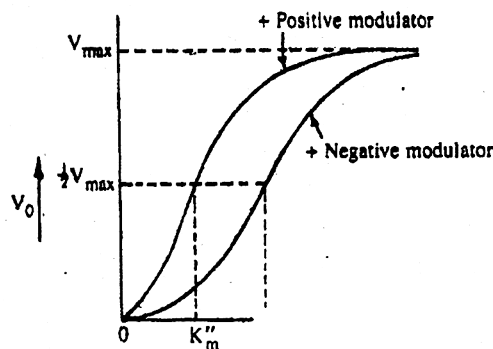


Figure 4.9 : Sigmoid curves of K-series in presence of allosteric modulators [From D. Das, Biochemistry, Academic Publishers, 2000]

(b) In *M or V series* of enzymes such as acetyle-CoA carboxylase, an allosteric activator shifts the curve to the left and raises its slope and summit, *enhancing the* V_{max} but *not chaniging the* K_m. An allosteric inhibitor shifts the curve to the right, lowers the summit as well as the slope, decreasing the V_{max} but keeping the K_m unaltered (Fig. 4.10).

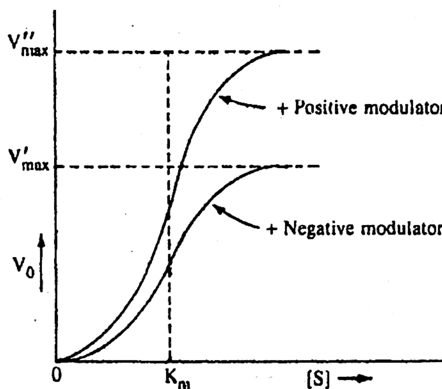


Figure 4.10 : Sigmoid curves of M-series in presence of allosteric modulators [From D. Das, Biochemistry, Academic Publishers, 2000]

4.6.3 Models of allosterism

Two models have been proposed to explain the molecular basis of allosteric regulation.

(a) *Monod's indirect concerted model :*

This model has been based on the *law of mass action.* Here, an allosteric enzyme is considered to be existing in two molecular forms in a dynamic equilibrium with one another. *(i)* The catalytically *active R form* of the enzyme presents optimally binding sites for the substrate and the allosteric activator; *(ii)* the *inactive T form* has sites binding optimally to the allosteric inhibitor, but is poor in optimal substrate-binding sites.

(i) If a substrate [S] binds to an optimal substrate-binding site of the R form to produce the RS complex, the equilibrium between free R and T forms is disturbed due to a decline in the concentration of free R molecules. To regain that equilibrium, some T molecules change into R molecules, increasing the availability of optimal substrate-binding sites and allowing more substrate molecules to bind to R molecules, consequently enhancing the enzyme sybstrate interaction. This may explain the *homotropic effect* of substrates due to *positive cooperativity* between them.

(ii) The concentration of free R form may also be lowered and the R : T equilibrium may be distrubed on the binding of an activator (A) to the optimal activator sites of R molecules to change them to the RA complex. To restore the R : T equilibrium, some T molecules now change into the R form, increasing the availability of optimal substrate-binding sites and the enzyme-substrate interaction. Thus, a *positive cooperativity* between substrate and activator *results in a heterotropic allosteric activation.*

(iii) An inhibitor (I), on the contrary, binds to the optimal inhibitor-binding site of T form of the enzyme, lowering the concentration of free T molecules and therby disturbing the R : T equilibrium. To restore the latter, some R molecules change into the T form, decreasing the availability of optimal substrate-binding sites and consequently lowering the rate of enzyme-substrate interactin. Thus, a *negative cooperativity* between the substrate and the inhibitor brings about a *heterotropic allosteric inhibition.*

(b) Koshland's direct sequential model :

Instead of assuming two distinct R and T forms of allosteric enzymes, Koshland has based his model on the concept of a *flexible conformation of enzyme molecules,* just as he proposed his induced fit model of enzyme-sybstrate interaction. He proposes that an allosteric enzyme molecule has only *partially accessible binding sites* for

substrates and allosteric modulators, and its three-midensional conformation changes on the initial binding of any of these ligands to the respective partially accessile binding sites, resulting in an altered accessibility of the substrate to the substrate-binding site.

(i) When the substrate binds initially to the partially accessible substrate-binding site of a peptide subunit of an enzyme molecule, conformational changes occur first in that subunit, but subsequently spread sequentially over other subunits also, making the partially accessible substrate binding sites of successive subunits more accessible to the substrate. So, progressively more substrate molecules, *bind sequentially* to those binding sites, leading to increased enzyme-substrate interaction and a *homotropic activation* of the enzyme in a *domino-type manner.*

(ii) Binding of an allosteric activator to the partially accessible binding site of a subunit of an enzyme molecule similarly changes the three-dimensional conformation of that subunit, which spreads over the other subunits in a domino-type effect to increase the accessibility of the substrate-binding sites on successive peptide units. This causes a *heterotropic allosteric activation* by increasing the enzyme substrate interaction in successive units of the enzyme molecule.

(iii) On the contrary, binding of an allosteric inhibitor to its partially accessible binding site on one subunit of an enzyme molecule produces such conformational changes, first in that subunit and then spreading sequentially to other subunits of the enzyme molecule, as to decrease the accessibility of its successive subunits to the substrate molecules in a domino-type effect. This brings about the *heterotropic allsoteric inhibition* of the enzyme.

4.7 Isozymes

In many cases, the same enzymic catalysis of an identical reactiion of the same sübtrates is carried out by more than one different protein. Such enzymes are called *isozymes* or *isoenzymes;* e.g., lactate dehydrogenase isozymes of chicken cariac and striated muscles, liver and bhrain; alkaline phosphatase isozymes of mouse small intestine; hexokinase isozymes of liver, brain and muscle; aldolase isozymes of muscle, liver and brain. For example, different lactate dehydrogenase isozymes catalyze the oxidation of lactate to pyruvate as well as the reduction of pyruvate in different tissues. Different isozymes are differentially distributed in different tissues and at different locations in the same cell. For example, hexokinase I, II and III occur mainly in extrahepatic tissues which hexokinase IV (glucokinase) occurs almost solely in the liver. Isozymes of the same enzyme differ in their physicochemical properties such as the types and combinations of peptide subunits in their molecules,

MW, sedimentation co-efficients, isolectric pH, thermolability and chemolability; so, they may be separated from each other by methods of protein separatiion such as ion-exchange chromatography, gel electrophoresis and isoelectrophoresis. They also differ in biological properties such as substrate-specificaties, substrate-affinities, K_m, optimum pH and optimum temperature, in the regulations by allosteric modulations, induction and repression, as also in their distributions in tissues. For example, hexokinase IV (glucokinase) of hepatocytes is far more substrate-specific for glucose but has a higher K_m than hexokinases I, II and III of extrahepatic tissues; lactate dehydrogenase (LDH) isozymes of different tissues are tetramers of H and M peptide subunits in different combinations; acid phosphatase isozymes of bones, liver and pancreas are ethanol-stable and tartrate-stable, while the isozyme of prostate and prostatic fluids is both tartrate-labile and elthanol-labile.

Differences between isozymes in their tissue distributions, kinetic properties and regulations enable the living organism to regulate metabolic activities differentially in different tissues, according to their metabolic needs. For example, hexokinases I, II and III of extrapatic tissues can continue their actions and help in glucose uptake by the tissues even when the blood sugar is low; but hexokinase IV of hepatocytes, because of its far higher K_m for glucose, suspends its action on glucose and thus keeps hepatic glucose uptake on a hold whenever the blood sugar declines.

4.8 Ribozymes

Ribozymes are *RNA molecules* with enzyme activities. They have been discovered in a few unicellular organisms, such as the *RNase P* of the bacterium *Escherichium coli,* and the intron segment of the pre-rRNA transcript of the protistan *Tetrahymena thermophila.* Some ribozyme molecules are made of both RNA and protein parts, but even then have their catalytic activity in the RNA part and not in the protein, and continue to function as an enzyme even after being isolated from the protein part. During post-transcriptional processing of some pre-tRNA and pre-rRNA transcripts of the organisms mentioned above, ribozymes have been shown to cleave the transcripts at specific sites to remove from the latter some inactive oligonucleotide segments as would not participate in the subsquent protein translation; the segments of the RNA transcript, flanking the cleaved segment on its two sides, are then *spliced* (joined) together to from the functional RNA molecule. The intron of the pre-rRNA transcript of *T. thermophila* is a ribozyme which follows the Michaelis-Menten hyperbolic substrate saturatiion Kinetics.

4.9 Rate-Limiting Enzymes

A *rate-limiting or commited enzyme* is such an enzyme of a metabolic pathway having more than one reaction step, as would determine the overall rate and direction of that pathway. In other words, the rate of a metabolic pathway may be regulated by regulating the activity of its rate-limiting enzyme by processes such as allosteric midulations, reversible covalent modifications, feedback inhibitions and competitive inhibitions, or by regulating the syntehsis of that enzyme by induction and repression. Rate limiting enzymes should have the following characteristics.

(a) The rate-limiting enzyme should have the *highest* K_m compared to the K_m values of other enzymes of that pathway. In other words, at saturating substrate concentrations for all those enzymes, the velocity of the committed step, catalyzed by the rate-limiting one, would be the lowest because of its lowest substrate affinity.

(b) The rate-limiting enzyme should catalyze the reaction of an *early step* in the metabolic pathway so that by regulating it, the rate of almost the entire pathway may be controlled, avoiding the unnecessary wastage of reactants and the unwanted accumulation of intermediates of preceeding steps.

(c) The rate-limiting enzyme should catalyze the reaction of the committed step in a *single specific direction* only and thus determine the overall direction of the pathway. The reverse reaction of that step should be carried out by a separate enzyme only.

(d) The rate-limiting enzyme has to be a *regulated enzyme* so that the rate of the metabolic pathway may be controlled by the activation inhibition, induction or repression of that enzyme.

Some rate-limiting enzymes are mentiioned below with the respective relevant pathways within parentheses : *(i)* mitochondrial carbamoyl phosphate synthase I (urea biosynthesis), *(ii)* cytoplasmic carbamoyl phosphate synthase II (pyrimidine biosynthesis), *(iii)* glucose 6-phosphate dehydrogenase (pentose phosphate pathway), *(iv)* phosphofructokinase-1 (glycolysis), (v) α-Ketoglutarate dehydrogenase (TCA cycle), *(vi)* glycogen synthase (glycogenesis), *(vii)* PEP Carboxykinase (gluconeogenesis), *(viii)* acetyl-CoA Carboxylas (fatty acid biosynthesis); *(ix)* HMG-CoA reductase (sterol synthesis); *(x)* AmLev synthase (porphyrin synthesis).

4.10 Summary

The active site of enzyme is constituted by some of its amino acid residues brought close together and in proper steric relations to each other by the coiled higher orders of structure of the enzyme molecule. It has a nonpolar cleft, accessible to the

substrate and containing specific amino acid sidechains forming the substrate-binding site. Specific amino acid residues at the active site must bear free sidechains for binding to and changing the substrate; for example, serine enzyme such as trypsin must possess a specific serine residue at the active site with its sidechain free.

The substrate binds to the substrate-binding site of the enzyme to form a highly reactive enzyme-sybstrate complex by noncovalent ionic, hydrogen, hydrophobic and van der Waals bonds or by covalent bonds such as thioester, thiohemiacetal and Schiff base bonds. The formation of the ES complex helps in catalysis by providing alternative reaction paths requiring much lower activation energy for the reaction. Fisher's template model proposes that the active site of the enzyme has a preformed, rigid, template-like conformation and readily fits without any change to the substrate, binding to the latter and catalyzing its change. But Koshaland proposes in his induced fit model that the active site is somewhat flexible |——| the initial loose binding of the substrate to that site induces its change into a more active and fully catalytic form.

Each enzyme can catalyze only a specific type of reaction of its substrate. Moreover, each enzyme can bind to and act on only one or a few substrate(s) bearing specific three-dimensional structural forms, specific groups and bonds, specific chain-lengths or specific configurations of double-bonds. Accordingly, enzymes show D-L stereospecificity, group specificity, **cis-trans** specificity, chan-length specificity and **d-l** optical specificity.

The initial velocity (V_o) of an enzymatic reaction initially rises linearly with the molar concentration [S] of the substrate, but its rate of rise declines progressively with subsequent further rises in [S], and ultimately reaches a maximum velocity (V_{max}), not surpassed by any further rise in [S]. Plotting the V_o against the corresponding [S] gives a rectangular hyperbolic curve obeying the Mischaelis-Menten equation, known as the rectangular hyperbolic substrate saturation kinetics; for a single-substrate reactiion, $V_o = (V_{max}$ [S] [S]/ (K_m + [S]), where K_m is the Michaelis constant. K_m is defined as that molar concentration of the substrate at which .... V_{max} is reached. K_m is a measure of the substrate-affinity of the enzyme |——| the lower the K_m, the higher is the substrate-affinity, while the higher the K_m, the lower is the substrate affinity. K_m varies from substrate to substrate for the same enzyme, and also from isozyme of the same enzyme. K_m is increased by competitive inhibition. It is also changed by allosteric modulatiions of K-series of allosteric enzymes. Three linear transformations of the Michel-Menten hyperbolic plot, viz., Lineweaver-Burk plot, Eadie-Hofstee plot and Wolf-Hanes plot, have also been described in this unit.

Specific proteases of pH changes may cause irreversible covalent activations of some inactive proenzymes into active proteases by the hydrolytic removal of specific

segments of their peptide chains. Some enzymes are reversible activated or inhibited by enzyme-catalyzed addition or removal of phosphate or adenylate groups.

Some enzymes undergo activation or inhibitiion when specific ligands (allosteric modulators) bind to their allosteric sites, distinct from their substrate binding sites. Such allosteric enzymes obey Hill's sigmoid saturatin Kinetics : $V_o = (V_{max} [S]^n) / (K' + [S]^n)$ where K' is a constant and n is the Hill coefficient serving as a measure of cooperativity between different ligands of the enzyme. Plotting V_o against [S] gives a sigmoid curve indicating such cooperativity between ligands. Allosteric enzymes bind to more than one substrate molecule on as many substrate-binding sites |——| binding of each substrate molecule promotes the binding of subsequent substrate molecules and their catalytic changes due to positive cooperativity between the substrates (homotropic effect). Binding of an allosteric activator or an allosteric inhibitor to their respective allosteric sites shifts the sigmoid curve respectively to the left and the right, because of respectively their positive and negative cooperativities with the substrate (heterotrophic effects). Allosteric activators decrease the K_m of the K-series of allosteric enzymes, and increase the V_{max} of the M-series of enzymes.

Allosteric inhibitors increase the K_m of K-enzymes and decrease the V_{max} of M-enzymes.

Isozymes are different proteins catalyzing the same reaction of the same substrates. Isozymes of an enzyme differe from each other in physicochemical properties such as substrate specificity, K_m , allosteric modulations, induction and repression.

Ribozymes are RNA molecules having enzyme activiteis. A few ribozymes, occurring in prokaryotes and unicellular eukaryotes, catalyze post-transcriptional modifications of their pre-RNA transcripts.

A rate-limiting enzyme of a metabolic pathway is a regulated enzyme catalyzing the reactiion of an initial step of the pathway in a single specific directin, having lower substrate-affinity than other enzymes of that pathwaym and determinign the overall rate of the entire pathway.

4.11 Terminal questions

1. (a) Describe the process of irreversible covalent activation, using the examples of two gastro-intestinal enzymes.

   (b) Explain how enzymes may be subjected to reversible covalent modifications by phosphorylation and dephosphorylation, describing such modulations of two suitable enzymes.

2. (a) Discuss the characteristics of allowsteric modulations.

(b) Describe the sigmoid saturation Kinetics, quoting the Hill equation.

(c) Mention the changes in the K- and M-series of enzymes during allosteric modulations.

3. (a) What is Michaelis Constant? Discuss its characteristics.

(b) How is the K_m indicated by the double-reciprocal, Eadie-Hofstee and Wolf-Hanes equations and their plots?

(c) What are rate-limiting enzymes? Mention the characteristics they should possess.

4. (a) Mention the bonds involved in forming enzyme-substrate complexes.

(b) Describe the transition state theory, using it to explain how the formation of the ES complex may help in catalysis.

(c) Discuss two contesting models for the formatin of ES complexes, indicating the superiority of one of them to the other.

5. (a) Describe the Michalis-Menten hyperbolic Kinetics of enzymes, quoting the Michaelis-Menten equation for a single-substrate reaction.

(b) Write how the Michaelis-Menten equation may be linearly transformed into the Lineweaver-Burk equatin, the Eadie-Hofstee equation and the Wolf-Hanes equatiion, quoting their respective equations.

(c) Describe the sigmoid saturation kinetics of enzymes, quoting the Hill equation. What is the Hill coefficient?

6. (a) Explain D-L stereospecificity and optical specificity of enzymes with an exmaple of each.

(b) Mention some experiemntal evidences in support of the formation of ES complexes.

(c) Differentiate between ES formations in singe-displacement and double-displacement bi-substrate reactions.

7. (a) Discuss two contesting models for the molecular basis of allosteric effects.

(b) Explain the cooperativities between allosteric modulators and substrate.

(c) What is homotropic effect in allosterism?

8. Write notes on the following :

(a) *cis-trans* stereospecificity and group specificity of enzymes.

(b) Isozymes

(c) K- and M-series of enzymes

(d) Ribozymes

(e) Induced fit model

(f) Indirect concerted model

4.12 Answers

1. (a) See Section 4.5.1.
   (b) See Sections 4.5.2.
2. (a) See section 4.6.1.
   (b) See Section 4.6.2.
   (c) See last part of Section 4.6.2
3. (a) See Section 4.4.1.
   (b) See Section 4.4.2.
   (c) See Section 4.9.
4. (a) See relevant part of Section 4.2.1.
   (b) See Section 4.2.4.
   (c) See Section 4.2.3.
5. (a) See Section 4.4.
   (b) See Section 4.4.2.
   (c) See Section 4.6.2.
6. (a) See paragraph (d) and (e) of Section 4.3.2.
   (b) See first paragraph of Section 4.2.1
   (c) See paragraph (b) of Section 4.2.1.
7. (a) See Section 4.6.3.
   (b) See paragraph (h) of Section 4.6.1.
   (c) See paragraph (j) of Section 4.6.1.
8. (a) See paragraph (a) and (c) of Section 4.3.2.
   (b) See Section 4.7.
   (c) See the last part of Section 4.6.2.
   (d) See Section 4.8.
   (e) See paragraph (b) of Section 4.2.3.
   (f) See paragraph (a) of Section 4.6.3.

Unit 5 ❒ Induction, Repression and Translation

Structure

5.1 Introduction

Objective

5.2 Induction and Repression of Enzymes

5.3 Translation of Proteins

5.4 Post-Translation Modifications

5.5 Summary

5.6 Terminal Questions

5.7 Answers

5.1 Introduction

You will read in this how the synthesis of enzymes is regulated by controlling the transcription of the mRNA molecules coding for them. In that context, you will get to learn about the operon concept of Monod and Jacoo, how it explains induction and repression of enzyme synthesis in prokaryotes, and also about similar regulations of enzyme synthesis in animals.

You will then proceed to read about the process of polysomal translation of proteins in animals, and how the nascent proteins turned out by translation undergo subsequent modifications into their final forms.

Objectives

Reading of this unit should enable you to :

- Understand the concept of operons in prokaryotes,
- Distinguish between inducible, repressible and constitutive enzymes.
- Describe the lac and ara operons of *E.coli* as examples of inducible catabolic operons,
- Describe the trp operon of *E.coli* as a repressible synthetic operon.
- Narrate induction and repression of enzymes in animals and other eukaryotes.
- Describe how amino acids get bound to the respective tRNA molecules before being incorporated in the peptide chain.

- Discuss the roles of polysomes in protein translation.
- Understand the events in eukaryotic translation.
- Narrate how a peptide chain is initiated, extended and terminated during its translation in animals.
- Describe different types of post-translational modifications of the translated protein.

5.2 Induction and Repression of enzymes

Induction and repression are processes for regulating the synthesis of enzymes as also of nonezymatic proteins. Many of the *regulated enzymes,* particularly the rate-limiting one, of various metabolic pathways are regulated by induction and/or repression.

Induction is the augmentation of enzyme synthesis by means of an increase in the gene transcription of the mRNA coding for that enzyme. It is brought by the effect of specific substances, called *inducers,* on the mRNA transcription; such an inducer, or some other molecule called a *gratuitous inducer* differing from the substrate but often possessing structural similarity with the latter. Synthesis of *inducible enzymes* ordinarily either remain suspended or take place at very low and insignificant rates; in presence of specific inducer(s), transcription of the mRNA bearing the genetic code for the relevant enzyme is manifold enhanced, augmenting thereby the translation of the latter. Many of the regulated enzymes of catabolic pathways are inducible enzymes.

Repression is the diminution or suspension of enzyme synthesis, brought about by the suppression of gene transcription of the mRNA bearing the genetic code for the relevant enzyme. It is brought about by the action of specific metabolites or products, called *repressors,* on the transcription of the particular mRNA. Syntheses of *repressible enzymes* ordinarily continue uninterrupted because of continuous transcription of their respective mRNAs; but in presence of specific repressor(s), gene transcription of the mRNA coding for the relevant enzyme gets suspended, leading to a suspension of translation of the latter. Many regulated enzyme of synthetic (anabolic) pathways are repressible enzymes. Such an enzyme for an initial step of the pathway is frequently repressed with the help of an intermediate of a subsequent step or the ultimate product of that pathway (*fee-back repression*). This ensures the prevention of unnecessary production and accumulation of metabolic intermediates and products the pathway. Sometimes, an initial enzyme of a pathway, which branches into several subsequent pathways, is repressed only in presence of intermediates of products of more than one such branches (*multivalent feed-back repression*).

There are many enzymes are synthesized continuously independent of and free from the effect of any repressor. These are called *constitutive enzymes.* Such constitutivity of enzymes frequently results from genetic mutations that may affect the bindings of repressors to their binding sites on the DNA sense strand.

Induction and repression may regulate enzyme synthesis in both prokaryotes and eukaryotes. But for the sake of simple descriptions of these phenomena, discussions about them are mainly restricted here to prokaryotes.

5.2.1 Operon concept

Monod and Jacob propounded the operon concept to explain the gene transcription of a prokaryotic *polycistronic mRNA.* According to this concept, the polycistronic mRNA is transcribe using as the template, called an *operon* of the DNA sense strand. The operon is described as such a specific segment of the DNA sense strand as consists of (*i*) a cluster of several *structural genes,* each carrying the genetic message for synthesizing a specific enzyme or one of its peptide subunits, (*ii*) an *operator gene* (O gene) adjoining the cluster of structural genes immediately on its 3′ or upstream side and operating the transcription of all those structural genes into a *polycistronic mRNA* strand bearing genetic codes for translating the respective peptides, and (*iii*) a *promoter site* (P site) adjoining the 3′ and of the O gene as also partly overlapping the latter and providing and initial binding site for RNA polymerase on the DNA sense strand (Fig. 5.1). In transcribing the DNA template of the operon, RNA polymerase bound initially to the P site, slides downstream in the 3′ → 5′ direction along the operator gene and then along the cluster of structural genes, transcribing the latter into a polycistronic mRNA strand, until it reaches the 5′ or down stream end of the operon and gets released from the DNA strand. Different segments of the polycistronic mRNA strand would subsequently code for the respective peptides during their translation.

A *regulator gene* is located on the same DNA strand that carries the operon, and either just next to or far away from the 3′ end of the later, but it is not a part of the operon. The regulator gene transcribes a *repressor mRNA* which in turn translates either an active *repressor* protein or an inactive *aporepressor,* that would participate in preventing the RNA polymerase from transcribing the structural genes of the operon (see below) and thus repress all the enzymes coded by those genes.

Three operons of *E. coli* are described below.

(*a*) ***Lac operon :***

The lac operon is an *inducible operon* of *E.coli* for transcribing a polycistronic mRNA bearing the genetic codes for three *enzymes of the lactose-catabolizing pathways* in the microbe (Fig. 5.1) A *promoter site* (P site) occurs at the 3′ or

upstream end of the operon, partly overlaps the 3′ end of the operator gene (O gene; next to it, and provides the initial binding site for RNA polymerase. On the 5′ or downstream side of the O gene, there occurs a cluster of three successive *structural genes,* viz, Z, Y and A genes in the 3′ → 5′ order, and coding respectively for β-galactosidase, galactoside permease and thiogalactoside transacetylase. A regulator gene (I gene) is situated immediately on the 3′ or upstream side of the P site, but is not considered as a part of the lac operon. The I gene transcribes a *repressor mRNA* which codes for an active tetrameric *repressor protein.* The latter directly binds to the O gene and prevents the RNA polymerase, bound to the P site, from moving across the O gene to the structural genes of the operon. So, the structural genes fail to be transcribed into any polycistronic mRNA which could code for and translate the three enzymes. Thus, all three enzymes remain *repressed.*

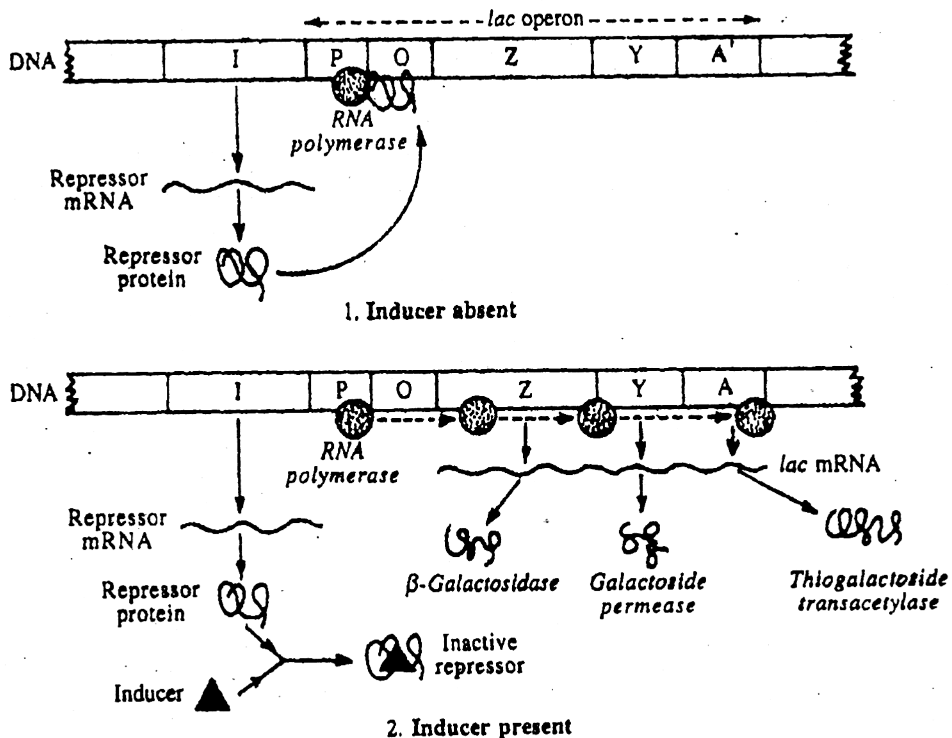


Figure 5.1 : Induction of Lac Operon [From D. Das, Biochemistry, Academic Publishers, 2000]

But in presence of lactose in the medium, small amounts of it enter the cell where it is changed into *1, 6-allolactose* by transglycosylation. This 1, 6-allolactose binds to the operator-bound repressor molecular to get it released from the O gene, and also to free repressor molecule to get it released from the O gene, and also to free repressor molecules to prevent their fresh binding to the O gene. This now allows the RNA

polymerase to bind effectively to the P site as also to slide across the O gene to reach and transcribe the structural genes into the polycistronic mRNA. The latter then starts translating the three enzymes coded by it. The 1, 6-allolactose acts as a *natural inducer* for the lac operon to bring about the *induction* of all three enzymes; in addition, the lac operon may also be induced by *gratuitous inducers* such ad β-methylgalactoside. Induction of lac operon serves as an example of *co-ordinate induction* of more than one enzyme by the effect of an inducer on a single operator gene.

(b) Ara operon :

This is another *inducible catabolic operon* of *E.coli* for transcribing a polycistronic mRNA coding for three *arabinose-catabolizing enzymes.* The operon consists of an *operator gene* (ara O gene), an adjoining *promoter* site (ara I), and just beside them, a cluster of three *structural genes,* viz., araB, araA and araD genes bearing genetic codes for the respective enzymes. A *regulator gene* (araC), situated on the other side of the ara O gene, transcribes a *repressor mRNA* which codes for a *repressor* protein. In the absense of arabinose in the cell, the repressor binds to the ara O gene and represses all three enzymes by blocking the RNA polymerase-catalysed transcription of the polycis ronic mRNA by the structural genes. Arabinose acts as an *inducer* |—| it changes the conformation of repressor, thereby enhancing RNA polymerase activity instead of blocking it. This induces the synthesis of all the three enzymes.

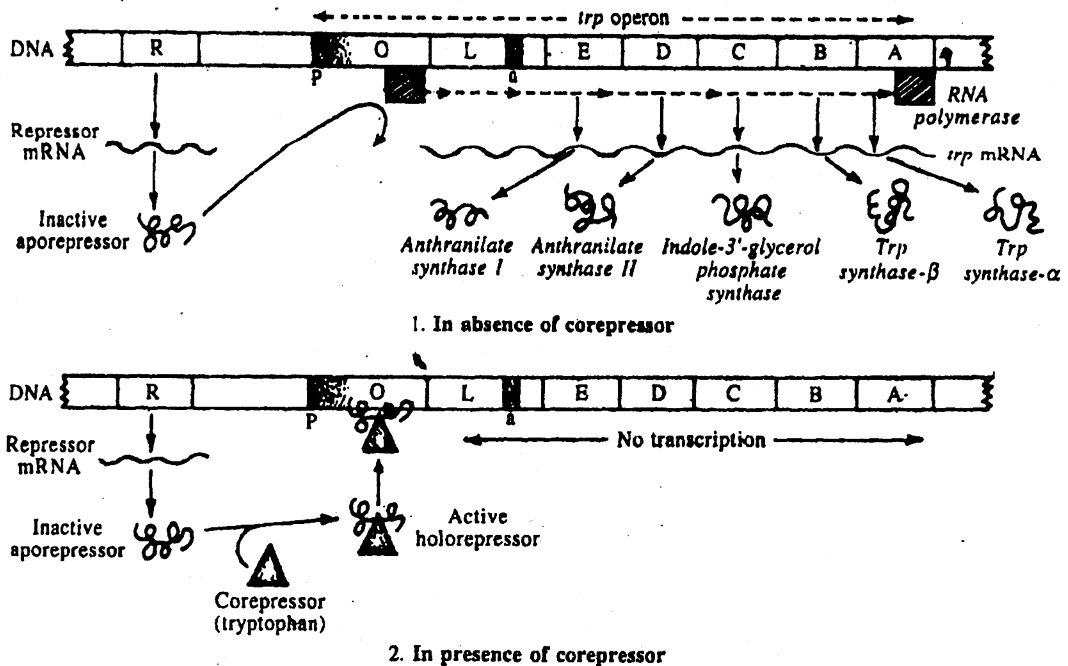


Figure 5.2 : Repression of trp operon [From D. Das, Biochemistry, Academic Publishers, 2000]

(c) Trp operon :

It is *repressible operon* of *E.coli* for transcribing a polycistronic mRNA coding for five peptides which participate in *tryptophan synthesis* in the organism (Fig. 5.2). From its 3 end to its 5 end (upstream to downstream) this operon consists of (*i*) an *operator* (O) *gene* partly overlapping a *promoter site* (P) that provides an initial binding site to RNA polymerase, (*ii*) a *leader gene* (L gene) on the 5′ side of O gene and tanscribing a 5′-leader sequence in the polycistronic mRNA to be transcribed by the structural genes, and (*iii*) five *structural genes*, viz, E, D, C, B and A gens, in the 3′ → 5′ direction along the DNA sense strand for tanscribing the polycistronic mRNA coding respectively for anthranilate synthases I and II, indole-3′-glycerophosphate synthase, and Trp synthases β and α |——| these five peptides would later combine with each other to from three enzymes for tryptophan synthesis.

A *regular gene* (R gene) occurs far away the 3′ or upstream end of the O gene of the operon on the same DNA sense strand. It trancribes a *repressor mRNA* which codes for an inactive dimeric *apopressor protein*, unable to bind as such to the O gene for bringing about repression. Consequently, so long as the tryptophan concentration is low in the cell, all five peptides continue to be translated; but when significantly high amounts of tryptophan occur in the cell, tryptophan binds as the *corepressor* prosthetic group to the inactive aporepressor to form an active *holorepressor* which in turn binds to the O gene, blocking the RNA-catalyzed transaction of structural genes and repressing all the peptides coded by them. Repression of trp operon is a case of *coordinate repression* of more than one enzyme or peptide by the effect of the repressor on a operator gene. It is also an example of feed-back repression.

5.2.2 Induction and repression in eukaryotes

In animals and other eukaryotes, enzymes as well as non-enzymatic proteins are coded by *monocistronic mRNAs,* each of which bears the genetic code for a single specific protein or peptide. So the concept of operons, each with a cluster of several structural genes transcribed simultaneously into a polycistronic mRNA does not hold good for animals and other eukaryotes. Still, induction and repression are important mechanisms for regulating enzyme synthesis in eukaryotes also; however, in the later, induction or repression affects each monocistronic mRNA individually and separately and thus controls the synthesis of individual enzymes independent of each other.

Several hormones such as corticosteroids, sex hormones and thyroid hormones as well as 1,25-dihydroxycholecalciferol affect the synthesis of many enzymes in animals by induction or repression of specific genes. Many catabolic enzymes of animals such as phospho-fructokinase-1, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, adipose tissue lapse and tryptophan 2-3,-

dioxygenase are inducible enzymes; many anabolic enzymes such as HMG-CoA reductase, PEP carboxykinase, PRPP synthase and fructose, 1,6-bisphosphatase are repressible enzymes.

The repression of ALA synthase, an enzyme of the porphyrin synthesis pathway in animals is an interesting example of both *aporrepressor-corepressor combination* and *feed-back repression.* This enzyme is repressed by an active *holorepressor* formed by the binding of the end product here as a *corepressor* to an inactive *aporepressore* protein coded by the relevant repressor mRNA.

Syntheses of Ca^{2+} binding and Ca^{2+} carrier proteins or enzymes are induced by 1, 25-*dihydroxychole-calciferol,* an active derivative of vitamin D_3, in intestinal, renal and bone cells. On entering such target cells, 1,25-$(OH)_2D_3$ binds to a specific *mobile receptor protein* in the cytoplasm to form an active 1,25-$(OH)_2D_3$-receptor complex. The latter is translocated to the nucleus where it binds to some nuclear proteins bound to specific genes and induces the transcriptions of those genes to monocistronic mRNA molecules coding for specific Ca^{2+} -binding proteins (e.g. intestinal Ca^{2+} -ATPase) to enhance the synthesis of such proteins.

Similarly, on entering renal tubular and salivary duct cells, the mineralocorticoid hormone *aldosterone* binds to specific soluble but inactive mobile receptor proteins to form active steroid-receptor complexes. The steroid-receptor complex then binds to the *hormone responsive element* (HRE) on the 3′ or upstream side of the promoter site of a specific gene in the DNA strand (Fig. 5.3) and induces the transcription of the gene into a monocistronic mRNA coding for a specific Na^+-transporter for sodium pump mechanism. In a similar way, the glucocorticoid hormone *cortisol* induces the synthesis of some key gluconeogenic enzymes such as PEP carboxykinase and fructose, 1, 6-bisphosphatase, the rate-limiting enzyme glucose 6-phosphate dehydrogenase of the pentose phosphate pathway, and the rate-limiting enzyme adipose tissue lipase for lipolysis in adipocytes.

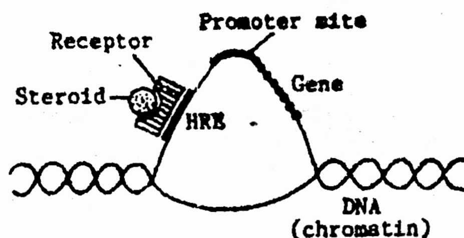


Figure 5.3 : Interaction of steroid-receptor complex with HRE on the 3' side of promoter site of a gene for induction [From D. Das, Biochemistry, Academic Publishers, 2000]

Induction of dihydroorotate dehydrogenase of the pyrimidine biosynthesis pathway by carbamoyl aspartate, an intermediate of an earlier step of the pathway, is an

example of *feed-back forward induction* in mammals, on the contrary, rpression of HMG-CoA reductase of sterol synthesis by the final product cholesterol of the same pathway is a *feed-back repression.*

5.3 Translation of Proteins

Translation is the polymerization of amino acids into a peptide chain by forming peptide bonds between the α-COOH and α-NH_2 groups of successive amino acids arranged serially on the mRNA strand obeying the codon sequence in the genetic code for that peptide. In conformity with the sequence of codons from the 5′ to the 3′ end of the genetic code, the message contained in the latter is *translated* with the help of tRNA molecules and ribosome particles into the amino acid sequence from the N-terminal end to the C-terminal end of the synthesized peptide. A peptide chain being translated grows in its N-terminal → C-terminal direction while the ribosome translates it by moving in the 5′ → 3′ direction along the mRNA. Animals and other eukaryotes translate their cytoplasmic proteins in the cytoplasm, some mitochondrial proteins in mitochondria, and membrane proteins and exportable (secretory) proteins on the membrane of rough Endoplasmic Reticulum (ER).

5.3.1 Formation of amino acyl-tRNA

Each *amino acyl-tRNA synthetase* first catalyzes the binding of the α-COOH group of a specific amino acid by an ester bond to either the 3′-OH or the 2′-OH group of the 3′-terminal ribose residue of either a specific tRNA molecule or one of several specific tRNAs (*cognate tRNAs*) at the cost of *two high-energy bonds* of ATP, producing an amino acyl-tRNA complex (Fig. 5.4).

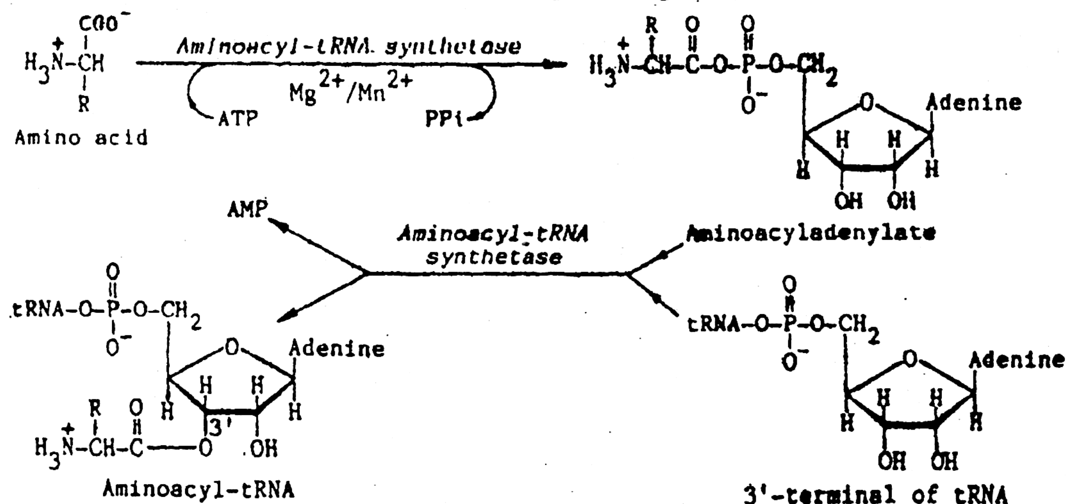
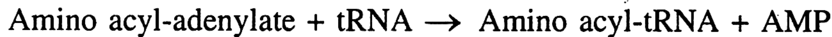
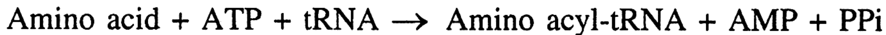


Figure 5.4 : Formation of aminoacyl-tRNA [From D. Das, Biochemistry, Academic Publishers, 2000]

Amino acid + ATP ⇄ Amino acyl-adenylate + PPi


Amino acyl-adenylate + tRNA → Amino acyl-tRNA + AMP


Amino acid + ATP + tRNA → Amino acyl-tRNA + AMP + PPi

The PPi is hydrolyzed by *inorganic pyrophosphatase* to two Pi molecules.

5.3.2 Polysomes in translation

From the cytoplasm, first a 40S ribosomal submit and next a 60S submit come and bind to an mRNA strand over the 5′-codon (first codon) of its genetic code to constitute an eukaryotic 80S ribosome particle on the mRNA (Fig. 5.6). The ribosome would then move in the 5′ → 3′ direction along the mRNA strand, extending the growing peptide chain by incorporating successive amino acids in that chain while passing over the successive codons. However, a number of ribosomes bind successively to the same mRNA strand and flow along it with small intervening gaps, translating as many peptide chains. This combination of the mRNA and several moving ribosomes is called a *polysome* or *polyribosome.*

An *aminoacyl or A site* and a *peptidyl of P site* have been constitued in the three-dimesional form of each ribosome when the latter has been formed on the mRNA by the binding together of its 40S and 60S ribosomal subunits (see above). These two sites can hold respectively an incoming aminocyl-tRNA and a peptidyl-tRNA formed at the preceding step of translation (Fig. 5.5). Each movement of ribosome down the mRNA places its vacant A site opposite the next codon yet to be translated, while its P site comes to lie over the preceding codon and is occupied by the peptidyl-tRNA still held by the ribosome. Next an aminoacyl-tRNA, bearing anticodon bases

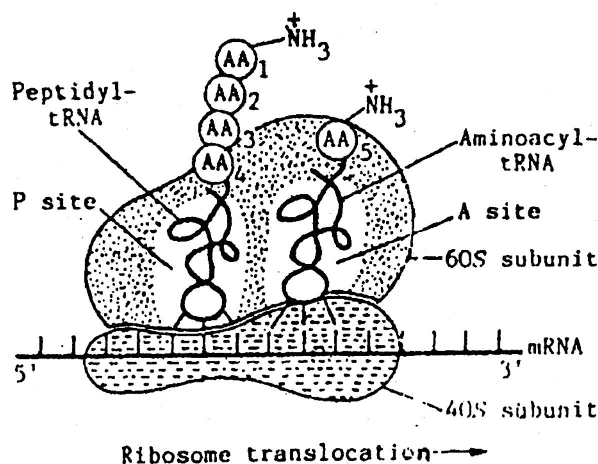


Figure 5.5 : The P and A sites of eukaryotic ribosome [From D. Das, Biochemistry, Academic Publishers, 2000]

complementary to the bases of the codon under the A site of the ribosome, gets hydrogen-bonded to the latter codon and comes to occupy the A site. The peptidyl group of the P-site peptidyl -tRNA is now transferred from the latter by a ribosomal *peptidyl transferase* to the α-amino group of the new A-site amino acyl-tRNA and a peptide bond is formed between the α-carbonyl carbon of the transferred peptidyl group and the α-amino nitrogen of the A-site amino acyl-tRNA. This extends the peptidyl group, now held by the ribosomal A site as the new peptidyl-tRNA, by one amino acid residue, while leaving the deacylated tRNA still in the P site (Fig. 5.7). A ribosome-dependent GT Pause, called *translocase*, next shifts the ribosome in the 5′ → 3′ direction along the mRNA towards the next 3′-codon on the latter. This ejects the deacylated tRNA from its P site which now gets occupied by the new peptidyl-tRNA still hydrogen-bonded to the preceding codon, makes the ribosomal A site vacant and places the latter over the next codon (Fig. 5.7). A new aminoacyl-tRNA now enters the vacant A site and gets hydrogen-bonded to the anticodon bases of the codon under that site. This starts the next cycle of adding another amino acid to the growing peptidyl group the events outlined above. Such events are repeated till the ribosome reaches the 3′-end of the genetic code. Then, the nascent peptide is released from the ribosomal which also falls apart from the mRNA strand in the form of two ribosomal subunits.

Because each tRNA can bind to a specific amino acid only, and also gets H-bonded to a specific codon depending on the base-complementarity between the latter and the tRNA anticodon, amino acids are brought to he ribosome and get peptide-bonded to each other in the Ne-terminal → C-terminal order in the peptide, as determined by the 5′ → 3′ order of codons in the relevant genetic code. So, the latter determines the amino acid sequence or primary structure of the "nascent" peptide.

5.3.3 Events in eukaryotic translation

Peptides are translated in eukaryotes including animals with the participation polysomes and many nonribosomal eukaryotic factors, some of the latter acting as GTP Pauses when bound to ribosomes (ribosome-dependent GT Pases).

1. *Peptide chain initiation :*

(*a*) Nonribosomal *eukaryotic initiation factors* or eIF proteins, viz., eIF-3 and eIF-4C, interact with 80S ribosome released from mRNA to dissociate the ribosome into a 60S ribosomal subunit and a 40S-subunit, eIF-3-4 Complex (Fig. 5.6).

(*b*) *Methionyl-tRNA* (Met-$tRNA_i^{Met}$), eIF-2, GTP and 40S.EIF-3-4C complex bind successively to each other to give an *entry complex.*

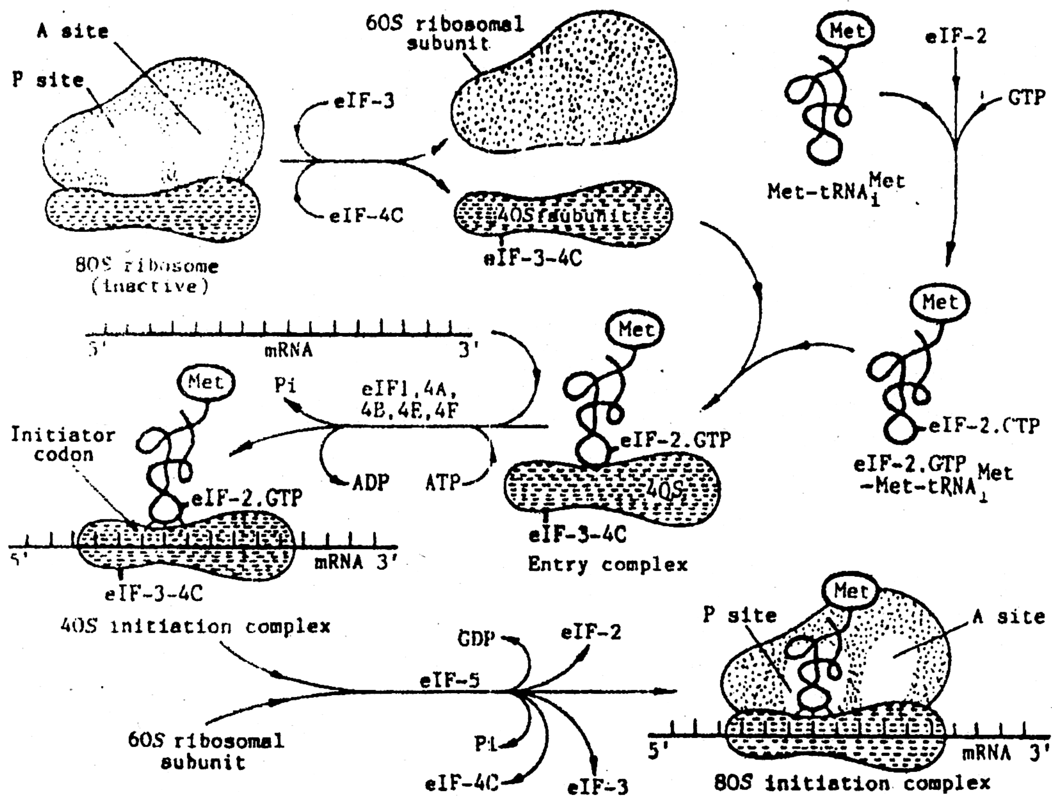


Figure 5.6 : Initiation of eukaryotic translation [From D. Das, Biochemistry, Academic Publishers, 2000]

(*c*) At the cost of high-energy bond of ATP and with the participation of eIF 1, 4A, 4B, 4E and 4F, the entry complex binds initially near the 5′-end of the mRNA stand to form a 40S *initiation complex;* the latter, then moves in the downstream or 5′ ⟶ 3′ direction along the mRNA strand to reach the *chain initiator codon* (AUG). On the latter, and gets hydrogen bonded to it by codon-anticondon reaction between the codon bases and the anticodon bases of tRNA$_i^{Met}$ in the 40S initiation complex.

(*d*) eIF-5 next helps in binding a 60S ribosomal subunit to the mRNA-bound 40S initiation complex to form an mRNA-bound 80S *ribosome* with its vacant A site opposite the second codon of the genetic code and its P site accommodating the AUG-bound methionyl-tRNA$_i^{Met}$. All the eIF molecules leave the ribosome during this process. *A ribosome-dependent GTP ase* almost similtaneously hydrolysis the GTP, so long bound to the inititation complex, to release GDP and Pi.

2. *Peptide chain elongation :*

(*a*) Stepwise interactions between a nonribosomal *eukaryotic elongation factor* (eEF1), a GTP molecule and a new amino acyl-tRNA, having base complementarity

of its anticodon with the second codon of the genetic code, release Pi and an eEF-1. GDP complex and result in the codon-anticodon hydrogen-bonding of the second codon with the new aminocyl-tRNA which now gets accommodated in the A site of the ribosome (Fig. 5.7).

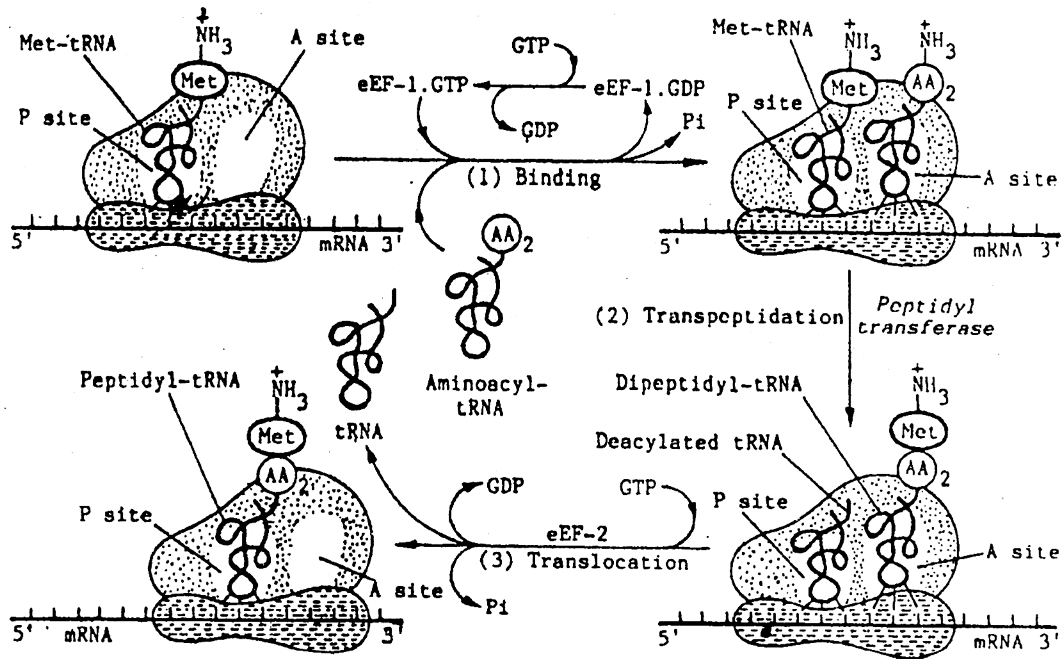


Figure 5.7 : Elongation of peptide chain with a second amino-acid [From D. Das, Biochemistry, Academic Publishers, 2000]

(*b*) *A peptidyl transferase* of the 60S ribosomal subunit next transfers the first amino acid (methionine) from the Met-tRNA$_i^{Met}$ at the P-site to the α-amino group of the amino acid held by the A-site aminoacyl-tRNA, forming a peptide bond between the α-carbonyl carbon of the transferred methionine and the α-amino nitrogen of the amino acid of the A site. This changes the A-site aminoacyl-tRNA into a dipeptidyl-tRNA at the A site while the P-site tRNA becomes deacylated.

(*c*) *A ribosome-dependent GTPase,* called the eEF-2 or *translocase,* hydrolyzes a GTP into GDP and Pi, and uses the released energy in catalyzing the downstream or 5′ → 3′ translocation of the 80S ribosome along the mRNA by one codon. This ejects the deacylated tRNA from the P site, shifts the dipeptidyl-tRNA still bound to the second codon from the A site to the vacated P site, and places the A site thus emptied over the next (third) codon.

This is followed by the next elongation cycle starting with the entry of a new

aminoacyl-tRNA into the vacated A site, as described in step (*a*) above. Subsequent reactions of the elongation cycle then follow.

Repetitions of such elongation cycles elongate the peptidyl-tRNA by one amino acid restidu percycle until the translocation of the ribosome places its A site against a *chain-terminator codon* at the 3′ end of the genetic code of the mRNA.

3. *Peptide chain termination :*

No tRNA can identify or get hydrogen-bonded to any of the chain-terminator codosn, viz., UAA, UAG and UGA. So, when the A site of the ribosome hase

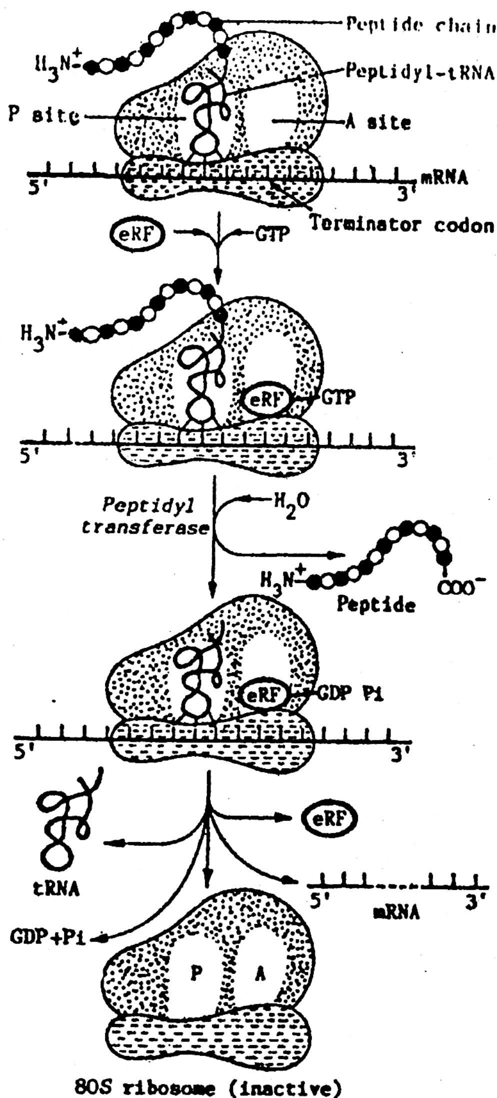


Figure 5.8 : Termination of peptide translation [From D. Das, Biochemistry, Academic Publishers, 2000]

reached a chain-terminator codon, a nonribosomal but ribosome dependent GTP ase called the *eukaryotic release factor* (eRF) binds, along with a GTP, to the chain-terminator codon, instead of any aminoacyl-tRNA (Fig. 5.8). The eRF hydrolyzes the GTP to GDP and Pi, and that energy is utilized by the ribosomal *peptidyl transferase* to release the peptide from the peptidyl-tRNA, along with deacylated tRNA and the eRF from the mRNA.

5.4 Post-Translational Modifications

Before and after its release from the ribosome from the ribosome, an "nascent" peptide is often considerably modified by different process.

5.4.1 Proteolytic cleavages

Specific amino acids or segments of peptides are very frequently removed from the nascent peptide chain by the hydrolysis of specific peptide bonds in the latter.

(*a*) The *N-terminal methionine residue,* with which the translation of each peptide was initiated, is removed either single or as an N-terminal peptide segment from the nascent peptide by peptidase-catalyzed hydrolysis of specific peptide bonds.

(*b*) Some peptide hormones, translated as a single *polyprotein* macromolecule, are released as separate hormone molecules in different endocrine tissues by the post-translationsl hydrolysis of such a polyprotein by tissu-specific peptidase. For example, *pro-opiomelampcprtin* (POMC) translated in both anterior and intermediate lones of pituitary is hydrolyzed at specific peptide bonds in those tisses to yield mainly ACTH and liptropins in the anterior pituitary, but to give α-and β-MSH, endorphins, lipotropins and corticotropin-like intermediate lobe peptide in the intermediate lobe.

(*c*) The short, *N-terminal signal or leader sequence* of nascent exportable and membrane proteins, translated by polysomes on the rough ER membrane, is hydrolyzed away by a microsomal *signal peptidase* even before the protein is released from its ribosome. For example, hydrolysis by signal peptidase removes the N-terminal leader sequence from *preproparathormone* (prepro PTH) initially translated, changing it to shoter prohormone (proPTH).

(*d*) After removal of the signal sequence from some nascent preproteins, additional terminal amino acid sequence may by further removed by other peptidase. For example, after proPTH has been formed by microsomal signal peptidase action on prepro PTH, the N-terminal hexapeptide segment of proPTH is hydrolyzed away by clipase *B* of the Golgi membrane to change proPTH to PTH.

5.4.2 Modification of amino acids

Some unusual amino acids such as pyroglutamate, γ-carbox glutamate, 4-hydrozyproline, phosphoserine and tyrosine 4-O-sulfate, though present in many proteins, are not directly coded by any codon. Such amino acid residues are produced in the protein by post-translational modification of specific coded amino acids already inserted in the protein during its translation.

(*a*) The N-terminal glutamate (Glu) residue of some peptide hormones, such as gastrin TRH and GnRH, is cyclized by *γ–glutamyl cyclase* into a *pyroglytamate* (pGlu) residue through a peptide bonding between its α-NH_2 and γ-COOH groups (Fig. 5.9).

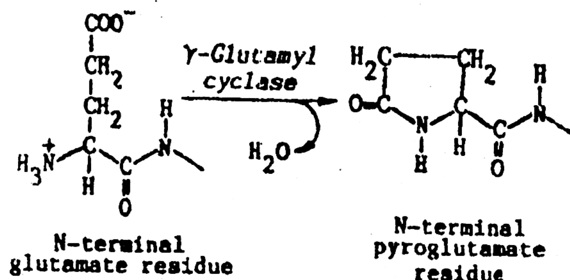


Figure 5.9 : Cyclization of Glu to pGlu residue [From D. Das, Biochemistry, Academic Publishers, 2000]

(*b*) Specific glumate residuess (Glu) of some Ca^{2+} binding proteins such as prothrombin are carboxylated in many tissues like lever and bones at the sidechain γ-carbon by microsomal *protein carboxylase*, modifying those resides to *γ-carboxyglutamate* (Gla) residues with the help vitamin K (Fig. 5.10).

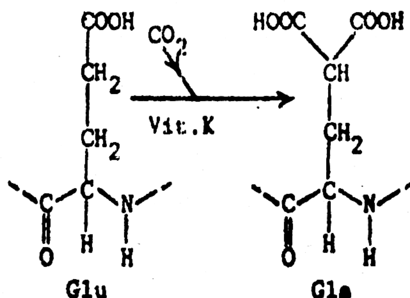


Figure 5.10 : Carbodylation of Glu to Gla residue [From D. Das, Biochemistry, Academic Publishers, 2000]

(*c*) C-terminal *amino acid amide* residues like glycin-amide and prolinamid are produced in many peptides and proteins such as gastrin, oxytocin, vasopressin and calcitonin by the amidation of their C-terminal amino acids. For example, a *peptidylflycine α-amidating monooxygenase* oxidizes the C-terminal glycine residue of a protein to form a double-bond between its α-carbon and α-amino nitrogen; this

double-bond is subsequently hydrolyzed to release glyxylic acid, leaving the nitrogen of glycine as the carboxamide group of the next amino acid; the latter thus becomes the new C-*terminal amino acid amide* residue such as valinamide, glycinamide and prolinamide (Fig. 5.11). The reaction uses vitamin C as a cofactor.

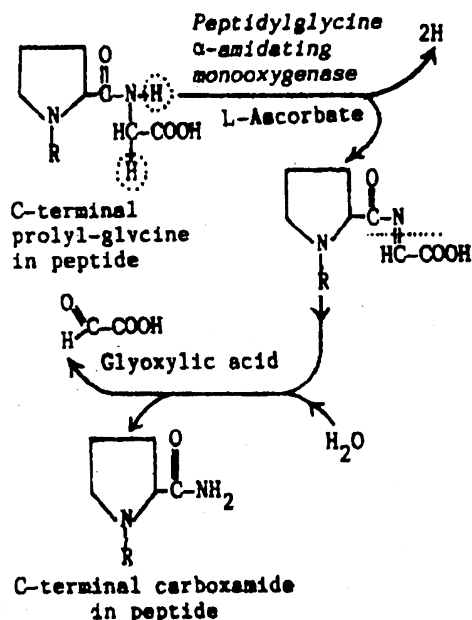


Figure 5.11 : Amidation of peptidylglycine [From D. Das, Biochemistry, Academic Publishers, 2000]

(*d*) The sidechain OH groups of specific tyrosine residues of some proteins, e.g. Tyr^{33} of cholecystokinin (CCK-39), get sulfated to form *tyrosine 4-0-sulfate* residues with the help of adenosine- 3′-phosphate -3′-phosphosulfate (active sulfate).

(*e*) The sidechain OH groups of specific tyrosine residues of some proteins like insulin receptos, and of specific serine residues of some other proteins like glycogen phosphorylase get phosphorylated by *protein Kinases* and ATP, changing then into *phosphotyrosine* and *phosphoserine* residues, respectively.

(*f*) Specific proline and lysine residues of some proteins like procollagen are hydroxylated by enzymes such as *procollagen prolyl hydroxylase* and *procollagen lysyl hydroxylase,* changing them respectively to *4-hydroxyproline* and *5-hydroxylysine* residues (Fig. 5. 12). The reaction uses vitamin C as a cofactor.

(*g*) *Methlyferases* transger the labile methul group of S-adenosylmethionine (active methionine) to the side chain amino groups of specific lysine, histidine and arginine residues modifying them respectively to methyllysine, N^3-methylhisitidine and ε-N-methylarginine residues.

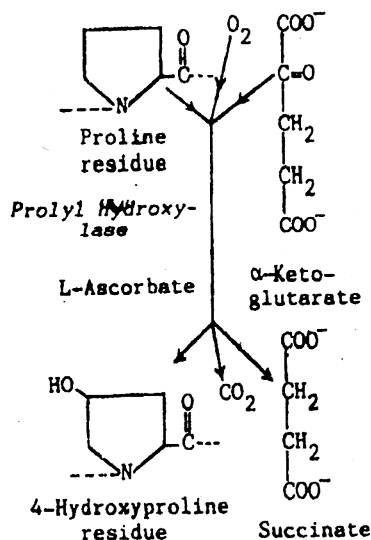


Figure 5.12 : Hydroxylation of proline to hydroxybroline residue [From D. Das, Biochemistry, Academic Publishers, 2000]

(*h*) The sudechain ε-NH_2 group of specific lysine residues in collagens and elastins may be oxidatively deaminated by *lysyl oxidase* into ε-aldehyde group, thus modifying them to *allysine* residues (Fig, 5.13.). Lysine, allysine and histidine residues of different peptide chains may then be cross-linked and further modified to residues like desmosine, allysine aldol, lysinonorleucine and aldol histidine.

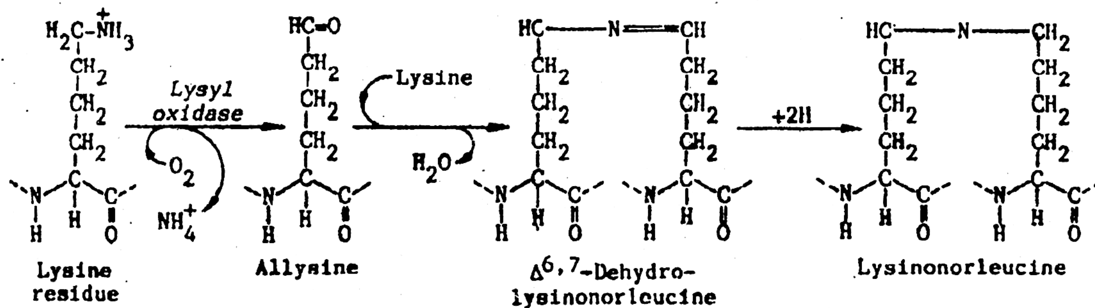


Figure 5.13 : Formation of lysine derivatives n peptides [From D. Das, Biochemistry, Academic Publishers, 2000]

5.4.3 Terminal additions

The peptide chain, left after post-translation removal of the N-terminal methionine and other specific amino acid sequences, may be extended at either of its ends by the addition of new amino acids. For example, the peptide chain of tubulin may be

extended by adding a tyrosine molecule at its C-terminal end by ATP-dependent *tblin-tyrosine ligase.*

5.4.4 Glycosylation

Using *dolichol phosphate* and *nucleotide-sugar complexes* such as UDP-glucose, UDP N-acteylglucosamine. GDP-mannose and CMP-sialic acid, microsomal and Golgi-membrane *glycosyltransferases* transfer monosaccharides and heteroglycans to many membrane and exportable proteins to form their oligosaccharide prosthetic groups, bond to the serine, therenine, arginine or hydroxylysine residues of those proteins by N-or O-glycosidic bonds.

5.5 Summary

Operon is such a segment of prokaryotic DNA that acts as a unit transcribing polycistronic mRNA with genetic codes for more than one peptide. From its 3′ to 5′ end, the opeon consists of a promoter (P) site for the initial binding of RNA polymerase, an operator (O) gene operating the operon, and a cluster of more than one structural genes transcribing together a polycistronic mRNA strand. The operon is regulated by a regulator gene either close to or far away from the 5′ -end of the operon. Lac operon, ara operon and trp operon of *E. coli* have been been briefly described in this unit.

Induction is the augmentation of the gene transcription of an mRNA leading to the enhanced translation of the peptide code by it. Repression is the decline or suspension of such transcription. In inducible operons such as the lac operon, the reglator gene transcribes a repressor mRNA that tranlates an active repressor; the latter ordinarily remains bound to the O gene to block the passage of the P site-bound RNA polymerase past the O gene and along the successive structural genes; the transcription of the latter into a polycistronic mRNA thus remains repressed. Binding of specific ligands (indcers) to the repressor inactivates the latter and allows the RNA polymerase to pass along the O gene and then along the structural genes, inducing their transcription into the polycistromic mRNA that translates all the petides coded by it.

For repressibe operons like the trp operon, the repressor protein (aporepressor) is inactive by itself so that the structural genes ordinarily continue transcribing the corresponding plocistronic mRNA and all the coded peptides continue to be translated, But the binding of a specific ligand (corepressor) to the aporepressor changes it to an active holorepressor which can now bind to the O gene and repress the polycistronic mRNA and the peptides coded by it.

Induction and repression of many ekaryotic enzymes have also been described in this unit although the operon concept is not applicable to eukaryotes.

Polysomes consist of several successive 80S eukaryotic ribosomes passing along each mRNA strand, each of those ribosome translating a peptide molecule by incorporating amino acids in the latter in a specific sequence according to the sequence of codons along the mRNA. For this, each amino acid binds to a specific tRNA to form an amino acyl-tRNA which binds to a specific codon of the mRNA according to the base-complimentarity between the codon and the anticodon of the tRNA. Each ribosome in the polysome assembly has an A site for receiving a new incoming amino acyl-tRNA and a P site holding the already formed peptidyl-tRNA complex of the preceding step. The transfer of the peptidyl group from the latter to the amino acyl group of A-site tRNA by a ribosomal peptidyl transferase at each step elongates the peptidyl group by one amino acid residue. A GTP-dependent translocase shifts the ribosome after each such step by one codon along the $5' \rightarrow 3'$ direction on the mRNA. Initiation, elongation and termination of translation depend also on specific nonribosomal proteins called eukaryotic initiation, elongation and termination factors, respectively. The energy for the endergonic process of translation is supplied by the exergonic hydrolysis of GTP and ATP molecules.

The translated 'nascent' peptide is subsequently modified into its final form by various post-translational modifications, such as modifications of some amino acid residues into unusual amino acids like hydroxyproline, allysine, γ-carboxyglumate, methylhistidine, pyroglutamate and prolinamide, hydrolytic removal of N-terminal methionine residue and N-terminal leader sequence, glycosylation of exportable and membrane proteins, and terminal addition of new amino acids at either and of the peptide chain.

5.6 Terminal questions

1. (a) Give an account of the role of polysomes in translating peptides.
   (b) Describes with the help of suitable diagrams how γ-carboxyglutamate and pyroglutamate residues are formed in a translated peptide chain.
2. (a) Describe the lac operon of *E. coil* and its induction, with a suitable diagram.
   (b) Using a flowchart, explain the action of an amino acy-tRNA synthetase.
   (c) Describe the initiation of translation of a peptide chain in eukaryotes with the help of diagram, mentioing the participation of ribosomal subunits and nonribosomal eukaryotic factors in the process.

3. (a) Describe the operon concept of Monod and Jacob.
   (b) Using a suitable diagram, describe the trp operon of *E.Coli.* and explain its repression.
   (c) Write how corticosteroids induce specific proteins in animals.
4. (a) Describe the events in the initiation and elongation of peptide chains in eukaryotes. Using suitable diagrams and mentioning the roles of ribosome, and nonribosomal eukaryotic factors in those events.
   (b) Discuss how the 'nascent' peptide chain undegoes post-translation modifications by different proteolytic cleavages.
5. (a) Discuss the phenomena of induction and repression in eukaryotes with examples.
   (b) Describe with the help of a suitable diagram how the trp operon is repressed in *E. coli* to illustrate prokaryotic repression in terms of the operon concept.
   (c) State what you understand by gratuitous inducers, coordinate induction, feed back repession and coordinate repression, citing one example of each.
6. (a) What are the roles of peptidyl transferase and translocase in eukaryotic translation?
   (b) Narrate the events in the initiation and termination of peptide translation in eukaryotes, with suitable diagrams.
   (c) What is the fate the N-terminal methionine residue of translated 'nascent' peptides in eukaryotes?
7. (a) Describe the post-translational modifications of amino acid residues in a 'nascent' peptide to give rise to unusual amino acid residues, with suitable examples and diagrams.
   (b) Narrate with the help of suitable diagram how a peptide chain is elongated in each cycle of its translation in eukaryotes.

5.7 Answers

1. (a) See Section 5.3.2
   (b) See paragraphs (a) and (b) of Section 5.4.2
2. (a) See paragraph (a) of Section 5.2.1
   (b) See Section 5.3.1
   (c) See No. 1 of Section 5.3.3

3. (a) See the first two paragraphs of Section 5.2.1
   (b) See paragraph (c) of 5.2.1
   (c) See the penultimate paragraph of Section 5.2.2
4. (a) See Nos. 1 and 2 of Section 5.3.3
   (b) See Section 5.4.1
5. (a) See Section 5.2.2
   (b) See paragraph (c) of Section 5.2.1
   (c) See second and third paragraphs of Section 5.2, last two sentences of paragraph (a) of Section 5.2.1, and last two sentences of paragraph (c) of Section 5.2.1.
6. (a) See paragraphs (b) and (c) under No. 2 of Section 5.3.3
   (b) See Nos. 1 and 3 of Section 5.3.3
   (c) See paragraph (a) Section 5.4.1
7. (a) See Section 5.4.2.
   (b) See No. 2 of Section 5.3.3

Unit 6 ❑ Adipose Tissue Profile

Structure

6.1 Introduction

Objectives

6.2 Triacylglycerol Synthesis

6.3 Adipose Tissue Lipolysis

6.4 Phospholipid Synthesis

6.5 Lipoproteins

6.6 Fatty liver

6.7 Summary

6.8 Terminal Questions

6.9 Answers

6.1 Introduction

You will read in this unit about different roles of adipose tissues in the metabolism of lipids. You have already learnt in Unit 1 about the beta-oxidation of fatty acids. Here, you will read about how fatty acids are used in adipocytes in synthesizing triacylglycerol fats for storage in the adipore tissue, how fatty acids are also used in synthesizing phospholipids, how the adipose tissue fat is hydrolyzed to release the fatty acids, and how they are released in the blood for their mobilization to other tissues for oxidation.

You will also read how lipoproteins are synthesized using fatty acids, fats, phosphopids, cholesterol and proteins. Fats and fatty acids are mobilized in blood and lymph in the form of lipoproteins. You will know about their circlation and metabolism. You will learn how cholesterol bound to two forms of lipoproteins of low-density and high-density, has different fates in circulation, and its relation to atherosclerosis. You will also read how the nonavailability of phospholipids and lipoproteins too much lipolysis in adipoacytes, and toxic effects of chemicals on the liver may lead to a high accumulation of fat in the liver.

Objectives

After reading this unit, you should be able to :

- Describe how triacylglycerols are synthesized from fatty acids for storage as fats in adipocytes.
- Narrate the synthesis of phospholipids from fatty acids.
- Understand the process and regulation of lipolysis of adipose tissue fat to fatty acids.
- Understand the composition and functions of different types of plasma lipoproteins.
- Narrate the circulation and fate of different plasma lipoproteins.
- Understand the relations between LDL-cholesterol, HDL-cholesterol and atherosclerosis,
- Explain how fatty liver may result due to decreased mobilization of fat from the liver because of varios factors.

6.2 Triacylglycerol Synthesis

Fatty acids, dietary as well as endogenous, are stored principally as fats in the adipose tissue of the body. For such storage, fatty acids are converted by smooth ER enzymes, and to a lesser extent by mitochondrial enzymes, into tracylglycerol fats in adipocytes and subsequently stored in those cells. Adipocytes occur mainly in the subcutaneous adipose tissue, mesentery, omentum and peritoneum of abdominal cavity, mammary gland and muscles.

Significance of triacylglycerol synthesis :

Whenever energy-producing nutrients, viz., fatty acids and carbohydrates, are available in the body in excess of their immediate requirement in energy production, they are converted to triacylgylcerols which are not much mobilization in that form and instead, are stored as fat in adipose tissues.

(*i*) When on high-fat diet, the dietary fatty acids in excess of their immediate metabolic need are converted to triacylglycerols and stored as adipore tissue fat for future use in energy production.

(*ii*) The mobilization of dietary fatty acids from the liver to the adipose tissues, where they are changed to fats for storage, decreases the fat-load of the liver, maintains its numerous other normal functions and prevents the formation of fatty liver (vide 6.6.).

(*iii*) When on a high-carbohydrate dite, citrate is formed as a TCA cycle intermediate from the glycolysis-endrproduct pyruvate, in excess of the need for its immediate aerobic oxidation. This excess amount of citrate is translocated to the

cytoplasm where it yields acetyl-CoA used in synthesizing fatty acids for forming adipose tissue fat. Moreover, glycerol 3-phosphae, used in tri-acylglycerol synthesis, is also largely formed from glycolytic intermediates like dihydroxyacetone phosphate. So, triacylglycerol synthesis also helps to convert the surplus carbohydrate metabolites into storage fats to be used subsequently for energy production when needed.

(*iv*) Hibernating mammals like polar bears have high rate of triacylglycerol synthesis from dietary nutrients such as fatty acids in the pre-hibernation period, for utilizing the stored fat for energy during hibernation. Similar is the case of nesting female birds preparatory to their nesting. Aquatic mammals such as whales, seals and walruses also have a high rate of riacylglycerol synthesis to form thick fatty outer coats of the body like the blubber of whales which acts as a thermoinsulator.

6.2.1 Thioesterification of fatty acids

Since fatty acids can participate in triacylglycerol synthesisi as acyl-CoA thioesters only, thioesterification of fatty acids must precede their use in the process. *Fatty acid thiokinases,* enzymes showing *chain-length specificites,* catalyze the thioesterifications of fatty acids of specific chain-length |——| some acting only on long-chain fatty acids such as palmtic (C_{16}) and steric (C_{18}) acids, some others only on medium-chain fatty acids (C_4-C_{12}) like caproic (C_6), octanoic (C_8) and capric (C_{10}) acids, and still others on short-chain (C_2-C_3) acids such as acetic (C_2) and propionic (C_3) acids. A thiokinase first transfers the adenyl (AMP) group of an ATP to the carboxyl group of the fatty acids, forming an acyladenylate (acyl-AMP) intermediate and releasing PPi, and next replaces the adenylate group of that intermediate by a coenzyme A molecule, forming acyl-CoA and realing AMP (Fig. 6.1). The PPi released is immediately hydrolyzed by *morganic pyrophosphatase* into two Pi molecules Overall :

Fatty acid + ATP + CoA + H_2O $\rightarrow$ acyl-CoA + AMP + 2Pi

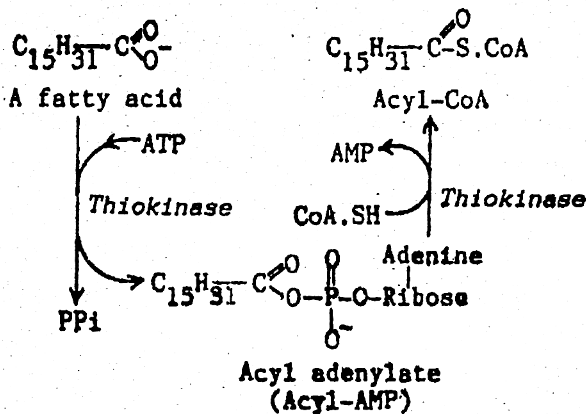


Figure 6.1 : Action of fatty acid this kinase

6.2.2 Glycerophosphate pathway

Acyl-CoA molecules are mostly converted to triacylglycrols by this pathway in the liver, mammary glands, adipore tissue and muscles, using smooth ER enzymes.

(*i*) In the adipocytes of mammary glands and in hepatocytes, cytoplasmic *glycerol kinase* phosphorylates glycerol to glycerol 3-phosphate, using ATP (Fig. 6.2). But adipocycles of subcutaneous fats, abdominal fats and muscle fats as well as enterocytes of intestine form glycerol 3-phosphate by reducing glycolytic dihydroxyacetone phosphate with NADH and cytoplasmic *dihydroxyacetone phosphate dehydrogenase.* The two alternatives are summarized below.

Glycerol + ATP → Glycerol 3-phosphate + ADP.

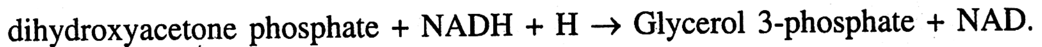

dihydroxyacetone phosphate + NADH + H → Glycerol 3-phosphate + NAD.

Figure 6.2 : Glycerophosphate pathway of triacylglycerol synthesis

(*ii*) The acyl group of a long-chained *saturated* acyl-CoA molecule (palimitoyl-CoA in mose cases) in next transferred by *glycerol 3-phosphate acyl-transferase* to the C^1 of glycerol 3-phosphate, changing the latter to lysophosphatidate and relasing coenzyme A.

(*iii*) The acyl-group of a long-chain *unsaturated* acyl-CoA (mostly mono-unsaturated oleyl-CoA and in many cases, polyenoic linoleyl-, linolenyl-and arachidonyl-CoA) is next transferred by **lysophosphatidate acyltransferase** to the C^2 of lysophosphatidate, changing the latter to phosphstidate and releasing another CoA.

(*iv*) The phosphate group of phosphatidate in released by the hydrolysis of its phosphoester bond with C^3 of the phosphatidate by *phosphatidate phosphohydrolase* to change the latter to 1, 2-diacylglycerol.

(*v*) The acyl group of another long-chain **saturated** acyl-CoA molecule is next transferred to the C^3 of 1,2-deacylglycerol by **diacylglycerol acyltransferase,** changing the latter to a triacylglycerol and releasing another CoA.

Overall : Glycerol 3-phosphate + 3acyl-CoA + HO → triacylglycerol + 3CoA + Pi

6.2.3 Dihydroxyacetone phosphate pathway

This is an alternative pathway for the microsomal and peroxisomal synthesis of triacyglycerol by subcutaneous, abdominal and muscle adipocytes.

(*i*) Dihydroxyacetone phosphate, produced in glycolysis, is acylated to 1-acyldihydroxyacetone phosphate by the esterification of its C^1 – OH group with an acyl group from an acyl-CoA molecule by *dihydroxyacetone phosphate acyltransferase* (Fig. 6.3). The coenzyme A of acyl-CoA is released free.

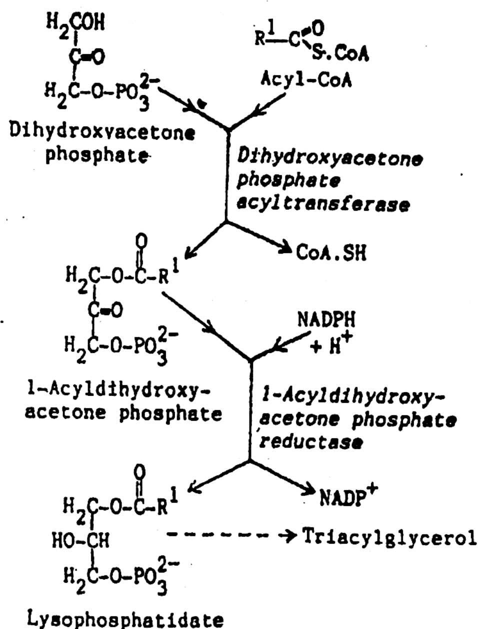


Figure 6.3 : Dihydroxyacetone phosphate pathway for triacylglycerol synthsis [From D. Das, Biochemistry, Academic Publishers, 2000]

(*ii*) 1-Acyldihydroxyacetone phosphate is next reduced to lysophosphatidate by receiving electrons from NADPH under the action of *I-acyldihydrozyacetone phosphate reductase.*

(*iii*) Lysophosphatidate is next changed to triacylglycerol through the successive reactions described in paragraphs (*iii*), (*iv*) and (*v*) of the glycerophsphate pathway (wide 6.2.2).

Overall : Dihydroxyacetone phosphate + 3acyl-CoA + NADPH + H^+ + H_3O → triacylglycerol + 3CoA + $NADP^+$ + Pi

6.3 Adipose Tissue Lipolysis

In diabetes, carbohydrate deprivation or starvation, there happens a dearth of carbohydrate metabolites for immediate energy production in tissues. In such conditions, the stored adipose tissue fats need to be hydrolyzed into free fatty acids for tissue oxidation for energy. This is accomplished in adipocytes by the successive hydrolysis of the stored triacylglycerols by three lipases into free fatty acids and glycerol, followed by the *mobilization of the fatty acids* to other tissues through the blood and their *enhanced oxidation.*

(*i*) Triacylglycerol molecules of adipocytes are first hydrolyzed, each into a free fatty acid and a diacylglycerol, by the *adipose tissue triacylglycerol lipase,* also known as the *hormone-sensitive triacylglycerol lipase* because of its regulation by different hormones (Fig. 6.4.).

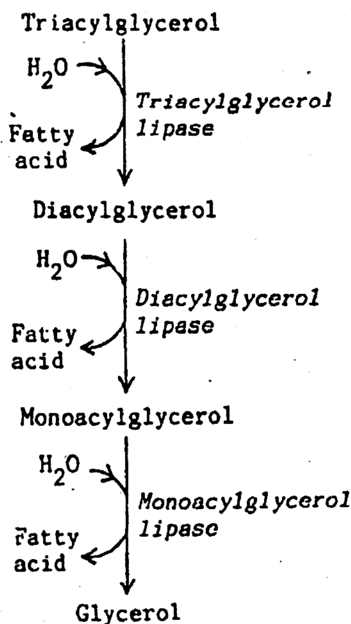


Figure 6.4 : Adipose tissue lipolysis [From D. Das, Biochemistry, Academic Publishers, 2000]

(*ii*) Diacylglycerol is next hydrolyzed by *diacylgtycerol lipase* into another fatty acid and a monoacylglycerol.

(*iii*) Finally, monoacylglycerol is hydrolyzed into glycerol and a third fatty acid by *monoacylglycerol lipase.*

The fatty acids thus released from adipose tisssue may then pass through blood to other tissues. Adipocytes maintain two somewhat distinct *metabolic pools* of fatty acids. One of these pools consists of fatty acids collected by the adipocyte after their release by the action of membrane *lipoprotein lipase* on plasma chylomicron and VLDL particles coming respectively from liver and intestine. The sources of the fatty acids of this pool thus consist of either the absorbed dietary acylglycerols and fatty acids, or the acylglycerols synthesized in enterocytes from dietary fatty acids. The immediate fate of the fatty acids of this pool is mostly the synthesis of triacylglycerols and their storage as adipose tissue fats. The second fatty acid pool in adipocytes consists of fatty acids, released by lipolysis of the stored triacylglycerols and destined to be caried by blood as *albumin-fatty acid complexes* to other tissues, mostly for oxidation.

Regulation of adipose tissue lipolysis :

The rate of adipose tissues lipolysis is enhanced during starvation, carbohydrate deprivation and stress, and is decreased when on high-carbohydrate or high-fat diets. Such regulation is carried out mainly by regulating the *hormone-sensitive triancylglycerol lipase,* the *rate-limiting enzyme* of adipose tissue lipolysis.

(*a*) Fall in blood sugar owing to poor-carbohydrate diets or starvation stimulates secretions of glucagon, adrenaline and glucocoricoids. Conditions of stress also enhance adrenaline and glucocorticoid secretions. (*i*) *Glucocorticoids* such as cortisol induce the synthesis of triacylglycerol lipase and consequently enhance adipose tissue lipolysis. (*ii*) *Glucagon and adrenaline* increase *cyclic AMP* in adipocytes, which activites a *protein kinase;* the latter uses ATP to phosphorylate inactive triacylglycerol lipase *b* to *active triacylglycerol lipase a,* thus enhancing adipose tissue lipolysis. Such lipolytic hormones, therefore, mobilize more fatty acids from the stored fat to raise the plama free fatty level in fasting, carbohydrate deprivation or stress.

(*b*) *Growth hormone and thyroid hormones* also enhance lipolysis and blood fatty acid level. The former brings about a rise in cAMP in the adipocyte by inducing some proteins involved in its formation, while the latter hormones facilitate the lipolytic actions of other hormones.

(*c*) High fat intake increases the plasma FFA level, which inhibits adenylate cyclase in adipocytes, thus decreases the formation and concentration of cAMP in those cells and consequently decreases adipose tissue lipolysis.

(*d*) The rise in blood sugar, when on high-carbohydrate diets, stimulates the secretion of insulin, an *anti-lipolytic* hormone. *Insuline (i)* decrease cAMP formation in adipocytes by inhibiting adenylate cyclase, and (*ii*) activates *phosphodiesterase* which hydrolyzes cAMP to inactive 5'–AMP; both these actions lower cAMP concentration in adipocytes and consequently decrease the action of adipose tissue lipase. (*iii*) Moreover, insulin inhibits the induction of the latter by glucocorticoids. All these lead to a decline in adipose tissue lipolysis and in plasma FFA.

6.4 Phospholipid Synthesis

Cells such as hepatocytes entercytes and adipocytes, playing some roles in fat mobilization into the blood, synthesize significant amounts of phospholipids for helping in the transport of nonpolar lipids in aqueous body fluids. These phospholipids are synthesized by enzymes of smooth ER membranes.

6.4.1 Phosphatidylcholines and phosphatidylethanolamines

(*a*) Choline is phosphorylated to phosphocholine (PC) by *choline Kinase,* using ATP (Fig. 6.5). Similarly, ethanolamine is phosphorylated to phosphoethanolamine (PE) by *ethanolamine 'inase,* using ATP.

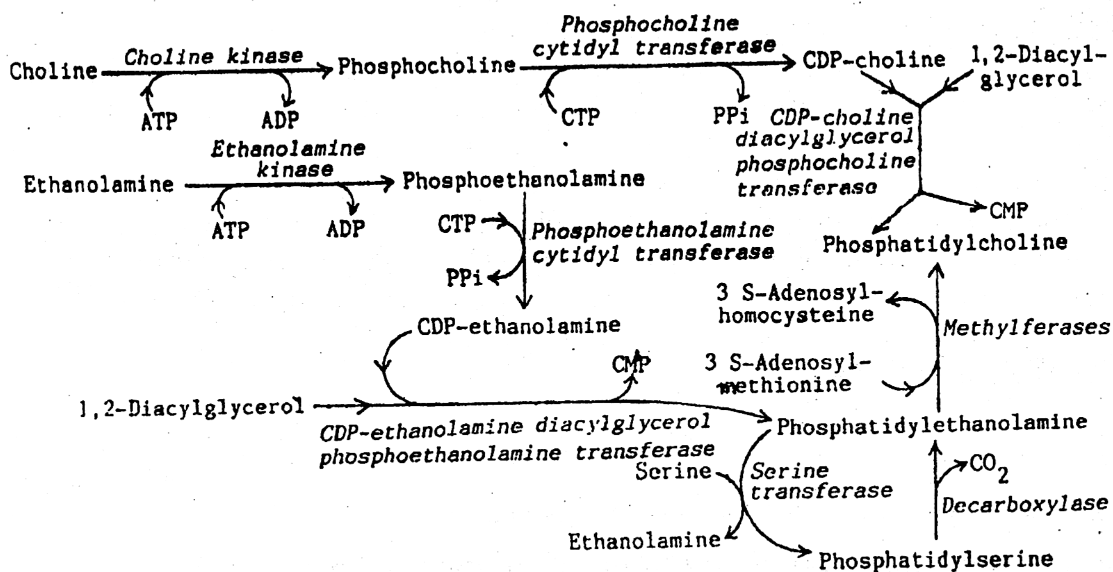


Figure 6.5 : Synthesis of phosphatidylcholines, phosphatidyl ethanolamines and phosphatidyl serines.

(*b*) Cytidylic acid (AMP) is transferred from cytidine triphosphate (CTP) to phosphocholine by *PC cytidyltransferase,* and to phosphoethanolamine by *PE cytidyl*

transferase, changing them respectively to cytidyldiphosphocholine (CDP-choline) and CDP-ethanolamine, with the release of PPi in each case.

(*c*) *CDP-ethanlamine diacylglycerol phosphoethanolamine transferase* next transfers phosphoethanolamine from CDP-ethanolamine to, 1, 2-diacylglycerol, producing phosphatidyl ethanolamine and releasing CMP. Similarly, *CDP-choline diacylglycerol phosphocholine transferase* transfers phosphocholine from CDP-choline to, 1, 2-diacylglycerol, changing the latter to phosphatidylcholine and releasing CMP; alternatively, phosphatidylcholine may also be formed from phosphatidylethanolamine by *methylferase*-catalyzed transfer of three labile methyl groups from three successive S-adenosyl–methionine molecules to the ethanolamine residue of phosphatidylethanolamine.

6.4.2 Phosphatidylserines

Phosphatidylserine may be formed from phosphatidylethanolamine by a *serine transferase* replacing the ethanol amine residue of the latter with serine.

6.4.3 Significance in lipid mobilization

Phospholipids have *amphipathic molecules,* each with a polar headgroup and two nonpolar hydrocarbon tails, and occur in different amounts (5–30%) in different types of lipoprotein particles. Because of their amphipathic molecules, a single layer of phospholipd molecules forms a *surface monolayer* over the lipid core of each lipoprotein particle in plasma and lymph (Fig. 6.6). In that surface monolayer, the monpolar tails of each phospholipid molecule are directed towards the nonpolar lipid core of the lipoprotein particle while its polar headgroup is oriented towards the aqueous phase adjoining the outer surface of the particle. Sûch orientation phospholipid molecules in the monolayer at the water-lipid interface enables nonpolar lipids to be transported in the core of lipoprotein particles in plasma and lymph. So, phospholipids are *essential for fat mobilization* in aqueous body fluids.

6.5 Lipoproteins

Lipoproteins are the principal vehicles for the mobilization and transport of nonpolar lipids such as triacylglycerols, cholesterol esters and free fatty acids in aqueous body fluids such as plasma and lymph.

6.5.1 Structure and composition of lipoprotein particles

A plasma lipoprotein particle may be smaller than 6 nm and even more than 800 nm in diameter (Fig. 6.6). Each such particle has a ~2 nm thick *surface monolayer* of amphipathic lipids such as phospholipids, glycolipids and free cholesterol. These amphipathic lipid molecules from a single layer on the outer surface of the particle, with ther polar head-groups oriented on the water-adjoining surface of the particle

and their nonpolar tails or groups towards the nonpolar **central core** of the particle. The nonpolar lipids in the central core consist largely of traicylglycerols and cholesteryl esters. Each particle bears on its surfaces some easily extractable apolipoproteins which can be easily transferred to other lipoprotein particles; they are called *peripheral apolipoproteins;* e.g. apo-A, apo-C, apo-D and apo-E. Some others like apo-B100 are deeply embedded in the surface monolayer of the particle, reachin its central lipid core, and are called *integral apolipoproteins.*

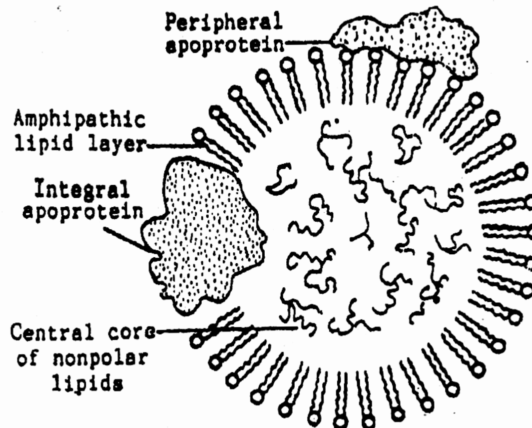


Figure 6.6 : Structure of a lipoprotein particle [From D. Das, Biochemistry, Academic Publishers, 2000]

Lipoproteins possess different densities |——| the higher the lipid percentage, the lower is the density and the larger is the diameter. According to their separation by utlracentrifugation, lipoproteins are classified into chylomicrons, very low-density lipoproteins, (VLDL), low-density lipoproteins (LDL), intermediate-density lipoproteins (IDL) and high-density lipoproteins (HDL). Compositions of different types of lipoproteins are smmarized in Table 6.1.

Table 6.1 Compositions of different types of lipoproteins

	Chylomicron	VLDL	IDL	LDL	HDL
Diameter (nm)	80-100	30-85	25-34	20-25	6-20
Density (g/cm^3)	<0.95	0.96-1.006	1.006-1.019	1.020-1.063	1.063-1.210
Proteins (%)	1.5-2.5	8-10	16-20	21-25	43-55
Triacylglycerols (%)	85-90	50-60	20-24	8-10	4-6
Phospholipids (%)	6-9	16-18	22-24	15-20	20-40
Cholesterylesters (%)	3-4	11-15	30-32	36-40	10-12
Free cholesterol (%)	1-2	4-10	7-8	7-10	3-5
Apoproteins	A-I, A-II, A-IV B-48, C-I, C-II C-III, E	B-100, C-I C-II, C-III, E	B-100, C-III C-III, E	B-100	A-I, A-II, A-IV C-I, C-II, C-III D, E

6.5.2 Functions of plasma lipoproteins

(a) Chylomicrons :

They transport mainly *triacylglycerols and* small amounts of cholesteryl esters, phospholipids and free cholesterol from the interstine to the live, adipose tissue and muscles. The carry both dietary traiacylglycerols and triacylglycerols synthesized in enterocytes from the intestine to the *adipose tissue* for *storage as fats, and to some extent to muscles* also. They are also involved in delivering *exogenous* (dietary) *cholesterol* and its esters to hepatocytes.

(b) VLDL :

They mobilize mainly *endogenous triacylglycerols,* synthesized in hepatocytes, from the liver to the *adipose tissue* in particular for storage as fats; smaller amounts may also be transported to other extrahepatic tissues like *muscles.* High-carbohydrate diets, high insulin : glucagon ratio or any other factor raising the triacylglycerol synthesis in hepatocytes enhances the synthesis of VLDL in the liver to accelerate the transfer of endogenous triacylglycerols from hepatocytes to adipocytes.

(c) LDL :

They transport mainly the *endogenous cholesterol* and its esters, synthesized by hepatocytes, and some *exogenous* (dietary) *cholesterol* also to *extrahepatic tissues* such as muscles glands, connective and lymphoid tissues. So, high plasma LDL may lead to cholesterol deposition in the vascular walls of such tissues.

(d) BHDL :

The serve mainly as *cholesterol scavangers* by transporting cholesterol and cholesteryl esters back from peripheral extrahepatic tissues to liver, diminishing thereby the possibility of cholesterol plaque fromation in peripheral vascular walls. HDL particles also collec *apo-A, apo-C* and *apo-E* proteins from other types of lipoprotein particles, recycle some of those apolipoproteins by transferring them to VLDL and chylomicrons, and return the remainder to the liver.

6.5.3 Metabolism of plasma lipoproteins

(a) Chylomicrons and VLDL :

Polysomes on the rough ER membranes of hepatocytes and enterocytes translate B-100 and B-4 apolipoproteins repectively. They are subsequently incorporated into 'nascent' lipoprotein particles assembled from triacylglycerols, cholesterol and phospholipids in the smooth ER of those cells. After the glycosylation of the apolipoproteins in the Golgi cisternae, secretory vesicles contaning the 'nascent'

lipoproteins are budded of from those cistermae and translocated to the cell membrane, where the vesicles fuse with the latter to release 'nascent' VLDL and chylomicrons respectively from hepatocytes and enterocytes. VLDL particles thereafter enter the blood by passing through the fenestrated walls of hepatic sinusoids; chylomicrons first enter the lymph and through it, pass into the blood. In the blood, these 'nascent' lipoproteins mature to VLDL and chylomicrons by receiving apo-C and apo-E proteins from circulating HDL particles (Fig. 6.7).

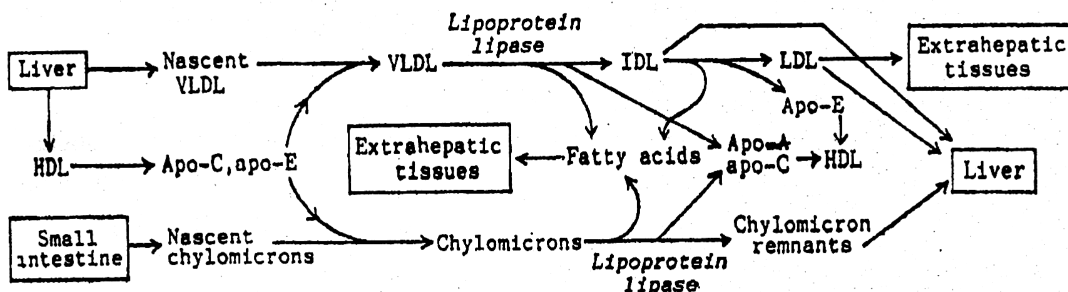


Figure 6.7 : Metabolic fates of Chylomicrons and VLDL [From D. Das, Biochemistry, Academic Publishers, 2000]

In course of their circulation in the blood, VLDL and chylomicron particles come to stick to the capillary endothelial membrane of adipose tissue, mammary glands, cardiac and striated muscles, lungs, renal medulla and other extrahepatic tissues. *Lipoprotein lipase* of the endothelial membrane then hydrolyzes the triacylglycerols of adherent lipoprotein particles through di-and mono-acylglycerols to fatty acids and glycerol. Most of the released fatty acids are taken up by extrahepatic tissue cells, the *adipose tissue* ordinarily taking up the major bulk of those fatty acids for the synthesis and storage of triacylglycerols. The differential uptake of fatty acids from these lipoproteins by different extrahepatic tissues at different times, is accomplished largely by *regulating the lipprotein lipase*. Phospholipids and apo-CII proteins of VLDL and chylomicrons activate this enzyme for its short-term regulation; its long-term regulation depends on the induction of its synthesis by insulin. Lipoproein lipase activity rises in adipocytes after food intake and declines on starvation; but starvation augments its activity in striated and cardiac muscles. So, adipocytes decrease their uptake of fatty acids from lipoproteins during starvation, sparing them for muscles while the latter still continue such uptake from lipoproteins at a higher rate due to enhanced lipoprotein lipase activity there. Prolonged high calory-intake brings about a significant rise in adipose tissue fat due to the induction of the lipase by insulin secreted in that condition.

Lipoprotein lipase action on VLDL and chylomicron particles decreases |——| halves — their triacylgylcerol percentages and diameters, the enhances the percentages

of cholesterol and cholesteryl esters about tow-fold, also increase the phospholipid percentage to about 1.5 times, and transfers some of their apo-A and apo-C proteins to the plasma HDL particles. VLDL and *chylomicron remnants* which get released from the end thelial membrane to reenter circulation.

The circulating chylomicron remmants ultimately bind by their apo-E proteins to *remmant-receptors* (E-receptors) on the hepaocyte membrane, thereby get concentrated on the latter, and are taken up by *adsorptive pinocytosis* into hepatocytes for further metabolism of triacylglycerols, cholesterol and its esters. Some IDL particles are also removed from the circulation in the same way.

(b) Low-density lipoproteins (LDL) :

Most of the circulating IDL particles lose their apo-E proteins by their transfer to HDL particles. They also lose some triacylglycerols to hepatocytes during their intermittent bindings to the E-respectors on the hepatocyte membrane (Fig. 6.7). These change the IDL particles to *LDL particles,* possessing somewhat smaller diameters, higher densities, lower percentages of total lipids, triacylglycerols and phospholipids, but *higher cholesteryl ester percentages* than IDL particles. As LDL circulates in the blood, it acts a *vehicle for delivering cholesterol,* particulartly to extrahepatic tissues such as lymphoid, muscular and vascular connective tissues. Most circulating LDL particles get gradually concentrated on those tissues cells by the binding of their *B-100 apoproteins* to specific *LDL receptors* (B-100 receptors) on the cell membrane and are then internalized into those cells by *adsorptive pinocytosis.* The remaining circulating LDL particles may be similarly pinocytized by hepatic cells, and their cholesterol is mostly excerted in bile as cholesterol, its esters, and bile acids formed from them in hepatocytes.

(c) High-density lipoproteins (HDL) :

HDL particles are formed in hepaocytes and enterocytes by the polysomal

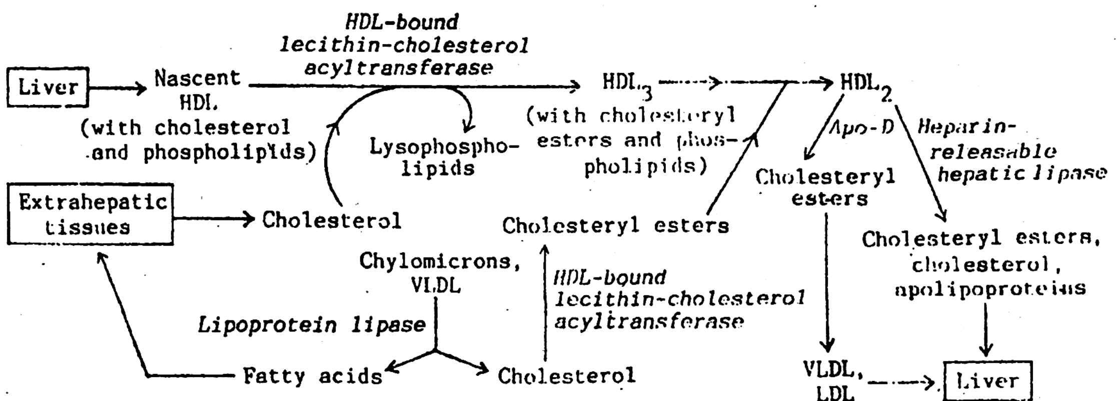
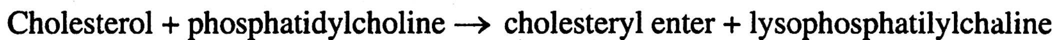


Figure 6.8 : Metabolic fates of HDL [From D. Das, Biochemistry, Academic Publishers, 2000]

translation of A, C and E apoproteins on their rough ER membranes and their incorporation into the 'nascent' assembly of triacylglycerols, phospholipids, cholesterol and its esters, made in the smooth ER (Fig. 6.8). The 'nascent' HDL particle thus constituted is usually a discoid or elliptical particle composed largely of bilayer of phospholipids and cholesterol along with some triacylglycerols and the apoproteins.

As the 'nascent' discoid HDL particles circulate in the blood, their A-I and C-I apolipoproteins activate plasma *lecithin-cholesterol acyltransferase* to transfer unsaturated acyl groups |——| particulary linoleyl goups |——| from the phosphatidylcholine molecules of HDL to the cholesterol molecules of its surface monolayer, esterifying amphipathic cholesterol into nonpolar cholesterylesters, mainly cholesteryl linoleate; phosphaticholine is simultaneously converted to lysophosphatidylcholine by the LCAT action.


Cholesterol + phosphatidylcholine → cholesteryl enter + lysophosphatilylchaline

While lysophosphatidylcholine formed in this reaction is released from HDL to the plasma, the change of amphipathic cholesterol to nonpolar cholesteryl ester makes the latter sink from the surface monolayer of the HDL particle to its nonpolar central core. Such reactions progressively change the elliptical or discoid 'nascent' HDL particles into more and more spherical HDL_3 particles with an amphipathic lipid-protein monolayer enclosing a nonpolar central lipid core. Simultaneously, the decline in cholesterol concentration in the surface monolayer leads to the progressive entry of free cholesterol from the plasma, other circulating lipoprotein particles and extrahepatic tissues into the surface monolayer of HDL particles. Thus, circulating HDL particles *gather cholesterol* from palsma and extrahepatic tissues, change it to cholesteryl esters with the help of LCAT, and carry them in its central lipid core. This changes the 'nscent' HDL particle stepwise into HDL_3 and HDL_2 particles with progressively larger diameters, lower densities, lower triacylglycerol percentage and higher percentage of cholesteryl esters in them. Some C and E apoproteins are transferred from HDL to circulating chylomicrons and VLDL particles. Some cholesteryl esters are also transferred from HDL to VLDL particles with the help of apo-D of HDL, acting as the *cholesteryl ester transfer protein.*

In this way, HDL particles, serve to *mop up cholesterol* from extrahepatic tissues and plasma. From the circulation, HDL particles get bound to specific HDL receptors on the membrane of liver cells and are then taken up by those cells by *adsorptive pinocytosis.* Cholesterol and cholesteryl esters of HDL are then eleminated in the bile either as such or after catabolism into bile acids.

6.5.4 Lipoproteins and atherosclerosis

Because triacyglycerols are mobilized from the liver to the extrahepatic tissues mainly in VLDL particle, a prolonged rise in plasma VLDL produces a sustained rise ain plasma triacylglycerols.

Cholesterol is mobilized from the liver to extrahepatic tissues mainly in LDL particles, and on the contrary, is collected from plasma and extrahepatic tissues mainly by HDL particles and brought back to liver. So, a sustained rise in the plasma LDL, LDL-cholesterol or LDL-cholesterol : HDL-cholesterol ratio, as may happen in habitual high-cholesterol intake, nicotine abuse, hypothyroidism and diabetes mellitus, results in the protracted rise in serum cholesterol level. Repression of synthesis of LDL-receptors (B-100 receptors) resulting from high dietary intake of cholesterol also heightens the plasma LDL-cholesterol by decreasing the removal of LDL from the blood. *Familial hypercholesterolemia* with high plasma LDL-cholesterol is an inborn error of LDL metabolism in humans, resulting from a dominant autosomal genetic defect of LDL-receptors.

High LDL-cholesterol or high LDL : HDL ratio in the plasma leads to the deposition of cholesterol-enriched lipids in vascular walls in the form of fibrous plaques, with consequent thickning and roughening of arterial walls (*altherosclerosis*) and formation of blood clots. Cholesterol plaques and blood clots gradually occlude the vessel, decreasing and finally stopping the blood flow in the affected tissue (*infarction*). Coronary occlusions and myocardial infarctions arise in this way.

High plama level of HDL and HDL-cholesterol would enhance the *scavnging of cholesterol* from extrahepatic tissues and plasma, and tend to lower serum cholesterol. Indeed, high dietary cholesterol has been fond to enhance the formation of HDL; the apo-E protein of the latter wold bind to the E-receptors on hepatocytes, followed by the adsorptive pinocytosis of HDL particle by the liver cells for cholesterol catabolism and excretion. By lowering serum cholesterol HDL prevents the formation of cholesterol plaques in vascular tissues.

6.6 Fatty Liver

Failure in synthesizing required amounts of phospholipids and VLDL in the liver decreases the mobilization of both dietary and endogenous lipids from the liver to extrahepatic tissues including the adipose tissue. The liver consequently gets loaded with accumulated lipids (*fatty liver*) and suffers from fibrosis, cirrhosis and functional failures.

(*a*) Fatty liver may be produced if the liver either receives from food or synthesizes

far more lipids than the amounts of phospholipids and VLDL it can sunthesize for their mobilization to extrahepatic tissues including the adipose tissue. This may happen due to sustainged high-fat diets or heightened adipose tissue lipolysis—free fatty acids rise in the plasma, leading to their enhanced hepatic uptake and conversion to fat which cannot be fully mobilized to adipose tissue in absence of the required enhance amount of VLDL.

(*b*) Fatty liver may result form a failure in hepatic formation of VLDL owing to the dietary deficeincy of essential fatty acids or choline which are constituents of the phospholipids of VLDL particle, or of methionine used as a methyl donor for choline synthesis in the liver. Choline, betaine, methionine, inositol and essential fatty acids are known as *lipostropic factors* as they contribute to the synthesis of phospholipids and VLDL, thus promoting fat mobilization from the liver to the adipose tissue.

(*c*) Protraced high cholesterol intake may also result in fatty liver, because cholesterol is esterified with essential fatty acids, particularly linoleic acid, in the body and therby decreases the availability of essential fatty acids for phospholipid and VLDL synthesis.

(*d*) Toxic effects of chloroform, carbon tetrachloride, puromyci, arsenic, lead and phosphorus prevent the synthesis of apo-B-100 in the liver, which is a constituent of VLDL. This leads to a failure in VLDL formation and a consequent production of fatty liver. Carbon tetrachloride also produces free radicals which peroxidate the lipids of hepatic smooth ER membranes and consequently prevent the formation and release of VLDL.

(*e*) Orotate prevents the release of VLDL from hepatocytes by inhibiting the glycosylation of apo-B-100 of VLDL in the hepatic Golgi cisternae, thus leading to fatty liver.

6.7 Summary

Adipose tissue plays a pivotal role in the metabolism of lipds. Dietary fatty acids, in excess of their immediate requirement for energy production, are used in synthesizing triacylglycerols in adipocytes and stored as adipose tissue. Even carbohydrate metabolites, in excess of their immediate need for energy production, are largely converted to facts and stored in adipose tissue. When needed for energy production, the stored triacylglycerols of adipose tissue undergo lipolysis to release fatty acids for beta-oxidation and energy production. The rate of adipose tissue lipolysis is controlled by regulating the synthesis and activity of its hormone-sensitive rate-limiting enzyme, triacylglycerol lipase.

Nonpolar lipids are transported in aqueous body fluids between the liver, small intestine and other extrahepatic tissues including the adipose tissue by forming phospholipids and lipoproteins. Phospholipids are synthesized in many tissues such as the liver, small intestinal mucosa and adipocytes, and used in forming lipoproteins. Lipoprotein particles are assembled with triacylglycerols, fatty acids, cholesterol and its esters in hepatocytes and enterocytes, with a surface monolayer of amphipathic phospholipids; some intergral and peripheral apolipopreteins are also incorporated into the lipoprotein particles.

According to their densities, lipoproteins are categorised into chylomicrons, very low-density lipoproteins low-density lipopreteins, intermediate density-lipoproteins and high-density lipoproteins. Chylomicrons mainly transport dietary triacylglycerols as well as triacylglycerolglycerols synthesized enterocytes through the lymph and blood from small intestine to adipose tissue and live. VLDL mainly carry triacylglycerols, snthesized inhepatocytes, through the blood to extrahepatic tissues including the adipose tissue. LDL acts as a vehicle for transporting cholesterol and its esters, synthesized in the liver, and also some dietary cholesterol, through the blood to extrahepatic tissues such as connective, lymphoid and vascular tissues. Excess of serum LDL-cholesterol can lead to the formation of cholesterol plaques on vessel walls, and corronary occlusions. HDL functions mainly as cholesterol scavangers, mopping up cholesterol and its esters from peripheral tissues and circulation and delivering them to the liver for catabolism or biliary excertion. HDL thus decreases the tendency of formation of cholesterol plaques in blood vessels. The courses of circulation and metabolism of different plasma lipoproteins have been elaborated in this unit. Fatty liver results from high accumulation of lipids in the liver due to the failure in VLDL formation for their mobilization to extraheptic tissues because of various reasons like dietary deficiencies of essential fatty acids and methionine, high intakes of fats and choles erol, and exposure to toxic chemical. The uptake of fatty acids from VLDL and chylomicrons by extrahepatic tissues depends upon the hydrolysis of their triacyglycerols by regulated enzyme, lipoprotein lipase, of the endothelial membrane.

6.6 Terminal Questions

1. (a) Describes how stored fats are hydrolyzed into fatty acids in the adipose tissue, mentioning the enzyme actions and using a suitable flow chart.

   (b) Name the rate-limiting enzyme for adipose tissue lipolysis and describe its regulation *in vivo.*

   (c) Name some lipotropic factors and their significance.

2. (a) Summarize the biological roles of plasma lipoproteins.
   (b) Describe the circulation and metabolism of plasma HDL and LDL with suitable flow charts.
   (c) Discuss how LDL-cholesterol and HDL-cholesterol are related to atherosclerpsis.
3. (a) Write how fatty acids are enzymatically thioesterified prior to their use in metabolic roles.
   (b) Describe using suitable flowcharts how triacylglycerols are synthesized by the enzymes of glycerophosphate and dihydroxyacetone phosphate pathways.
   (c) What are the significances of triacylglycerol synthesis?
4. (a) Describe using a suitable flowchart how phospholipids are synthesized, mentioning the actions of the enzymes involved.
   (b) Explain the significances of phospholipid synthesis in fat mobilization.
   (c) Discuss various factors which can give rise to fatty liver.
5. (a) Describe the structure of a typical lipoprotein particle with a suitable sectional sketch and mentioning its principal constituents.
   (b) Give a brief classification and average composition of plasma lipoproteins of different classes.
   (c) How are chylomicrons and VLDL particles circulated in the plasma and metabolized? Describe it with a suitable flow chart.
6. (a) Discuss using suitable flowcharts how different plasma lipoproteins are circulated and metabolized.
   (b) Explain briefly how he LDL : HDL ratio in the plasma influences of deposition in cholesterol in peripheral vessels.
7. Write notes on the following :
   (a) Lipoprotein lipase
   (b) Hormone-sensitive adipose tissue lipase.
   (c) Chylomicrons.
   (d) Lipotropic factors.

6.9 Answers

1. (a) See Section 6.3
   (b) See Section 6.3
   (c) See paragraph (b) of Section 6.6

2. (a) See Section 6.5.2
   (b) See (b) and (c) of Section 6.5.3
   (c) See Section 6.5.4
3. (a) See Section 6.2.1
   (b) See Sections 6.2.2 and 6.2.3.
   (c) See second paragraph of Section 6.2
4. (a) See Sections 6.4.1 and 6.4.2
   (b) See Section 6.4.3
   (c) See Section 6.6
5. (a) See Section 6.5.1
   (b) See Section 6.5.1 and Table 6.1
   (c) See (a) of Section 6.5.3
6. (a) See Section 6.5.3
   (b) See Section 6.5.4
7. (a) See second paragraph of (a) of Section 6.5.3
   (b) See Section 6.3
   (c) See Sections 6.5.1 – 6.5.3
   (b) See third paragraph of Section 6.6

Unit 7 ❐ Genetic Disorders of Phenylalanine, Tyrosine and Glycogen Metabolisms

Structure

7.1 Introduction

Objectives.

7.2 Genetic errors of Phenylalanine and Tyrosine Metabolisms

7.3 Genetic errors of Glycogen Metabolism

7.4 Summary

7.5 Terminal Questions

7.6 Answers

7.1 Introduction

You have already read in the preceding units of Group A (2/2) about some important pathways of metabolism of carbohydrates, lipids, proteins and purines. You have also learnt about the kinetics, modulations, inductions and repressions of enzymes.

In this unit, you will read about the discorders of the metabolisms of phenylalanine, tyrosine and glycogen owing to genetic errors.

Genetic errors may result offen from the substitution, omission or addition of one or more mucleotides in the DNA strand of a single gene, and are then called *single-gendisorders* such as albinism. In contrast, *polygenic traits* such as clift lip-and-palate may arise from simultaneous mutations in more than one gene. The genetic disorder may be inherited as either a modinant or a recesive or a codominant trait according as the mutant gene is a Mendelian *dominant, recessive* or codominant one; examples of such traits are respecitively familial hypercholesterolemia, albinism and sickle cell anemia. A mutant gene located in autosome is called and *autosomal trait.* Genetic mutation of a gene affects the protein coded by it and consequently produces disorders in the synthesis, functions and metabolism of such substrates as need that protein for their syntheses and biological roles. Here, in this unit, you will read about such genetic errors of two amino acids and a polysaccharide.

Objectives

Study of this unit should enable you to :

- Understand the natures of inherited defects of two important amino acids, viz., phenylalanine and tyrosine,
- Explain how such genetic errors of these two amino acids affect their metabolism,
- Describe the symptoms of the consequent diseases,
- Know about the causes of important inherited defects of glycogen metabolism.
- Explain how such genetic errors affect the storage of glycogen in animal tissues,
- Describe the principal symptoms of such genetic errors of glycogen metabolism.

7.2 Genetic Errors of Phenylalanine and Tyrosine Metabolisms

You may recall that phenylalanine may be directly incorporated in peptide chains as their phenylalanine residues during translation of tissue proteins. But for all other metabolic functions and fates, phenylalanine has to be converted first to tyrosine which then may be used in synthesizing melanins, catecholamines and iodotyrosines or in being catabolized to fumarate and acetoacetate.

Genetic errors of these two amino acids result from mutations of genes encoding different proteins involved in their metabolism. Such errors are inherited as life-long metabolic defects because of the abnormal or missing proteins whose synthesis and functions have been affected by mutations of their genes. An outline of such genetic errors of Phe and Tyr metabolism is given below.

7.2.1 Hyperphenylalaninemias

Inborn failures to convert phenylalanine to tyrosine many result from genetic defects of several proteins involved in this conversion (Fig. 7.1). In each such case, the inability or deficiency in changing phenylalanine to tyrosine prevents the normal metabolism of phenylalanine through tyrosine in some way or other. Consequently, blood phenylalanineries (hyperphenylalaninemia) and blood tyrosine declines.

(a) ***Hyperphenylalaninemia type I (phenylketonemia) :***

This serius genetic error of phenylalanine metabolism is inherited as an autosomal recessive defect and consists of an inborn deficiency of *phenylalanine hydroxylase,* the enzyme catalyzing the hydroxylation of phenylalanine to tyrosine using molecular O_2 and *tetrahydrobiopterin* as an electron-donor. Failure to change phenylalanine to

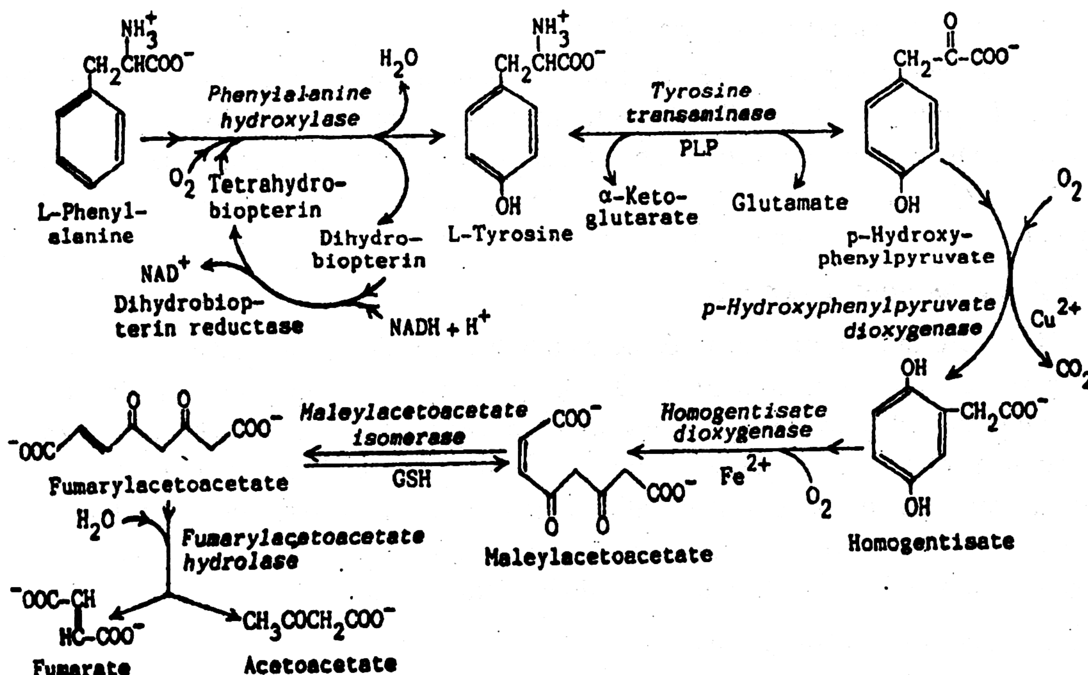


Figure 7.1 : Catabolism of phenylatemine to fumarate and acetoacetate [From D. Das, Biochemistry, Academic Publishers, 2000]

tyrosine leads to high blood levels are urinary eliminations of phenylalanine and its abnormal metabolites such as phenylpyruvate and phenyllactate, severe mental retradation, very low IQ, severe psychosis, seizures and eczematons skin lesions. High blood phenylalanine inhibits *tyrosine hydroxylase* which would have normally hydroxylated tyrosine to dopa for its further metabolism; poor availability of tyrosine for forming melanin pigments leads to decreaed pigmentations of skin and hair.

(b) Hyperphenylalaninemia types II and III :

These rare genetic errors arise from inborn defects of *dihydrobiopterin reductase* which normally would have used NADH to reduce dihydrobiopterin back to tetrahydrobiopterin for continuing phenylalanine hydroxylase activity (Fig. 7.1). Evidently, any genetic defect of this reductase decreases the availability of tetrahydrobiopterin and consequently blocks the hydroxylation of phenylalanine to tyrosine. Symptoms include high blood and urinary levels of phenylalanine, phenylpyruvate and phenyllactate, low blood tyrosine, severe, mental retardation, neurological symptoms, psychosis and seizures. Because tetrahydrobiopterin is also the electron-donor for the synthesis of noradrenaline and serotonin, the decreased synthesis of these neurotransmitters also contribute to neurological symptoms.

(c) Hyperphenylalaminemia types IV and V :

These rare inborn errors results from a genetic deficiencies of *some enzymes* for *dihydrobiopterin synthesis.* This leads to the nonavailability to tetrahydrobiopterin as an electron-donor for converting phenylalanine to tyrosine and consequently blocks phenylalanine metabolism. Symptoms include hyperphenylalaninemia and other symptoms of phenyketonuria, and neurological lesions.

7.2.2 Tyrosinemia type II

This rare inborn error of tyrosine metabolism results from a genetic deficiency of *tyrosine transaminase,* a PLP-dependent enzyme for converting tyrosine to p-hydroxy-phenylpyruvate by transamination (Fig.7.1). This blocks the normal catabolism of tyrosine, and so of phenylalanine also, resulting in high blood and urinary levels of tyrosine, urinary elimination of abnormal tyrosine catabolites like tyramine and N-acetyltyrosine, neuromuscular incoordination, mental retardation, skin lesions, opthalmic nerve lesion and self mutilating tendency.

7.2.3 Neonatal tyrosinemia

This genetic disorder of tyrosine metabolism results from the inborn defect of *p-hydroxpyphenylpyruvate dioxygenase,* the enzyme for oxidizing p-hydroxyphenylpyruvate (see 7.2.2) to homogentisic acid at the next step (Fig. 7.1). Consequently, normal catabolisms of both tyrosine and phenylalanine are blocked, leading to their high blood levels and urinary eliminations, enhancement of their alternative Catbolic pathways, and the resulting urinary eliminations of their abnormal catabolites, such as tyramine, N-acetyltyrosine p-hydroxyphenyllactate and p-hydroxyphenylacetate.

7.2.4 Alkaptonuria

This is a relatively mild autosomal recessive trait. It results from a genetic defect of *homogentisate dioxygenase* the enzyme for oxidizing homogentisate to maleylacetoacetate at the next step of tyrosine catabolism. As this blocks the catabolism of homogentisate, the latter is deposited in connective tissues like cartilages, causing their abnormal pigmentation and leading to rheumatoid arthritis in advanced age. Homogentisate, eliminated in the urine, gets oxidized to a block pigment on expopsure to air, thus turning the urine black.

7.2.5 Tyrosinemia type I

This inborn error of tyrosine catabolism is also called *tyrosinosis.* It produces symptoms like high blood and urinary tyrosine levels, gastrointestinal symptoms

such as nausea, vomiting and diarrhoea, and ultimately death due to hepatic nonfunction. It results from the genetic deficiency either of *maleylacetoacetate isomerase* for isomerizing maleylacetoacetate, a tyrosine catabolite, to fumary lacetoacetate, or of *fumarylacetoacetate hydrolase* which normally hydrolyzes the latter to fumarate and acetoacetate (Fig. 7.1).

7.2.6 Albinisms

These are mild genetic errors of tyrosine metabolism. Melanins, protein-bound polymeric pigments of skin, hair and pigment cells of iris, choroid and retina, are synthesized form tyrosine by a copper enzyme *tyrosinase* in sunlight.

Tyrosine → Dopa → Dopa quinone → Melanins.

Genetic deficiences of malanins may cause two types of albinism with a lack of melanin pigmentation of skin, hair and eye tissues, and the formation of melanomas.

(a) Oculocutaneous albinism :

This autosomal recessive trait results mostly from a genetic dificiency of *tyrosinase.* Skin, hair and eyes have almost no melanin pigmentation in most cases; photophobia is also common and results from the lack of iris pigment. However, some cases may have light cutaneous and iris pigmentation.

(b) Ocular albinism :

This genetic error of melanin formation is inherited as either X-linked or an autosomal trait. Melanin is absent in retina only, but is present in skin and hair. The metabolic defect underlying it is not properly known.

7.3 Genetic Errors of Glycogen Metabolism

Glycogenosis or *glycogen storage diseases* are inherited defects of glycogen metabolism due to the genetic deficiencies of several enzymes for synthesis and breakdown of glycogen.

7.3.1 Type I. von Gierke's disease

You may recall that liver stores glycogen which, when needed, is converted by *glycogenolysis* to glucose for mobilization by blood to extrahepatic tissues. *Type I glycogenosis* is an inborn disorder of glycogenolysis due to a genetic dificiency of *glucose 6-phosphatase,* which catalyzes the hydrolytic dephosphorylation of glucose 6-phosphate to glucose at the final step of that pathway. In this disease, a genetic defect of that enzyme is inherited as an autosomal recessive trait and causes a failure in mobilizing stored glycogen as glucose to extrahepatic tissues. This results in the storage of too much glycogen in hepatic and renal cells, very low blood glucose,

massive hepatic enlargemenmt, no rise in glycogenolysis and consequently in blood sugar even after adrenaline administration, hyperlipidemia and Ketosis—the last two symptoms are due to greater adipose tissue lipolysis and higher fatty acid oxidation because of insufficient glucose supplied to tisues for energy production.

7.3.2 Type II. Pompe's disease

This autosomal recessive trait is characterized by a genetic deficiency of *lysosomal α-1, 4-glucosidases* which normally participate in the lysosomal hydrolysis of polyglycans including glycogen. The defect results in excess glycogen accumulation in tissues and causes infant mortality from respiratory and cardiac failures.

7.3.3 Type III. Cori's disease or limit dextrinosis

In this autosomal recessive trait, *amylo-1, 6-glycosidase* activity of the *glycogen-debranching enzyme* is genetically deficient n the liver. This enzyme activity normally hydrolyzes the α-1, 6-glycosidic bonds at the branching points of glycogen molecules during glycogenolysis. So, a genetic deficiency of this activity causes a failure of hydrolysis of glycosidic bonds at the branching points of glycogen chains. So, *glycogenolysis* remains incomplete, limit-dextrin-type highly-branched polysaccharide molecules accumulate in hepatic cells and muscles, blood sugar remains low, and the liver gets enlarged.

7.3.4 Type IV. Adersen's disease or amylopectinosis

You have read in Unit 3 how glucose is changed to glycogen in the liver, muscles and many other tissues by the processes of *glycogenesis.* In the autosomal recessive trait called amylopectinosis, a genetic deficiency of *amylo-1 4 $\rightarrow$ 1, 6-transglycosylase* (branching enzyme) of that pathway causes a failure in forming α-1 6-glycosidic bonds between growing oligosaccharide chains and a consequent inability to introduce branchings in glycogen molecules. As a result, abnormal amylose-like unbranched long-chain polysaccharide molecules accumulate in liver, muscles and spleen, leading to infant mortality due to either hepatic cirrhosis or cardiac failure.

7.3.5 Type V. McArdle's disease

You have read about the EMP pathway of *glycolysis* of glycogen in muscles. In type V glycogenosis, an autosomal recessive trait, a genetic deficiency of the *muscle glycogen phosphorylase,* the very first enzyme of theat pathway, birngs about in inability to *glycolyze* muscle glycogen. This results in an increase in muscle glycogen, poor post-exercise blood lactate loevel, severe muscle cramps and inability for heavy exercise.

7.3.6 Type VI. Hers' disease

In this autosomal recessive trait, liver fails to carry out *glycogenolysis* of liver glycogen to glucose because of a genetic error of the *hepatic glycogen phosphorylase* the very first and the rate-limiting enzyme of that pathway. This leads to the accumulation of excessive glycogen in the liver, hepatic enlargement, low blood sugar due to decreased mobilization of glucose from liver into blood, poor hyperglycemic response to epinephrine administration, and Ketosis owing to enhanced fatty acid oxidation because of insufficient availability of glucose for energy production.

7.3.7 Type VII. Taru's disease

Phosphofructokinase I, the rate-limiting enzyme for glycolysis, is genetically deficient in muscles and erythrocytes in this autosomal recessive trait. The consequent failure of glycolysis in those tissus raises the muscles glycogen, decreases the blood lactate, towers the muscular work capacity, and produces muscles camps.

7.3.8 Type VIII. Phosphorylase Kinase deficiency

This disease, inherited as a sec-linked recessive trait, is characterized by a genetic deficiency of the *hepatic phosphorylase kinase* which normally would have phosphorylated and activated the rate-limiting *glycogenphosphorylase* of *glycogenolysis*. This results in poor hepatic glycogenolysis, consequent accumulation of glycogen in the river, hepatic enlargement and mild decline in the blood glucose level.

7.3.9 Type IX. Glycogen synthase deficiency

In this autosomal recessive trait, the rate-limiting *hepatic glycogen synthase* of glycogenesis in the liver bring about a failure in *hepatic glycogenesis* and a consequent decline in the liver glycogen content.

7.4 Summary

Five types of hyperphenylalaninemias result from the genetic errors of different enzymes/cofactors participating in phenylalanine metabolism. They are characterized by high blood and urinary levels of phenylalanine and its abnormal metabolities as well as neurological and mental symptoms.

Out of the genetic disorders of tyrosine metabolism, caused by the genetic errors of different enzymes of the latter, albinisms are characterized by lack of melanin

pigmentation of skin, hair and eye tissues due to genetic failures in melanin synthesis, alkaptonuria is characterized by black urine containing a tyrosine metabolic called homogentisate, and three types of tyrosinemias show symptoms including high blood and uninary levels of tyrosin, and its abnormal metabolites.

Nine types of glycogenoses result from genetic errors of glycogen metabolism and affect the storage of glycogen in liver and extrahepatic tissues. Glycogenoses of types I, III, VI and VIII result from genetic errors of different glycogenolytic enzymes, types IV and IX from genetic deficiencies of glycogenic enzymes, types V and VII from genetic errors of glycolytic enzymes, and type II from genetic defects of lysosomal enzymes for glycogen breakdown.

7.5 Terminal Questions

1. (a) Describe the genetic causes and symptoms of different types of tyrosinemia.
   (b) What is glycogenosis? Describe type IX glycogenosis.
   (c) Describe the inbron errors of glycogen storage owing to genetic defects of glycogenolysis.
2. (a) Discuss the causes and symptoms of different types of albinism.
   (b) Describe the inborn errors of glycogen metabolism due to genetic defects of different enzymes for glycogenesis.
   (c) Describe the genetic cause and symptoms of Pompe's disease.
   (d) What are the symptoms and causes of alkaptonuria.
3. (a) Discuss different inborn deficiencies of metabolism of phenolic amino acids, their genetic causes and effects.
   (b) Give an account of the inborn errors arising from genetic defects in the branching and debranching of glycogen chains.
4. (a) Describe the negetic causes and symptoms of such glycogen storage diseases which result from inherited defects of glycolysis.
   (b) Give an account of the genetic causes and symptoms of different types of hyperphenylalaninemia.

7.6 Answers

1. (a) See Sections 7.2.2, 7.2.3 and 7.2.5.

   (b) See first paragraph of Section 7.3 and 7.3.9.

   (c) See Sections 7.3.1, 7.3.3, 7.3.6 and 7.3.8.

2. (a) See Section 7.2.6.

   (b) See Sections 7.3.4 and 7.3.9.

   (c) See Section 7.3.2.

   (d) See Section 7.2.4.

3. (a) See Sections 7.2.1-7.2.6.

   (b) See Sections 7.3.3 and 7.3.4.

4. (a) See Sections 7.3.5 and 7.3.7.

   (b) See Section 7.2.1.