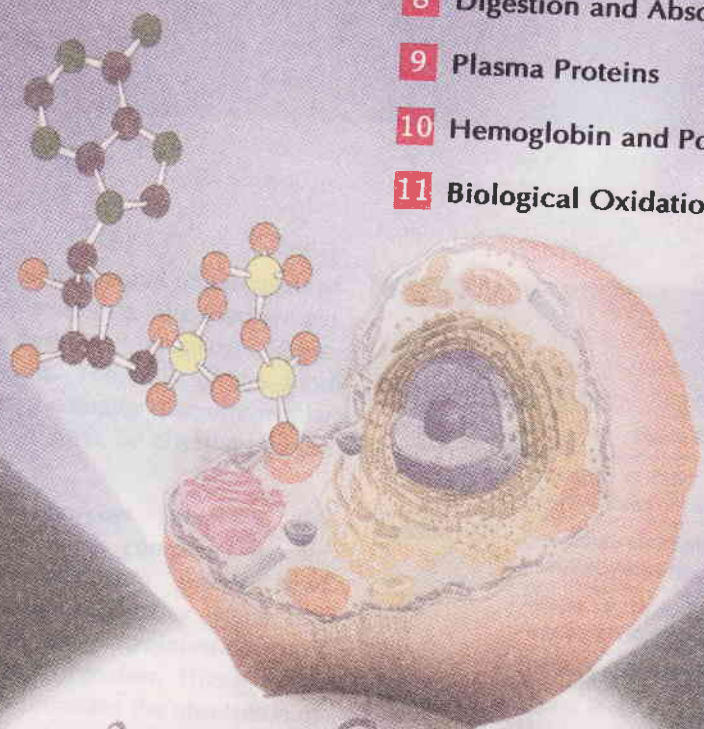
PHYSIOLOGICAL BIOCHEMISTRY



Section II

8 Digestion and Absorption

The natural foodstuffs speak :

"Complex is the ingested food,
But digested to simpler products,
Absorbed by intestinal mucosal cells,
Assimilated and utilized by all cells."

Food is the basic and essential requirement of man for his very existence. The food we eat consists of carbohydrates, proteins, lipids, vitamins and minerals. The bulk of the food ingested is mostly in a complex macromolecular form which cannot, as such, be absorbed by the body.

Digestion is a process involving the hydrolysis of large and complex organic molecules of foodstuffs into smaller and preferably water-soluble molecules which can be easily absorbed by the gastrointestinal tract for utilization by the organism. Digestion of macromolecules also promotes the absorption of fat soluble vitamins and certain minerals.

Cooking of the food, and mastication (in the mouth) significantly improve the digestibility of foodstuffs by the enzymes.

Table 8.1
Organs of gastrointestinal tract with their major functions in digestion and absorption

Organ	*Major function(s)*
Mouth	Production of saliva containing α-amylase; partial digestion of polysaccharides
Stomach	Elaboration of gastric juice with HCl and proteases; partial digestion of proteins
Pancreas	Release of $NaHCO_3$ and many enzymes required for intestinal digestion
Liver	Synthesis of bile acids
Gall bladder	Storage of bile
Small intestine	Final digestion of foodstuffs; absorption of digested products
Large intestine	Mostly absorption of electrolytes; bacterial utilization of certain non-digested and/or unabsorbed foods

Gastrointestinal tract

Digestion as well as absorption are complicated processes that occur in the gastro-intestinal tract (GIT) involving many organs. The diagrammatic representation of GIT is depicted in ***Fig.8.1,*** and the essential organs with their respective major functions are given in ***Table 8.1***. The digestive organs possess a large

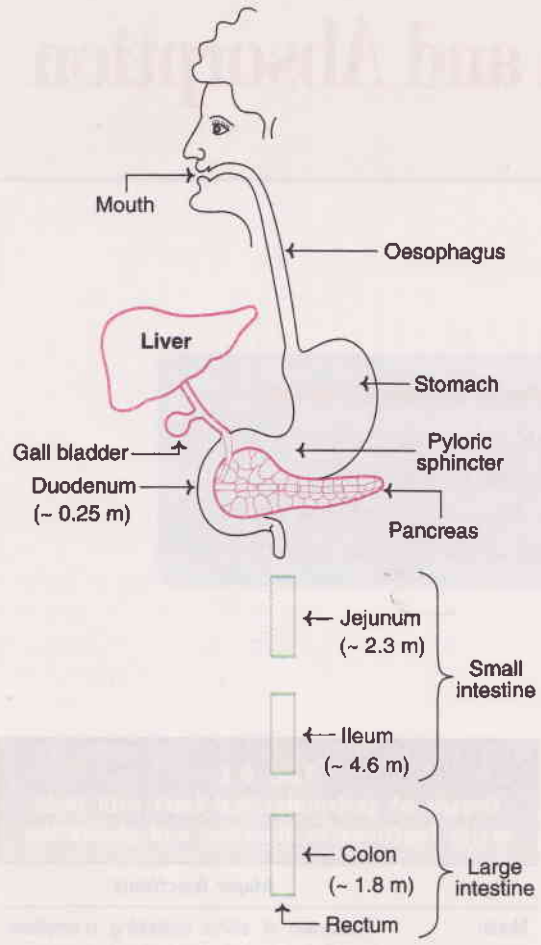


Fig. 8.1 : Diagrammatic representation of gastrointestinal tract.

reserve capacity. For instance, pancreas secretes enzymes 5-10 fold higher than required for digestion of foods normally ingested.

The digestion and absorption of individual foods, namely carbohydrates, proteins, lipids and nucleic acids, is described here. The gastrointestinal hormones are discussed under hormones (***Chapter 19***).

CARBOHYDRATES

The principal dietary carbohydrates are polysaccharides (starch, glycogen), disaccharides (lactose, sucrose) and, to a minor extent, monosaccharides (glucose, fructose). The structures of carbohydrates are described in ***Chapter 2***.

Digestion

The digestion of carbohydrates occurs briefly in mouth and largely in the intestine. The polysaccharides get hydrated during heating which is essential for their efficient digestion. The hydrolysis of glycosidic bonds is carried out by a group of enzymes called ***glycosidases*** (***Fig.8.2***). These enzymes are specific to the bond, structure and configuration of monosaccharide units.

Digestion in the mouth : Carbohydrates are the only nutrients for which the digestion begins in the mouth to a significant extent. During the process of mastication, ***salivary α-amylase (ptyalin)*** acts on starch randomly and cleaves α-1,4-glycosidic bonds. The products formed include α-limit dextrins, (containing about 8 glucose units with one or more α-1,6-glycosidic bonds) maltotriose and maltose.

Carbohydrates not digested in the stomach : The enzyme salivary amylase is inactivated by high acidity (low pH) in the stomach. Consequently, the ongoing degradation of starch is stopped.

Digestion in the small intestine : The acidic dietary contents of the stomach, on reaching small intestine, are neutralized by bicarbonate produced by pancreas. The ***pancreatic α-amylase*** acts on starch and continues the digestion process. Amylase specifically acts on α-1,4-glycosidic bonds and not on α-1,6-bonds.

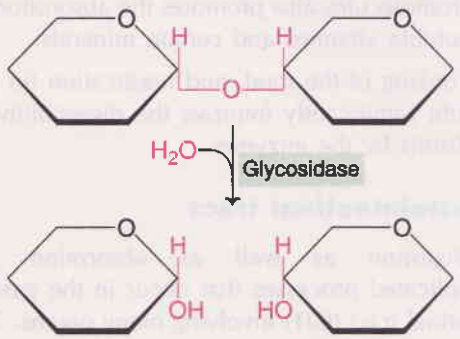


Fig. 8.2 : Hydrolysis of a glycosidic bond.

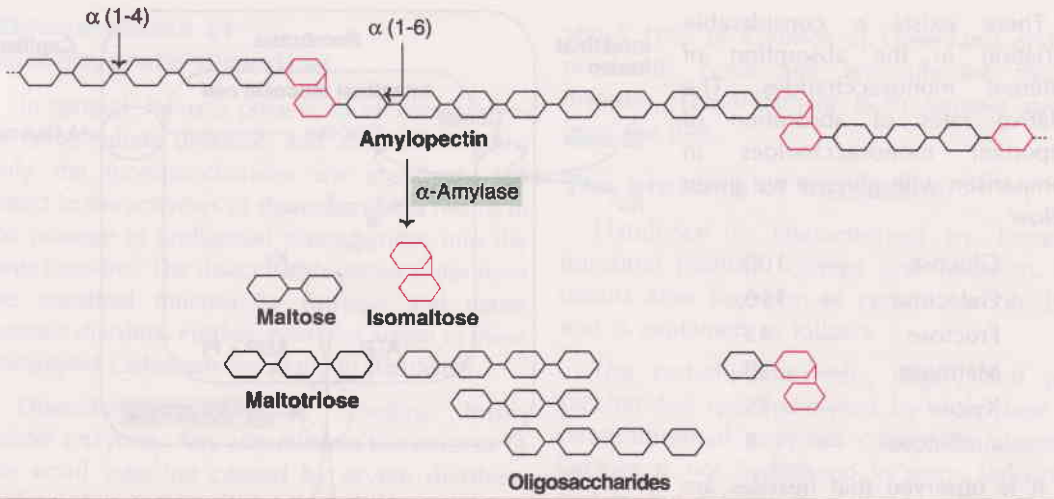


Fig. 8.3 : Degradation of amylopectin by salivary or pancreatic α-amylase.

The resultant products are disaccharides (maltose, isomaltose) and oligosaccharides (***Fig.8.3***).

The final digestion of di- and oligosaccharides to monosaccharides (***Fig.8.4***) primarily occurs at the mucosal lining of the upper jejunum. This is carried out by ***oligosaccharidases*** (e.g. glucoamylase acting on amylose) ***and disaccharidases*** (e.g. maltase, sucrase, lactase). The enzyme ***sucrase*** is capable of hydrolysing a large quantity of table sugar (sucrose). In contrast, ***lactase (β-galactosidase)*** is the rate-limiting, and, consequently, the utilization of milk sugar (lactose) is limited in humans.

Absorption of monosaccharides

The principal monosaccharides produced by the digestion of carbohydrates are glucose, fructose and galactose. Of these, glucose accounts for nearly 80% of the total monosaccharides. The absorption of sugars mostly takes place in the ***duodenum*** and ***upper jejunum*** of small intestine.

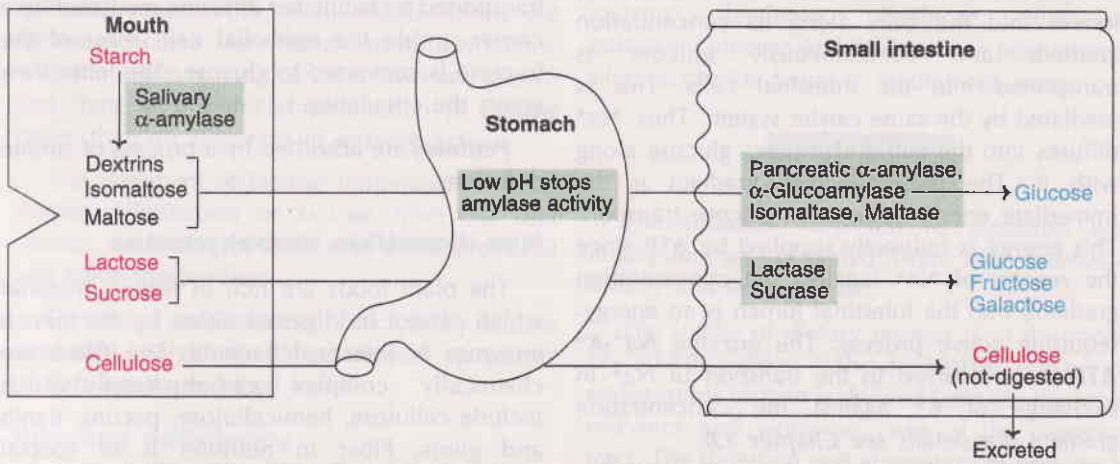


Fig. 8.4 : Overview of digestion of carbohydrates.

There exists a considerable variation in the absorption of different monosaccharides. The relative rates of absorption of important monosaccharides in comparison with glucose are given below

Glucose	—	100
Galactose	—	110
Fructose	—	43
Mannose	—	20
Xylose	—	15
Arabinose	—	9

It is observed that hexoses are more rapidly absorbed than pentoses. Further, among the monosaccharides, ***galactose is most efficiently absorbed*** followed by glucose and fructose. Insulin has no effect on the absorption of sugars.

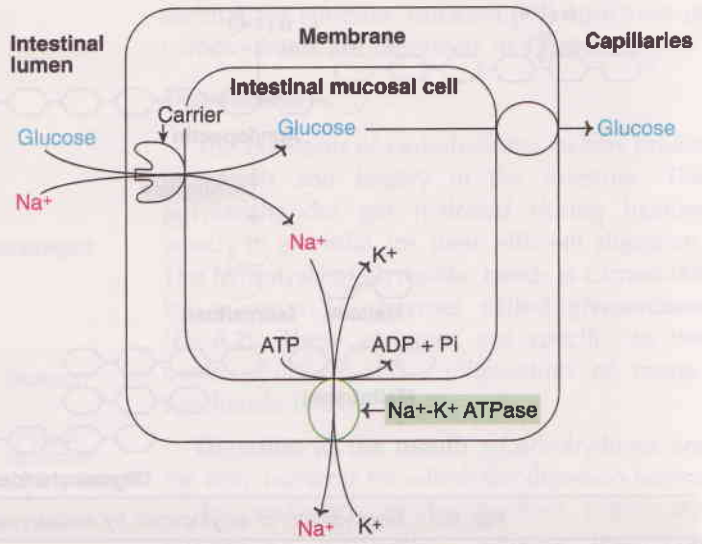


Fig. 8.5 : *Transport of glucose across intestinal epithelium* ***(Note :*** *Transport of amino acids also occurs by a similar mechanism; replace glucose in figure by amino acid).*

Mechanism of absorption

Different sugars possess different mechanisms for their absorption. Glucose is transported into the intestinal mucosal cells by a carrier mediated and energy requiring process (***Fig.8.5***).

Glucose and ***Na+*** share the same transport system (***symport***) which is referred to as sodium-dependent glucose transporter. The concentration of Na^+ is higher in the intestinal lumen compared to mucosal cells. Na^+, therefore, moves into the cells along its concentration gradient and simultaneously glucose is transported into the intestinal cells. This is mediated by the same carrier system. Thus, Na^+ diffuses into the cell and it drags glucose along with it. The intestinal Na^+ gradient is the immediate energy source for glucose transport. This energy is indirectly supplied by ATP since the reentry of Na^+ (against the concentration gradient) into the intestinal lumen is an energy-requiring active process. The enzyme ***Na^+-K^+ ATPase*** is involved in the transport of Na^+ in exchange of K^+ against the concentration gradient (for details ***see Chapter 33***).

Oral rehydration therapy (ORT) : *ORT is the* most common treatment of diarrhea. The oral rehydration fluid contains glucose and sodium. Intestinal absorption of sodium is facilitated by the presence of glucose.

The mechanism of absorption of galactose is similar to that of glucose. The inhibitor ***phlorizin*** blocks the Na^+ dependent transport of glucose and galactose.

Absorption of fructose : Fructose absorption is relatively simple. It does not require energy and is independent of Na^+ transport. Fructose is transported by facilitated diffusion mediated by a carrier. Inside the epithelial cell, most of the fructose is converted to glucose. The latter then enters the circulation.

Pentoses are absorbed by a process of simple diffusion.

Non-digestible carbohydrates

The plant foods are rich in fibrous material which cannot be digested either by the human enzymes or intestinal bacteria. The ***fibers*** are chemically complex carbohydrates which include cellulose, hemicellulose, pectins, lignin and gums. Fiber in nutrition is of special importance which is described under nutrition (***Chapter 23***).

Abnormalities of carbohydrate digestion

In general, humans possess an efficient system of carbohydrate digestion and absorption. Since only the monosaccharides are absorbed, any defect in the activities of ***disaccharidases*** results in the passage of undigested disaccharides into the large intestine. The disaccharides draw water from the intestinal mucosa by osmosis and cause osmotic diarrhea. Further, bacterial action of these undigested carbohydrates leads to flatulence.

Disaccharidases are the intestinal brush border enzymes. Any alteration in the mucosa of the small intestine caused by severe diarrhea, malnutrition, intestinal diseases or drug therapy will lead to a temporary acquired deficiency of disaccharidases. The patients with such disorders are advised to restrict the consumption of sucrose and lactose.

Hereditary disorders with deficiency of individual disaccharidases in infants and children cause intolerance of specific disaccharides.

Lactose intolerance

Defect in the enzyme ***lactase*** (β-galactosidase) is the most common disaccharidase deficiency in humans. It is estimated that more than half of the world's adult population is affected by lactose intolerance. It is more commonly found in Africans (blacks) and Asians compared to Europeans. Surprisingly, according to a recent estimate, about 90% of the adult Asians are lactase deficient. The mechanism of how lactase is lost in adults is not clear. It is however, known that there is a reduced production of lactase rather than an alteration in enzyme activity.

The treatment of lactose intolerance is quite simple. Elimination of lactose from the diet (severe restriction of milk and dairy products) will solve the problem.

Continued consumption of lactose by lactose intolerant individuals causes typical symptoms of ***flatulence*** (described later).

Sucrase deficiency

The deficiency of the enzyme sucrase causes intolerance to dietary sucrose. It is estimated that about 10% of Eskimos of Greenland and 2% of North Americans are affected by this disorder. The treatment is to remove sucrose from the diet.

The problem of flatulence

Flatulence is characterized by increased intestinal motility, cramps and irritation. This occurs after ingestion of certain carbohydrates and is explained as follows.

The carbohydrates (di-, oligo-, and polysaccharides) not hydrolysed by α-amylase and other intestinal enzymes cannot be absorbed. Lactose is not hydrolysed in some individuals due to the deficiency of lactase. The di-, and oligosaccharides can be degraded by the bacteria present in ileum (lower part of small intestine) to liberate monosaccharides. The latter can be metabolized by the bacteria.

During the course of utilization of monosaccharides by the intestinal bacteria, the gases such as ***hydrogen, methane and carbon dioxide***—besides lactate and short chain fatty acids—are released. These compounds cause flatulence.

The occurrence of flatulence after the ingestion of ***leguminous*** seeds (bengal gram, redgram, beans, peas, soya bean) is very common. They contain several ***nondigestible oligosaccharides*** by human intestinal enzymes. These compounds are degraded and utilised by intestinal bacteria causing flatulence. ***Raffinose*** containing galactose, glucose and fructose is a predominant oligosaccharide found in leguminous seeds.

PROTEINS

The proteins subjected to digestion and absorption are obtained from two sources—dietary and endogenous.

The intake of dietary protein is in the range of 50-100 g/day. About 30-100 g/day of endogenous protein is derived form the digestive enzymes and worn out cells of the digestive tract. The digestion and absorption of proteins is very efficient in healthy humans, hence very little protein (about 5-10 g/day) is lost through feces.

Dietary proteins are denatured on cooking and therefore, easily digested.

Proteins are degraded by a class of enzymes—namely ***hydrolases***—which specifically cleave the peptide bonds, hence known as ***peptidases***. They are divided into two groups

1. **Endopeptidases (proteases)** which attack the internal peptide bonds and release peptide fragments, e.g. pepsin, trypsin.

2. **Exopeptidases** which act on the peptide bonds of terminal amino acids. Exopeptidases are subdivided into ***carboxypeptidases*** (act on C-terminal amino acid) and ***aminopeptidases*** (act on N-terminal amino acid).

The proteolytic enzymes responsible for the digestion of proteins are produced by the stomach, the pancreas and the small intestine. Proteins are not digested in the mouth due to the absence of proteases in saliva.

I. Digestion of proteins by gastric secretion

Protein digestion begins in the stomach. Gastric juice produced by stomach contains hydrochloric acid and a protease proenzyme namely pepsinogen.

Hydrochloric acid : The pH of the stomach is < 2 due to the presence of HCl, secreted by parietal (oxyntic) cells of gastric gland. This acid performs two important functions-denaturation of proteins and killing of certain microorganisms. The denatured proteins are more susceptible to proteases for digestion.

Pepsin : Pepsin (*Greek* : pepsis—digestion) is produced by the serous cells of the stomach as pepsinogen, the inactive zymogen or proenzyme. Pepsinogen is converted to active pepsin either by autocatalysis, brought about by other pepsin molecules or by gastric HCl (pH < 2). Removal of a fragment of polypeptide chain (44 amino acids in case of pig enzyme) makes the inactive enzyme active after attaining a proper conformation.

Pepsin is an acid-stable endopeptidase optimally active at a very low pH (2.0). The active site of the enzyme contains two carboxyl groups, which are maintained at low pH. Pepsin

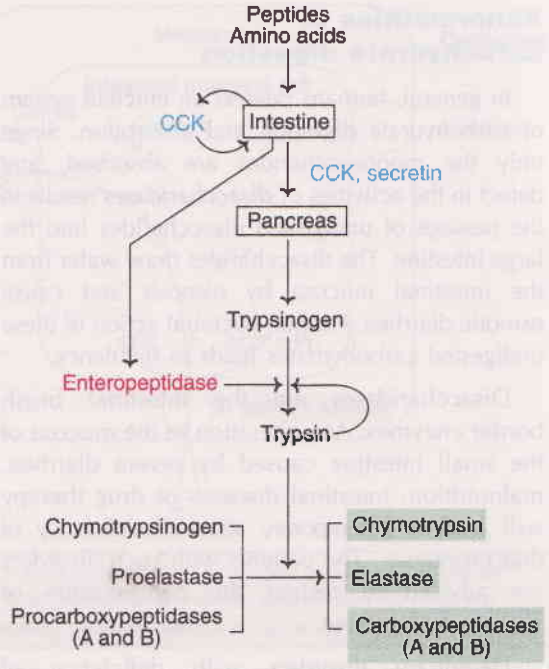


Fig. 8.6 : Formation and activation of pancreatic proteases (CCK-Cholecystokinin).

A is the most predominant gastric protease which preferentially cleaves peptide bonds formed by amino groups of phenylalanine or tyrosine or leucine.

Pepsin digestion of proteins results in peptides and a few amino acids which act as stimulants for the release of the hormone cholecystokinin from the duodenum.

Rennin : This enzyme, also called chymosin, is found in the stomach of infants and children. Rennin is involved in the curdling of milk. It converts milk protein casein to calcium paracaseinate which can be effectively digested by pepsin. Rennin is absent in adults.

II. Digestion of proteins by pancreatic proteases

The proteases of pancreatic juice are secreted as zymogens (proenzymes) and then converted to active forms. These processes are initiated by the release of two polypeptide hormones, namely ***cholecystokinin*** and ***secretin*** from the intestine (***Fig.8.6***).

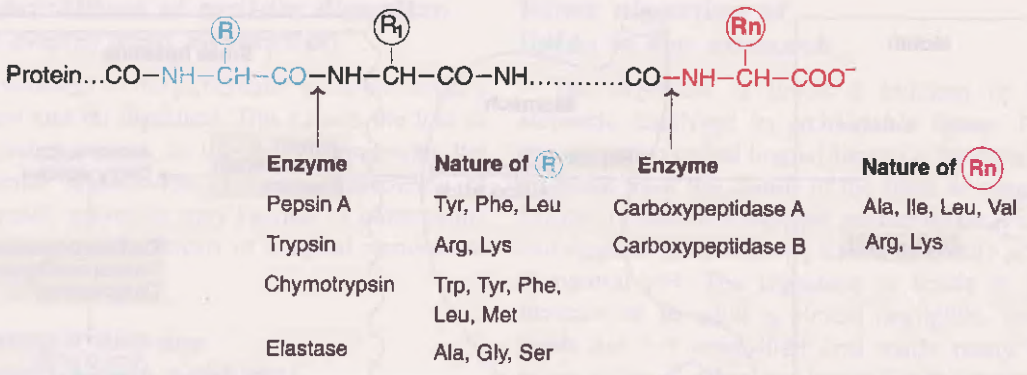


Fig. 8.7 : Digestion of proteins—Specificity of enzyme cleavage of peptide bonds. (R_1 can be from any amino acid)

Release and activation of zymogens : The key enzyme for activation of zymogen is ***enteropeptidase*** (formerly enterokinase) produced by intestinal (mostly duodenal) mucosal epithelial cells. Enteropeptidase cleaves off a hexapeptide (6 amino acid fragment) from the N-terminal end of trypsinogen to produce trypsin, the active enzyme. ***Trypsin***, in turn, activates other trypsinogen molecules (autocatalysis). Further, trypsin is the common activator of all other pancreatic zymogens to produce the active proteases, namely chymotrypsin, elastase and carboxypeptidases (A and B).

Specificity and action of pancreatic proteases : Trypsin, chymotrypsin and elastase are endopeptidases active at neutral pH. Gastric HCl is neutralized by pancreatic $NaHCO_3$ in the intestine and this creates favourable pH for the action of proteases.

The substrate specificity of pancreatic proteases is depicted in ***Fig.8.7***. For instance, trypsin cleaves the peptide bonds, the carbonyl (–CO–) group of which is contributed by arginine or lysine.

The amino acid serine is essential at the active centre to bring about the catalysis of all the three pancreatic proteases, hence these enzymes are referred to as ***serine proteases***.

Action of carboxypeptidases : The pancreatic carboxypeptidases (A and B) are metalloenzymes that are dependent on Zn^{2+} for their catalytic activity, hence they are sometimes called ***Zn-proteases***. They also possess certain degree of substrate specificity in their action. For example, carboxypeptidase B acts on peptide bonds of COOH-terminal amino acid, the amino group of which is contributed by arginine or lysine (***Fig.8.7***).

The combined action of pancreatic proteases results in the formation of free amino acids and small peptides (2-8 amino acids).

III. Digestion of proteins by small intestinal enzymes

The luminal surface of intestinal epithelial cells contains ***aminopeptidases*** and ***dipeptidases***. Aminopeptidase is a non-specific exopeptidase which repeatedly cleaves N-terminal amino acids one by one to produce free amino acids and smaller peptides. The dipeptidases act on different dipeptides to liberate amino acids (***Fig.8.8***).

Absorption of amino acids and dipeptides

The free amino acids, dipeptides and to some extent tripeptides are absorbed by intestinal epithelial cells.

The di- and tripeptides, after being absorbed are hydrolysed into free amino acids in the cytosol of epithelial cells. The activities of dipeptidases are high in these cells. Therefore, ***after a protein meal, only the free amino acids are found in the portal vein.***

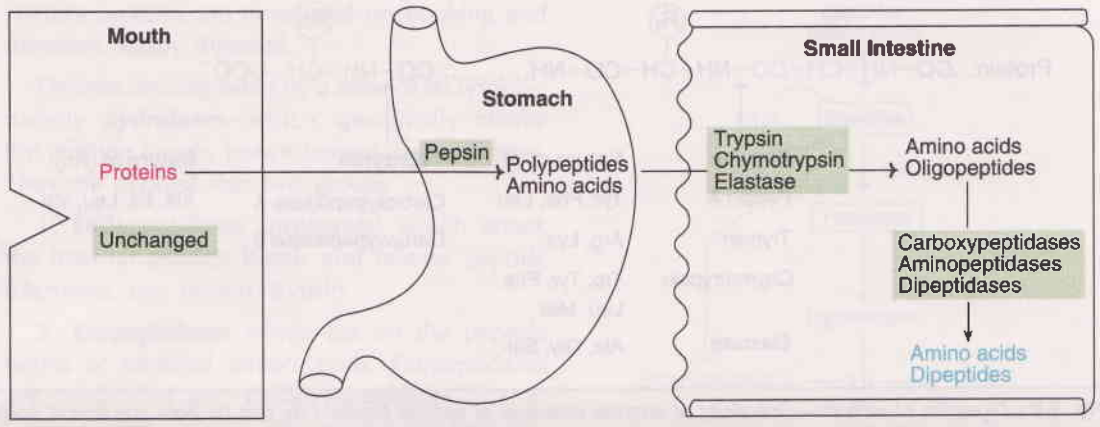


Fig. 8.8 : Overview of digestion of proteins.

The small intestine possesses an efficient system to absorb free amino acids. L-Amino acids are more rapidly absorbed than D-amino acids. The transport of L-amino acids occurs by an active process (against a concentration gradient), in contrast to D-amino acids which takes place by a simple diffusion.

Mechanism of amino acid absorption

Amino acids are primarily absorbed by a similar mechanism, as described for the transport of D-glucose. It is basically a Na^+-dependent active process linked with the transport of Na^+. As the Na^+ diffuses along the concentration gradient, the amino acid also enters the intestinal cell. Both Na^+ and amino acids share a common carrier and are transported together. The energy is supplied indirectly by ATP (for details, ***see*** absorption of monosaccharides and ***Fig.8.5***).

A Na^+-independent system of amino acid transport across intestinal cells has also been identified. The compound ***cytochalasin B*** inhibits Na^+-independent transport system.

Another transport system to explain the mechanism of amino acid transfer across membrane in the intestine and kidney has been put forth. This is known as ***γ-glutamyl cycle or Meister cycle*** and involves a tripeptide namely ***glutathione*** (γ-glutamylcysteinylglycine). Three ATP are utilized for the transport of a single amino acid by this cycle. For this season, Meister cycle is not a common transport system for amino acid. However, this cycle is operative for rapid transport of cysteine and glutamine.

The γ-glutamyl cycle appears to be important for the metabolism of glutathione, since this tripeptide undergoes rapid turnover in the cells. There may be more physiological significance of γ-glutamyl cycle.

Absorption of intact proteins and polypeptides

For a short period, immediately after birth, the small intestine of infants can absorb intact proteins and polypeptides. The uptake of proteins occurs by a process known as ***endocytosis*** or ***pinocytosis***. The macromolecules are ingested by formation of small vesicles of plasma membrane followed by their internalization. The direct absorption of intact proteins is very important for the transfer of maternal immunoglobulins (γ-globulins) to the offspring.

The intact proteins and polypeptides are not absorbed by the adult intestine. However, the macromolecular absorption in certain individuals appears to be responsible for antibody formation that often causes ***food allergy***.

Abnormalities of protein digestion and amino acid absorption

Any defect in the pancreatic secretion impairs protein and fat digestion. This causes the loss of undigested protein in the feces along with the abnormal appearance of lipids. Deficiency of pancreatic secretion may be due to pancreatitis (see later), cystic fibrosis or surgical removal of pancreas.

Hartnup's disease (neutral amino aciduria)

Hartnup is the name of the family in whom this disease was first discovered. It is characterized by the ***inability*** of intestinal and renal epithelial cells ***to absorb neutral amino acids***. ***Tryptophan*** absorption is most severely affected with a result that typical symptoms of ***pellagra*** are observed in the patients of Hartnup's disease. This is related to the impairment in the conversion of tryptophan to NAD^+ and $NADP^+$, the coenzymes of niacin.

LIPIDS

There is considerable variation in the daily consumption of lipids which mostly depends on the economic status and dietary habits. The intake of lipids is much less (often < 60 g/day) in poorer sections of the society, particularly in the less developed countries. In the developed countries, an adult ingests about 60-150 g of lipids per day. Of this, more than 90% is fat (triacylglycerol). The rest of the dietary lipid is made up of phospholipids, cholesterol, cholesteryl esters and free fatty acids.

Lipids are insoluble or sparingly soluble in aqueous solution. The digestive enzymes, however, are present in aqueous medium. This poses certain problems for the digestion and absorption of lipids. Fortunately, the digestive tract possesses specialized machinery to

1. Increase the surface area of lipids for digestion;
2. Solubilize the digested products for absorption.

Minor digestion of lipids in the stomach

The digestion of lipids is initiated in the stomach, catalysed by ***acid-stable lipase***. This enzyme (also called lingual lipase) is believed to originate from the glands at the back of tongue. Stomach contains a separate ***gastric lipase*** which can degrade fat containing short chain fatty acids at neutral pH. The digestion of lipids in the stomach of an adult is almost negligible, since lipids are not emulsified and made ready for lipase action. Further, the low pH in the stomach is unfavourable for the action of gastric lipase.

In case of infants, the milk fat (with short chain fatty acids) can be hydrolysed by gastric lipase to some extent. This is because the stomach pH of infants is close to neutrality, ideal for gastric lipase action.

Emulsification of lipids in the small intestine

Emulsification is the phenomenon of dispersion of lipids into smaller droplets due to reduction in the surface tension. This is accompanied by increase in the surface area of lipid droplets. Emulsification is essential for effective digestion of lipids, since the enzymes can act only on the surface of lipid droplets. More correctly, lipases act at the interfacial area between the aqueous and lipid phase.

The process of emulsification occurs by three complementary mechanisms

1. Detergent action of bile salts;
2. Surfactant action of degraded lipids;
3. Mechanical mixing due to peristalsis.

1. **Bile salts :** The terms bile salts and bile acids are often used interchangeably. At physiological pH, the bile acids are mostly present as anions. Bile salts are the biological detergents synthesized from cholesterol in the liver. They are secreted with bile into the duodenum. Bile salts possess steroid nucleus, the side chain of which is attached to either ***glycine (glycocholic acid)*** or ***taurine (taurocholic acid)***. For the synthesis and other details on bile acids, refer cholesterol metabolism (***Chapter 14***). Bile salts are the most effective biological emulsifying

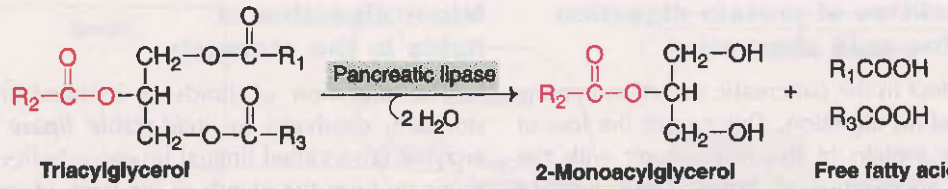


Fig. 8.9 : Enzymatic cleavage of dietary fat.

agents. They interact with lipid particles and the aqueous duodenal contents and convert them into smaller particles (emulsified droplets). Further, bile salts stabilize the smaller particles by preventing them from coalescing.

2. **Surfactant action of degraded lipids :** The initial digestive products of lipids (catalysed by lipase) namely free fatty acids, monoacylglycerols promote emulsification. These compounds along with phospholipids are known as surfactants. They are characterized by possessing polar and non-polar groups. Surfactants get absorbed to the water-lipid interfaces and increase the interfacial area of lipid droplets. Thus the initial action of the enzyme lipase helps in further digestion of lipids.

3. Besides the action of bile salts and surfactants, the ***mechanical mixing due to peristalsis*** also helps in the emulsification of lipids. The smaller lipid emulsion droplets are good substrates for digestion.

Digestion of lipids by pancreatic enzymes

The pancreatic enzymes are primarily responsible for the degradation of dietary triacylglycerols, cholesteryl esters and phospholipids.

Degradation of triacylglycerols (fat)

Pancreatic lipase is the major enzyme that digests dietary fats. This enzyme preferentially cleaves fatty acids (particularly long chain, above 10 carbons) at position 1 and 3 of triacylglycerols. The products are ***2-monoacylglycerol*** (formerly 2-monoglyceride) and ***free fatty acids*** (***Fig.8.9***). The activity of pancreatic lipase is inhibited by bile acids which are present along with the enzyme in the small intestine. This problem is overcome by a small protein, ***colipase*** (mol. wt. 12,000). It is also secreted by pancreas as procolipase and converted to active form by trypsin. Colipase binds at the lipid-aqueous interface and helps to anchor and stabilize lipase.

Lipid esterase is a less specific enzyme present in pancreatic juice. It acts on monoacylglycerols, cholesteryl esters, vitamin esters etc. to liberate free fatty acids. The presence of bile acids is essential for the activity of lipid esterase.

Degradation of cholesteryl esters

A specific enzyme namely pancreatic ***cholesterol esterase*** (cholesteryl ester hydrolase) cleaves cholesteryl esters to produce cholesterol and free fatty acids (***Fig.8.10***).

Degradation of phospholipids

Phospholipases are enzymes responsible for the hydrolysis of phospholipids. Pancreatic juice is rich in phospholipase A_2 which cleaves the fatty acid at the 2nd position of phospholipids. The products are a free fatty acid and a lysophospholipid. Phospholipase A_2 is secreted as a zymogen which is activated in the intestine by the action of trypsin.

An overview of the digestion of lipids is given in ***Fig.8.11***.

Absorption of lipids

The former and present theories to explain the absorption of lipids are briefly described hereunder

1. **Lipolytic theory put forth by Verzar :** According to this, fats are completely hydrolysed to glycerol and free fatty acids. The latter are absorbed either as soaps or in association with bile salts.

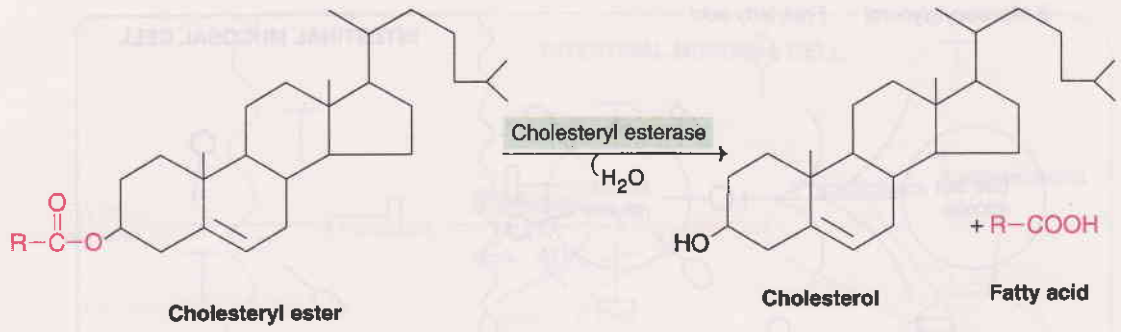


Fig. 8.10 : Enzymatic cleavage of cholesteryl ester.

2. **Partition theory proposed by Frazer :** This theory states that the digestion of triacylglycerols is partial and not complete. The partially digested triacylglycerols, in association with bile salts, form emulsions. The lipids are taken up by the intestinal mucosal cells. As per this theory, resynthesis of lipids is not necessary for their entry into the circulation.

3. **Bergstrom theory :** This is a more recent and comprehensive theory to explain lipid absorption. It has almost replaced the earlier theories, and is briefly described hereunder

The primary products obtained from the lipid digestion are 2-monoacylglycerol, free fatty acids and free cholesterol.

Role of bile salts in lipid absorption

Besides their participation in digestion, bile salts are essential for absorption of lipids. Bile salts form ***mixed micelles*** with lipids. These micelles are smaller in size than the lipid emulsion droplets (utilized for digestion, described above). The micelles have a disk like shape with lipids (monoacylglycerol, fatty acids, cholesterol and phospholipids) at the interior and bile salts at the periphery. The hydrophilic groups of the lipids are oriented to the outside (close to the aqueous environment) and the hydrophobic groups to the inside. In this fashion, the bile salt micelles exert a solubilizing effect on the lipids.

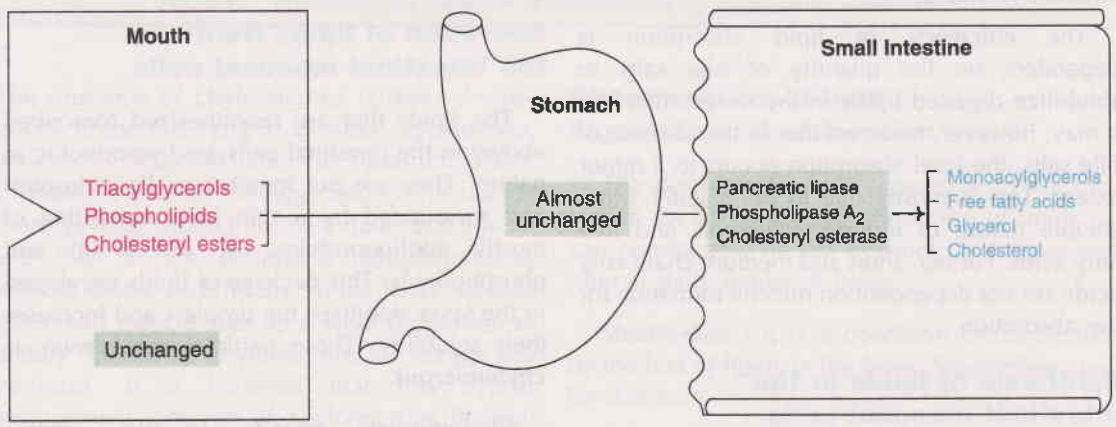


Fig. 8.11 : Overview of digestion of lipids.

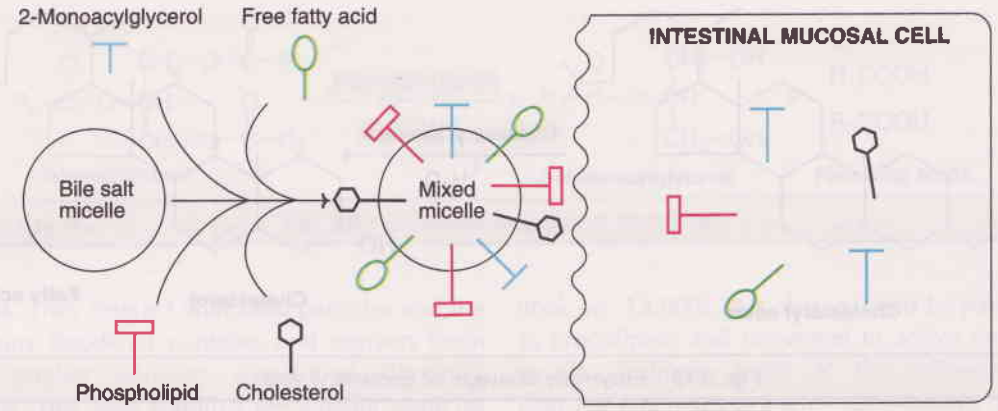


Fig. 8.12 : Absorption of lipids by intestinal mucosal cell.

Mechanism of lipid absorption

The mixed micelles serve as the major vehicles for the transport of lipids from the intestinal lumen to the membrane of the intestinal mucosal cells, the site of lipid absorption. The lipid components pass through the unstirred fluid layer and are absorbed through the plasma membrane by diffusion (***Fig.8.12***). Absorption is almost complete for monoacylglycerols and free fatty acids which are slightly water soluble. However, for water insoluble lipids, the absorption is incomplete. For instance, less than 40% of the dietary cholesterol is absorbed.

The micelle formation is also essential for the absorption of fat soluble vitamins, particularly vitamins A and K.

The efficiency of lipid absorption is dependent on the quantity of bile salts to solubilize digested lipids in the mixed micelles. It may, however, be noted that in the absence of bile salts, the lipid absorption occurs to a minor extent. This is mostly due to the slightly water soluble nature of monoacylglycerols and free fatty acids. Further, short and medium chain fatty acids are not dependent on micelle formation for the absorption.

Synthesis of lipids in the intestinal mucosal cells

The fatty acids of short and medium chain length (< 10 carbons), after their absorption into the intestinal cells, do not undergo any modification. They enter the portal circulation and are transported to the liver in a bound form to albumin.

The long chain fatty acids are activated by ***thiokinase*** (fatty acyl CoA synthetase) in the intestinal cells. The acyl CoA derivatives so formed combine with 2-monoacylglycerols to produce ***triacylglycerols***. These reactions are catalysed by a group of enzymes, namely acyltransferases (***Fig.8.13***). Further, within the intestinal cells, cholesterol is converted to cholesterylester, and phospholipids are regenerated from the absorbed lysophospholipids. The newly synthesized lipids are usually different from those consumed in the diet.

Secretion of lipids from the intestinal mucosal cells

The lipids that are resynthesized (described above) in the intestinal cells are hydrophobic in nature. They are put together as lipid droplets and surrounded by a thin layer consisting of mostly apolipoproteins (A_I and B-48) and phospholipids. This package of lipids enveloped in the layer stabilizes the droplets and increases their solubility. These particles are known as ***chylomicrons.***

Chylomicrons migrate to the plasma membrane of intestinal mucosal cells. They are released into the lymphatic vessels by exocytosis.

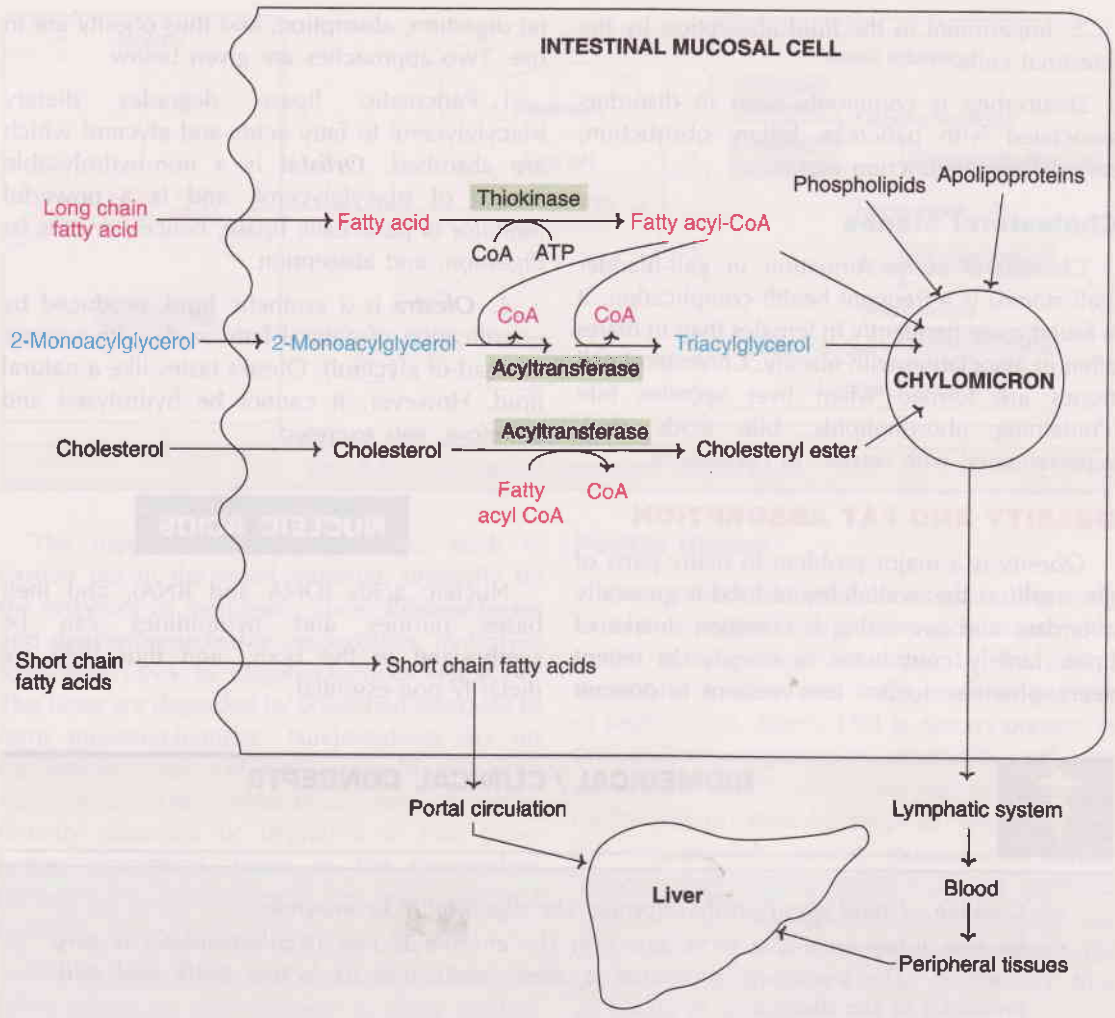


***Fig. 8.13** : Formation and secretion of chylomicrons by intestinal mucosal cells.*

The presence of chylomicrons (*Greek:* chylos–juice) gives the lymph a milky appearance, which is observed after a lipid-rich meal. Chylomicrons enter the large body veins via the thoracic duct. Blood from here flows to the heart and then to the peripheral tissues (muscle, adipose tissue) and, finally, to the liver. Adipose tissue and muscle take up a large proportion of dietary lipids from chylomicrons for storage and transport. It is believed that this bypass arrangement (passage of chylomicrons through peripheral tissues) protects the liver from a lipid overload after a meal.

Abnormalities of lipid digestion and absorption

The gastrointestinal tract possesses an efficient system for digestion and absorption of lipids. It can comfortably handle as much as 4 times the normal daily intake of lipids.

Steatorrhea : It is a condition characterized by the loss of lipids in the feces. Steatorrhea may be due to

1. A defect in the secretion of bile or pancreatic juice into the intestine;

2. Impairment in the lipid absorption by the intestinal cells.

Steatorrhea is commonly seen in disorders associated with pancreas, biliary obstruction, severe liver dysfunction etc.

Cholesterol stones

Cholesterol stone formation in gall-bladder (gall stones) is a frequent health complication. It is found more frequently in females than in males often in association with obesity. Cholesterol gall stones are formed when liver secretes bile (containing phospholipids, bile acids etc.), supersaturated with respect to cholesterol.

OBESITY AND FAT ABSORPTION

Obesity is a major problem in many parts of the world as the availability of food is generally abundant and overeating is common. Intake of lipids largely contributes to obesity. In recent years, pharmacological interventions to prevent fat digestion, absorption, and thus obesity are in use. Two approaches are given below

1. Pancreatic lipase degrades dietary triacylglycerol to fatty acids and glycerol which are absorbed. ***Orlistat*** is a non-hydrolysable analog of triacylglycerol, and is a powerful inhibitor of pancreatic lipase, hence prevents fat digestion, and absorption.

2. ***Olestra*** is a synthetic lipid, produced by esterification of natural fatty acids with sucrose (instead of glycerol). Olestra tastes like a natural lipid. However, it cannot be hydrolysed and therefore, gets excreted.

NUCLEIC ACIDS

Nucleic acids (DNA and RNA), and their bases purines and pyrimidines can be synthesized in the body, and thus they are dietarily non-essential.

BIOMEDICAL / CLINICAL CONCEPTS

- *Cooking of food significantly improves the digestibility by enzymes.*
- *Lactose intolerance due to a defect in the enzyme lactase (β-galactosidase) is very common. The treatment advocated is severe restriction of lactose (milk and milk products) in the diet.*
- *Flatulence, occurring after ingestion of certain non-digestible oligosaccharides, is characterized by increased intestinal motility, cramps and irritation.*
- *Direct intestinal absorption of proteins and polypeptides is observed in the infants, immediately after birth. This is important for the transfer of maternal immunoglobulins (via breast-feeding) to the offspring.*
- *In some adults, macromolecular (protein) absorption by intestine is responsible for antibody formation, often causing food allergy.*
- *Emulsification of lipids is essential for their effective digestion, since lipases can act only on the surface of lipid droplets. Bile salts are the most efficient biological emulsifying agents.*
- *Pharmacological interventions (e.g. Orlistat, Olestra) to block fat digestion and/or absorption so as to prevent obesity are in recent use.*
- *Steatorrhea, characterized by the loss of lipids in feces is commonly associated with impaired pancreatic function and biliary obstruction.*
- *Gastric ulcers are mainly caused by the bacterium* H. pylori. *The antibiotics that eliminate this bacterium are effective in the treatment.*
- *Acute pancreatitis is caused by autodigestion of pancreas while chronic pancreatitis is associated with excessive consumption of alcohol.*

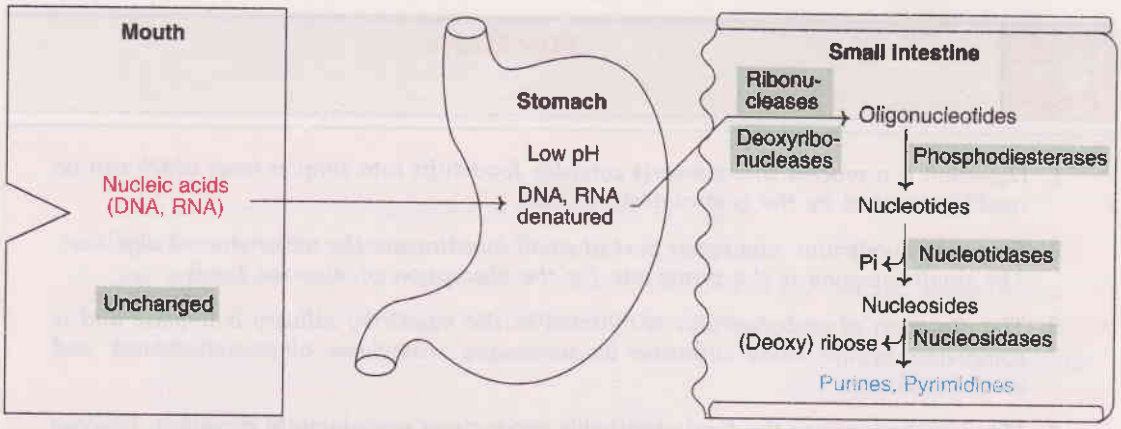


Fig. 8.14 : Overview of digestion of nucleic acids.

The digestion of dietary nucleic acids is carried out in the small intestine, primarily by the enzymes of pancreatic juice. ***Ribonucleases*** and ***deoxyribonucleases***, respectively, hydrolyse RNA and DNA to oligonucleotides (***Fig.8.14***). The latter are degraded by phosphodiesterases to form mononucleotides. Nucleotidases act on nucleotides to liberate phosphate and nucleosides. The nucleosides may be either directly absorbed or degraded to free bases before absorption. Some of the unabsorbed purines are metabolized by the intestinal bacteria.

The dietary purines and pyrimidines are not of much utility for the synthesis of tissue nucleic acids. Further, the purines after their absorption are mostly converted to uric acid by the intestinal mucosal cells and excreted in the urine.

ABNORMALITIES RELATED TO DIGESTION AND ABSORPTION

The following are the major abnormalities (of interest to biochemists) concerned with digestion and absorption of food in the gastrointestinal tract.

Lactose intolerance, deficiency of sucrase, Hartnup's disease and steatorrhea have already been described. Peptic ulcer and pancreatitis are other important disorders associated with digestive system.

Peptic ulcers

Gastric and duodenal ulcers are collectively known as peptic ulcers. Ulceration occurs due to the autodigestion of mucosa by the gastric secretions (pepsin and HCl). In the patients of peptic ulcer, gastric HCl is always present in the pyloric regions of stomach and the duodenum. Gastic ulcers are mainly caused by the bacterium *Helicobacter pylori* which lives in the nutrient-rich gastric mucosa. *H. pylori* induces chronic inflammation in the stomach tissues, which gets exposed to acid damage. For this reason, the best mode of ***treatment for gastric ulcers is the use of antibiotics that eliminate H. pylori.***

Achlorhydria is a less serious disorder involving the failure to secrete gastric HCl.

Pancreatitis

Inflammation of the pancreas is known as pancreatitis. Acute pancreatitis is caused by the autodigestion of pancreas due to the unusual conversion of zymogens into the active enzymes by trypsin. In normal circumstances, this is prevented by trypsin inhibitor.

Acute pancreatitis is a life-threatening disorder. Measurement of serum ***amylase*** (highly elevated) is used ***in the diagnosis of pancreatitis.*** Excessive ***consumption of alcohol*** over a long period is blamed as the prime cause of chronic pancreatitis.

SUMMARY

1. *Digestion is a process that converts complex foodstuffs into simpler ones which can be readily absorbed by the gastrointestinal tract.*
2. *Stomach, duodenum and upper part of small intestine are the major sites of digestion. The small intestine is the prime site for the absorption of digested foods.*
3. *The digestion of carbohydrates is initiated in the mouth by salivary α-amylase and is completed in the small intestine by pancreatic α-amylase, oligosaccharidases and disaccharidases.*
4. *Monosaccharides are the final absorbable products of carbohydrate digestion. Glucose is transported into the intestinal mucosal cells by a carrier mediated, Na^+-dependent energy requiring process.*
5. *Lactose intolerance due to a defect in the enzyme lactase (β-galactosidase) resulting in the inability to hydrolyse lactose (milk sugar) is the common abnormality of carbohydrate digestion.*
6. *Protein digestion begins in the stomach by pepsin, which is aided by gastric HCl. Pancreatic proteases (trypsin, chymotrypsin and elastase) and intestinal amino-peptidases and dipeptidases complete the degradation of proteins to amino acids and some dipeptides.*
7. *The intestinal absorption of amino acids occurs by different transport systems (at least six known). The uptake of amino acids is primarily a Na^+-dependent energy requiring process.*
8. *Digestion of lipids occurs in the small intestine. Emulsification of lipids, brought about by bile salts, is a prerequisite for their digestion. Pancreatic lipase aided by a colipase degrades triacylglycerol to 2-monoacylglycerol and free fatty acids. Cholesterol esterase and phospholipases, respectively, hydrolyse cholesteryl esters and phospholipids.*
9. *Lipid absorption occurs through mixed micelles, formed by bile salts in association with products of lipid digestion (primarily 2-monoacylglycerol, cholesterol and free fatty acids). In the intestinal mucosal cells, lipids are resynthesized from the absorbed components and packed as chylomicrons which enter the lymphatic vessels and then the blood.*
10. *Dietary nucleic acids (DNA and RNA) are digested in the small intestine to nucleosides and/or bases (purines and pyrimidines) which are absorbed.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Write an account of the digestion and absorption of lipids.
2. Describe briefly the digestion of carbohydrates and proteins.
3. Give an account of the Na^+ dependent intestinal transport of glucose and amino acids.
4. Describe the role of intestine in the digestion of foodstuffs.
5. Write briefly on the enzymes of gastrointestinal tract involved in the digestion of foodstuffs.

II. Short notes

(a) Mixed micelles, (b) Lactose intolerance, (c) Salivary amylase, (d) Disaccharidases, (e) γ-Glutamyl cycle, (f) Zymogens, (g) Specificity of proteases, (h) Bile salts, (i) Synthesis of chylomicrons in the intestinal mucosal cells, (j) Pancreatic juice.

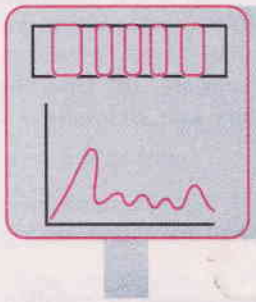
III. Fill in the blanks

1. Cellulose is not digested in humans due to lack of the enzyme that hydrolyses _____ bonds.
2. The most important carbohydrate associated with flatulence caused by ingestion of leguminous seeds _____.
3. Lactose intolerance is caused by the deficiency of the enzyme _____.
4. The non-digested carbohydrates are collectively called _____.
5. Gastric HCl is secreted by _____.
6. Name of the peptide believed to be involved in the transport of amino acids _____.
7. The disease characterized by impairment in the absorption of neutral amino acids _____.
8. Trypsin hydrolyses peptide bonds, the carbonyl group of which is contributed by the amino acids _____ or _____.
9. The inhibition of the enzyme pancreatic lipase by bile salts is overcome by a protein, namely _____.
10. The vehicles for the transport of lipids from the intestinal lumen to the membrane of mucosal cells _____.

IV. Multiple choice questions

11. Transport of glucose from the lumen to the intestinal mucosal cells is coupled with diffusion of
(a) Na^+ (b) K^+ (c) Cl^- (d) HCO_3^-.
12. The key enzyme that converts trypsinogen to trypsin is
(a) Secretin (b) Chymotrypsin (c) Elastase (d) Enteropeptidase.
13. The products obtained by the action of pancreatic lipase on triacylglycerols are
(a) Glycerol and free fatty acids (b) 1-Acylglycerol and free fatty acids (c) 2-Acylglycerol and free fatty acids (d) 3-Acylglycerol and free fatty acids.
14. The lipoproteins synthesized in the intestinal mucosal cells from the absorbed lipids are
(a) High density lipoproteins (b) Chylomicrons (c) Low density lipoproteins (d) Very low density lipoproteins.
15. Salivary α-amylase becomes inactive in the stomach primarily due to
(a) Inactivation by low pH (b) Degradation by gastric pepsin (c) Inhibition by Cl^- (d) Inhibition by peptides.

9 Plasma Proteins



The official 'spokesperson' of plasma proteins, albumin, speaks :

"I am the most abundant plasma protein;
Produced exclusively by the liver;
Perform osmotic, transport and nutritive functions;
Estimated in lab to assess liver function."

The plasma is the liquid medium of blood (55-60%), in which the cell components—namely erythrocytes, leukocytes, platelets—are suspended. If blood containing anticoagulants (e.g. heparin, potassium oxalate) is centrifuged, the plasma separates out as a supernatant while the cells remain at the bottom. The packed cell volume or ***hematocrit*** is about 45%.

The term serum is applied to the liquid medium which separates out after the blood clots (coagulates). Serum does not contain fibrinogen and other clotting factors. Thus, the main ***difference between plasma and serum is the presence or absence of fibrinogen.***

Importance of blood

The total volume of blood in an adult is around 4.5 to 5 liters. Blood performs several diversified functions. These include respiration, excretion, acid-base maintenance, water balance, transport of metabolites, hormones and drugs, body defense and coagulation.

Separation of plasma proteins

The total concentration of plasma proteins is about 6-8 g/dl. The plasma is a complex mixture of proteins, and several techniques are employed to separate them. An age-old technique is based on the use of varying concentrations of ammonium sulfate or sodium sulfate. By this method, which is known as ***salting out process,*** the plasma proteins can be separated into three groups—namely albumin, globulins and fibrinogen.

Electrophoresis : This is the most commonly employed analytical technique for the separation of plasma (serum) proteins. The basic principles of electrophoresis are described in ***Chapter 43***. Paper or agar gel electrophoresis with vernol buffer (pH-8.6) separates plasma proteins into 5 distinct bands namely ***albumin,*** α_1, α_2, β ***and*** γ ***globulins*** (***Fig.9.1***). The concentration of each one of these fractions can be estimated by a densitometer.

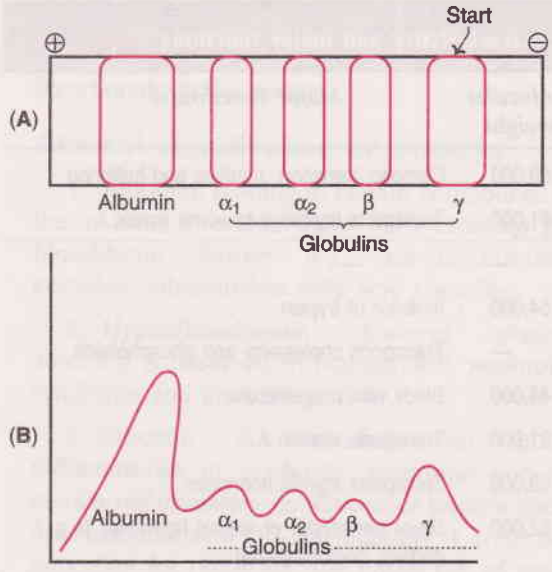


Fig. 9.1 : Electrophoresis of plasma proteins— (A) Separated bands, (B) Densitometer scanning

Abnormal electrophoretic pattern

Electrophoresis of serum proteins is conveniently used for the diagnosis of certain diseases

1. **Multiple myeloma :** A sharp and distinct M band appears in the γ-globulin fraction.

2. **Acute infections :** α_1- and α_2- globulins are increased.

3. **Nephrotic syndrome :** Decreased albumin with sharp and prominent α_2-globulin.

4. **Primary immune deficiency :** Diminished γ-globulin band.

5. α_1-**Antitrypsin deficiency :** Diminished α_1-globulin band.

Albumin/globulin (A/G) ratio : The albumin concentration of plasma is 3.5 to 5.0 g/dl while that of total globulins is 2.5 to 3.5 g/dl. The ***normal A/G ratio is 1.2 to 1.5 : 1.*** The A/G ratio is lowered either due to decrease in albumin or increase in globulins, as found in the following conditions

1. Decreased synthesis of albumin by liver—usually found in liver diseases and severe protein malnutrition.

2. Excretion of albumin into urine in kidney damage.

3. Increased production of globulins associated with chronic infections, multiple myelomas etc.

Components of plasma proteins

The important plasma proteins along with their characteristics (based on electrophoretic pattern) and major functions are given in ***Table 9.1.*** Some selected plasma proteins are discussed hereunder.

ALBUMIN

Albumin is the major constituent (60%) of plasma proteins with a concentration of 3.5–5.0 g/dl. Human albumin has a molecular weight of 69,000, and consists of a single polypeptide chain of 585 amino acids with 17 disulfide bonds.

Synthesis of albumin

Albumin is exclusively synthesized by the liver. For this reason, measurement of serum albumin concentration is conveniently used to assess liver function (synthesis decreased in liver diseases). Liver produces about 12 g albumin per day which represents 25% of the total hepatic protein synthesis. Albumin has an half-life of 20 days.

Functions of albumin

Plasma albumin performs osmotic, transport and nutritive functions

1. **Osmotic function :** Due to its high concentration and low molecular weight, albumin contributes to 75–80% of the total plasma osmotic pressure (25 mm Hg). Thus, albumin plays a predominant role in maintaining blood volume and body fluid distribution. Decrease in plasma albumin level results in a fall in osmotic pressure, leading to enhanced fluid retention in tissue spaces, causing edema. The edema observed in ***kwashiorkor***, a disorder

Table 9.1 Important plasma proteins, characteristics and major functions

Protein	*Plasma concentration*	*Molecular weight*	*Major function(s)*
Albumin	3.5–5.0 g/dl	69,000	Osmotic, transport, nutritive and buffering
Prealbumin	25–30 mg/dl	61,000	Transports thyroxine to some extent
α_1-Globulins	0.3–0.5 g/dl	—	—
α_1-Antitrypsin	< 0.2 g/dl	54,000	Inhibitor of trypsin
α_1-Lipoproteins (HDL)	0.2–0.3 g/dl	—	Transports cholesterol and phospholipids
Orosomucoid	< 0.1 g/dl	44,000	Binds with progesterone
Retinol binding protein (RBP)	3–6 mg/dl	21,000	Transports vitamin A
Thyroxine binding globulin (TBG)	1–2 mg/dl	58,000	Transports thyroid hormones
Transcortin or cortisol binding protein (CBG)	3–4 mg/dl	52,000	Major transporter of steroid hormones (e.g. cortisol, corticosterone)
α_2-Globulins	0.4–0.8 g/dl	—	—
α_2-Macroglobulin	0.2–0.3 g/dl	800,000	Antitrypsin and antiplasmin activity
Haptoglobins (Hp 1-1; Hp 2-1 and Hp 2-2)	< 0.3 g/dl	90,000	Binds with plasma free hemoglobin and prevents its excretion
Prothrombin	< 0.02 g/dl	63,000	Participates in blood coagulation
Ceruloplasmin	< 0.03 g/dl	150,000	Transport of copper; oxidation of Fe^{2+} to Fe^{3+}.
β-Globulins	0.6–1.1 g/dl	—	—
β-Lipoproteins (LDL)	0.2–0.5 g/dl	—	Transports triacylglycerols and cholesterol
Transferrin	0.2–0.3 g/dl	76,000	Transports iron
Hemopexin	< 0.1 g/dl	57,000	Transports heme
Plasminogen	< 0.05 g/dl	140,000	Forms plasmin, involved in fibrinolysis
γ-Globulins	0.8–1.8 mg/dl	—	Antibody functions
(Immunoglobulins—IgG, IgA, IgM, IgD and IgE; refer ***Table 9.2*** for details)			
Fibrinogen	0.2–0.4 g/dl	340,000	Participates in blood coagulation

of protein-energy malnutrition, is attributed to a drastic reduction in plasma albumin level.

2. **Transport functions :** Plasma albumin binds to several biochemically important compounds and transports them in the circulation. These include free fatty acids, bilirubin, steroid hormones, calcium and copper.

[**Note :** Besides albumin, there are several other ***plasma transport proteins.*** These include prealbumin, retinol binding protein, thyroxine binding protein, transcortin and others as stated in the functions of plasma proteins in ***Table 9.1***].

3. **Nutritive functions :** Albumin serves as a source of amino acids for tissue protein synthesis to a limited extent, particularly in nutritional deprivation of amino acids.

4. **Buffering function :** Among the plasma proteins, albumin has the maximum buffering

capacity. However, the buffering action of albumin in plasma is not significant compared to bicarbonate buffer system.

Clinical significance of albumin

1. Albumin, binding to certain compounds in the plasma, prevents them from crossing the blood-brain barrier e.g. albumin-bilirubin complex, albumin-free fatty acid complex.

2. ***Hypoalbuminemia*** (lowered plasma albumin) is observed in malnutrition, nephrotic syndrome and cirrhosis of liver.

3. Albumin is excreted into urine (***albuminuria***) in nephrotic syndrome and in certain inflammatory conditions of urinary tract. ***Microalbuminuria*** (30-300 mg/day) is clinically important for predicting the future risk of renal diseases (***Refer Chapter 36***).

4. Albumin is therapeutically useful for the treatment of burns and hemorrhage.

GLOBULINS

Globulins constitute several proteins that are separated into four distinct bands (α_1, α_2, β and γ-globulins) on electrophoresis (***See Fig.9.1***). Globulins, in general, are bigger in size than albumin. They perform a variety of functions which include transport and immunity. In ***Table 9.1***, the important globulins are given, some of them are discussed hereunder.

α_1-Antitrypsin

α_1-Antitrypsin, more recently called as ***α-anti-proteinase***, is a glycoprotein with 394 amino acids and a molecular weight of 54,000. It is a major constituent of α_1-globulin fraction of plasma proteins with a normal concentration of about 200 mg/dl. α_1-Antitrypsin is a serine protease inhibitor. It combines with trypsin, elastase and other protease enzymes and inhibits their activity.

Clinical significance of α_1-antitrypsin

α_1-Antitrypsin deficiency has been implicated in two diseases, namely, ***emphysema*** and ***α_1-AT deficiency liver disease.***

Emphysema (*Greek* : emphusan—to inflate) is a term used to represent the abnormal distension of lungs by air. At least 5% of emphysema cases are due to the deficiency of α_1-AT. This is associated with lung infections (e.g. pneumonia) and increase in the activity of macrophages to release elastase that damages lung tissues. In the normal circumstances, elastase activity is inhibited by α_1-AT.

Effect of smoking on α_1-AT : The amino acid methionine at position 358 of α_1-AT is involved in binding with proteases. Smoking causes oxidation of this methionine to methionine sulfoxide. As a result, α_1-AT with ***methionine sulfoxide*** cannot bind and inactivate proteases. Emphysema is more commonly associated with heavy smoking and the situation becomes worse in persons with α_1-AT deficiency.

α_1-Antitrypsin deficiency and liver disease : This is due to the accumulation of a mutant α_1-AT which aggregates to form polymers. These polymers, in turn—by an unknown mechanism—cause liver damage (hepatitis) followed by accumulation of collagen resulting in fibrosis (cirrhosis).

α_2-Macroglobulin

It is a high molecular weight (8,00,000) protein and is a major constituent of α_2-fraction. α_2-Macroglobulin inhibits protease activity and serves as an anticoagulant. Its concentration in plasma is elevated in ***nephrotic syndrome***. This is due to the fact that majority of the low molecular weight proteins are lost in urine (***proteinuria***) in this disorder.

HAPTOGLOBIN

Haptoglobin (Hp) is a plasma glycoprotein with an approximate molecular weight of 90,000. Hp is an ***acute phase protein*** since its plasma concentration is increased in several inflammatory conditions.

Functions of haptoglobin

Haptoglobin binds with the free hemoglobin (known as extra-corpuscular hemoglobin) that spills into the plasma due to hemolysis. The

haptoglobin-hemoglobin (Hp-Hb) complex (mol. wt. 155,000) cannot pass through glomeruli of kidney while free Hb (mol. wt. 65,000) can. Haptoglobin, therefore, prevents the loss of free Hb into urine.

Clinical significance of Hp : Hemolytic anemia is associated with decreased plasma concentration of haptoglobin. This is explained as follows. *The half-life of Hp is about 5 days* while that of Hp-Hb complex is 90 min. In hemolytic anemia, free Hb in plasma is elevated leading to increased formation of Hp-Hb complex. This complex, in turn, is rapidly cleared from the plasma resulting in decreased Hp levels.

CERULOPLASMIN

Ceruloplasmin is a blue coloured, copper—containing α_2-globulin with a molecular weight of 150,000. Its plasma concentration is about 30 mg/dl. Ceruloplasmin binds with almost 90% of plasma copper (6 atoms of Cu bind to a molecule). This binding is rather tight and, as a result, copper from ceruloplasmin is not readily released to the tissues. Albumin carrying only 10% of plasma copper is the major supplier of copper to the tissues. Ceruloplasmin possesses oxidase activity, and it is associated with ***Wilson's disease*** which is discussed under copper metabolism (***Chapter 18***).

TRANSFERRIN

Transferrin (Tf) is a glycoprotein with a molecular weight of 76,000. It is associated with β-globulin fraction. Tf is a transporter of iron in the circulation.

ACUTE PHASE PROTEINS

Acute phase response refers to a non-specific response to the stimulus of infection, injury, various inflammatory conditions (affecting tissue/organs), cancer etc. This phase is associated with a characteristic pattern of changes in certain plasma proteins, collectively referred to as acute phase proteins e.g. α_1-antitrypsin, ceruloplasmin, complement proteins, C-reactive protein. During the acute phase, synthesis of certain plasma proteins decreases, and they are regarded as negative acute phase reactants e.g. albumin, transferrin.

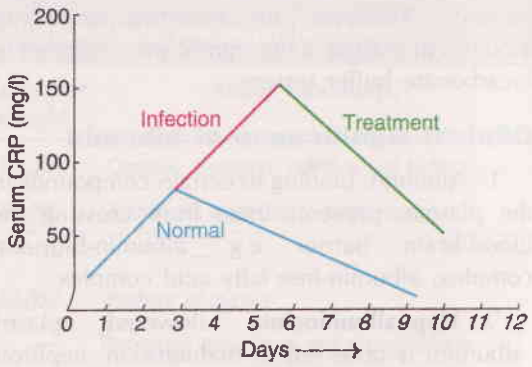


Fig. 9.2 : *The response of C-reactive protein (CRP) in response to surgery (The normal acute phase is depicted by blue line, the development of infection by red line and the response after treatment by green line).*

C-reactive protein (CRP)

CRP is a ***major component of acute phase proteins.*** It is produced in the liver and is present in the circulation in minute concentration (< 1 mg/dl). C-reactive protein (***C*** strands for ***c***arbohydrate to which it binds on the capsule of pneumococci) is involved in the promotion of immune system through the activation of complement cascade.

Estimation of CRP in serum is important for the evaluation of acute phase response. The response of CRP to surgery is depicted in ***Fig.9.2.*** In a normal surgery, serum CRP increases and returns to normal level within 7-10 days. If the recovery is complicated by any infection, it will be reflected by the continuous elevation of CRP which requires further treatment.

IMMUNOGLOBULINS

The higher vertebrates, including man, have evolved a defense system to protect themselves against the invasion of foreign substances—a virus, a bacterium or a protein. The defense

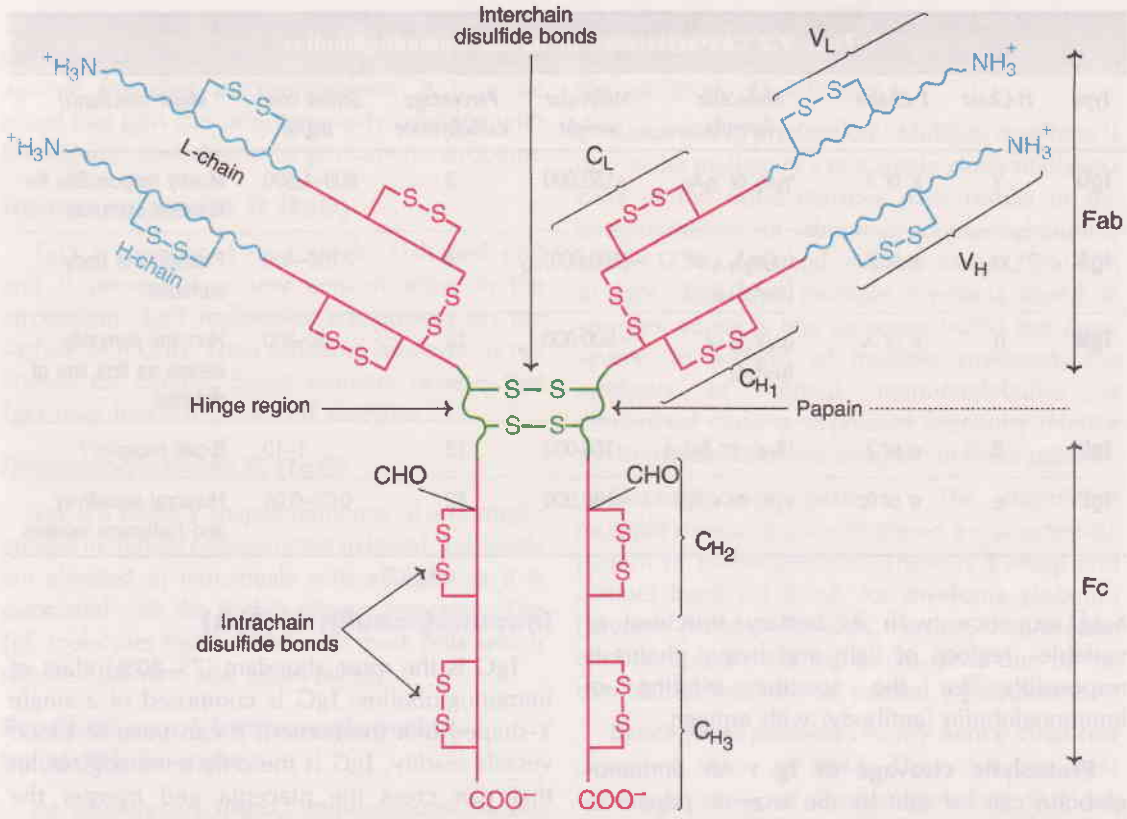


Fig. 9.3 : Diagrammatic representation of human IgG molecule (V—variable region, C—constant region; CHO—carbohydrate; Each heavy chain is composed of four units—V_H, C_{H_1}, C_{H_2}, C_{H_3} while light chain consists of two units—V_L, C_L).

strategies of the body are collectively referred to as ***immunity***, and are briefly described under immunology (***Chapter 42***). Immunoglobulins (or antibodies) are described here.

Immunoglobulins—basic concepts

Immunoglobulins, a specialised group of proteins are mostly associated with ***γ-globulin fraction*** (on electrophoresis) of plasma proteins. Some immunoglobulins however, separate along with β and α-globulins. Therefore, it should be noted that γ-globulin and immunoglobulin are not synonymous. ***Immunoglobulin is a functional term*** while ***γ-globulin is a physical term.***

Structure of immunoglobulins

All the ***i**mmuno**g**lobulin* (***Ig***) molecules basically consist of two identical ***heavy (H) chains*** (mol. wt. 53,000 to 75,000 each) and two identical ***light (L) chains*** (mol. wt. 23,000 each) held together by disulfide linkages and non-covalent interactions (***Fig.9.3***). Thus, immunoglobulin is a Y-shaped tetramer (H_2L_2). Each heavy chain contains approximately 450 amino acids while each light chain has 212 amino acids. The heavy chains of Ig are linked to carbohydrates, hence immunoglobulins are ***glycoproteins***.

Constant and variable regions : Each chain (L or H) of Ig has two regions (domains), namely the constant and the variable. The amino terminal half of the light chain is the variable region (V_L) while the carboxy terminal half is the constant region (C_L). As regards heavy chain, approximately one-quarter of the amino terminal region is variable (V_H) while the remaining three-quarters is constant (C_{H_1}, C_{H_2}, C_{H_3}). The amino

Table 9.2 Characteristics of human immunoglobulins

Type	H-Chain	L-Chains	Molecular formula	Molecular weight	Percentage carbohydrate	Serum conc. mg/dl	Major function(s)
IgG	γ	κ or λ	$\gamma_2\kappa_2$ or $\gamma_2\lambda_2$	~150,000	3	800–1,500	Mostly responsible for humoral immunity
IgA	α	κ or λ	$(\alpha_2\kappa_2)_{1-3}$ or $(\alpha_2\lambda_2)_{1-3}$	~$(160,000)_{1-3}$	8	150–400	Protects the body surfaces
IgM	μ	κ or λ	$(\mu_2\kappa_2)_5$ or $(\mu_2\lambda_2)_5$	~900,000	12	50–200	Humoral immunity, serves as first line of defense
IgD	δ	κ or λ	($\delta_2\kappa_2$ or $\delta_2\lambda_2$)	~180,000	13	1–10	B-cell receptor?
IgE	ε	κ or λ	$\varepsilon_2\kappa_2$ or $\varepsilon_2\lambda_2$	~190,000	12	0.02–0.05	Humoral sensitivity and histamine release.

acid sequence (with its tertiary structure) of variable regions of light and heavy chains is responsible for the specific binding of immunoglobulin (antibody) with antigen.

Proteolytic cleavage of Ig : An immunoglobulin can be split by the enzyme ***papain*** to their fragments. These are two identical antigen binding fragments (Fab) and one crystallizable fragment (Fc). Papain cleaves the immunoglobin molecule at the site between C_{H1} and C_{H2} regions which is referred to as ***hinge region.***

CLASSES OF IMMUNOGLOBULINS

Humans have five classes of immunoglobulins—namely ***IgG, IgA, IgM, IgD*** and ***IgE***—containing the heavy chains γ, α, μ, δ and ε, respectively. The type of heavy chain ultimately determines the class and the function of a given Ig.

Two types of light chains—namely kappa (κ) and lambda (λ)—are found in immunoglobulins. They differ in their structure in C_L regions. An immunoglobulin (of any class) contains two κ or two λ light chains and never a mixture. The occurrence of κ chains is more common in human immunoglobulins than λ chains.

The characteristics of the 5 classes of human immunoglobulins are given in ***Table 9.2.***

Immunoglobulin G (IgG)

IgG is the most abundant (75–80%) class of immunoglobulins. IgG is composed of a single Y-shaped unit (monomer). It can traverse blood vessels readily. IgG is the only immunoglobulin that can cross the placenta and transfer the mother's immunity to the developing fetus. IgG triggers ***foreign cell destruction*** mediated by complement system.

Immunoglobulin A (IgA)

IgA occurs as a single (monomer) or double unit (dimer) held together by J chain. It is mostly found in the body secretions such as saliva, tears, sweat, milk and the walls of intestine. IgA is the most predominant antibody in the colostrum, the initial secretion from the mother's breast after a baby is born. The IgA molecules bind with bacterial antigens present on the body (outer epithelial) surfaces and remove them. In this way, IgA ***prevents the foreign substances*** from entering the body cells.

Immunoglobulin M (IgM)

IgM is the largest immunoglobulin composed of 5 Y-shaped units (IgG type) held together by a J polypeptide chain. Thus IgM is a pentamer. Due to its large size, IgM cannot traverse blood vessels, hence it is restricted to the blood stream.

IgM is the first antibody to be produced in response to an antigen and is the most ***effective against invading microorganisms.*** It may be noted that IgM can simultaneously combine with 5 antigenic sites due to its pentameric structure.

Immunoglobulin D (IgD)

IgD is composed of a single Y-shaped unit and is present in a low concentration in the circulation. IgD molecules are present on the surface of B cells. Their function, however, is not known for certain. Some workers believe that IgD may function as ***B-cell receptor.***

Immunoglobulin E (IgE)

IgE is a single Y-shaped monomer. It is normally present in minute concentration in blood. IgE levels are elevated in individuals with ***allergies*** as it is associated with the body's allergic responses. The IgE molecules tightly bind with mast cells which release histamine and cause allergy.

Production of immunoglobulins by multiple genes

As already discussed, immunoglobulins are composed of light and heavy chains. Each light chain is produced by 3 separate genes, namely a variable region (V_L) gene, a constant region (C_L) gene and a joining region (J) gene. Each heavy chain is produced by at least 4 different genes—a variable region (V_H) gene, a constant region (C_H) gene, a joining region (J) gene and diversity region (D) gene. Thus multiple genes are responsible for the synthesis of any one of the immunoglobulins.

Antibody diversity : A person is capable of generating antibodies to almost an unlimited range of antigens (more than one billion!). It should, however, be remembered that humans do not contain millions of genes to separately code for individual immunoglobulin molecules. The antibody diversity is achieved by two special processes, namely ***combination of various structural genes*** and ***somatic mutations.***

MULTIPLE MYELOMA

Multiple myeloma, a ***plasma cell cancer,*** constitutes about 1% of all cancers affecting the population. Females are more susceptible than males for this disorder and it usually occurs in the age group 45-60 years.

Abnormal Ig production : Multiple myeloma is due to the malignancy of a single clone of plasma cells in the bone marrow. This results in the overproduction of abnormal immunoglobulins, mostly (75%) ***IgG*** and in some cases (25%) IgA or IgM. IgD type multiple myeloma found in younger adults is less common (<2%) but more severe. In patients of multiple myeloma, the synthesis of normal immunoglobulins is diminished causing depressed immunity. Hence recurrent infections are common in these patients.

Electrophoretic pattern : The plasma of multiple myeloma patients shows a characteristic pattern of electrophoresis. There is a sharp and distinct band (M band, for myeloma globulin) between β-and γ-globulins. Further, this ***M band*** almost replaces the γ-globulin band due to the diminished synthesis of normal γ-globulins.

Bence Jones proteins : Henry Bence Jones first described them in 1847. These are the light chains (κ or λ) of immunoglobulins that are synthesized in excess. Bence Jones proteins have a molecular weight of 20,000 or 40,000 (for dimer). In about 20% of the patients of multiple myeloma, Bence Jones proteins are excreted in urine which often damages the renal tubules.

Amyloidosis is characterized by the deposits of light chain fragments in the tissue (liver, kidney, intestine) of multiple myeloma patients.

The presence of Bence Jones proteins in urine can be detected by specific tests.

1. **Electrophoresis** of a concentrated urine is the best test to detect Bence Jones proteins in urine.

2. **The classical heat test** involves the precipitation of Bence Jones proteins when slightly acidified urine is heated to 40-50°C. This precipitate redissolves on further heating of urine to boiling point. It reappears again on cooling urine to about 70°C.

3. **Bradshaw's test** involves layering of urine on concentrated HCl that forms a white ring of precipitate, if Bence Jones proteins are present.

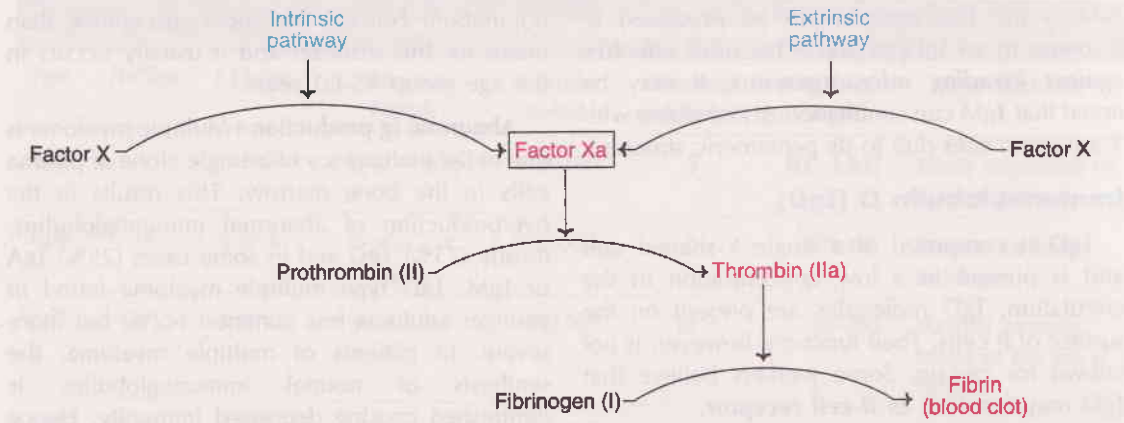


Fig. 9.4 : Overview of blood clotting with the final common pathway.

BLOOD CLOTTING

The term ***hemostasis*** is applied to the sequence of physiological responses to stop bleeding (loss of blood after an injury). This is carried out by blood clotting.

Blood clotting or coagulation is the body's major ***defense mechanism against blood loss***. A blood clot is formed as a result of a series of reactions involving nearly 20 different substances, most of them being ***glycoproteins, synthesized by the liver***.

Blood clotting process involves two independent pathways

1. **The extrinsic pathway** is the initial process in clotting and involves the factors that are not present in the blood (hence the name).

2. **The intrinsic pathway** involves a series of reactions participated by the factors present in the blood.

Strictly speaking, the extrinsic and intrinsic pathways are not independent, since they are coupled together. Further, the final reactions are identical for both pathways that ultimately lead to the activation of prothrombin to thrombin and the conversion of fibrinogen to fibrin clot (***Fig.9.4***).

The blood coagulation factors in human plasma along with their common names and molecular weights are listed in ***Table 9.3***. All but two of these factors are designated by a Roman numeral. It should, however, be noted that the numbers represent the order of their discovery and not the order of their action. The cascade of blood clotting process is depicted in ***Fig.9.5*** and the salient features are discussed below. The active form of a factor is designated by a subscript ***a***. The active clotting factors (with exception of fibrin) are serine proteases.

Conversion of fibrinogen to fibrin

Fibrinogen (factor I) is a soluble glycoprotein that constitutes 2-3% of plasma proteins (plasma concentration 0.3 g/dl). Fibrinogen consists of 6 polypeptide chains-two A α, two B β and two γ making the structure $(A\ \alpha)_2\ (B\ \beta)_2\ \gamma_2$.

Fibrinogen undergoes proteolytic cleavage catalysed by thrombin to release small ***fibrinopeptides (A and B)***. This results in the formation of fibrin monomers which can stick together to form hard clots (***Fig.9.6***). Clot formation is further stabilized by covalent cross-linking between glutamine and lysine residues. This reaction cross-links fibrin clots and is catalysed by fibrin stabilizing factor (XIII). The red colour of the clot is due to the presence of red cells entangled in the fibrin cross-links.

Conversion of prothrombin to thrombin

Prothrombin (II) is the inactive zymogen form of thrombin (IIa). The activation of prothrombin

occurs on the platelets and requires the presence of factors Va and Xa, besides phospholipids and Ca^{2+}.

The extrinsic pathway

The extrinsic pathway is very rapid and occurs in response to ***tissue injury***. This pathway essentially involves the conversion of proconvertin (VII) to its active form (VIIa) and the generation factor Xa. The tissue factor (III), found to be necessary to accelerate the action VIIa on a factor X, is present in lung and brain.

The intrinsic pathway

The intrinsic pathway is rather slow. It involves the participation of a contact system (wounded surface) and a series of factors to generate factor Xa.

The Hageman factor (XII) is activated (XIIa) on exposure to activating wound surface containing collagen or platelet membranes. The formation of XIIa is accelerated by kallikrein and HMK. The activated Hageman factor (XIIa) activates factor XI. The XIa activate the Christmas factor (IX). The Christmas factor is also activated by active proconvertin (VIIa).

In the next step, the Staurt factor (X) is activated by Christmas factor (IXa) and this reaction requires the presence of antihemophilic factor (VIIIa), Ca^{2+} and phospholipids.

The extrinsic and intrinsic pathways lead to the formation of factor Xa which then participates in the final common pathway to ultimately result in the formation of fibrin clot.

Anticoagulants

Several substances, known as anticoagulants, are in use to inhibit the blood clotting. Calcium is essentially required for certain reactions of blood coagulation. The substances which bind with Ca^{2+} are very effective as anticoagulants. These include ***oxalate***, ***fluoride***, ***EDTA*** and ***citrate***.

Heparin is an anticoagulant used to maintain normal hemostasis. It is a heteropolysaccharide found in many tissues including mast cells in the endothelium of blood vessels. Heparin combines with antithrombin III which in turn, inhibits the

Table 9.3 Blood coagulation factors in humans

Factor number	*Common name(s)*	*Subunit molecular weight*
I	Fibrinogen	340,000
II	Prothrombin	720,000
III	Tissue factor, thromboplastin	370,000
IV	Calcium (Ca^{2+})	—
V	Proaccelerin, labile factor	330,000
VII	Proconvertin, serum prothrombin conversion accelerator (SPCA)	50,000
VIII	Antihemophilic factor A, antihemophilic globulin (AHG)	330,000
IX	Christmas factor, antihemophilic factor B, Plasma thromboplastin component (PTC)	56,000
X	Staurt-Prower factor	56,000
XI	Plasma thromboplastin antecedent (PTA)	160,000
XII	Hageman factor	80,000
XIII	Fibrin-stabilizing factor (FSF), fibrinoligase, Liki Lorand factor	320,000
—	Prekallikrein	88,000
—	High molecular weight kininogen (HMK)	150,000

Note : *The numbers represent the order of their discovery and not the order of their action. Factor Va was once referred to as factor VI, hence there is no factor VI.*

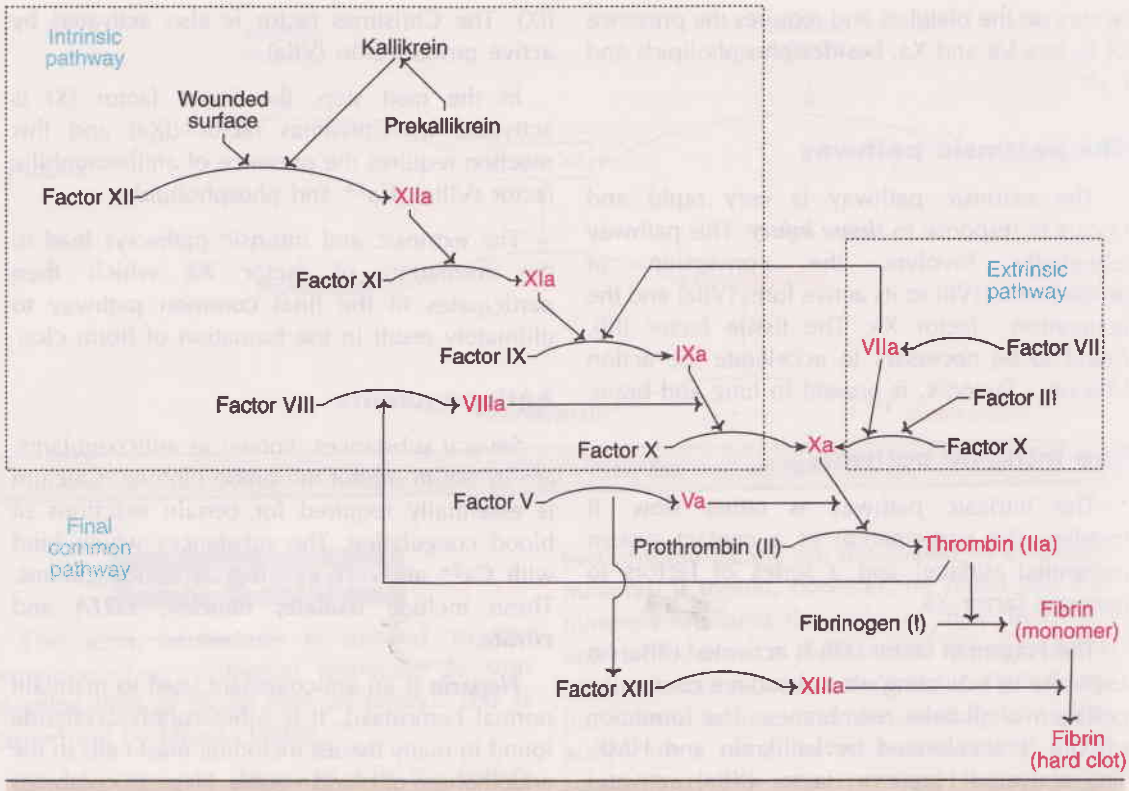


***Fig. 9.5** : The blood clotting cascade in humans (the active forms of the factors are represented in red with subscript 'a').*

clotting factors II, IX, X, XI, XII and kallikrein. Heparin can be administered to patients during and after surgery to retard blood clotting.

The blood contains another anticoagulant—namely ***protein C***—which is activated by thrombin. Active protein C hydrolyses and inactivates clotting factors V and VIII.

Warfarin, a vitamin K antagonist may be considered as an ***oral anticoagulant***. This acts by reducing the synthesis of certain clotting factors (II, VII, IX and X).

Fibrinolysis

The term fibrinolysis refers to the dissolution or lysis of blood clots. ***Plasmin*** is mostly responsible for the dissolution of fibrin clots. Plasminogen, synthesized in the kidney, is the inactive precursor of plasmin. Tissue plasminogen activator (TPA) and urokinase convert plasminogen to plasmin.

Streptokinase is a therapeutic fibrinolytic agent which activates plasminogen.

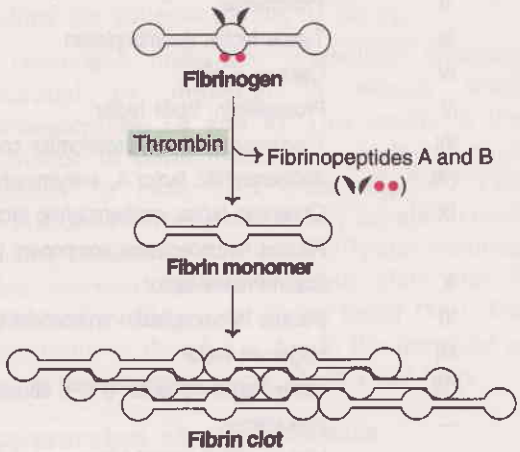


***Fig. 9.6** : Diagrammatic representation of fibrin clot formation from fibrinogen.*

Abnormalities in blood clotting

Several abnormalities associated with blood clotting are known. These are due to defects in ***clotting factors*** which may be inherited or acquired. Hemophilia, Von Willebrand's disease etc., are examples of inherited disorder while afibrinogenemia is an acquired disease.

Hemophilia A (classical hemophilia) : This is a sex-linked disorder transmitted by females affecting males. Hemophilia A is the most common clotting abnormality and is due to the ***deficiency of antihemophilic factor (VIII)***. The affected individuals have prolonged clotting time and suffer from internal bleeding (particularly in joints and gastrointestinal tract). Hemophilia A has gained importance due to the fact that the Royal families of Britain are among the affected individuals.

Hemophilia B (Christmas disease) : This is due to the deficiency of ***Christmas factor (IX)***. The clinical symptoms are almost similar to that found in hemophilia A.

Von Willebrand's disease : This disorder is characterized by failure of platelets to aggregate and is due to a defect in the platelet adherence factor.

BIOMEDICAL / CLINICAL CONCEPTS

- *Albumin, the most abundant plasma protein, is involved in osmotic function, transport of several compounds (fatty acids, steroid hormones), besides the buffering action.*
- *Hypoalbuminemia and albuminuria are observed in nephrotic syndrome.*
- *α_1-Antitrypsin deficiency has been implicated in emphysema (abnormal distension of lungs by air) which is more commonly associated with heavy smoking.*
- *Haptoglobin prevents the possible loss of free hemoglobin from the plasma through the kidneys by forming haptoglobin-hemoglobin complex.*
- *Immunoglobulins (antibodies), a specialized group of plasma globular proteins, are actively involved in immunity. IgG and IgM are primarily concerned with humoral immunity while IgE is associated with allergic reactions.*
- *Multiple myeloma, a plasma cell cancer disease of bone marrow, is characterized by overproduction of abnormal immunoglobulins (mostly IgG). Laboratory diagnosis of multiple myeloma can be made by the presence of a distinct M band on plasma/serum electrophoresis.*
- *Blood clotting or coagulation is the body's major defense mechanism against blood loss. Defects in clotting factors cause coagulation abnormalities such as hemophilia A (deficiency of factor VIII) and Christmas disease (deficiency of factor IX).*
- *Anticoagulants inhibit blood clotting. These include heparin, oxalate, fluoride, EDTA and citrate.*

SUMMARY

1. *The total concentration of plasma proteins is about 6-8 g/dl. Electrophoresis separates plasma proteins into 5 distinct bands, namely albumin, α_1, α_2, β and γ globulins.*
2. *Albumin is the major constituent (60%) of plasma proteins with a concentration 3.5 to 5.0 g/dl. It is exclusively synthesized by the liver. Albumin performs osmotic, transport and nutritive functions.*
3. *α_1-Antitrypsin is a major constituent of α_1 globulin fraction. α_1-Antitrypsin deficiency has been implicated in emphysema and a specific liver disease.*
4. *Haptoglobin (Hp) binds with free hemoglobin (Hb) that spills into the plasma due to hemolysis. The Hp-Hb complex cannot pass through the glomeruli, hence haptoglobin prevents the loss of free hemoglobin into urine.*
5. *Alterations in the acute phase proteins (e.g. α_1-antitrypsin, ceruloplasmin, C-reactive protein) are observed as a result of non-specific response to the stimulus of infection, injury, inflammation etc. Estimation of serum C-reactive protein is used for the evaluation of acute phase response.*
6. *Immunoglobulins are specialized proteins to defend the body against the foreign substances. They are mostly associated with γ-globulin fraction of plasma proteins. The immunoglobulins essentially consist of two identical heavy chains and two identical light chains, held together by disulfide linkages.*
7. *Five classes of immunoglobulins—namely IgG, IgA, IgM, IgD and IgE—are found in humans. IgG is most abundant and is mainly responsible for humoral immunity. IgA protects body surfaces. IgM serves as a first line of defense for humoral immunity while IgE is associated with allergic reactions.*
8. *Multiple myeloma is due to the malignancy of a single clone of plasma cells in the bone marrow. This causes the overproduction of abnormal IgG. The plasma of multiple myeloma patients on electrophoresis shows a distinct M-band.*
9. *Blood clotting is the body's major defense mechanism against blood loss. The extrinsic and intrinsic pathways lead to the formation of factor Xa which then participates in the final common pathway to activate prothrombin to thrombin. Fibrinogen is then converted to fibrin clot.*
10. *Plasmin is mostly responsible for the dissolution of fibrin clots. Plasminogen, synthesized by the kidney, is the inactive precursor of plasmin. Tissue plasminogen activator (TPA) and urokinase convert plasminogen to plasmin.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Describe the characteristics and major functions of plasma proteins.
2. Give an account of different types of immunoglobulins along with their functions.
3. Discuss the cascade of blood clotting process.
4. Describe the structure of different immunogloublins.
5. Discuss the role of acute phase proteins in health and disease.

II. Short notes

(a) Electrophoresis of plasma proteins, (b) Functions of albumin, (c) α_1-Antitrypsin, (d) Haptoglobin, (e) Immunoglobulin G, (f) Multiple myeloma, (g) Bence-Jones proteins, (h) Fibrinogen, (i) Anticoagulants, (j) Hemophilia.

III. Fill in the blanks

1. The difference between plasma and serum is the presence or absence of _____.
2. The most commonly employed technique for separation of plasma proteins _____.
3. Haptoglobin binds and prevents the excretion of the compound _____.
4. The cells responsible for the production of immunoglobulins _____.
5. The immunoglobulin that can cross the placenta and transfer the mother's immunity to the developing fetus _____.
6. The immunoglobulins that can bind with mast cells and release histamine _____.
7. Bence-Jones proteins are precipitated when urine is heated to _____.
8. The major component of acute phase proteins used for the evaluation of acute phase response _____.
9. The extrinsic and intrinsic pathways result in the formation of a common activated factor _____.
10. The factor mostly responsible for the lysis of blood clot _____.

IV. Multiple choice questions

11. Hemophilia A is due to the deficiency of clotting factor

    (a) X (b) V (c) VIII (d) II.

12. Plasma albumin performs the following functions

    (a) Osmotic (b) Transport (c) Nutritive (d) All of them.

13. The immunoglobulin present in most abundant quantity

    (a) IgG (b) IgA (c) IgM (d) IgE.

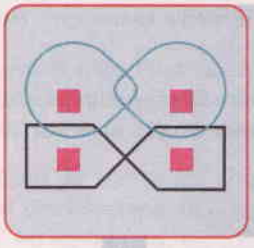
14. Name the immunoglobulin involved in body allergic reactions

    (a) IgA (b) IgE (c) IgD (d) IgM.

15. The following anticoagulant binds with Ca^{2+} and prevents blood clotting

    (a) Heparin (b) Oxalate (c) Protein C (d) All of them.

10 Hemoglobin and Porphyrins



The hemoglobin speaks :

"I am the red of blood, responsible for respiration;
Deliver O_2 to tissues and return CO_2 to lungs;
Influenced by factors pH, BPG and Cl^- in my functions;
Disturbed in my duties by structural abnormalities."

The structure, functions and abnormalities of hemoglobin, the synthesis and degradation of heme, the porphyrin containing compounds are discussed in this chapter.

HEMOGLOBIN

Hemoglobin (Hb) is the red blood pigment, exclusively found in ***erythrocytes*** (*Greek*: erythrose—red; kytos—a hollow vessel). The normal concentration of Hb in blood in males is 14–16 g/dl, and in females 13–15 g/dl. Hemoglobin performs two important biological functions concerned with respiration

1. Delivery of O_2 from the lungs to the tissues.

2. Transport of CO_2 and protons from tissues to lungs for excretion.

Structure of hemoglobin

Hemoglobin (mol. wt. 64,450) is a conjugated protein, containing ***globin***—the apoprotein part—and the ***heme***—the non-protein part (prosthetic group). Hemoglobin is a ***tetrameric allosteric protein*** (***Fig.10.1***).

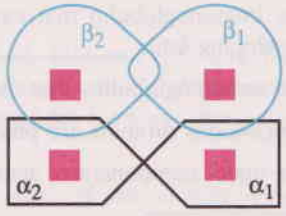


Fig. 10.1 : *Diagrammatic representation of hemoglobin with 2α and 2β chains (Red blocks–Heme).*

Structure of globin : Globin consists of four polypeptide chains of two different primary structures (monomeric units). The common form of adult hemoglobin (HbA_1) is made up of two α-chains and two β-chains ($\alpha_2\beta_2$). Some authors consider hemoglobin consisting of two identical dimers—$(\alpha\beta)_1$ and $(\alpha\beta)_2$. Each α-chain contains 141 amino acids while β-chain contains 146 amino acids. Thus HbA_1 has a total of 574

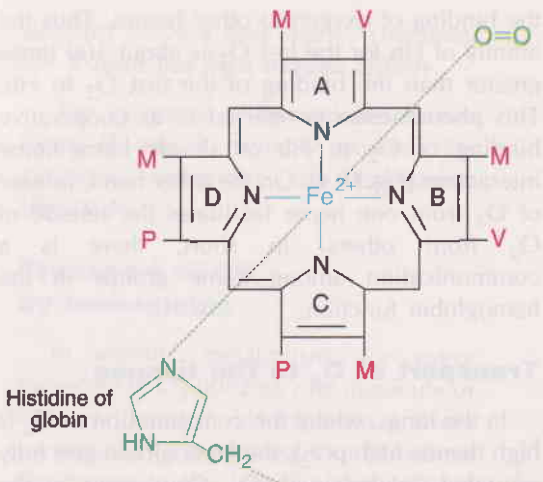


Fig. 10.2 : Structure of heme [M-Methyl (—CH_3); V-Vinyl (—$CH=CH_2$—); P-Propionyl (—$CH_2—CH_2—COOH$)

amino acid residues. The four subunits of hemoglobin are held together by non-covalent interactions primarily hydrophobic, ionic and hydrogen bonds. Each subunit contains a heme group.

Structure of heme : The characteristic red colour of hemoglobin (ultimately blood) is due to heme. Heme contains a porphyrin molecule namely ***protoporphyrin IX***, with iron at its center. Protoporphyrin IX consists of four ***pyrrole*** rings to which four methyl, two propionyl and two vinyl groups are attached (***Fig.10.2***).

Heme is common prosthetic group present in cytochromes, in certain enzymes such as catalase, tryptophan pyrolase, and chlorophyll (Mg^{2+}). In case of cytochromes, oxidation and reduction of iron ($Fe^{2+} \rightleftharpoons Fe^{3+}$) is essential for their biological function in electron transport chain.

Other forms of hemoglobin

Besides the adult hemoglobin (HbA_1) described above, other minor hemoglobins are also found in humans (***Table 10.1***). In adults a small fraction (< 5%) of hemoglobin, known as HbA_2 is present. HbA_2 is composed of two α and two δ (delta) chains. ***Fetal hemoglobin (HbF)*** is synthesized during the fetal development and a little of it may be present even in adults. ***Glycosylated hemoglobin (HbA_{1c})***, formed by covalent binding of glucose is also found in low concentration. It is increased in diabetes mellitus which is successfully utilized for the prognosis of these patients (details under Diabetes, in ***Chapter 36***).

Myoglobin

Myoglobin (Mb) is monomeric oxygen binding hemoprotein found in heart and skeletal muscle. It has a single polypeptide (153 amino acids) chain with heme moiety. Myoglobin (mol. wt. 17,000) structurally resembles the individual subunits of hemoglobin molecule. For this reason, the more complex properties of hemoglobin have been conveniently elucidated through the study of myoglobin.

Myoglobin functions as a ***reservoir for oxygen.*** It further serves as oxygen carrier that promotes the transport of oxygen to the rapidly respiring muscle cells.

Functions of hemoglobin

Hemoglobin is largely responsible for the transport of O_2 from lungs to tissues. It also helps to transport CO_2 from the tissues to the lungs.

Binding of O_2 to hemoglobin

One molecule of hemoglobin (with four hemes) can bind with four molecules of O_2. This is in contrast to myoglobin (with one heme) which can bind with only one molecule of oxygen. In other words, each heme moiety can bind with one O_2.

Table 10.1 Normal major types of hemoglobins

Type	*Composition and symbol*	*Percentage of total hemoglobin*
HbA_1	$\alpha_2\beta_2$	90%
HbA_2	$\alpha_2\delta_2$	< 5%
HbF	$\alpha_2\gamma_2$	< 2%
HbA_{1c}	$\alpha_2\beta_2$-glucose	< 5%

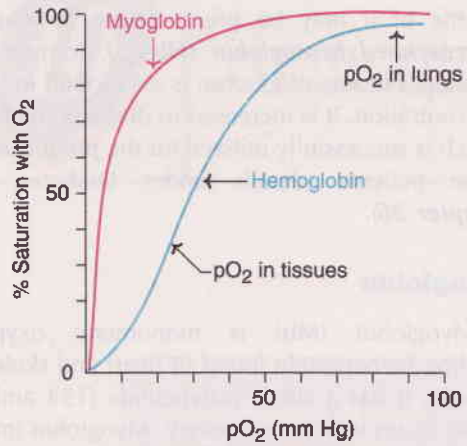


Fig. 10.3 : Oxygen dissociation curves of hemoglobin and myoglobin (pO_2—Partial pressure of oxygen).

Oxygen dissociation curve : The ***binding*** ability of ***hemoglobin with O_2*** at different partial pressures of oxygen (***pO_2***) can be measured by a graphic representation known as O_2 dissociation curve. The curves obtained for hemoglobin and myoglobin are depicted in ***Fig.10.3***.

It is evident from the graph that myoglobin has much higher affinity for O_2 than hemoglobin. Hence O_2 is bound more tightly with myoglobin than with hemoglobin. Further, pO_2 needed for half saturation (50% binding) of myoglobin is about 1 mm Hg compared to about 26 mm Hg for hemoglobin.

Cooperative binding of O_2 to hemoglobin

The oxygen dissociation curve for hemoglobin is sigmoidal in shape (***Fig.10.3***). This indicates that the binding of oxygen to one heme increases the binding of oxygen to other hemes. Thus the affinity of Hb for the last O_2 is about 100 times greater than the binding of the first O_2 to Hb. This phenomenon is referred to as cooperative binding of O_2 to Hb or simply heme-heme interaction (***Fig.10.4***). On the other hand, release of O_2 from one heme facilitates the release of O_2 from others. In short, there is a communication among heme groups in the hemoglobin function.

Transport of O_2 to the tissues

In the lungs, where the concentration of O_2 is high (hence high pO_2), the hemoglobin gets fully saturated (loaded) with O_2. Conversely, at the tissue level, where the O_2 concentration is low (hence low pO_2), the oxyhemoglobin releases (unloads) its O_2 for cellular respiration. This is often mediated by binding O_2 to myoglobin which serves as the immediate reservoir and supplier of O_2 to the tissues (***Fig.10.5***).

T and R forms of hemoglobin

The four subunits ($\alpha_2\beta_2$) of hemoglobin are held together by weak forces. The relative position of these subunits is different in oxyhemoglobin compared to deoxyhemoglobin.

T-form of Hb : The deoxy form of hemoglobin exists in a T or taut (tense) form. The hydrogen and ionic bonds limit the movement of monomers. Therefore, the T-form of Hb has ***low oxygen affinity***.

R-form of Hb : The binding of O_2 destabilizes some of the hydrogen and ionic bonds particularly between $\alpha\beta$ dimers. This results in a relaxed form or R-form of Hb wherein the

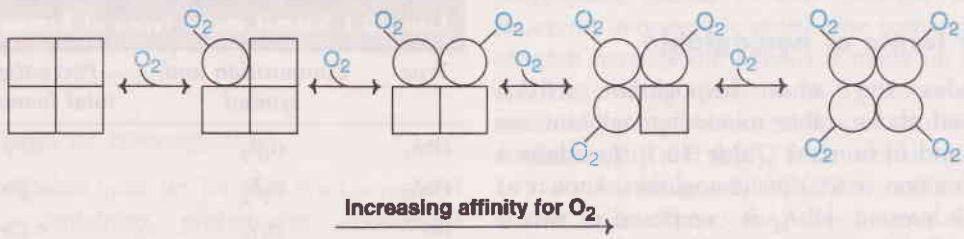


Fig. 10.4 : Cooperative binding of O_2 to hemoglobin (□ : T or taut form; ○ : R or relaxed form).

subunits move a little freely. Therefore, the ***R-form has high oxygen affinity***.

The existence of hemoglobin in two forms (T and R) suitably explains the allosteric behaviour of hemoglobin (***Fig.10.4***).

Transport of CO_2 by hemoglobin

In aerobic metabolism, for every molecule of O_2 utilized, one molecule of CO_2 is liberated. Hemoglobin actively participates in the transport of CO_2 from the tissues to the lungs. About 15% of CO_2 carried in blood directly binds with Hb. The rest of the tissue CO_2 is transported as bicarbonate (HCO_3^-).

Carbon dioxide molecules are bound to the uncharged α-amino acids of hemoglobin to form carbamyl hemoglobin as shown below

$$Hb-NH_2 + CO_2 \rightleftharpoons Hb-NH-COO^- + H^+$$

The oxyHb can bind 0.15 moles CO_2/mole heme, whereas deoxyHb can bind 0.40 moles CO_2/mole heme. The binding of CO_2 stabilizes the T (taut) form of hemoglobin structure, resulting in decreased O_2 affinity for Hb.

Hemoglobin also helps in the transport of CO_2 as bicarbonate, as explained below (***Fig.10.6***).

As the CO_2 enters the blood from tissues, the enzyme ***carbonic anhydrase*** present in erythrocytes catalyses the formation of carbonic acid (H_2CO_3). Bicarbonate (HCO_3^-) and proton (H^+) are released on dissociation of carbonic acid. Hemoglobin acts as a buffer and immediately binds with protons. It is estimated that for every 2 protons bound to Hb, 4 oxygen molecules are released to the tissues. In the lungs, binding O_2 to Hb results in the release of protons. The bicarbonate and protons combine to form carbonic acid. The latter is acted upon by carbonic anhydrase to release CO_2, which is exhaled.

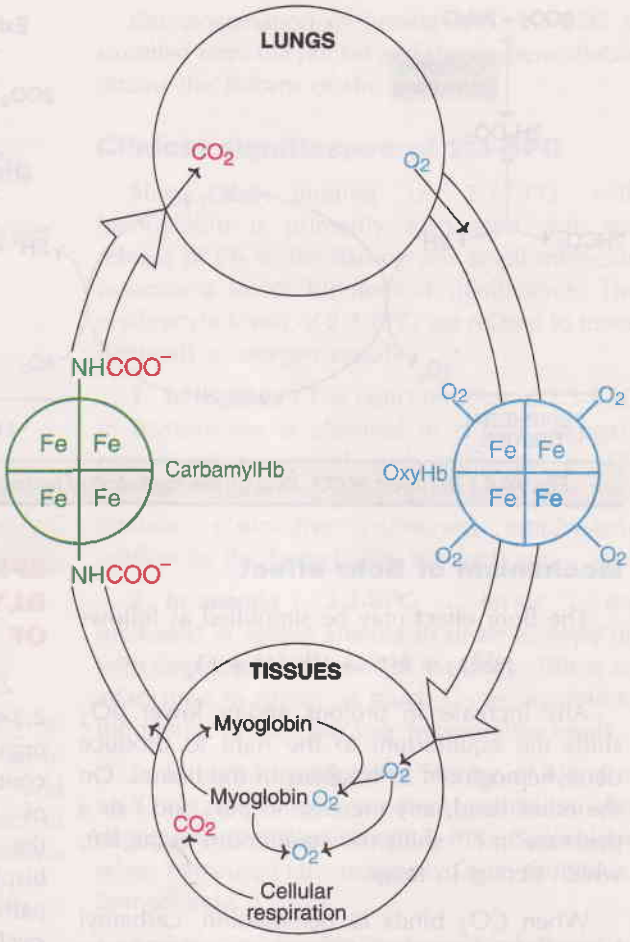


Fig. 10.5 : Diagrammatic representation of transport of O_2 and CO_2 by hemoglobin.

BOHR EFFECT

The binding of oxygen to hemoglobin decreases with increasing H^+ concentration (lower pH) or when the hemoglobin is exposed to increased partial pressure of CO_2 (pCO_2). This phenomenon is known as Bohr effect. It is due to a change in the binding affinity of oxygen to hemoglobin. Bohr effect causes a ***shift in the oxygen dissociation curve to the right*** (***Fig.10.7***).

Bohr effect is primarily responsible for the release of O_2 from the oxyhemoglobin to the tissue. This is because of increased pCO_2 and decreased pH in the actively metabolizing cells.

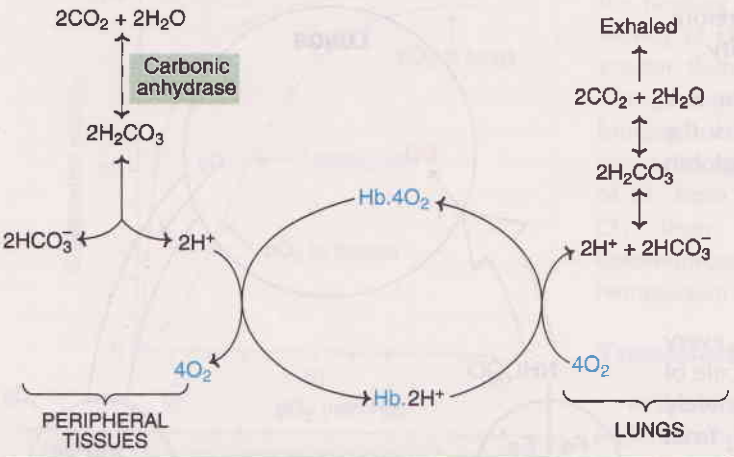


Fig. 10.6 : Transport of CO_2 through the mediation of hemoglobin.

Mechanism of Bohr effect

The Bohr effect may be simplified as follows

$$HbO_2 + H^+ \rightarrow Hb\ H^+ + O_2$$

Any increase in protons and/or lower pO_2 shifts the equilibrium to the right to produce deoxyhemoglobin as happens in the tissues. On the other hand, any increase in pO_2 and / or a decrease in H^+ shifts the equilibrium to the left, which occurs in lungs.

When CO_2 binds to hemoglobin, carbamyl hemoglobin is produced (details described under transport of CO_2). This causes the removal of protons from the terminal NH_2 group and stabilizes the structure of Hb in the T form (deoxyhemoglobin). Therefore, the binding of CO_2 promotes the release of oxygen (in tissues). On the other hand, when hemoglobin is oxygenated in lungs, CO_2 is released as it binds loosely with R-form of Hb.

Role of Cl^- in oxygen transport

Chloride (Cl^-) is bound more tightly to deoxyhemoglobin than to oxyhemoglobin. This facilitates the release of O_2 which is explained as follows

Bicarbonate (HCO_3^-) is freely permeable across the erythrocyte membrane. Once produced in the erythrocytes, HCO_3^- freely moves out and equilibrates with the surrounding plasma. In order to maintain neutrality, Cl^- enters the erythrocytes and binds with deoxyhemoglobin. The concentration of Cl^- is greater in venous blood than in arterial blood.

The four substances namely 2,3-bisphosphoglycerate (described below), CO_2, H^+ and Cl^- are collectively called as ***allosteric effectors***. They interact with the hemoglobin molecule and facilitate the release of O_2 from oxyhemoglobin.

EFFECT OF 2,3-BISPHOSPHOGLYCERATE ON O_2 AFFINITY OF Hb

2,3-Bisphosphoglycerate (***2,3-BPG***; formerly, 2,3-diphosphoglycerate) is the most abundant organic phosphate in the erythrocytes. Its molar concentration is approximately equivalent to that of hemoglobin. 2,3-BPG is produced in the erythrocytes from an intermediate (1,3-bisphosphoglycerate) of glycolysis. This short pathway, referred to as ***Rapaport-Leubering cycle***, is described in carbohydrate metabolism (***Chapter 13***).

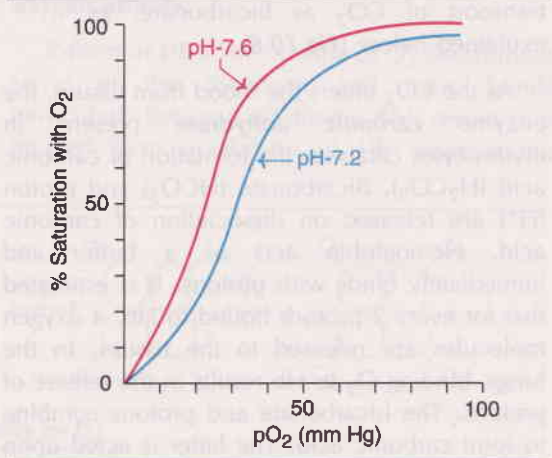


Fig. 10.7 : Effect of pH (Bohr effect) on oxygen dissociation curve (pO_2-Partial pressure of O_2).

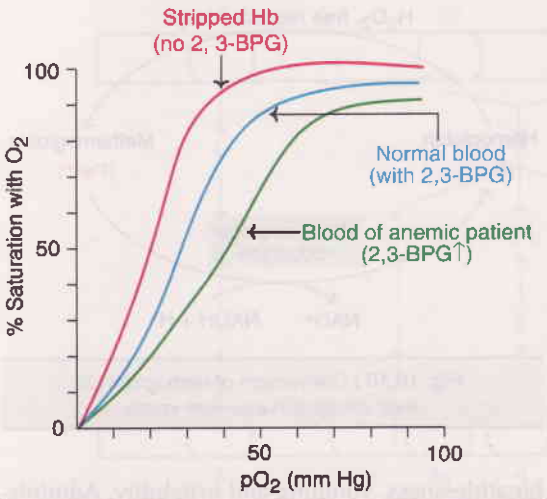


Fig. 10.8 : Effect of pH (Bohr effect) on oxygen dissociation curve (pO_2-Partial pressure of O_2).

Binding of 2,3-BPG to deoxyhemoglobin

2,3-BPG regulates the binding of O_2 to hemoglobin. It specifically binds to deoxyhemoglobin (and not to oxyhemoglobin) and decreases the O_2 affinity to Hb. The effect of 2,3-BPG on Hb may be summarized as follows

$$HbO_2 + \text{2,3-BPG} \longrightarrow \text{Hb-2,3-BPG} + O_2$$

OxyHb — DeoxyHb bound to 2,3-BPG

The reduced affinity of O_2 to Hb facilitates the release of O_2 at the partial pressure found in the tissues. This 2,3-BPG shifts the oxygen dissociation curve to the right (***Fig.10.8***).

Mechanism of action of 2,3-BPG

One molecule of 2,3-BPG binds with one molecule (tetramer) of deoxyhemoglobin in the central cavity of the four subunits. This central pocket has positively charged (e.g. histidine, lysine) two β-globin chains. Ionic bonds (salt bridges) are formed between the positively charged amino acids (of β globins) with the negatively charged phosphate groups of 2,3-BPG (***Fig.10.9***). The binding of 2,3-BPG ***stabilizes the deoxygenated hemoglobin*** (T-form) by cross-linking the β-chains.

On oxygenation of hemoglobin, 2,3-BPG is expelled from the pocket and the oxyhemoglobin attains the R-form of structure.

Clinical significance of 2,3-BPG

Since the binding of 2,3-BPG with hemoglobin is primarily associated with the release of O_2 to the tissues, this small molecule assumes a lot of biomedical significance. The erythrocyte levels of 2,3-BPG are related to tissue demands of oxygen supply.

1. **In hypoxia :** The concentration of 2,3-BPG in erythrocytes is elevated in chronic hypoxic conditions associated with difficulty in O_2 supply. These include adaptation to high altitude, obstructive pulmonary emphysema (airflow in the bronchioles blocked) etc.

2. **In anemia :** 2,3-BPG levels are increased in severe anemia in order to cope up with the oxygen demands of the body. This is an adaptation to supply as much O_2 as possible to the tissue, despite the low hemoglobin levels.

3. **In blood transfusion :** Storage of blood in acid citrate-dextrose medium results in the decreased concentration of 2,3-BPG. Such blood when transfused fails to supply O_2 to the tissues immediately.

Addition of ***inosine*** (hypoxanthine-ribose) to the stored blood prevents the decrease of 2,3-BPG. The ribose moiety of inosine gets phosphorylated and enters the hexose monophosphate pathway and finally gets converted to 2,3-BPG.

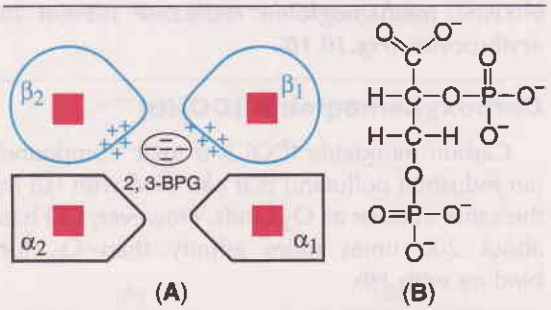


Fig. 10.9 : (A) Diagrammatic representation of binding of 2,3-BPG to deoxyhemoglobin; (B) Structure of 2,3-BPG.

4. **Fetal hemoglobin (HbF) :** The binding of 2,3-BPG to fetal hemoglobin is very weak. Therefore, HbF has higher affinity for O_2 compared to adult hemoglobin (HbA). This may be needed for the transfer of oxygen from the maternal blood to the fetus.

HEMOGLOBIN DERIVATIVES

Hemoglobin (specifically heme) combines with different ligands and forms hemoglobin derivatives. The normal blood contains oxyHb and deoxyHb. Besides these, ***methemoglobin*** (metHb) and ***carboxyhemoglobin*** are the other important Hb derivatives. The Hb derivatives have characteristic colour and they can be detected by absorption spectra.

Methemoglobin

For the biological function of hemoglobin—to carry oxygen—the iron should remain in the ferrous (Fe^{2+}) state. Hemoglobin (Fe^{2+}) can be oxidized to methemoglobin (Fe^{3+}). In normal circumstances, however, molecular oxygen does not oxidize Hb, it only loosely binds to form oxyhemoglobin.

The oxidation of hemoglobin to methemoglobin (metHb) may be caused in the living system by ***H_2O_2, free radicals and drugs.*** The methemoglobin (with Fe^{3+}) is unable to bind to O_2. Instead, a water molecule occupies the oxygen site in the heme of metHb.

In normal circumstances, the occasional oxidation of hemoglobin is corrected by the enzyme ***methemoglobin reductase*** present in erythrocytes (***Fig.10.10***).

Carboxyhemoglobin (COHb)

Carbon monoxide (CO) is a toxic compound (an industrial pollutant) that can bind with Hb in the same manner as O_2 binds. However, CO has about 200 times more affinity than O_2 for binding with Hb.

Clinical manifestations of CO toxicity are observed when the COHb concentration exceeds 20%. The symptoms include headache, nausea,

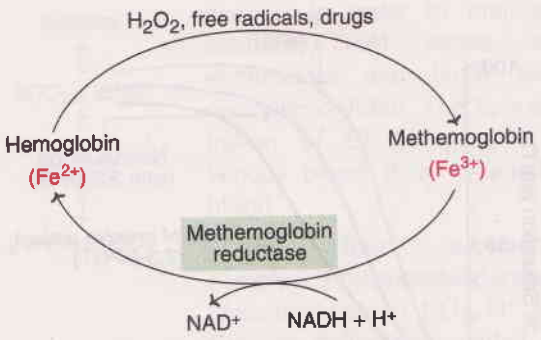


Fig. 10.10 : Conversion of hemoglobin to methemoglobin and vice versa.

breathlessness, vomiting and irritability. Administration of O_2 through oxygen masks will help to reverse the manifestations of CO toxicity.

ABNORMAL HEMOGLOBINS

Abnormal hemoglobins are the resultant of mutations in the genes that code for α or β chains of globin. As many as 400 mutant hemoglobins are known. About 95% of them are due to alteration in a single amino acid of globin.

Basic concepts of globin synthesis

For a better understanding of abnormal hemoglobins, it is worthwhile to have a basic knowledge of globin synthesis. The globin genes are organised into two gene families or clusters (***Fig.10.11***).

1. **α-Gene family :** There are two genes coding for α-globin chain present on each one of chromosome 16. The ζ-gene, other member of α-gene cluster is also found on chromosome 16 and is active during the embryonic development.

2. **β-Gene family :** The synthesis of β-globin occurs from a single gene located on each one of chromosome 11.

This chromosome also contains four other genes.

One ε-gene expressed in the early stages of embryonic development.

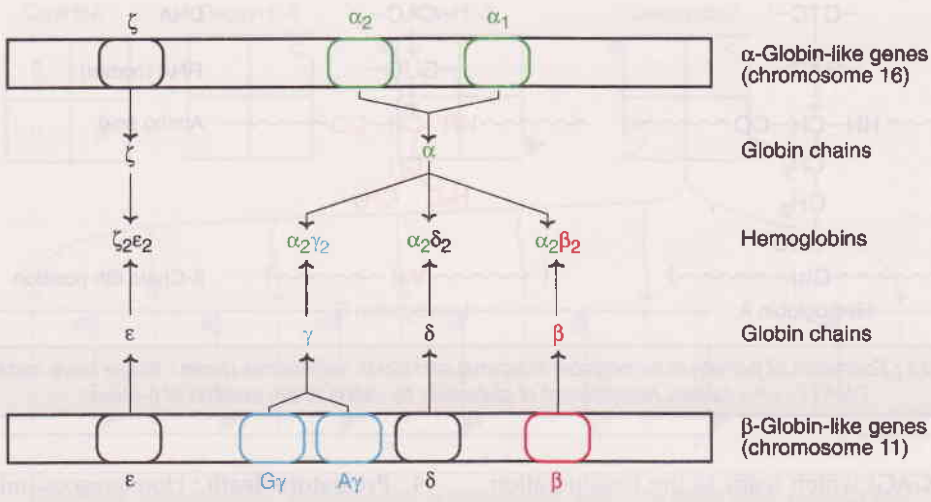


Fig. 10.11 : Diagrammatic representation of globin genes with the synthesis of globin chains and hemoglobins ($\zeta_2\varepsilon_2$-Hb Gower 1; $\alpha_2\gamma_2$-HbF; $\alpha_2\delta_2$-HbA_2; $\alpha_2\beta_2$-HbA_1).

Two γ-genes (Gγ and Aγ) synthesize γ-globin chains of fetal hemoglobin (HbF).

One δ-gene producing δ-globin chain found in adults to a minor extent (HbA_2).

Hemoglobinopathies

It is a term used to describe the disorders caused by the synthesis of abnormal hemoglobin molecule or the production of insufficient quantities of normal hemoglobin or rarely both.

Sickle-cell anemia (HbS) and ***hemoglobin C disease (HbC)*** are the classical examples of abnormal hemoglobins. ***Thalassemias***, on the other hand, are caused by decreased synthesis of normal hemoglobin.

SICKLE-CELL ANEMIA OR SICKLE-CELL HEMOGLOBIN

Sickle-cell anemia (HbS) is the most common form of abnormal hemoglobins. It is so named because the erythrocytes of these patients adopt a sickle shape (crescent like) at low oxygen concentration (***Fig.10.12***).

Occurrence of the disease

Sickle-cell anemia is largely confined to tropical areas of the world. It primarily occurs in the black population. It is estimated that 1 in 500 newborn black infants in the USA are affected by sickle-cell anemia.

Molecular basis of HbS

The structure of hemoglobin (as described already) contains two α-and two β-globin chains. In case of sickle-cell anemia, the hemoglobin (HbS) has two normal α-globin chains and two abnormal (mutant) β-globin chains. This is due to a difference in a single amino acid. In HbS, ***glutamate at sixth position of β-chain is replaced by valine*** (Glu $\beta_6 \rightarrow$ Val).

Sickle-cell anemia is due to a change (missense mutation) in the single nucleotide (thymine → adenine) of β-globin gene. This error causes the formation of altered codon (GUG in

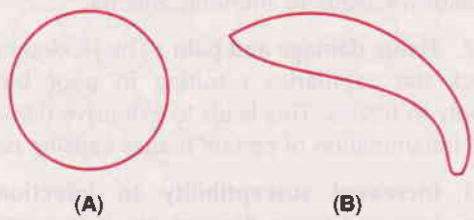


Fig. 10.12 : Erythrocytes : (A) From a normal person; (B) From a patient of sickel-cell anemia.

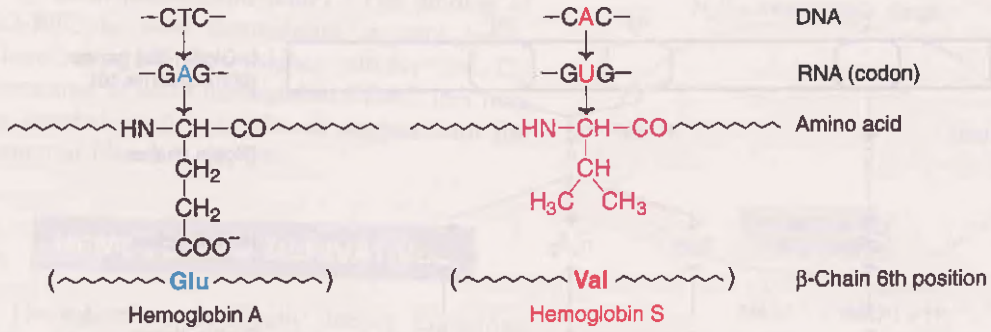


*Fig. 10.13 : Formation of β-chain of hemoglobin in normal and sickle cell anemia (**Note :** Single base mutation in DNA (T → A) causes replacement of glutamate by valine at 6th position of β-chain).*

place of GAG) which leads to the incorporation of valine instead of glutamate at the sixth position in β-chain (***Fig.10.13***).

Homozygous and heterozygous HbS : Sickle-cell anemia is said to be homozygous, if caused by inheritance of two mutant genes (one from each parent) that code for β-chains. In case of heterozygous HbS, only one gene (of β-chain) is affected while the other is normal. The erythrocytes of heterozygotes contain both HbS and HbA and the disease is referred to as ***sickle-cell trait*** which is more common in blacks (almost 1 in 10 are affected). The individuals of sickle-cell trait lead a ***normal life***, and do not usually show clinical symptoms. This is in contrast to homozygous sickle-cell anemia.

Abnormalities associated with HbS

Sickle-cell anemia is characterized by the following abnormalities

1. **Life-long hemolytic anemia :** The sickled erythrocytes are fragile and their continuous breakdown leads to life-long anemia.

2. **Tissue damage and pain :** The sickled cells block the capillaries resulting in poor blood supply to tissues. This leads to extensive damage and inflammation of certain tissues causing pain.

3. **Increased susceptibility to infection :** Hemolysis and tissue damage are accompanied by increased susceptibility to infection and diseases.

4. **Premature death :** Homozygous individuals of sickle-cell anemia die before they reach adulthood (< 20 years).

Mechanism of sickling in sickle-cell anemia

Glutamate is a polar amino acid and it is replaced by a non-polar valine in sickle-cell hemoglobin. This causes a marked decrease in the solubility of HbS in deoxygenated form (T-form). However, solubility of oxygenated HbS is unaffected.

Sticky patches and formation of deoxyhemoglobin fibres

The substitution of valine for glutamate results in a sticky patch on the outer surface of β-chains. It is present on oxy- and deoxyhemoglobin S but absent on HbA. There is a site or receptor complementary to sticky patch on deoxyHbS.

The sticky patch of one deoxyHbS binds with the receptor of another deoxyHbS and this process continuous resulting in the formation of long aggregate molecules of deoxyHbS (***Fig.10.14***). Thus, the polymerization of deoxy-HbS molecules leads to long fibrous precipitates (***Fig.10.15***). These stiff fibres distort the erythrocytes into a sickle or crescent shape (***Fig.10.12***). The sickled erythrocytes are highly vulnerable to lysis.

In case of oxyHbS, the complementary receptor is masked, although the sticky patch is

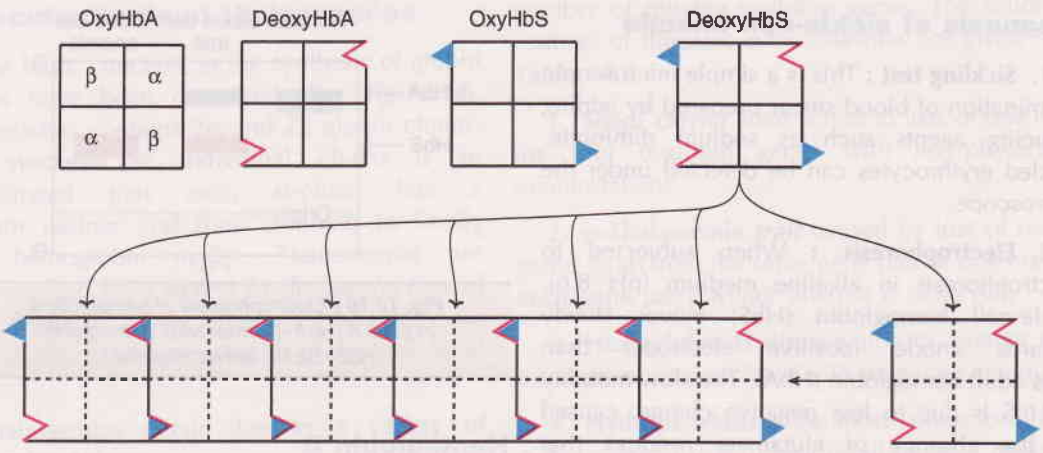


Fig. 10.14 : Diagrammatic representation of sticky patch (Blue) and sticky patch receptor (>) in the formation of long aggregates of deoxyhemoglobins.

present (***Fig.10.14***). Hence, the molecules of oxyHbS cannot bind among themselves or with the molecules of deoxyHbS.

Normal deoxyHbA lacks sticky patches but contains receptors. Absence of sticky patches does not allow the deoxyHbA to participate in the formation of aggregates.

As explained above, sickling is due to polymerization of deoxyHbS. Therefore, if HbS is maintained in the oxygenated form (or with minimum deoxyHbS), sickling can be prevented.

Sickle-cell trait provides resistance to malaria

The incidence of sickle-cell disease coincides with the high incidence of malaria in tropical areas of the world (particularly among the black Africans).

Sickle-cell trait (heterozygous state with about 40% HbS) provides resistance to malaria which is a major cause of death in tropical areas. This is explained as follows

1. Malaria is a parasitic disease caused by *Plasmodium falciparum* in Africa. The malarial parasite spends a part of its life cycle in erythrocytes. Increased lysis of sickled cells (shorter life span of erythrocytes) interrupts the parasite cycle.

2. More recent studies indicate that malarial parasite increases the acidity of erythrocytes (pH down by 0.4). The lowered pH increases the sickling of erythrocytes to about 40% from the normally occurring 2%. Therefore, the entry of malarial parasite promotes sickling leading to lysis of erythrocytes. Furthermore, the concentration of K^+ is low in sickled cells which is unfavourable for the parasite to survive.

Sickle-cell trait appears to be an adaptation for the survival of the individuals in malaria-infested regions. Unfortunately, homozygous individuals, the patients of sickle-cell anemia (much less frequent than the trait), cannot live beyond 20 years.

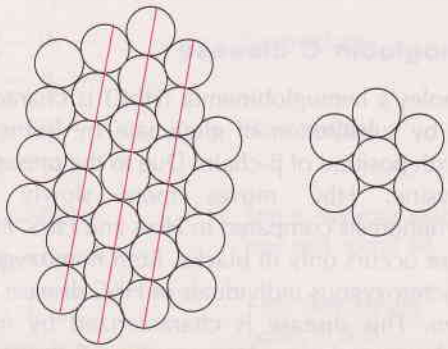


Fig. 10.15 : Diagrammatic representation a fibre of aggregated deoxyhemoglobin.

Diagnosis of sickle-cell anemia

1. **Sickling test :** This is a simple microscopic examination of blood smear prepared by adding reducing agents such as sodium dithionite. Sickled erythrocytes can be detected under the microscope.

2. **Electrophoresis :** When subjected to electrophoresis in alkaline medium (pH 8.6), sickle-cell hemoglobin (HbS) moves slowly towards anode (positive electrode) than does adult hemoglobin (HbA). The slow mobility of HbS is due to less negative charge, caused by the absence of glutamate residues that carry negative charge. In case of sickle-cell trait, the fast moving HbA and slow moving HbS are observed. The electrophoresis of hemoglobin obtained from lysed erythrocytes can be routinely used for the diagnosis of sickle-cell anemia and sickle-cell trait (***Fig.10.16***).

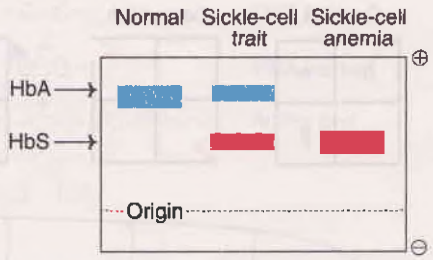


Fig. 10.16 : *Electrophoresis of hemoglobins at pH 8.6 (HbA–Normal adult hemoglobin; HbS–Sickle cell hemoglobin).*

Management of sickle-cell disease

Administration of ***sodium cyanate inhibits sickling of erythrocytes***, Cyanate increases the affinity of O_2 to HbS and lowers the formation of deoxyHbS. However, it causes certain side-effects like peripheral nerve damage.

In patients with severe anemia, repeated blood transfusion is required. This may result in iron overload and cirrhosis of liver.

Replacement of HbS with other forms of hemoglobins has been tried. Fetal hemoglobin (HbF) reduces sickling. Sickle-cell disease awaits gene-replacement therapy!

Hemoglobin C disease

Cooley's hemoglobinemia (HbC) is characterized by substitution of glutamate by lysine in the sixth position of β-chain. Due to the presence of lysine, HbC moves more slowly on electrophoresis compared to HbA and HbS. HbC disease occurs only in blacks. Both homozygous and heterozygous individuals of HbC disease are known. This disease is characterized by mild hemolytic anemia. No specific therapy is recommended.

Hemoglobin D

This is caused by the substitution of glutamine in place of glutamate in the 121st positioin of β-chain. Several variants of HbD are identified from different places indicated by the suffix. For instance, HbD (Punjab), HbD (Los Angeles). HbD, on electrophoresis moves along with HbS.

Hemoglobin E

This is the most common abnormal hemoglobin after HbS. It is estimated that about 10% of the population in South-East Asia (Bangladesh, Thailand, Myanmar) suffer from HbE disease. In India, it is prevalent in West Bengal. HbE is characterized by replacement of glutamate by lysine at 26th position of β-chain. The individuals of HbE (either homozygous or heterozygous) have no clinical manifestations.

THALASSEMIAS

Thalassemias are a group of hereditary hemolytic disorders characterized by impairment/imbalance in the synthesis of globin chains of Hb.

Thalassemias (*Greek*: thalassa-sea) mostly occur in the regions surrounding the Mediterranean sea, hence the name. These diseases, however, are also prevalent in Central Africa, India and the Far East.

Molecular basis of thalassemias

The basic concepts in the synthesis of globin chains have been described (***See Fig.10.14***). Hemoglobin contains 2α and 2β globin chains. The synthesis of individual chains is so coordinated that each α-chain has a β-chain partner and they combine to finally give hemoglobin ($\alpha_2\beta_2$). Thalassemias are characterized by a ***defect in the production of α-or β-globin chain***. There is however, no abnormality in the amino acids of the individual chains.

Thalassemias occur due to a variety of molecular defects

1. Gene deletion or substitution,
2. Underproduction or instability of mRNA,
3. Defect in the initiation of chain synthesis,
4. Premature chain termination.

α-Thalassemias

α-Thalassemias are caused by a ***decreased*** synthesis ***or*** total ***absence of α-globin chain of Hb***. There are four copies of α-globin gene, two on each one of the chromosome 16. Four types of α-thalassemias occur which depend on the number of missing α-globin genes. The salient features of different α-thalassemias are given in ***Table 10.2***.

1. **Silent carrier** state is due to loss of one of the four α-globin genes with no physical manifestations.

2. **α-Thalassemia *trait*** caused by loss of two genes (both from the same gene pair or one from each gene pair). Minor anemia is observed.

3. **Hemoglobin H disease**, due to missing of three genes, is associated with moderate anemia.

4. **Hydrops fetalis** is the most severe form of α-thalassemias due to lack of all the four genes. The fetus usually survives until birth and then dies.

β-Thalassemias

Decreased synthesis or total ***lack*** of the formation of ***β-globin chain*** causes β-thalassemias. The production of α-globin chain continues to be normal, leading to the formation of a globin tetramer (α_4) that precipitate. This causes premature death of erythrocytes. There are mainly two types of β-thalassemias (***Fig.10.17***)

Table 10.2 Summary of different types of α-thalassemias

Type of thalassemia	*Number of missing genes*	*Schematic representation of genes on chromosome 16*	*Clinical symptoms*
Normal	Nil	α_1 α_2 / α_1 α_2	Nil
Silent carrier	1	α_1 α_2 / α_1 α_2	No symptoms
α-Thalassemia trait (heterozygous form)	2	α_1 α_2 / α_1 α_2	Minor anemia
Hemoglobin H disease	3	α_1 α_2 / α_1 α_2	Mild to moderate anemia may lead normal life.
Hydrops fetalis	4	α_1 α_2 / α_1 α_2	Fetal death usually occurs at birth.

(: represent functional genes; : represent missing genes)

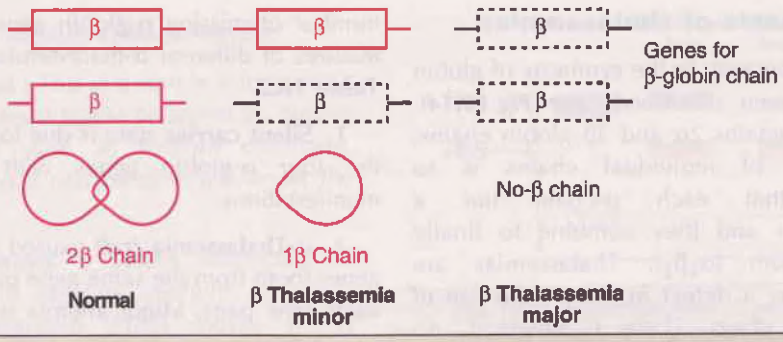


Fig. 10.17: Diagrammatic representation of gene deletions in β-thalassemias (each one of the chromosome pair of 11 has one gene for β-globin).

1. **β-Thalassemia minor :** This is an heterozygous state with a defect in only one of the two β-globin gene pairs on chromosome 11. This disorder, also known as ***β-thalassemia trait***, is usually asymptomatic, since the individuals can make some amount of β-globin from the affected gene.

2. **β-Thalassemia major :** This is a homozygous state with a defect in both the genes responsible for β-globin synthesis. The infants born with β-thalassemia major are healthy at birth since β-globin is not synthesized during the fetal development. They become severely anemic and die within 1-2 years. Frequent blood transfusion is required for these children. This is associated with iron overload which in turn may lead to death within 15-20 years.

PORPHYRINS

Porphyrins are cyclic compounds composed of 4 pyrrole rings held together by methenyl (=CH–) bridges (***Fig.10.18***). Metal ions can bind with nitrogen atoms of pyrrole rings to form complexes. Heme is an iron-containing porphyrin (***See Fig.10.2***) while chlorophyll is a magnesium-containing porphyrin. Thus heme and chlorophyll are the classical examples of metalloporphyrins.

Presentation and nomenclature of porphyrins

The structure of porphyrins ($C_{20}H_{14}N_4$) has four ***pyrrole rings*** namely I, II, III and IV. Naturally occurring porphyrins contain substituent groups replacing the 8 hydrogen atoms of the porphyrin nucleus.

Hans Fischer, the father of porphyrin chemistry, proposed a shorthand model for presentation of porphyrin structures. Accordingly, each pyrrole ring is represented as a bracket. Thus porphyrin has 4 closed brackets with the 8 substituent positions numbered as shown in ***Fig.10.18***.

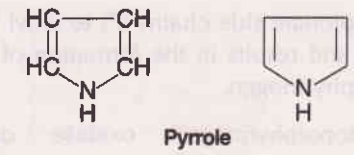
Type I porphyrins : When the substituent groups on the 8 positions are symmetrically arranged they are known as type I porphyrins, e.g. uroporphyrin I.

Type III porphyrins : They contain asymmetric groups at the 8 positions and are more common in the biological system. Originally, Fischer placed them as IX series hence they are more popularly known as type IX porphyrins. It may be observed that the structure of uroporphyrin is asymmetric since on ring IV, the order of substituent groups is reversed (P, A instead of A, P).

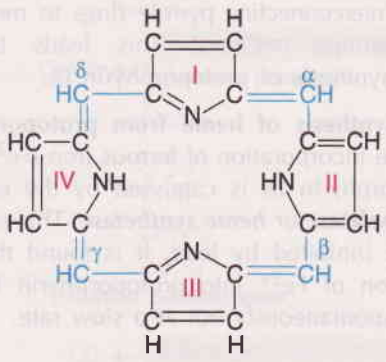
The Fischer's shorthand models of important porphyrins (uroporphyrin I and III; coproporphyrin I and III; protoporphyrin IX and heme) are depicted in ***Fig.10.19***.

Porphyrins in cancer therapy

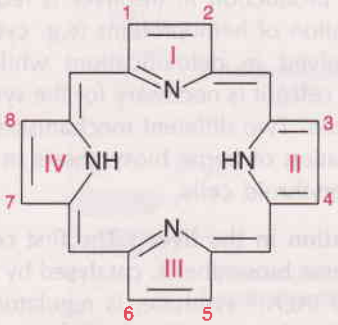
The photodynamic properties of porphyrins can be used in the treatment of certain cancers. This is carried out by a technique called ***cancer phototherapy***. Tumors are capable of taking up



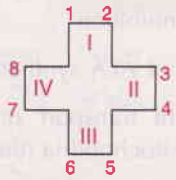
Pyrrole



Porphyrin



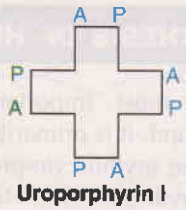
Porphyrin



Fischer's model

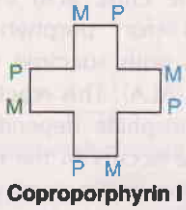
***Fig. 10.18** : Structures of pyrrole and porphyrin [I-IV are pyrrole rings; 1-8 are substituent positions; α, β, γ, δ are methylene (−CH=) bridges.]*

more porphyrins than normal tissues. The cancer phototherapy is carried out by administering ***hematoporphyrin*** (or other related compounds) to the cancer patient. When the tumor is exposed to an argon laser, the porphyrins get excited and produce cytotoxic effects on tumor cells.

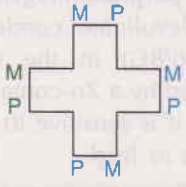


Uroporphyrin I

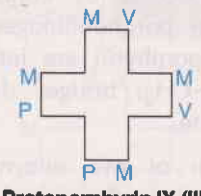
Uroporphyrin III



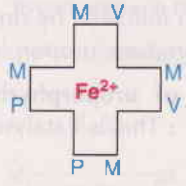
Coproporphyrin I



Coproporphyrin III



Protoporphyrin IX (III)



Heme

***Fig. 10.19** : Fischer's shorthand models of physiologically important porphyrins [A–Acetate (−CH_2COO^-); P–Propionyl (−$CH_2CH_2COO^-$); M–Methyl (−CH_3); V–Vinyl (−CH=CH_2)].*

BIOSYNTHESIS OF HEME

Heme is the most important porphyrin containing compound. It is primarily synthesized in the liver and the erythrocyte-producing cells of bone marrow (erythroid cells). Heme synthesis also occurs to some extent in other tissues. However, mature erythrocytes lacking mitochondria are a notable exception. Biosynthesis of heme occurs in the following stages (***Fig.10.20***).

1. **Formation of δ-aminolevulinate : *Glycine***, a non-essential amino acid and ***succinyl CoA***, an intermediate in the citric acid cycle, are the starting materials for porphyrin synthesis. Glycine combines with succinyl CoA to form δ-aminolevulinate (ALA). This reaction catalysed by a pyridoxal phosphate dependent ***δ-aminolevulinate synthase*** occurs in the mitochondria. It is a rate-controlling step in porphyrin synthesis.

2. **Synthesis of porphobilinogen :** Two molecules of δ-aminolevulinate condense to form porphobilinogen (PBG) in the cytosol. This reaction is catalysed by a Zn-containing enzyme ***ALA dehydratase***. It is sensitive to inhibition by heavy metals such as lead.

3. **Formation of porphyrin ring :** Porphyrin synthesis occurs by condensation of four molecules of porphobilinogen. The four pyrrole rings in porphyrin are interconnected by methylene ($-CH_2$) bridges derived from α-carbon of glycine.

The interaction of two enzymes—namely uroporphyrinogen I synthase and uroporphyrinogen III cosynthase—results in condensation of porphobilinogen followed by ring closure and isomerization to produce uroporphyrinogen III.

4. **Conversion of uroporphyrinogen III to protoporphyrin IX :** This is catalysed by a series of reactions

(a) Uroporphyrinogen decarboxylase decarboxylates all the four acetate (A) side chains to form methyl groups (M), to produce coproporphyrinogen.

(b) Coproporphyrinogen oxidase converts (oxidative decarboxylation) two of the propionate side chains (P) to vinyl groups (V) and results in the formation of protoporphyrinogen.

(c) Protoporphyrinogen oxidase oxidizes methylene groups ($-CH_2-$) interconnecting pyrrole rings to methenyl groups (=CH−). This leads to the synthesis of protoporphyrin IX.

5. **Synthesis of heme from protoporphyrin IX :** The incorporation of ferrous iron (Fe^{2+}) into protoporphyrin IX is catalysed by the enzyme ***ferrochelatase*** or ***heme synthetase***. This enzyme can be inhibited by lead. It is found that the induction of Fe^{2+} into protoporphyrin IX can occur spontaneously but at a slow rate.

Regulation of heme synthesis

Heme production in the liver is required for the formation of hemoproteins (e.g. cytochrome P_{450} involved in detoxification) while in the erythroid cells, it is necessary for the synthesis of hemoglobin. Two different mechanisms exist for the regulation of heme biosynthesis in the liver and the erythroid cells.

Regulation in the liver : The first committed step in heme biosynthesis, catalysed by δ-aminolevulinate (ALA) synthase, is regulatory. ***Heme*** or its oxidized product ***hemin*** (Fe^{3+}) controls this enzyme activity by three mechanisms

1. Feedback inhibition

2. Repression of ALA synthatase

3. Inhibition of transport of ALA synthase from cytosol to mitochondria (the site of action).

Effect of drugs on ALA synthase activity : The activity of ALA synthase is markedly ***increased by*** the administration of a large number of drugs e.g. ***phenobarbital, insecticides, carcinogens*** etc. This is expected since these compounds are mostly metabolized by a heme containing protein, cytochrome P_{450}. On administration of drugs, cellular levels of heme are depleted due to its increased incorporation into cytochrome P_{450}. The reduced heme concentration increases the synthesis (derepression) of ALA synthase to meet the cellular demands.

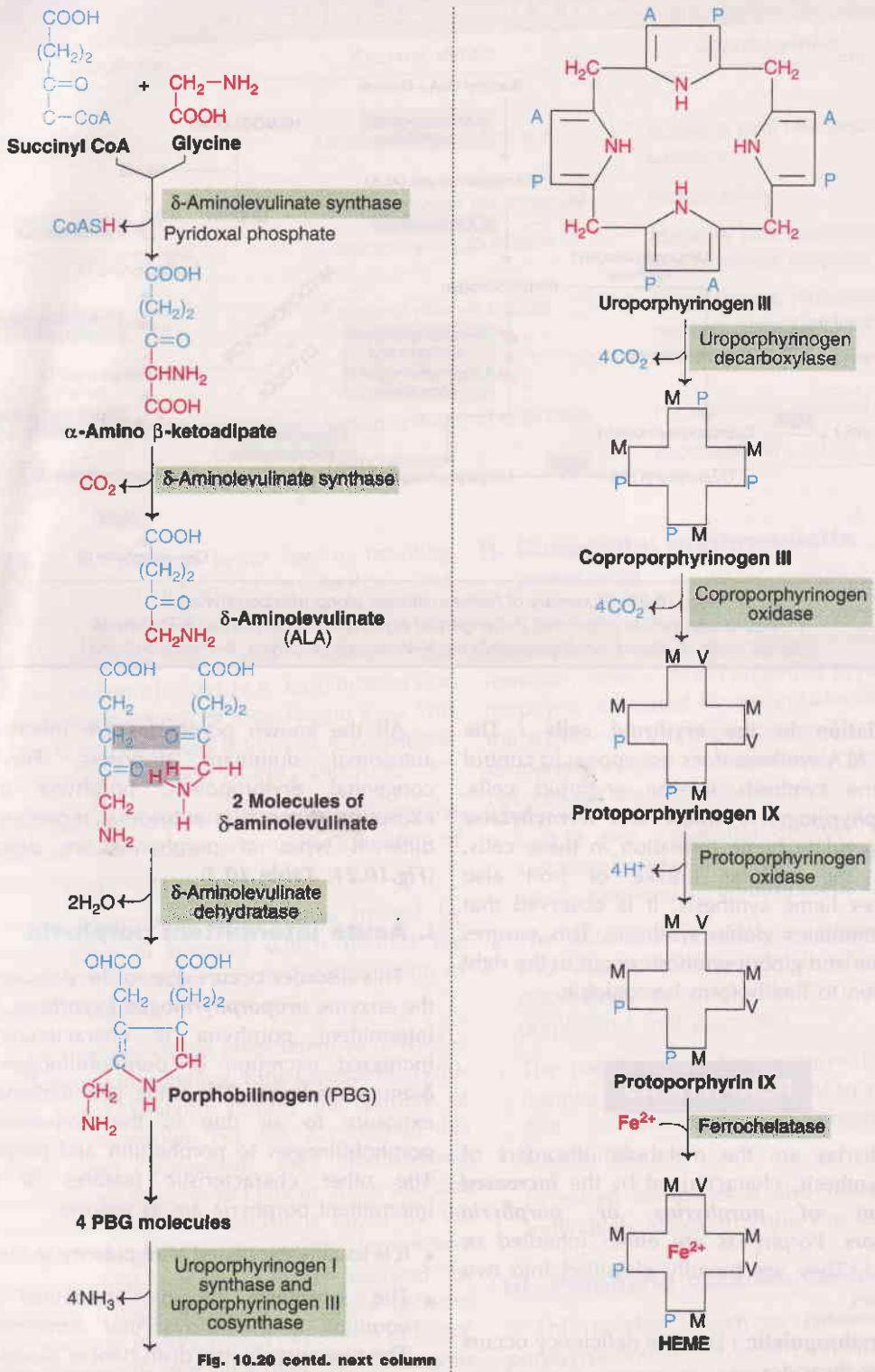


Fig. 10.20 contd. next column

Fig. 10.20 : Biosynthesis of heme [A–Acetyl (−CH_2−COO^-); P–Propionyl (−CH_2−CH_2−COO^-); M–Methyl (−CH_3); V–Vinyl (−CH=CH_2)].

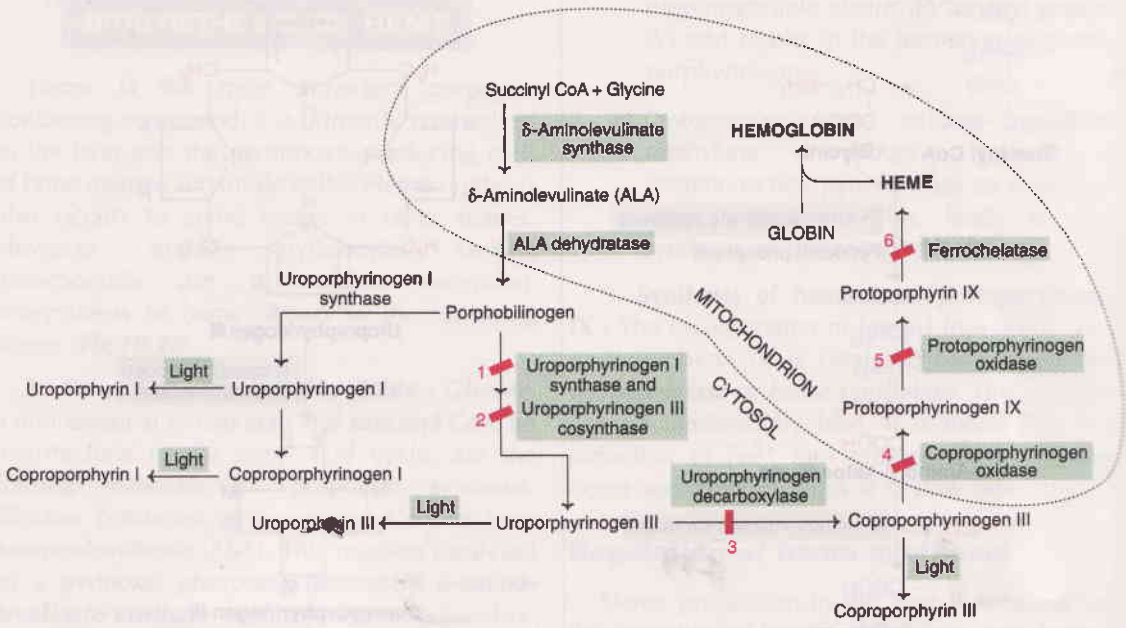


***Fig. 10.21** : Summary of heme synthesis along with porphyrias (1–Acute intermittent porphyria; 2–Congenital erythropoietic porphyria; 3–Porphyria cutanea tarda; 4–Hereditary coproporphyria; 5–Variegate porphyria; 6–Protoporphyria).*

Regulation in the erythroid cells : The enzyme ALA synthase does not appear to control the heme synthesis in the erythroid cells. ***Uroporphyrinogen synthase*** and ***ferrochelatse*** mostly regulate heme formation in these cells. Further, the cellular uptake of iron also influences heme synthesis. It is observed that heme stimulates globin synthesis. This ensures that heme and globin synthesis occur in the right proportion to finally form hemoglobin.

PORPHYRIAS

Porphyrias are the metabolic disorders of heme synthesis, characterized by the ***increased excretion of porphyrins or porphyrin precursors***. Porphyrias are either inherited or acquired. They are broadly classified into two categories

1. **Erythropoietic :** Enzyme deficiency occurs in the erythrocytes.

2. **Hepatic :** Enzyme defect lies in the liver.

All the known porphyrias are inherited as autosomal dominant disorders. However, congenital erythropoietic porphyria is an exception, since it is autosomal recessive. The different types of porphyrias are described (***Fig.10.21, Table 10.3***)

I. Acute intermittent porphyria

This disorder occurs due to the deficiency of the enzyme ***uroporphyrinogen I synthase.*** Acute intermittent porphyria is characterized by increased excretion of porphobilinogen and δ-aminolevulinate. The urine gets darkened on exposure to air due to the conversion of porphobilinogen to porphobilin and porphyrin. The other characteristic features of acute intermittent porphyria are as follows

- It is usually expressed after puberty in humans.
- The symptoms include abdominal pain, vomiting and cardiovascular abnormalities. The neuropsychiatric distrubances observed in these patients are believed to be due to reduced activity of tryptophan pyrrolase

Table 10.3 A general summary of porphyrias

Type of porphyria	*Enzyme defect*	*Characteristics*
Hepatic		
Acute intermittent porphyria	Uroporphyrinogen I synthase	Abdominal pain, neuropsychiatric symptoms
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	Photosensitivity
Hereditary coproporphyria	Corpoporphyrinogen oxidase	Abdominal pain, photosensitivity, neuropsychiatric symptoms
Variegate porphyria	Protoporphyrinogen oxidase	Abdominal pain, photosensitivity, neuropsychiatric symptoms
Erythropoietic		
Congenital erythropoietic porphyria	Uroporphyrinogen III cosynthase	Photosensitivity, increased hemolysis
Protoporphyria	Ferrochelatase	Photosensitivity

(caused by depleted heme levels), resulting in the accumulation of tryptophan and 5-hydroxytryptamine.

- The symptoms are more severe after administration of drugs (e.g. barbiturates) that induce the synthesis of cytochrome P_{450}. This is due to the increased activity of ALA synthase causing accumulation of PBG and ALA.
- These patients are not photosensitive since the enzyme defect occurs prior to the formation of uroporphyrinogen.

Acute intermittent porphyria is treated by administration of hematin which inhibits the enzyme ALA synthase and the accumulation of porphobilinogen.

[The disease—acute intermittent porphyria—has historical importance. King George III (1760-1820) ruled England during the period of American revolution. He was a victim of this disease and possessed the characteristic manifestations (such as red colour urine) and was considered mad. The decisions taken by the deranged King due to acute intermittent porphyria had led to a war followed by American Independence. It is widely believed that American history would have been different, had George III not inherited this metabolic disorder!]

II. Congenital erythropoietic porphyria

This disorder is due to a defect in the enzyme ***uroporphyrinogen III cosynthase***. Some workers, however, believe that congenital erythropoeitic porphyria is caused by an imbalance between the activities of uroporphyrinogen I synthase and uroporphyrinogen III cosynthase. This disease has certain characteristic features

- It is a rare congenital disorder caused by autosomal recessive mode of inheritance, mostly confined to erythropoietic tissues.
- The individuals excrete uroporphyrinogen I and coproporphyrinogen I which oxidize respectively to uroporphyrin I and coproporphyrin I (red pigments).
- The patients are photosensitive (itching and burning of skin when exposed to visible light) due to the abnormal prophyrins that accumulate.
- Increased hemolysis is also observed in the individuals affected by this disorder.

III. Porphyria cutanea tarda

This is also known as ***cutaneous hepatic porphyria*** and is the most common porphyria, usually associated with liver damage caused by

alcohol overconsumption or iron overload. The partial deficiency of the enzyme ***uroporphyrinogen decarboxylase*** appears to be responsible for the occurrence of porphyria cutanea tarda. The other characteristic features include

- Increased excretion of uroporphyrins (I and III) and rarely porphobilinogen.
- Cutaneous photosensitivity is the most important clinical manifestation of these patients.
- Liver exhibits fluorescence due to high concentration of accumulated porphyrins.

IV. Hereditary coproporphyria

This disorder is due to a defect in the enzyme ***coproporphyrinogen oxidase***. As a result of this, coproporphyrinogen III and other intermediates (ALA and PBG) of heme synthesis prior to the blockade are excreted in urine and feces. The victims of hereditary coproporphyria are photosensitive. They exhibit the clinical manifestations observed in the patients of acute intermittent porphyria.

Infusion of hematin is used to control this disorder. Hematin inhibits ALA synthase and thus reduces the accumulation of various intermediates.

V. Variegate porphyria

The enzyme ***protoporphyrinogen oxidase*** is defective in this disorder. Due to this blockade, protoporphyrin IX required for the ultimate synthesis of heme is not produced. Almost all the intermediates (porphobilinogen, coproporphyrin, uroporphyrin, protoporphyrin etc.) of heme synthesis accumulate in the body and are excreted in urine and feces. The urine of these patients is coloured and they exhibit photosensitivity.

VI. Protoporphyria

This disorder, also known as ***erythropoietic protoporphyria***, is caused by a deficiency of the enzyme ***ferrochelatase***. Protoporphyrin IX accumulates in the tissues and is excreted into urine and feces. Reticulocytes (young RBC) and skin biopsy exhibit red flourescence.

Acquired (toxic) porphyrias

The porphyrias, though not inherited, may be acquired due to the toxicity of several compounds. Exposure of the body to heavy metals (e.g. lead), toxic compounds (e.g. hexachlorobenzene) and drugs (e.g. griseofulvin) inhibits many enzymes in heme synthesis. These include ALA dehydratase, uroporphyrin I synthase and ferrochelatase.

DEGRADATION OF HEME TO BILE PIGMENTS

Erythrocytes have a life span of 120 days. At the end of this period, they are removed from the circulation. Erythrocytes are taken up and degraded by the macrophages of the reticuloendothelial (RE) system in the spleen and liver. The hemoglobin is cleaved to the protein part globin and non-protein heme. About 6 g of hemoglobin per day is broken down, and resynthesized in an adult man (70 kg).

Fate of globin : The globin may be reutilized as such for the formation of hemoglobin or degraded to the individual amino acids. The latter undergo their own metabolism, including participation in fresh globin synthesis.

Sources of heme : It is estimated that about 80% of the heme that is subjected for degradation comes from the erythrocytes and the rest (20%) comes from immature RBC, myoglobin and cytochromes.

Heme oxygenase : A complex microsomal enzyme namely heme oxygenase utilizes NADPH and O_2 and cleaves the methenyl bridges between the two pyrrole rings (A and B) to form ***biliverdin***. Simultaneously, ferrous iron (Fe^{2+}) is oxidized to ferric form (Fe^{3+}) and released. The products of heme oxygenase reaction are biliverdin (a green pigment), Fe^{3+} and carbon monoxide (CO). Heme promotes the activity of this enzyme.

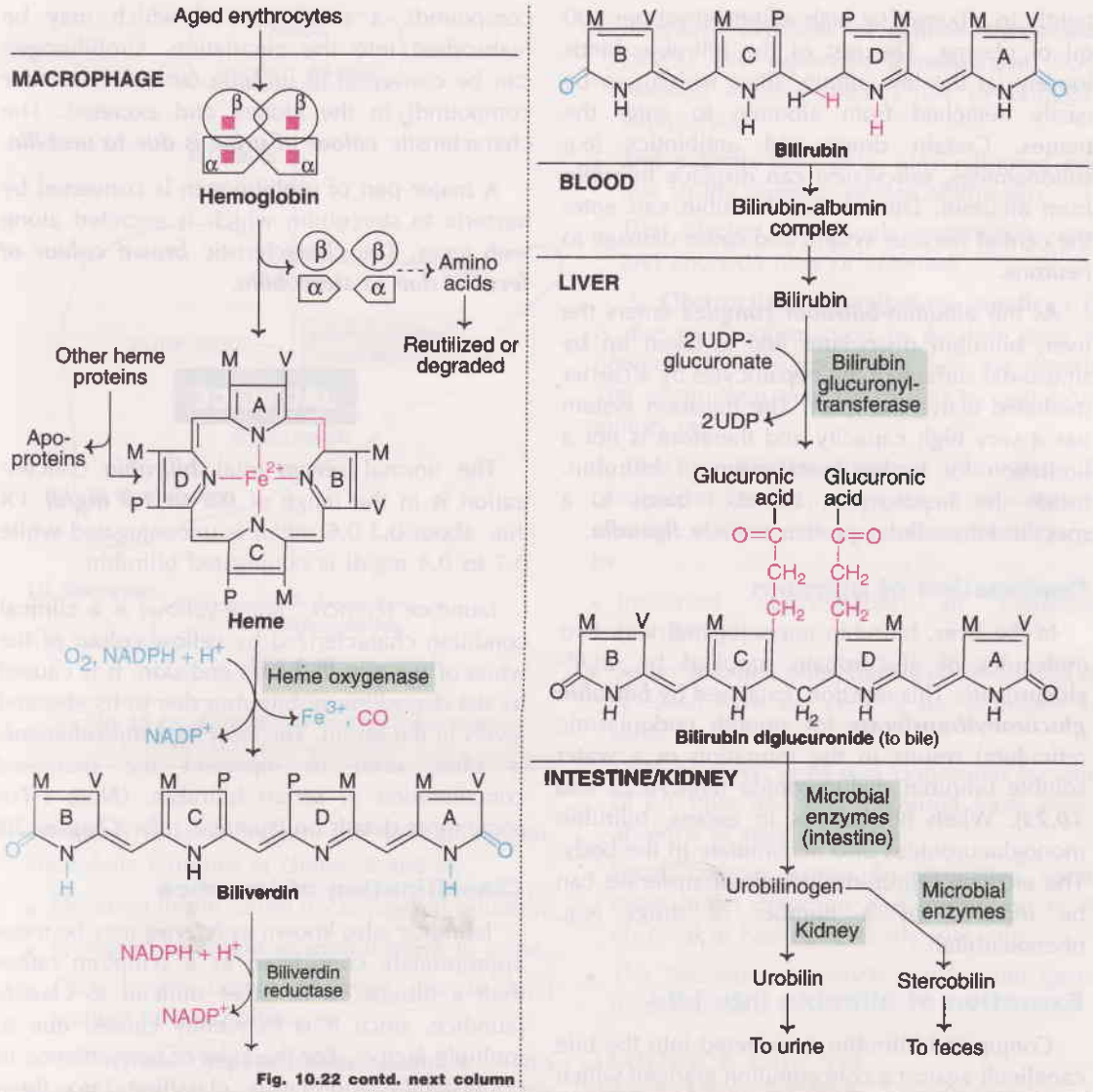


Fig. 10.22 contd. next column

***Fig. 10.22** : Degradation of heme to bile pigments*
***(Note** : Colours used in structures represent change in the specific reaction only).*

Biliverdin is excreted in birds and amphibia while in mammals it is further degraded.

Biliverdin reductase : Biliverdin's methenyl bridges (between the pyrrole rings C and D) are reduced to methylene group to form ***bilirubin*** (yellow pigment). This reaction is catalysed by an NADPH dependent soluble enzyme, biliverdin reductase (***Fig.10.22***). ***One gram of hemoglobin on degradation finally yields about 35 mg bilirubin***. Approximately 250-350 mg of bilirubin is daily produced in human adults. The term bile pigments is used to collectively represent bilirubin and its derivatives.

Transport of bilirubin to liver : Bilirubin is lipophilic and therefore insoluble in aqueous solution. Bilirubin is transported in the plasma in a bound (non-covalently) form to albumin. Albumin has two binding sites for bilirubin—a high affinity site and a low affinity site. Approximately 25 mg of bilirubin can bind

tightly to albumin (at high affinity sites) per 100 ml of plasma. The rest of the bilirubin binds loosely (at the low affinity sites) which can be easily detached from albumin to enter the tissues. Certain drugs and antibiotics (e.g sulfonamides, salicylates) can displace bilirubin from albumin. Due to this, bilirubin can enter the central nervous system and cause damage to neurons.

As the ***albumin-bilirubin complex*** enters the liver, bilirubin dissociates and is taken up by sinusoidal surface of the hepatocytes by a carrier mediated active transport. The transport system has a very high capacity and therefore is not a limitation for further metabolism of bilirubin. Inside the hepatocytes, bilirubin binds to a specific intracellular protein namely ***ligandin***.

Conjugation of bilirubin

In the liver, bilirubin is conjugated with two molecules of glucuronate supplied by UDP-glucuronate. This reaction, catalysed by ***bilirubin glucuronyltransferase*** (of smooth endoplasmic reticulum) results in the formation of a water soluble bilirubin diglucuronide (***Figs.10.22 and 10.23***). When bilirubin is in excess, bilirubin monoglucuronides also accumulate in the body. The enzyme bilirubin glucuronyltransferase can be induced by a number of drugs (e.g. phenobarbital),

Excretion of bilirubin into bile

Conjugated bilirubin is excreted into the bile canaliculi against a concentration gradient which then enters the bile. The transport of ***bilirubin diglucuronide*** is an active, energy-dependent and rate limiting process. This step is easily susceptible to any impairment in liver function. Normally, there is a good coordination between the bilirubin conjugation and its excretion into bile. Thus almost all the bilirubin (> 98%) that enters bile is in the conjugated form.

Fate of bilirubin

Bilirubin glucuronides are hydrolysed in the intestine by specific bacterial enzymes namely ***β-glucuronidases*** to liberate bilirubin. The latter is then converted to urobilinogen (colourless compound), a small part of which may be reabsorbed into the circulation. Urobilinogen can be converted to urobilin (an yellow colour compound) in the kidney and excreted. The characteristic ***colour of urine is due to urobilin***.

A major part of urobilinogen is converted by bacteria to stercobilin which is excreted along with feces. The characteristic ***brown colour of feces is due to stercobilin***.

JAUNDICE

The normal ***serum*** total ***bilirubin*** concentration is in the range of ***0.2 to 1.0 mg/dl***. Of this, about 0.2-0.6 mg/dl is unconjugated while 0.2 to 0.4 mg/dl is conjugated bilirubin.

Jaundice (*French* : *Jaune*-yellow) is a clinical condition characterized by yellow colour of the white of the eyes (sclerae) and skin. It is caused by the deposition of bilirubin due to its elevated levels in the serum. The term ***hyperbilirubinemia*** is often used to represent the increased concentration of serum bilirubin. (***Note :*** For some more details on jaundice, refer ***Chapter 20***)

Classification of jaundice

Jaundice (also known as ***icterus***) may be more appropriately considered as a symptom rather than a disease. It is rather difficult to classify jaundice, since it is frequently caused due to multiple factors. For the sake of convenience to understand, jaundice is classified into three major types—hemolytic, hepatic and obstructive.

1. **Hemolytic jaundice :** This condition is associated with ***increased hemolysis of erythrocytes*** (e.g. incompatible blood transfusion, malaria, sickle-cell anemia). This results in the overproduction of bilirubin beyond the ability of the liver to conjugate and excrete the same. It should, however be noted that liver possesses a large capacity to conjugate about 3.0 g of bilirubin per day against the normal bilirubin production of 0.3 g/day.

In hemolytic jaundice, more bilirubin is excreted into the bile leading to the increased

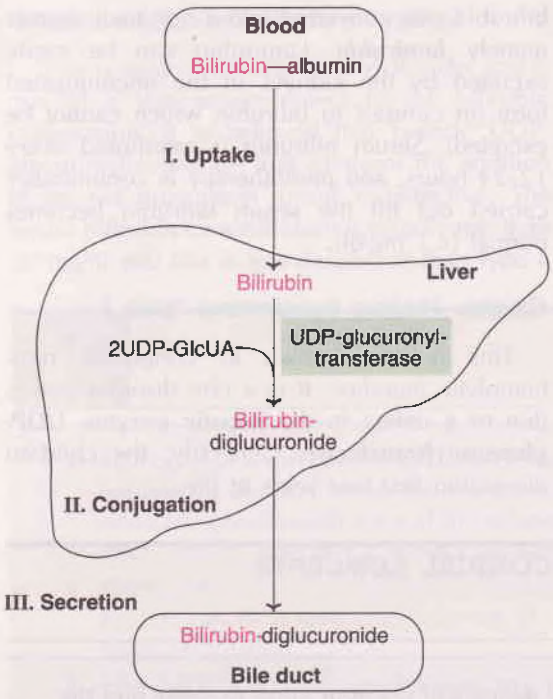


Fig. 10.23 : Summary of bilirubin metabolism (UDP-GlcUA—UDP-glucuronic acid).

formation of urobilinogen and stercobilinogen. Hemolytic jaundice is characterized by

- Elevation in the serum unconjugated bilirubin.
- Increased excretion of urobilinogen in urine.
- Dark brown colour of feces due to high content of stercobilinogen.

2. **Hepatic (hepatocellular) jaundice :** This type of jaundice is caused by ***dysfunction of the liver*** due to damage to the parenchymal cells. This may be attributed to viral infection (viral hepatitis), poisons and toxins (chloroform, carbon tetrachloride, phosphorus etc.) cirrhosis of liver, cardiac failure etc. Among these, viral hepatitis is the most common.

Damage to the liver adversely affects the bilirubin uptake and its conjugation by liver cells. Hepatic jaundice is characterized by

- Increased levels of conjugated and unconjugated bilirubin in the serum.
- Dark coloured urine due to the excessive excretion of bilirubin and urobilinogen.
- Increased activities of ***alanine transaminase*** (SGPT) and ***aspartate transaminase*** (SGOT) released into circulation due to damage to hepatocytes.
- The patients pass pale, clay coloured stools due to the absence of stercobilinogen.
- The affected individuals experience nausea and anorexia (loss of appetite).

3. **Obstructive (regurgitation) jaundice :** This is due to an obstruction in the bile duct that prevents the passage of bile into the intestine. The obstruction may be caused by gall stones, tumors etc.

Due to the blockage in bile duct, the conjugated bilirubin from the liver enters the circulation. Obstructive jaundice is characterized by

- Increased concentration of conjugated bilirubin in serum.
- Serum ***alkaline phosphatase*** is elevated as it is released from the cells of the damaged bile duct.
- Dark coloured urine due to elevated excretion of bilirubin and clay coloured feces due to absence of stercobilinogen.
- Feces contain excess fat indicating impairment in fat digestion and absorption in the absence of bile (specifically bile salts).
- The patients experience nausea and gastrointestinal pain.

JAUNDICE DUE TO GENETIC DEFECTS

There are certain hereditary abnormalities that cause jaundice.

Neonatal-physiologic jaundice

Physiological jaundice is not truly a genetic defect. It is caused by increased hemolysis coupled with immature hepatic system for the uptake, conjugation and secretion of bilirubin. The activity of the enzyme ***UDP-glucuronyl-transferase is low*** in the newborn. Further, there is a limitation in the availability of the substrate UDP-glucuronic acid for conjugation. The net effect is that in some infants the serum

uncojugated bilirubin is highly elevated (may go beyond 25mg/dl), which can cross the blood-brain barrier. This results in hyperbilirubinemic toxic encephalopathy or ***kernicterus*** that causes mental retardation. The drug phenobarbital is used in the treatment of neonatal jaundice, as it can induce bilirubin metabolising enzymes in liver. In some neonates, blood transfusion may be necessary to prevent brain damage.

Phototherapy : Bilirubin can absorb blue light (420–470 nm) maximally. Phototherapy deals with the exposure of the jaundiced neonates to blue light. By a process called ***photoisomerization***, the toxic native unconjugated bilirubin gets converted into a non-toxic isomer namely ***lumirubin***. Lumirubin can be easily excreted by the kidneys in the unconjugated form (in contrast to bilirubin which cannot be excreted). Serum bilirubin is monitored every 12–24 hours, and phototherapy is continuously carried out till the serum bilirubin becomes normal (< 1 mg/dl).

Crigler-Najjar syndrome type I

This is also known as congenital non-hemolytic jaundice. It is a rare disorder and is due to a defect in the hepatic enzyme ***UDP-glucuronyltransferase***. Generally, the children die within first two years of life.

BIOMEDICAL / CLINICAL CONCEPTS

- *Hemoglobin is primarily responsible for the delivery of O_2 from lungs to tissue and the transport of CO_2 from tissues to lungs.*
- *Increased erythrocyte 2,3-BPG levels in anemia and chronic hypoxia facilitate the release of more O_2 from the oxyhemoglobin to the tissues.*
- *Storage of blood causes a decrease in the concentration of 2,3-BPG. This can be prevented by the addition of ionosine.*
- *Hemoglobin (Fe^{2+}) on oxidation by H_2O_2, free radicals or drugs, forms methemoglobin (Fe^{3+}) which cannot transport O_2.*
- *Carboxyhemoglobin is produced when carbon monoxide, an industrial pollutant, binds to hemoglobin. The clinical manifestations of CO toxicity (> 20% COHb) include headache, nausea, breathlessness and vomiting.*
- *Sickle cell hemoglobin (HbS) causes hemolytic anemia, increased susceptibility to infection and premature death. However, HbS offers protection against malaria.*
- *Thalassemias are hemolytic disorders caused by impairment/imbalance in the synthesis of globin chains of Hb. These include α-thalassemia trait, hydrops fetalis and β-thalassemias.*
- *Administration of porphyrins can be used in the treatment certain cancers by phototherapy.*
- *Abnormalities in heme synthesis cause porphyrias which may be erythropoietic (enzyme defect in RBC) or hepatic (enzyme defect in liver). Porphyrias are associated with elevated excretion of porphyrins, neuropsychiatric disturbances and cardiovascular abnormalities.*
- *Jaundice is caused by elevated serum bilirubin (normal < 0.8 mg/dl) levels and is characterized by yellow coloration of white of the eyes, and skin.*
- *Phototherapy (by exposure to blue light) is used in to control severe cases of neonatal physiologic jaundice.*

Crigler-Najjar syndrome type II

This is again a rare hereditary disorder and is due to a less severe defect in the bilirubin conjugation. It is believed that hepatic UDP-glucuronyltransferase that catalyses the addition of second glucuronyl group is defective. The serum bilirubin concentration is usually less than 20 mg/dl and this is less dangerous than type I.

Gilbert's disease : This is not a single disease but a combination of disorders. These include

1. A defect in the uptake of bilirubin by liver cells.
2. An impairment in conjugation due to reduced activity of UDP-glucuronyltransferase.
3. Decreased hepatic clearance of bilirubin.

SUMMARY

1. *Hemoglobin (HbA_1, mol. wt. 64,450) is a conjugated protein containing globin, the apoprotein and the heme, the nonprotein moiety (prosthetic group). It is a tetrameric, allosteric protein with 2α and 2β polypeptide chains held by non-covalent interactions. Each subunit contains a heme with iron in the ferrous state.*
2. *Hemoglobin is responsible for the transport of O_2 from lungs to the tissues. Each heme (of Hb) can bind with one molecule of O_2 and this is facilitated by cooperative heme-heme interaction.*
3. *Hemoglobin actively participates in the transport of CO_2 from tissues to lungs. Increased partial pressure of CO_2 (pCO_2) accompanied by elevated H^+ decreases the binding of O_2 to Hb, a phenomenon known as Bohr effect.*
4. *The four compounds namely 2,3-bisphosphoglycerate, CO_2, H^+ and Cl^- are collectively known as allosteric effectors. They interact with hemoglobin and facilitate the release of O_2 from oxyHb.*
5. *Sickle-cell anemia (HbS) is a classical example of abnormal hemoglobins. It is caused when glutamate at 6th position of β-chain is replaced by valine. HbS is characterized by hemolytic anemia, tissue damage, increased susceptibility to infection and premature death. Sickle-cell anemia, however offers resistance to malaria.*
6. *Thalassemias are a group of hereditary hemolytic disorders characterized by impairment/imbalance in the synthesis of globin (α or β) chain of Hb. Hydrops fetalis, the most severe form of α-thalassemia is characterized by the death of infant at birth. β-Thalassemia major is another serious disorder with severe anemia and death of child within 1-2 years.*
7. *Heme is the most important porphyrin compound, primarily synthesized in the liver from the precursors-glycine and succinyl CoA. Heme productioin is regulated by δ-aminolevulinate synthase.*
8. *Porphyrias are the metabolic disorders of heme synthesis, characterized by the increased excretion of porphyrins or their precursors. Acute intermittent porphyria occurs due to the deficiency of the enzyme uroporphyrinogen I synthase and is characterized by increased excretion of porphobilinogen and δ-aminolevulinate. The clinical symptoms include neuropsychiatric disturbances and cardiovascular abnormalities.*
9. *Heme is degraded mainly to bilirubin, an yellow colour bile pigment. In the liver, it is conjugated to bilirubin diglucuronide, a more easily excretable form into bile.*
10. *Jaundice is a clinical condition caused by elevated serum bilirubin concentration (normal <1.0 mg/dl). Jaundice is of three types-hemolytic (due to increased hemolysis), hepatic (due to impaired conjugation) and obstructive (due to obstruction in the bile duct).*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Describe the structure of hemoglobin and discuss oxygen transport.
2. Write an account of hemoglobinopathies with special reference to sickle-cell anemia.
3. Discuss the biosynthesis of heme. Add a note on the regulation of heme synthesis.
4. What are porphyrias? Describe any three porphyrias in detail.
5. Write an account of the degradation of heme to bile pigments. Add a note on jaundice.

II. Short notes

(a) Methemoglobin, (b) Heme—heme interaction, (c) Bohr effect, (d) 2,3—BPG, (e) Sickle cell anemia and malaria, (f) Thalassemias, (g) Acute intermittent porphyria, (h) Heme oxygenase, (i) Bilirubin diglucuronide, (j) Carboxyhemoglobin.

III. Fill in the blanks

1. The total number of amino acids present in adult hemoglobin _____.
2. The oxidation of ferrous (Fe^{2+}) iron to ferric (Fe^{3+}) iron in hemoglobin results in the formation of a compound namely _____.
3. The enzyme that catalyses the formation of carbonic acid _____.
4. Name the compound that is increased in RBC of anemic patients to facilitate the supply of O_2 to the tissues _____.
5. Sickling of RBC in sickle-cell anemia is due to polymerization of _____.
6. The disorders characterized by decreased synthesis or total absence of globin chains of hemoglobin are collectively known as _____.
7. The intermediate of citric acid cycle that is involved in heme synthesis _____.
8. The enzyme defect in acute intermittent porphyria _____.
9. The enzyme that is regulated by feedback inhibition in heme synthesis is _____.
10. The product formed when heme oxygenase cleaves heme _____.

IV. Multiple choice questions

11. The characteristic red colour of hemoglobin is due to

    (a) Heme (b) α-Globin (c) β-Globin (d) All of them.

12. The number of heme groups present in myoglobin

    (a) 1 (b) 2 (c) 3 (d) 4.

13. The patients of sickle-cell anemia are resistant to

    (a) Filaria (b) Malaria (c) Diabetes (d) Trypanosomiasis.

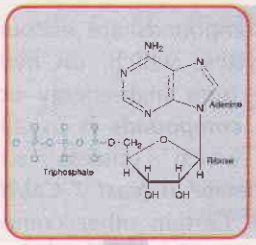
14. The compound that facilitates the release of O_2 from oxyhemoglobin

    (a) 2, 3-BPG (b) H^+ (c) $C1^-$ (d) All of them.

15. Name the amino acid that directly participates in the synthesis of heme

    (a) Methionine (b) Aspartate (c) Glycine (d) Tryptophan.

11 Biological Oxidation



The high energy compound, ATP speaks :

"I am the energy currency of the cell !
Continuous consumption and regeneration is my thrill;
Without me, all biochemical functions come to a standstill;
Existence of life is unimaginable without my will."

For a better understanding of biological oxidation, it is worthwhile to have a basic knowledge of bioenergetics and the role of high-energy compounds in biological processes.

BIOENERGETICS

Bioenergetics or ***biochemical thermodynamics*** deals with the study of energy changes (transfer and utilization) in biochemical reactions. The reactions are broadly classified as ***exergonic*** (energy releasing) and ***endergonic*** (energy consuming). Bioenergetics is concerned with the initial and final states of energy component of the reactants and not the mechanism of chemical reactions.

Free energy

The energy actually ***available to do work*** (utilizable) is known as free energy. Changes in the free energy (ΔG) are valuable in predicting the feasibility of chemical reactions. The reactions can occur spontaneously if they are accompanied by decrease in free energy.

During a chemical reaction, heat may be released or absorbed. ***Enthalpy (ΔH)*** is a measure of the change in heat content of the reactants, compared to products.

Entropy (ΔS) represents a change in the randomness or disorder of reactants and products. Entropy attains a maximum as the reaction approaches equilibrium. The reactions of biological systems involve a temporary decrease in entropy.

The relation between the changes of free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) is expressed as

$$\Delta G = \Delta H - T\Delta S$$

T represents the absolute temperature in Kelvin (K = 273 + °C).

The term ***standard free energy*** represented by ΔG° (note the superscript°) is often used. It indicates the free energy change when the reactants or products are at a concentration of 1 mol/l at pH 7.0.

Negative and positive ΔG

If free energy change (ΔG) is represented by a negative sign, there is a loss of free energy. The reaction is said to be ***exergonic***, and proceeds spontaneously. On the other hand, a positive ΔG indicates that energy must be supplied to the reactants. The reaction cannot proceed spontaneously and is ***endergonic*** in character.

The hydrolysis of ATP is a classical example of exergonic reaction

$$ATP + H_2O \longrightarrow ADP + Pi \ (\Delta G^\circ = -7.3 \text{ Cal/mol})$$

The reversal of the reaction (ADP + Pi → ATP) is endergonic and occurs only when there is a supply of energy of at least 7.3 Cal/mol (ΔG° is positive).

The free energy change becomes zero (ΔG = 0) when a reaction is at equilibrium.

At a constant temperature and pressure, ΔG is dependent on the actual concentration of reactants and products. For the conversion of reactant A to product B (A → B), the following mathematical relation can be derived

$$\Delta G = \Delta G^\circ + RT \ln \frac{[B]}{[A]}$$

where ΔG° = Standard free energy change

R = Gas constant (1.987 Cal/mol)

T = Absolute temperature (273 + °C)

ln = Natural logarithm

[B] = Concentration of product

[A] = Concentration of reactant.

ΔG° is related to equilibrium constant (Keq)

When a reaction $A \rightleftharpoons B$ is at equilibrium (eq), the free energy change is zero. The above equation may be written as

$$\Delta G = 0 = \Delta G^\circ + RT \ln \frac{[B] \text{ eq.}}{[A] \text{ eq.}}$$

Hence $\Delta G^\circ = -RT \ln Keq.$

ΔG is an additive value for pathways

Biochemical pathways often involve a series of reactions. For such reactions, free energy change is an additive value. The sum of ΔG is crucial in determining whether a particular pathway will proceed or not. As long as the sum of ΔGs of individual reactions is negative, the pathway can operate. This happens despite the fact that some of the individual reactions may have positive ΔG.

HIGH-ENERGY COMPOUNDS

Certain compounds are encountered in the biological system which, on hydrolysis, yield energy. The term high-energy compounds or ***energy rich compounds*** is usually applied to substances which possess sufficient free energy to liberate ***at least 7 Cal/mol*** at pH 7.0 (***Table 11.1***). Certain other compounds which liberate less than 7.0 Cal/mol (lower than ATP hydrolysis to ADP + Pi) are referred to as low-energy compounds.

Table 11.1 Standard free energy of hydrolysis of some important compounds

Compounds	*ΔG° (Cal/mol)*
High-energy phosphates	
Phosphoenol pyruvate	−14.8
Carbamoyl phosphate	−12.3
Cyclic AMP	−12.0
1,3-Bisphosphoglycerate	−11.8
Phosphocreatine	−10.3
Acetyl phosphate	−10.3
S-Adenosylmethionine*	−10.0
Pyrophosphate	−8.0
Acetyl CoA**	−7.7
ATP → ADP + Pi	−7.3
Low-energy phosphates	
ADP → AMP + Pi	−6.6
Glucose 1-phosphate	−5.0
Fructose 6-phosphate	−3.8
Glucose 6-phosphate	−3.3
Glycerol 3-phosphate	−2.2

* *Sulfonium compound*

** *Thioester*

Table 11.2 High-energy compounds

Class	Bond	Example(s)
Pyrophosphates	—C—Ⓟ—Ⓟ	ATP, pyrophosphate
Acyl phosphates	O ‖ —C—O~Ⓟ	1,3-Bisphosphoglycerate, carbamoyl phosphate, acetyl phosphate
Enol phosphates	—CH ‖ —C—O~Ⓟ	Phosphoenol pyruvate
Thiol esters (thioesters)	C ‖ —C—O~S—	Acetyl CoA, acyl CoA
Guanidio phosphates (Phosphagens)	—N~P	Phosphocreatine, phosphoarginine

All the high-energy compounds—when hydrolysed—liberate more energy than that of ATP. These include ***phosphoenol pyruvate, 1,3-bisphosphoglycerate, phosphocreatine*** etc. Most of the high-energy compounds contain phosphate group (exception acetyl CoA) hence they are called high-energy phosphate compounds.

Classification of high-energy compounds

There are at least 5 groups of high-energy compounds.

1. Pyrophosphates e.g. ATP.
2. Acyl phosphates e.g. 1,3-bisphosphoglycerate.
3. Enol phosphates e.g. phosphoenolpyruvate.
4. Thioesters e.g. acetyl CoA.
5. Phosphagens e.g. phosphocreatine.

Table 11.2 gives some more details on the high-energy compounds, including the high-energy bonds present in each category.

High-energy bonds : The high-energy compounds possess ***acid anhydride bonds*** (mostly phosphoanhydride bonds) which are formed by the condensation of two acidic groups or related compounds. These bonds are referred to as high-energy bonds, since the free energy is liberated when these bonds are hydrolysed. Lipmann suggested use of the ***symbol ~ to represent high-energy bond.*** For instance, ATP is written as AMP~P~P.

ATP - the most important high-energy compound

Adenosine triphosphate (ATP) is a unique and the most important high-energy molecule in the living cells. It consists of an adenine, a ribose and a triphosphate moiety (***Fig.11.1***). ATP is a high-energy compound due to the presence of two phosphoanhydride bonds in the triphosphate unit. ATP serves as the ***energy currency of the cell*** as is evident from the ATP-ADP cycle.

ATP-ADP Cycle

The hydrolysis of ATP is associated with the release of large amount of energy.

$$ATP + H_2O \longrightarrow ADP + Pi + 7.3 \text{ Cal.}$$

The energy liberated is utilized for various processes like muscle contraction, active transport etc. ATP can also act as a donor of high-energy phosphate to low-energy compounds, to make them energy rich. On the other hand, ADP can accept high-energy phosphate from the compounds possessing higher free energy content to form ATP.

ATP serves as an immediately available energy currency of the cell which is constantly

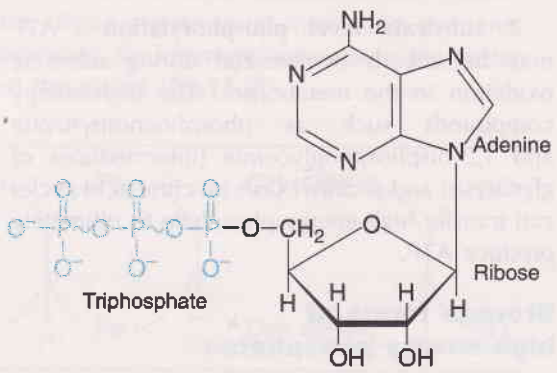


Fig. 11.1 : Structure of ATP.

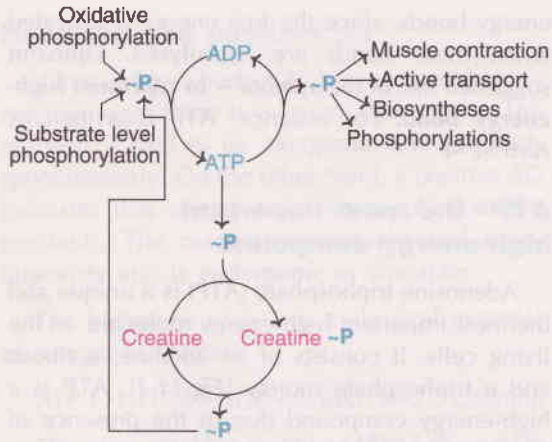


Fig. 11.2 : ATP-ADP cycle along with sources and utilization of ATP (Note that ~P does not exist in free form, but is only transferred).

being utilized and regenerated. This is represented by ATP-ADP cycle, the fundamental basis of energy exchange reactions in living system (***Fig.11.2***). The turnover of ATP is very high.

ATP acts as an ***energy link between the catabolism*** (degradation of molecules) and ***anabolism*** (synthesis) in the biological system.

Synthesis of ATP

ATP can be synthesized in two ways

1. **Oxidative phosphorylation :** This is the major source of ATP in aerobic organisms. It is linked with the mitochondrial electron transport chain (details described later).

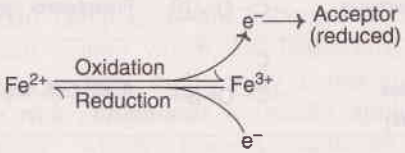
2. **Substrate level phosphorylation :** ATP may be directly synthesized during substrate oxidation in the metabolism. The high-energy compounds such as phosphoenolpyruvate and 1,3-bisphosphoglycerate (intermediates of glycolysis) and succinyl CoA (of citric acid cycle) can transfer high-energy phosphate to ultimately produce ATP.

Storage forms of high-energy phosphates

Phosphocreatine (creatine phosphate) stored in vertebrate muscle and brain is an energy-rich compound. In invertebrates, phosphoarginine (arginine phosphate) replaces phosphocreatine.

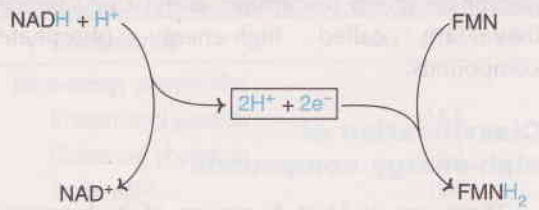
BIOLOGICAL OXIDATION

Oxidation is defined as the loss of electrons and reduction as the gain of electrons. This may be illustrated by the interconversion of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}).



The electron lost in the oxidation is accepted by an acceptor which is said to be reduced. Thus the oxidation-reduction is a tightly coupled process.

The general principle of oxidation-reduction is applicable to biological systems also. The oxidation of NADH to NAD^+ coupled with the reduction of FMN to $FMNH_2$ is illustrated



In the above illustration, there are two redox pairs $NADH/NAD^+$ and $FMN/FMNH_2$. The redox pairs differ in their tendency to lose or gain electrons.

Redox potential (E_0)

The ***oxidation-reduction*** potential or, simply, redox potential, is a quantitative measure of the tendency of a redox pair to lose or gain electrons. The redox pairs are assigned specific ***standard redox potential*** (E_0 volts) at pH 7.0 and 25°C.

The redox potentials of some biologically important redox systems are given in ***Table 11.3***. The more negative redox potential represents a greater tendency (of reductant) to lose electrons.

Table 11.3 Standard redox potential (E_0) of some oxidation-reduction systems

Redox pair	*E_0 Volts*
Succinate/α-ketoglutarate	– 0.67
$2H^+/H_2$	– 0.42
$NAD^+/NADH$	– 0.32
$NADP^+/NADPH$	– 0.32
$FMN/FMNH_2$ (enzyme bound)	– 0.30
Lipoate (ox/red)	– 0.29
$FAD/FADH_2$	– 0.22
Pyruvate/lactate	– 0.19
Fumarate/succinate	+ 0.03
Cytochrome b (Fe^{3+}/Fe^{2+})	+ 0.07
Coenzyme Q (ox/red)	+ 0.10
Cytochrome c_1 (Fe^{3+}/Fe^{2+})	+ 0.23
Cytochrome c (Fe^{3+}/Fe^{2+})	+ 0.25
Cytochrome a (Fe^{3+}/Fe^{2+})	+ 0.29
$\frac{1}{2}O_2/H_2O$	+ 0.82

On the other hand, a more positive redox potential indicates a greater tendency (of oxidant) to accept electrons. The electrons flow from a redox pair with more negative E_0 to another redox pair with more positive E_0.

The redox potential (E_0) is directly related to the change in the free energy (ΔG°).

ELECTRON TRANSPORT CHAIN

The energy-rich carbohydrates (particularly glucose), fatty acids and amino acids undergo a series of metabolic reactions and, finally, get oxidized to CO_2 and H_2O. The reducing equivlents from various metabolic intermediates are transferred to coenzymes NAD^+ and FAD to produce, respectively, NADH and $FADH_2$. The latter two reduced coenzymes pass through the electron transport chain (ETC) or ***respiratory chain*** and, finally, reduce oxygen to water. The passage of electrons through the ETC is associated with the loss of free energy. A part of this free energy is utilized to generate ATP from ADP and Pi (***Fig.11.3***).

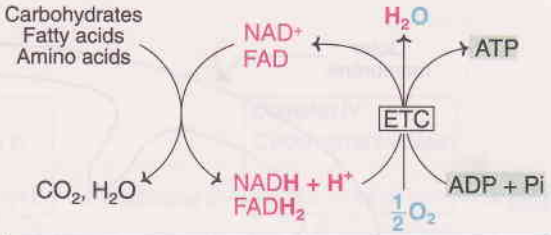


Fig. 11.3 : *Overview of biological oxidation (ETC-Electron transport chain).*

An overview of the ETC is depicted in ***Fig.11.4***.

Mitochondria – the power houses of cell

The mitochondria are the centres for metabolic oxidative reactions to generate reduced coenzymes (NADH and $FADH_2$) which, in turn, are utilized in ETC to liberate energy in the form of ATP. For this reason, mitochondrion is appropriately regarded as the ***power house of the cell***.

Mitochondrial organization

The mitochondrion consists of five distinct parts. These are the outer membrane, the inner membrane, the intermembrane space, the cristae and the matrix (***Fig.11.5***).

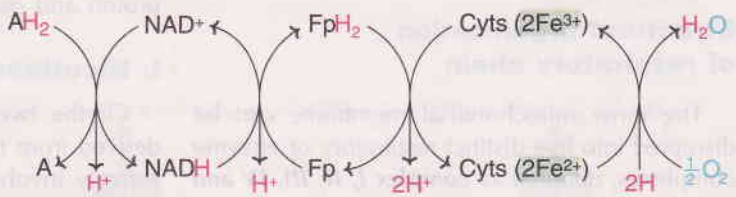


Fig. 11.4 : *Overview of electron transport chain (A–Substrate; F_p–Flavoprotein; Cyts–Cytochromes).*

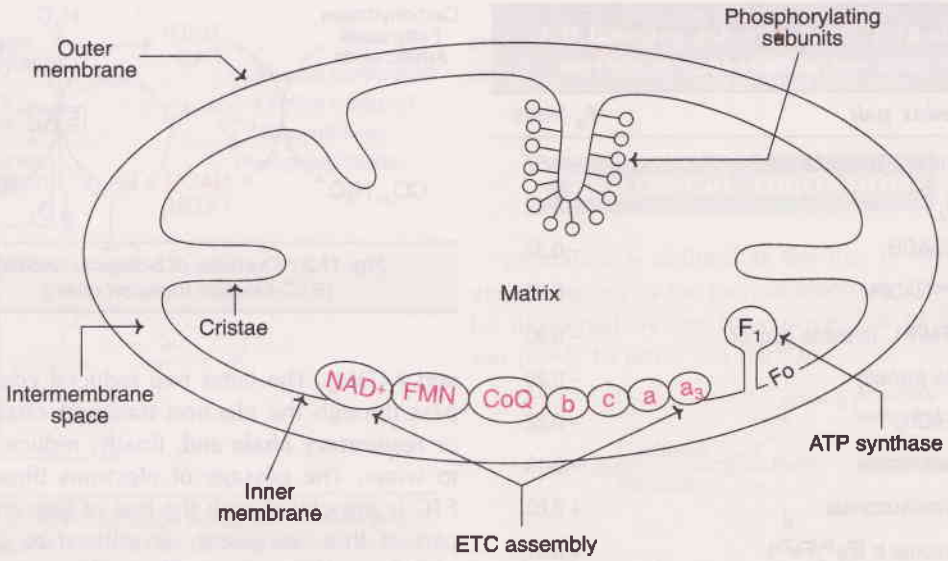


Fig. 11.5 : Structure of mitochondrion depicting electron transport chain (ETC) (F_o, F_1—Protein subunits).

Inner mitochondrial membrane : The electron transport chain and ATP synthesizing system are located on the inner mitochondrial membrane which is a specialized structure, rich in proteins. It is impermeable to ions (H^+, K^+, Na^+) and small molecules (ADP, ATP). This membrane is highly folded to form ***cristae***. The surface area of inner mitochondrial membrane is greatly increased due to cristae. The inner surface of the inner mitochondrial membrane possesses specialized particles (that look like lollipops), the ***phosphorylating subunits*** which are the centres for ATP production.

Mitochondrial matrix : The interior ground substance forms the matrix of mitochondria. It is rich in the ***enzymes*** responsible for the ***citric acid cycle, β-oxidation of fatty acids*** and ***oxidation of amino acids.***

Structural organization of respiratory chain

The inner mitochondrial membrane can be disrupted into five distinct respiratory or enzyme complexes, denoted as ***complex I, II, III, IV and V*** (***Fig.11.6***). The complexes I-IV are carriers of electrons while complex V is responsible for ATP synthesis. Besides these enzyme complexes, there are certain mobile electron carriers in the respiratory chain. These include NADH, coenzyme Q, cytochrome C and oxygen.

The enzyme complexes (I-IV) and the mobile carriers are collectively involved in the transport of electrons which, ultimately, combine with oxygen to produce water. The largest proportion of the oxygen supplied to the body is utilized by the mitochondria for the operation of electron transport chain.

Components and reactions of the electron transport chain

There are five distinct carriers that participate in the electron transport chain (ETC). These carriers are sequentially arranged (***Fig.11.7***) and are responsible for the transfer of electrons from a given substrate to ultimately combine with proton and oxygen to form water.

I. Nicotinamide nucleotides

Of the two coenzymes NAD^+ and $NADP^+$ derived from the vitamin niacin, NAD^+ is more actively involved in the ETC. NAD^+ is reduced to ***NADH + H^+*** by dehydrogenases with the removal of two hydrogen atoms from the substrate (AH_2). The substrates include

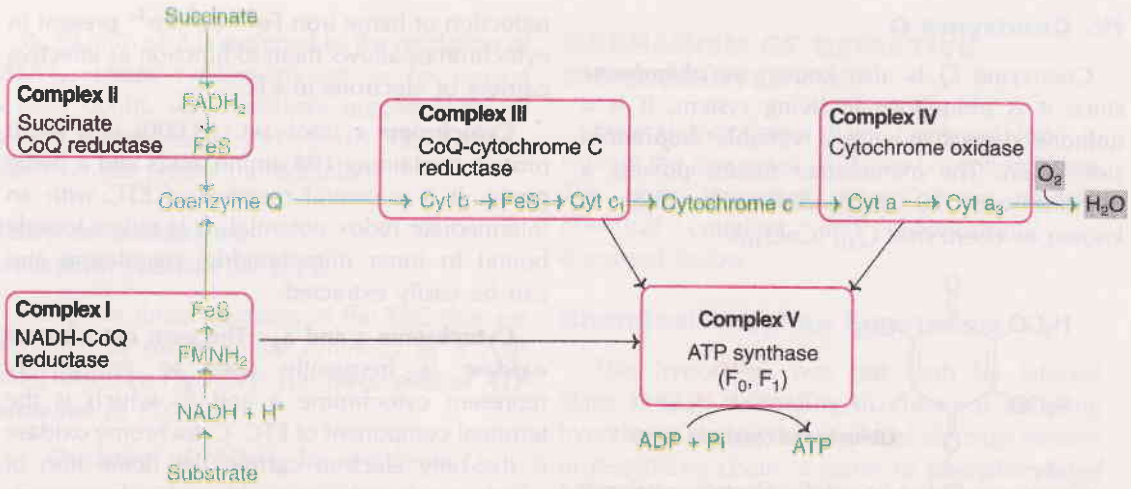


Fig. 11.6 : Multiprotein complexes in electron transport chain.

glyceraldehyde-3 phosphate, pyruvate, isocitrate, α-ketoglutarate and malate.

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

$NADPH + H^+$ produced by $NADP^+$-dependent dehydrogenase is not usually a substrate for ETC. NADPH is more effectively utilized for anabolic reactions (e.g. fatty acid synthesis, cholesterol synthesis).

II. Flavoproteins

The enzyme NADH dehydrogenase (NADH-coenzyme Q reductase) is a flavoprotein with FMN as the prosthetic group. The coenzyme FMN accepts two electrons and a proton to form $FMNH_2$. ***NADH dehydrogenase*** is a complex enzyme closely associated with non-heme iron proteins (NHI) or iron-sulfur proteins (FeS).

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

Succinate dehydrogenase (succinate-coenzyme Q reductase) is an enzyme found in the inner mitochondrial membrane. It is also a flavoprotein with FAD as the coenzyme.This can accept two hydrogen atoms ($2H^+ + 2e^-$) from succinate.

$$Succinate + FAD \longrightarrow Fumarate + FADH_2$$

III. Iron-sulfur proteins

The iron-sulfur (FeS) proteins exist in the oxidized (Fe^{3+}) or reduced (Fe^{2+}) state. About half a dozen FeS proteins connected with respiratory chain have been identified. However, the mechanism of action of iron-sulfur proteins in the ETC is not clearly understood.

One FeS participates in the transfer of electrons from FMN to coenzyme Q. Other FeS proteins associated with cytochrome b and cytochrome c_1 participate in the transport of electrons.

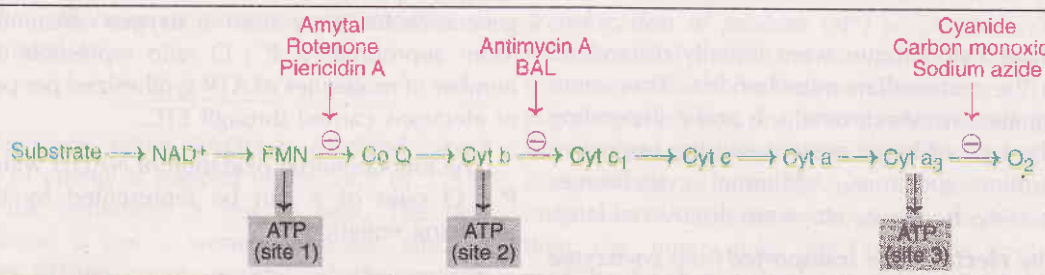
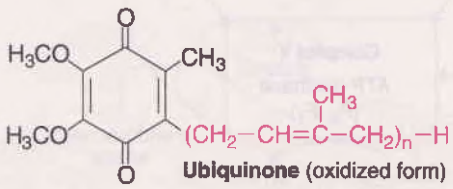


Fig. 11.7 : Electron transport chain with sites of ATP synthesis and inhibitors (BAL–British antilewisite).

IV. Coenzyme Q

Coenzyme Q is also known as ***ubiquinone*** since it is ubiquitous in living system. It is a quinone derivative with a variable ***isoprenoid*** side chain. The mammalian tissues possess a quinone with 10 isoprenoid units which is known as coenzyme Q_{10} (CoQ_{10}).



Ubiquinone (oxidized form)

Coenzyme Q is a lipophilic electron carrier. It can accept electrons from $FMNH_2$ produced in the ETC by NADH dehydrogenase or $FADH_2$ produced outside ETC (e.g. succinate dehydrogenase, acyl CoA dehydrogenase).

Coenzyme Q is not found in mycobacteria. Vitamin K performs similar function as coenzyme Q in these organisms. Coenzyme Q has no known vitamin precursor in animals. It is directly synthesized in the body. (Refer cholesterol biosynthesis, ***Chapter 14***)

V. Cytochromes

The cytochromes are conjugated proteins containing heme group. The latter consists of a porphyrin ring with iron atom. The heme group of cytochromes differ from that found in the structure of hemoglobin and myoglobin. The iron of heme in cytochromes is alternately oxidized (Fe^{3+}) and reduced (Fe^{2+}), which is essential for the transport of electrons in the ETC. This is in contrast to the heme iron of hemoglobin and myoglobin which remains in the ferrous (Fe^{2+}) state.

Three cytochromes were initially discovered from the mammalian mitochondria. They were designated as cytochrome a, b and c depending on the type of heme present and the respective absorption spectrum. Additional cytochromes such as c_1, b_1, b_2, a_3 etc. were discovered later.

The ***electrons*** are ***transported*** from ***coenzyme Q to cytochromes*** (in the order) ***b, c_1, c, a and a_3***. The property of reversible oxidation-reduction of heme iron $Fe^{2+} \rightleftharpoons Fe^{3+}$ present in cytochromes allows them to function as effective carriers of electrons in ETC.

Cytochrome c (mol. wt. 13,000) is a small protein containing 104 amino acids and a heme group. It is a central member of ETC with an intermediate redox potential. It is rather loosely bound to inner mitochondrial membrane and can be easily extracted.

Cytochrome a and a_3 : The term ***cytochrome oxidase*** is frequently used to collectively represent cytochrome a and a_3 which is the terminal component of ETC. Cytochrome oxidase is the only electron carrier, the heme iron of which can directly react with molecular oxygen. Besides heme (with iron), this oxidase also contains copper that undergoes oxidation-reduction ($Cu^{2+} \rightleftharpoons Cu^{+}$) during the transport of electrons.

In the final stage of ETC, the transported electrons, the free protons and the molecular oxygen combine to produce water.

OXIDATIVE PHOSPHORYLATION

The transport of electrons through the ETC is linked with the release of free energy. The process of ***synthesizing ATP from ADP and Pi coupled with the electron transport chain*** is known as oxidative phosphorylation. The complex V (***See Fig.11.6***) of the inner mitochondrial membrane is the site of oxidative phosphorylation.

P : O Ratio

The P : O ratio refers to the number of ***inorganic phosphate*** molecules utilized for ATP generation for every atom of ***oxygen*** consumed. More appropriately, P : O ratio represents the number of molecules of ATP synthesized per pair of electrons carried through ETC.

The mitochondrial oxidation of NADH with a P : O ratio of 3 can be represented by the following equation :

$$NADH + H^+ + \frac{1}{2}O_2 + 3ADP + 3Pi \longrightarrow NAD^+ + 3ATP + 4H_2O$$

P : O ratio of 2 is assigned to the oxidation of $FADH_2$. (***Note*** : Although yet to be proved beyond doubt, some workers suggest a P : O ratio of 2.5 for NADH + H^+, and 1.5 for $FADH_2$, based on the proton translocation).

Sites of oxidative phosphorylation in ETC

There are three reactions in the ETC that are exergonic to result in the synthesis of 3 ATP molecules (***See Fig.11.7***). The ***three sites of ATP formation*** in ETC are

1. Oxidation of $FMNH_2$ by coenzyme Q.
2. Oxidation of cytochrome b by cytochrome c_1.
3. Cytochrome oxidase reaction.

Each one of the above reactions represents a ***coupling site*** for ATP production. There are only two coupling sites for the oxidation of $FADH_2$ (P : O ratio 2), since the first site is bypassed.

Energetics of oxidative phosphorylation

The transport of electrons from redox pair $NAD^+/NADH$ ($E_0 = -0.32$) to finally the redox pair $\frac{1}{2}O_2/H_2O$ ($E_0 = +0.82$) may be simplified and represented in the following equation

$$\frac{1}{2}O_2 + NADH + H^+ \longrightarrow H_2O + NAD^+$$

The redox potential difference between these two redox pairs is 1.14 V, which is equivalent to an energy 52 Cal/mol.

Three ATP are synthesized in the ETC when NADH is oxidized which equals to 21.9 Cal (each ATP = 7.3 Cal).

The efficiency of energy conservation is calculated as

$$\frac{21{\cdot}9 \times 100}{52} = 42\%.$$

Therefore, when NADH is oxidized, about 42% of energy is trapped in the form of 3 ATP and the remaining is lost as heat. The heat liberation is not a wasteful process, since it allows ETC to go on continuously to generate ATP. Further, this heat is necessary to ***maintain body temperature.***

MECHANISM OF OXIDATIVE PHOSPHORYLATION

Several hypotheses have been put forth to explain the process of oxidative phosphorylation. The most important among them—namely, chemical coupling, and chemiosmotic—are discussed below.

Chemical coupling hypothesis

This hypothesis was put forth by Edward Slater (1953). According to chemical coupling hypothesis, during the course of electron transfer in respiratory chain, a series of ***phosphorylated high-energy intermediates*** are first produced which are utilized for the synthesis of ATP. These reactions are believed to be analogous to the substrate level phosphorylation that occurs in glycolysis or citric acid cycle. However, this hypothesis lacks experimental evidence, since all attempts, so far, to isolate any one of the high-energy intermediates have not been successful.

Chemiosmotic hypothesis

This mechanism, originally proposed by Peter Mitchell (1961), is now widely accepted. It explains how the transport of electrons through the respiratory chain is effectively utilized to produce ATP from ADP + Pi. The concept of chemiosmotic hypothesis is comparable with energy stored in a battery separated by positive and negative charges.

Proton gradient : The inner mitochondrial membrane, as such, is impermeable to protons (H^+) and hydroxyl ions (OH^-). The transport of electrons through ETC is coupled with the ***translocation of protons (H^+)*** across the inner mitochondrial membrane (coupling membrane) from the matrix to the intermembrane space. The pumping of protons results in an ***electrochemical or proton gradient.*** This is due to the accumulation of more H^+ ions (low pH) on the outer side of the inner mitochondrial membrane than the inner side (***Fig.11.8***). The proton gradient developed due to the electron flow in the respiratory chain is sufficient to result in the synthesis of ATP from ADP and Pi.

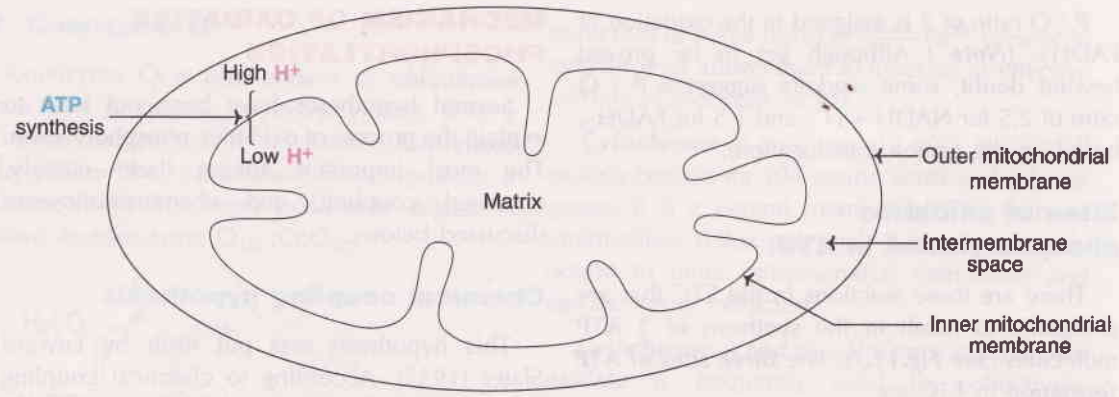


Fig. 11.8 : Outline of chemiosmotic hypothesis for oxidative phosphorylation.

Enzyme system for ATP synthesis : ATP synthase, present in the complex V, utilizes the proton gradient for the synthesis of ATP. This enzyme is also known as ***ATPase*** since it can hydrolyse ATP to ADP and Pi. ***ATP synthase*** is a complex enzyme and consists of two functional subunits, namely F_1 and F_0 (***Fig.11.9***). Its structure is comparable with 'lollipops'.

The protons that accumulate on the intermembrane space re-enter the mitochondrial matrix leading to the synthesis of ATP.

Rotary motor model for ATP generation

Paul Boyer in 1964 proposed (Nobel Prize, 1997) that a conformational change in the

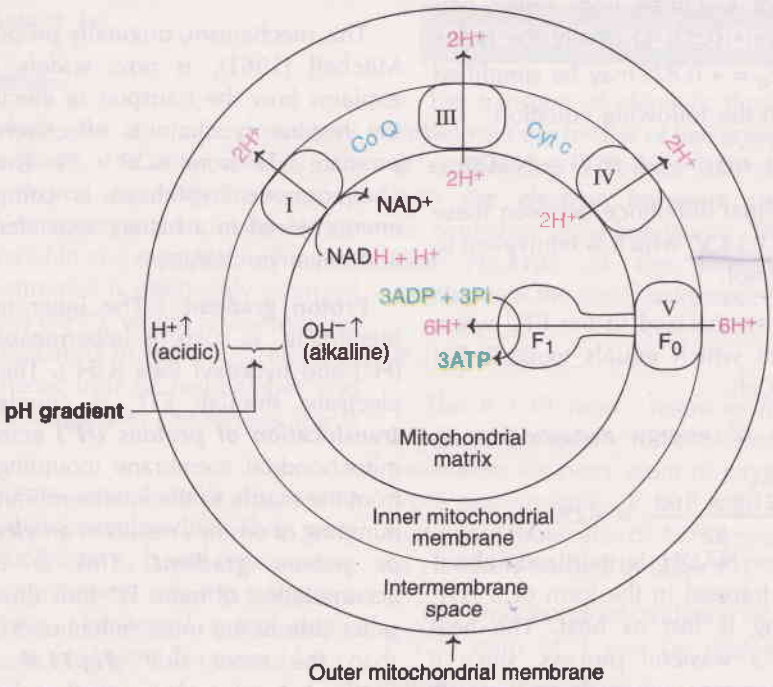


Fig. 11.9 : Diagrammatic representation of chemiosmotic hypothesis for oxidative phosphorylation (I, III, IV and V–Respiratory chain complexes; F_o, F_1–Protein subunits for phosphorylation).

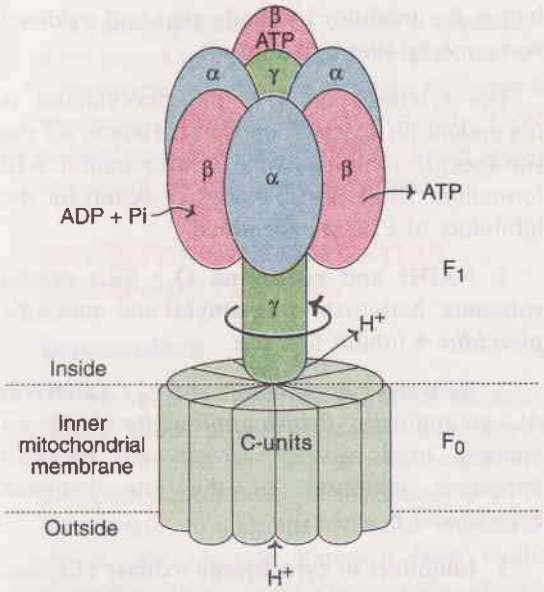


Fig. 11.10 : Structure of mitochondrial ATP synthase (F_0F_1) complex (C units-channel protein subunits; α, β, and γ are the subunits of F_1-ATP synthase).

mitochondrial membrane proteins leads to the synthesis of ATP. The original Boyer hypothesis, now considered as ***rotary motor/engine driving model*** or ***binding change model***, is widely accepted for the generation ATP.

The enzyme ATP synthase is F_0F_1 complex (of complex V). The F_0 subcomplex is composed of channel protein 'C' subunits to which F_1-ATP synthase is attached (***Fig.11.10***). F_1-ATP synthase consists of a central γ subunit surrounded by alternating α and β subunits (α_3 and β_3).

In response to the proton flux, the γ subunit physically rotates. This induces conformational changes in the β_3 subunits that finally lead to the release of ATP.

According to the binding change mechanism, the three β subunits of F_1-ATP synthase adopt different conformations. One subunit has ***open*** (***O***) conformation, the second has ***loose*** (***L***) conformation while the third one has ***tight*** (***T***) ***conformation*** (***Fig.11.11***).

By an unknown mechanism, protons induce the rotation of γ subunit, which in turn induces conformation changes in β subunits. The substrates ADP and Pi bind to β subunit in L-conformation. The L site changes to T conformation, and this leads to the synthesis of ATP. The O site changes to L conformation which binds to ADP and Pi. The T site changes to O conformation, and releases ATP. This cycle of conformation changes of β subunits is repeated. And three ATP are generated for each revolution (***Fig.11.11***).

It may be noted that the ATP release in O conformation is energy dependent (and not ATP synthesis) and very crucial in rotary motor model for ATP generation.

The enzyme ATP synthase acts as a proton-driving motor, and is an example of rotary catalysis. Thus, ***ATP synthase*** is the world's smallest ***molecular motor.***

Inherited disorders of oxidative phosphorylation

It is estimated that about 100 polypeptides are required for oxidative phosphorylation. Of these, 13 are coded by mitochondrial DNA (mtDNA) and synthesized in the mitochondria, while the rest are produced in the cytosol (coded by nuclear DNA) and transported. ***mtDNA is***

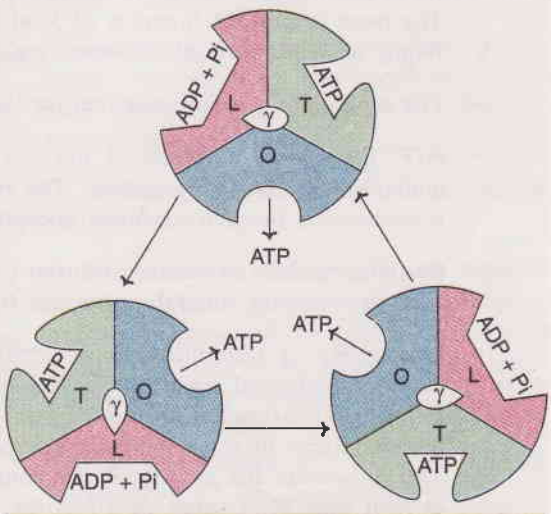


Fig. 11.11 : The binding change model (rotary motor/ engine driving model) for ATP synthesis by F_1-ATP synthase.

maternally inherited since mitochondria from the sperm do not enter the fertilized ovum.

Mitochondrial DNA is about 10 times more susceptible to mutations than nuclear DNA. mtDNA mutations are more commonly seen in tissues with high rate of oxidative phosphorylation (e.g. central nervous system, skeletal and heart muscle, liver).

Leber's hereditary optic neuropathy is an example for mutations in mtDNA. This disorder is characterized by loss of bilateral vision due to neuroretinal degeneration.

Inhibitors of electron transport chain

Many site-specific inhibitors of ETC have contributed to the present knowledge of mitochondrial respiration. Selected examples of these inhibitors haven been given in ***Fig.11.7***. The inhibitors bind to one of the components of ETC and block the transport of electrons. This causes the accumulation of reduced components before the inhibitor blockade step and oxidized components after that step.

The synthesis of ATP (phosphorylation) is dependent on electron transport. Hence, all the site-specific inhibitors of ETC also inhibit ATP formation. Three possible sites of action for the inhibitors of ETC are identified

1. **NADH and coenzyme Q :** Fish poison ***rotenone***, barbituate drug ***amytal*** and antibiotic ***piercidin A*** inhibit this site.

2. **Between cytochrome b and c_1 :** ***Antimycin A*** —an antibiotic, ***British antilewisite (BAL)***—an antidote used against war-gas—are the two important inhibitors of the site between cytochrome b and c_1.

3. **Inhibitors of cytochrome oxidase :** Carbon monoxide, cyanide, hydrogen sulphide and azide effectively inhibit cytochrome oxidase. ***Carbon monoxide*** reacts with reduced form of the cytochrome while ***cyanide*** and ***azide*** react with oxidized form.

BIOMEDICAL / CLINICAL CONCEPTS

- *The most important function of food is to supply energy to the living cells. This is finally achieved through biological oxidation.*
- *The supply of O_2 is very essential for the survival of life (exception—anaerobic bacteria).*
- *ATP, the energy currency of the cell, acts as a link between the catabolism and anabolism in the living system. The major production of body's ATP occurs in the mitochondria through oxidative phosphorylation coupled with respiration.*
- *Respiratory chain or electron transport chain (ETC) is blocked by site specific inhibitors such as rotenone, amytal, antimycin A, BAL, carbon monoxide and cyanide.*
- *Uncoupling of respiration from oxidative phosphorylation under natural conditions assumes biological significance. The brown adipose tissue, rich in electron carriers, brings about oxidation uncoupled from phosphorylation. The presence of active brown adipose tissue in some individuals is believed to protect them from becoming obese. This is because the excess calories consumed by these people are burnt and liberated as heat instead of being stored as fat.*
- *Inherited disorders of oxidative phosphorylation caused by the mutations in mitochondrial DNA have been identified e.g. Leber's hereditary optic neuropathy.*

Cyanide poisoning : Cyanide is probably the most potent inhibitor of ETC. It binds to Fe^{3+} of cytochrome oxidase blocking mitochondrial respiration leading to cell death. Cyanide poisoning causes death due to tissue asphyxia (mostly of central nervous system).

INHIBITORS OF OXIDATIVE PHOSPHORYLATION

Uncouplers

The mitochondiral transport of electrons is tightly coupled with oxidative phosporylation (ATP synthesis). In other words, oxidation and phosphorylation proceed simultaneously. There are certain compounds that can uncouple (or delink) the electron transport from oxidative phosphorylation. Such compounds, known as uncouplers, increase the permeability of inner mitochondrial membrane to protons (H^+). The result is that ATP synthesis does not occur. The energy linked with the transport of electrons is dissipated as heat. ***The uncouplers allow*** (often at accelerated rate) ***oxidation of substrates*** (via NADH or $FADH_2$) ***without ATP formation.***

The uncoupler, ***2,4-dinitrophenol (DNP)***, has been extensively studied. It is a small lipophilic molecule. DNP is a proton-carrier and can easily diffuse through the inner mitochondrial membrane. In the people seeking to lose weight, DNP was used as a drug. However, this is now discontinued, as it produces hyperthermia and other side effects. In fact, Food and Drug Administration (USA) has banned the use of DNP.

The other uncouplers include dinitrocresol, pentachlorophenol, trifluorocarbonylcyanide phenylhydrazone (FCCP). The last compound (FCCP) is said to be 100 times more effective as an uncoupler than dinitrophenol. When administered ***in high doses***, the drug ***aspirin*** acts as an uncoupler.

Physiological uncouplers : Certain physiological substances which act as uncouplers at higher concentration have been identified. These include ***thermogenin***, ***thyroxine*** and ***long chain free fatty acids***. The unconjugated bilirubin is also believed to act as an uncoupler. This is, however, yet to be proved beyond doubt.

Significance of uncoupling

Uncoupling of respiration from oxidative phosphorylation under natural conditions assumes biological significance. The maintenance of body temperature is particularly important in hairless animals, hibernating animals and the animals adapted to cold. These animals possess a specialized tissue called ***brown adipose tissue*** in the upper back and neck portions. The mitochondria of brown adipose tissue are rich in electron carriers and are specialized to carry out an ***oxidation uncoupled from phosphorylation***. This causes liberation of heat when fat is oxidized in the brown adipose tissue. Brown adipose tissue may be considered as a site of ***non-shivering thermogenesis***. The presence of active brown adipose tissue in certain individuals is believed to protect them from becoming obese. The excess calories consumed by these people are burnt and ***liberated as heat***, instead of being stored as fat.

Thermogenin (or uncoupling protein, UCP) is a natural uncoupler located in the inner mitochondrial membrane of brown adipose tissue. It acts like an uncoupler, blocks the formation of ATP, and liberates heat.

Ionophores : The term 'ionophores' is used to collectively represent the lipophilic substances that promote the transport of ions across biological membranes.

All the uncouplers (described above) are, in fact, proton ionophores.

The antibiotics ***valinomycin*** and ***nigercin*** act as ionophores for K^+ ions. Both these compounds are also capable of dissipating proton gradient across the inner mitochondrial membrane and inhibit oxidative phosphorylation.

Other inhibitors of oxidative phosphorylation

Oligomycin : This antibiotic prevents the mitochondrial oxidation as well as phosphorylation. It ***binds with the enzyme ATP***

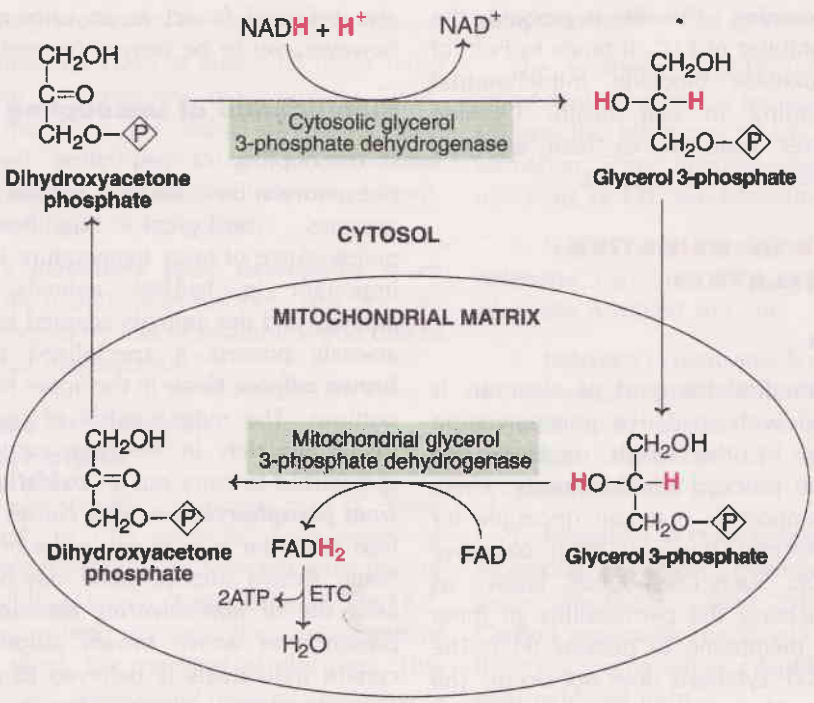


Fig. 11.12 : Glycerol-phosphate shuttle (reducing equivalents transported are shown in Blue).

synthase and blocks the proton (H^+) channels. It thus prevents the translocation (re-entry) of protons into the mitochondrial matrix. Due to this, protons get accumulated at higher concentration in the intermembrane space. Electron transport (respiration) ultimately stops, since protons cannot be pumped out against steep proton gradients.

Atractyloside : This is a plant toxin and inhibits oxidative phosphorylation by an indirect mechanism. Adenine nucleotide carrier system facilitates the transport of ATP and ADP. Atractyloside inhibits adenine nucleotide carrier and, thus, ***blocks the adequate supply of ADP,*** thereby preventing phosphorylation.

TRANSPORT OF REDUCING EQUIVALENTS—SHUTTLE PATHWAYS

The inner mitochondrial membrane is impermeable to NADH. Therefore, the NADH produced in the cytosol cannot directly enter the mitochondria. Two pathways—namely ***glycerol-phosphate shuttle*** and ***malate-aspartate shuttle***—are operative to do this job. They transport the reducing equivalents from cytosol to mitochondria and not vice versa.

I. Glycerol-phosphate shuttle

Cytosolic glycerol 3-phosphate dehydrogenase oxidizes NADH to NAD^+. The reducing equivalents are transported through glycerol 3-phosphate into the mitochondria. Glycerol 3-phosphate dehydrogenase—present on outer surface of inner mitochondrial membrane—reduces FAD to $FADH_2$. Dihydroxyacetone phosphate escapes into the cytosol and the shuttling continues as depicted in ***Fig.11.12.*** $FADH_2$ gets oxidized via ETC to generate ***2 ATP.***

II. Malate-aspartate shuttle

In the cytosol, oxaloacetate accepts the reducing equivalents (NADH) and becomes malate. Malate then enters mitochondria where it is oxidized by mitochondrial malate dehydro-

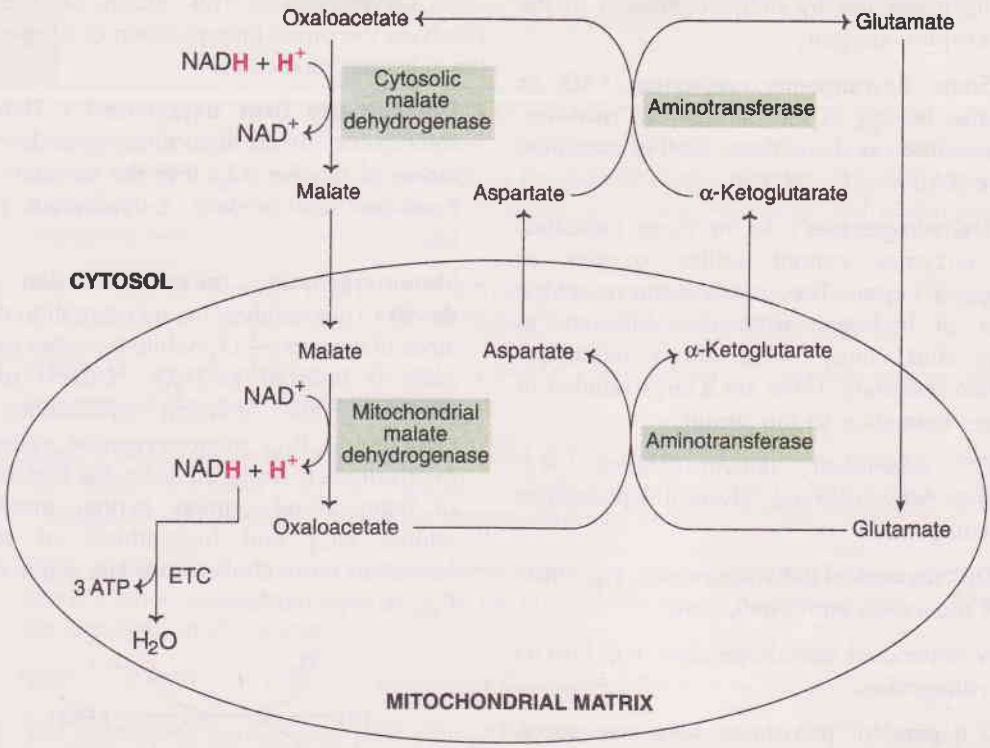


Fig. 11.13 : Malate-aspartate shuttle.

genase. In this reaction, NADH and oxaloacetate are regenerated. NADH gets oxidized via electron transport chain and ***3 ATP*** are produced. This is in contrast to glycerol-phosphate shuttle where only 2 ATP are produced.

In the mitochondria, oxaloacetate participates in transamination reaction with glutamate to produce aspartate and α-ketoglutarate. The aspartate enters the cytosol and transaminates with α-ketoglutarate to give oxaloacetate and glutamate. The malate-aspartate shuttle is shown in ***Fig.11.13.***

Shuttle pathways and tissues

Liver and heart utilize malate-aspartate shuttle, and yield 3 ATP per mole of NADH. Most of the other tissues, however, employ glycerol-phosphate shuttle and liberate 2 ATP from NADH.

ENZYMES INVOLVED IN BIOLOGICAL OXIDATION

All the enzymes participating in biological oxidation belong to the class ***oxidoreductases.*** These are further grouped into four categories

1. Oxidases
2. Dehydrogenases
3. Hydroperoxidases
4. Oxygenases.

1. **Oxidases :** These enzymes catalyse the elimination of hydrogen from the substrates which is accepted by oxygen to form mostly water, e.g. cytochrome oxidase, tyrosinase, monoamine oxidase (H_2O_2 formed instead of H_2O).

Cytochrome oxidase, the terminal component of electron transport chain, transfers electrons (obtained from the oxidation of

substrate molecules by dehydrogenases) to the final acceptor, oxygen.

Some flavoproteins containing FAD or FMN also belong to the category of oxidases. e.g., L-amino acid oxidase (FMN), xanthine oxidase (FAD).

2. **Dehydrogenases :** As the name indicates, these enzymes cannot utilize oxygen as hydrogen acceptor. They catalyse the reversible transfer of hydrogen from one substrate to another and, thus, bring about oxidation-reduction reactions. There are a large number of enzymes belonging to this group

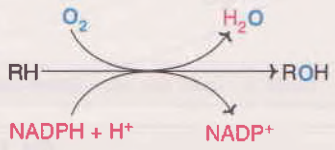
- NAD^+ dependent dehydrogenases, e.g. alcohol dehydrogenase, glycerol 3-phosphate dehydrogenase.
- $NADP^+$ dependent dehydrogenases, e.g. HMG CoA reductase, enoyl reductase.
- FMN dependent dehydrogenases, e.g. NADH dehydrogenase.
- FAD dependent dehydrogenases, e.g. succinate dehydrogenase, acyl CoA dehydrogenase.
- The cytochromes : All the cytochromes of electron transport chain (b, c_1 and c) except the terminal cytochrome oxidase ($a+a_3$) belong to this group.

3. **Hydroperoxidases :** Hydrogen peroxide is the substrate for these enzymes. There is a constant production of H_2O_2 in the reactions catalysed by the aerobic dehydrogenases. The harmful effects of H_2O_2 are prevented by hydroperoxidases, e.g. peroxidase and catalase.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

4. **Oxygenases :** This group of enzymes catalyses the direct incorporation of oxygen into the substrate molecules.

- **Dioxygenases (true oxygenases) :** They are responsible for the incorporation of both the atoms of oxygen (O_2) into the substrate, e.g. homogentisate oxidase, L-tryptophan pyrrolase.
- **Monooxygenases (mixed function oxidases) :** They catalyse the incorporation of one atom of oxygen ($\frac{1}{2}O_2$) while the other oxygen atom is reduced to H_2O. NADPH usually provides the reducing equivalents, e.g. cytochrome P_{450} monooxygenase system of microsomes is responsible for the metabolism of many drugs (amino pyrine, morphine, aniline etc.) and biosynthesis of steroid hormones (from cholesterol). The action of Cyt P_{450} is depicted here.



Electron transport in prokaryotes

In contrast to eukaryotes, the prokaryotes lack mitochondria. However, prokaryotes possess a separate system for biological oxidation. A set of electron carriers (different from that found in mitochondria) and enzymes of oxidative phosphorylation are bound to the inner cell membrane in prokaryotes. This arrangement of oxidative machinery is one of the reasons to believe that mitochondria of higher organisms have descended from prokaryotic cells.

SUMMARY

1. *Bioenergetics deals with the study of energy changes in biochemical reactions. Change in free energy (ΔG) is valuable in predicting the feasibility of a reaction. A negative and a positive ΔG, respectively, represent an exergonic (energy-releasing) and endergonic (energy-consuming) reactions.*

2. *High-energy compounds (ΔG > –7.0 Cal/mol) play a crucial role in the energy transfer of biochemical reactions (e.g. ATP, phosphocreatine, phosphoenolpyruvate).*

3. *ATP is the energy currency of the cell. ATP-ADP cycle acts as a connecting energy link between catabolic and anabolic reactions.*

4. *Respiratory chain or electron transport chain (ETC) located in the inner mitochondrial membrane represents the final stage of oxidizing the reducing equivalents (NADH and $FADH_2$) derived from the metabolic intermediates to water.*

5. *ETC is organized into five distinct complexes. The complexes I to IV are electron carriers while complex V is responsible for ATP production. The components of ETC are arranged in the sequence*

   $$NAD^+ \longrightarrow FMN \longrightarrow CoQ \longrightarrow Cyt\ b \longrightarrow Cyt\ c_1 \longrightarrow Cyt\ c \longrightarrow Cyt\ a + a_3 \longrightarrow O_2$$

6. *The process of synthesizing ATP from ADP and Pi coupled with ETC is known as oxidative phosphorylation. NADH oxidation with a P : O ratio 3 indicates that 3 ATP are synthesized while $FADH_2$ oxidation (P : O ratio 2) results in the production of 2 ATP.*

7. *Among the hypotheses put forth to explain the mechanism of oxidative phosphorylation, the chemiosmotic hypothesis (of Mitchell) is widely accepted. The rotary motor model (of Boyer) involving the conformation changes in the β-subunits of ATP synthase explains the ATP generation.*

8. *NADH produced in the cytosol cannot directly enter mitochondria. Glycerol-phosphate shuttle (generates 2 ATP) and malate-asparate shuttle (generates 3 ATP) operate to overcome the difficulty.*

9. *There are many inhibitors of electron transport chain (rotenone, amytal, antimycin, CO,-CN, H_2S etc.) and oxidative phosphorylation (oligomycin, atractyloside). Uncouplers (e.g. dinitrophenol) are the substances that delink ETC from oxidative phosphorylation.*

10. *The enzymes participating in biological oxidation belong to the class oxidoreductases. There are five groups, namely oxidases, aerobic dehydrogenases, anaerobic dehydrogenases, hydroperoxidases and oxygenases.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Write an account of the high-energy compounds in metabolism
2. Describe the components of electron transport chain and discuss the oxidation of NADH.
3. Define oxidative phosphorylation. Discuss chemiosmotic hypothesis in detail.
4. Give an account of the enzymes involved in biological oxidation.
5. Discuss about the inhibitors of ETC and oxidative phosphorylation.

II. Short notes

(a) High-energy bonds, (b) Uncouplers, (c) P : O ratio, (d) Redox loops, (e) ATP synthase, (f) Cytochromes, (g) Sites of oxidative phosphorylation, (h) Coenzyme Q, (I) Redox potential, (j) ATP as energy currency.

III. Fill in the blanks

1. The relation between the change of free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) is expressed by the equation _____.
2. A negative sign of free energy indicates that the reaction is _____.
3. The bonds responsible for a majority of high-energy compounds are _____.
4. The storage form of high-energy compound in invertebrates is _____.
5. A more negative redox potential represents a greater tendency to lose _____.
6. The electron transport chain is located in _____.
7. The prosthetic group present in cytochromes _____.
8. The component of electron transport chain which can directly react with molecular oxygen _____.
9. The site of ETC inhibited by cyanide _____.
10. Superoxide is converted to H_2O_2 by the enzyme _____.

IV. Multiple choice questions

11. Name the compound with the greatest standard free energy.

    (a) ATP (b) Phosphocreatine (c) Cyclic AMP (d) Phosphoenolpyruvate.

12. One of the following components of ETC possesses isoprenoid units

    (a) Coenzyme Q (b) Cytochrome (c) Cytochrome b (d) Non-heme iron.

13. The P : O ratio for the oxidation of $FADH_2$ is

    (a) 1 (b) 2 (c) 3 (d) 4.

14. Inner mitochondrial membrane is impermeable to

    (a) H^+ (b) K^+ (c) OH^- (d) All of them.

15. ATP synthase activity is associated with the mitochondrial enzyme complex

    (a) V (b) III (c) IV (d) I.