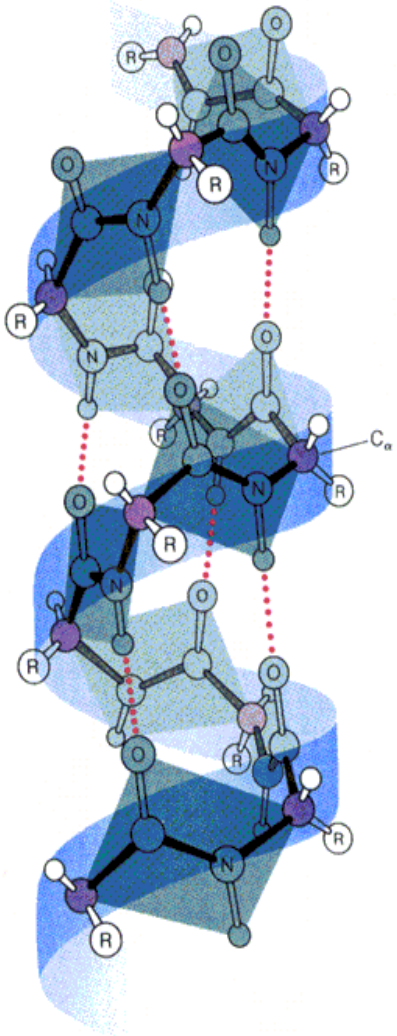
E.O. DANCHENKO

BIOCHEMISTRY



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Danchenko E.O.
Д 19 Biochemistry: course of lectures. Gomel: VSMU Press, 2004. – 360 p., 119 fig., 9 tab.

The course of the lectures is designed for students of higher medical educational establishments. The lectures can be used as an integrated curriculum in which ready access to biochemical information is required.

The course of lectures corresponds with typical educational plan and program proved by Ministry of Health Care of Republic of Belarus.

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LECTURE 1

INTRODUCTION TO BIOCHEMISTRY. STRUCTURE AND FUNCTION OF PROTEINS

Biochemistry seeks to describe the structure, organization, and functions of living matter in molecular terms. It can be divided into three principal areas:

1. The structural chemistry of the components of living matter and the relationship of biological function to chemical structure.

2. Metabolism — the totality of chemical reactions that occur in living matter.

3. The chemistry of processes and substances that store and transmit biological information.

The medical students who acquire a sound knowledge of biochemistry will be in a position to confront, in practice and research, the 2 central concerns of the health sciences: (1) the understanding and **maintenance of health** and (2) the understanding and effective **treatment of disease.**

The major **objective** of biochemistry is the complete understanding at the molecular level of all of the chemical processes associated with living cells. To achieve this objective, biochemists have sought to **isolate the numerous molecules** found in cells, **determine their structures, and analyze how they function.**

All diseases are manifestations of **abnormalities of molecules, chemical reactions, or processes**. The major factors responsible for causing diseases in animals and humans are follows

1. **Physical agents:** Mechanical trauma, extremes of temperature, sudden changes in atmospheric pressure, radiation, electric shock.
2. **Chemical agents and drugs:** Certain toxic compounds, therapeutic drugs, etc.
3. **Biologic agents:** Viruses, rickettsiae, bacteria, fungi, higher forms of parasites.
4. **Oxygen lack:** Loss of blood supply, depletion of the oxygen-carrying capacity of the blood, poisoning of the oxidative enzymes.
5. **Genetic:** Congenital, molecular.
6. **Immunologic reactions:** Anaphylaxis, autoimmune disease.
7. **Nutritional imbalances:** Nutritional deficiencies, nutritional excesses.
8. **Endocrine imbalances:** Hormonal deficiencies, hormonal excesses.

HISTORY OF BIOCHEMISTRY

Biochemistry's roots as a distinct field of study date to the early **19th century**, with the pioneering work of **Friedrich Wöhler**. Prior to that time, it was believed that the substances in living matter were somehow qualitatively different from those in nonliving matter and did not behave according to the known laws of physics and chemistry. In **1828 Wöhler** showed that **urea**, a substance of biological origin, could be synthesized in the laboratory from the inorganic compound ammonium cyanate.

Chromosomes were discovered in **1875** by **Walter Flemming** and identified as genetic elements by **1902**. The development of the electron microscope, between about **1930** and **1950,** provided a whole new level of insight into cellular structure. With it, subcellular organelles like mitochondria and chloroplasts could be studied, and it was realized that specific biochemical processes were localized in these subcellular particles.

Nucleic acids had been isolated in **1869** by **Friedrich Miescher**, but their chemical structures were poorly understood, and in the early **1900s** they were thought to be simple substances, fit only for structural roles in the cell. The idea of the gene, a unit of hereditary information, was first proposed in the mid-nineteenth century by **Gregor Mendel**. By about **1900**, cell biologists realized that genes must be found in chromosomes, which are composed of proteins and nucleic acids. Most biochemists believed that only the proteins were structurally complex enough to carry genetic information.

That belief was dead wrong. Experiments in the **1940s** and early **1950s** proved conclusively that deoxyribonucleic acid (DNA) is the bearer of genetic information. One of the most important advances in the history of science occurred in **1953**, when James Watson and Francis Crick described the double-helical structure of DNA. This concept immediately suggested ways in which information could be encoded in the structure of molecules and transmitted intact from one generation to the next.

Biochemistry draws its major themes from

1. Organic chemistry, which describes the properties of biomolecules.

2. Biophysics, which applies the techniques of physics to study the structures of biomolecules.

3. Medical research, which increasingly seeks to understand disease states in molecular terms.

4. Nutrition, which has illuminated metabolism by describing the dietary requirements for maintenance of health.

5. Microbiology, which has shown that single-celled organisms and viruses are ideally suited for the elucidation of many metabolic pathways and regulatory mechanisms.

6. Pharmacology and pharmacy rest on a sound knowledge of biochemistry and physiology; in particular, most drugs are metabolized by enzyme-catalyzed reactions.

7. Physiology which investigates life processes at the tissue and organism levels.

8. Cell biology, which describes the biochemical division of labor within a cell.

9. Genetics, which describes mechanisms that give a particular cell or organism its biochemical identity.

USES OF BIOCHEMISTRY

1. The results of **biochemical research** are used extensively in the world outside the laboratory — in **agriculture, medical sciences, nutrition, and many other fields**. In clinical chemistry, **biochemical measurements** on people help **diagnose illnesses** and **monitor responses** to treatment.

2. Pharmacology and **toxicology** are concerned with the effects of external chemical substances on metabolism. Drugs and poisons usually act by interfering with specific metabolic pathways.

3. A particularly exciting prospect in contemporary **biochemistry** is that of creating so-called **designer drugs**. If the target site for action of a drug is a protein enzyme or receptor, determining the detailed molecular structure of that target allows us to design inhibitors that bind to it with great selectivity.

4. Herbicides and **pesticides**, in many instances, act in similar ways — by blocking enzymes or receptors in the target organism. **Biochemistry** is involved in understanding the actions of herbicides and pesticides, in increasing their selectivity, and in understanding and dealing with mechanisms by which target organisms become resistant to them. Thus, **biochemistry** has become an important component of environmental science.

BIOCHEMISTRY AS A CHEMICAL SCIENCE

In order to understand the impact of biochemistry on biology, one must understand the **chemical elements** of living matter and the complete structures of many biological compounds — **amino acids**, **sugars, lipids**, **nucleotides, vitamins, and hormones** — and their behavior during metabolic reactions. Essential understanding of biochemistry requires knowledge of the stoichiometry and mechanisms of a large number of reactions. In addition, an understanding of the basic thermodynamic principles is essential for learning how plants derive energy from sunlight (photosynthesis) and how animals derive energy from food (catabolism).

Living creatures on the earth are composed mainly of a very few elements, principally carbon, hydrogen, oxygen, and nitrogen (**C, H, O, N**). Life is not built on these four elements alone. Many other elements are necessary for terrestrial organisms. A "second tier" of essential elements includes sulfur and phosphorus, which form covalent bonds, and the ions Na^+, K^+, Mg^{2+}, Ca^{2+}, and Cl^-.

PROTEINS

Proteins are the most abundant organic molecules of the living system. They occur in every part of the cell and constitute about 50% of cellular dry weight. Proteins form the fundamental basis of structure and function of life.

The term “protein” is derived from a *Greek* word “**proteios**”, meaning “holding the first place”. **Berzelius** (Swedish chemist) suggested the name proteins to the group of organic compounds that are utmost important to life. **Mudler** (Dutch

chemist) in 1838 used the term "proteins" for the high molecular weight nitrogen-rich and most abundant substances present in animals and plants.

Function of proteins

Proteins perform a great variety of specialized and essential functions in the living cells. These functions may be broadly grouped as **static** (structural) and **dynamic**.

1. Structural functions. Certain proteins perform "brick and mortal" roles and are primarily responsible for structure and strength of body. These include **collagen** and **elastin** found in bone matrix, vascular system and other organ and **α-keratin** present in epidermal tissues.

2. Dynamic function. The dynamic functions of proteins are more diversified in nature. These include proteins acting as **enzyme, hormones, blood clotting factors, immunoglobulins**, membrane receptors, storage proteins, besides their function in genetic control, muscle contraction, respiration etc.

Elemental composition of proteins

Proteins are predominantly constituted by five major elements in the following proportion: carbon — 50—55%, hydrogen — 6—7.3%, oxygen — 19—24%, nitrogen — 13—19%, sulfur — 0—4%. Besides the above, proteins may also contain other elements such as P, Fe, Cu, I, Mg, Mn, Zn etc. The content of **nitrogen**, an essential component of proteins, on an average is 16%.

Structure

1. Proteins are linear, unbranched polymers constructed from 20 different α-amino acids that are encoded in the DNA of the genome.

2. All living organisms use the same 20 amino acids and, with few exceptions, the same genetic code.

Size

Proteins are diverse in size. The mass of single-chain proteins is typically 10-50 kilodaltons (kdal), although proteins as small as 350 dal and greater than 1000 kdal are known to exist. Multichain protein complexes of greater than 200 kdal are frequently encountered.

Protein classification

Proteins are classified in several ways. There are three major types of classifying proteins based on their function, chemical nature and solubility properties and nutritional importance.

1. Functional classification of proteins.

a. Enzymes or catalytic proteins (most enzymes are proteins).

b. Transport proteins (hemoglobin, serum albumin).

c. Structural proteins (keratin of hair and nails, collagen of bone).

d. Hormonal proteins (insulin, growth hormone).

e. Contractile proteins (actin, myosin).

f. Storage proteins (ovalbumin, glutelin).

g. Genetic proteins (nucleoproteins).

h. Defense proteins (snake venoms, immunoglobulins)

i. Receptors proteins for hormones, viruses.

2. Protein classification based on chemical nature and solubility.

This is a more comprehensive and popular classification of proteins. It is based on the amino acid composition, structure, shape and solubility properties. Proteins are classified into 3 major groups: simple, conjugated or derived:

a. Simple protein (such as serum albumin and keratin) contains **only amino acids.**

(1) Fibrous proteins are long **rod-shaped** molecules that are **insoluble** in water and physically tough. They have **structural and protective functions**.

(2) Globular proteins are compact **spherical** molecules that are usually **water-soluble**. Globular proteins have **dynamic functions** (for example, enzymes, immunoglobulins and the transport proteins hemoglobin and albumin).

b. Conjugated protein consists of a simple protein combined with a nonprotein component. The nonprotein component is called a **prosthetic group (or conjugated group)**. A protein without its prosthetic group is called an **apoprotein.** A protein molecule combined with its prosthetic group is referred to as a **holoprotein.** Prosthetic groups typically play an important, even crucical role in the function of individual protein. Conjugated proteins are classified according to the nature of their prostetic groups: **glycoproteins** (contain a carbohydrate component), **lipoproteins** (contain lipid molecules), **nucleoproteins** (contain nucleic acids), **metalloproteins** (contain metal ion), **phosphoproteins** (contain phosphate groups), **hemoproteins** (contain heme groups).

c. Derived proteins are the denatured or degraded products of simple and conjugated proteins.

3. Nutritional classification of protein. The nutritive value of proteins is determined by the composition of essential amino acids. From the nutritional point of view, proteins are classified into 3 categories:

a. Complete proteins. These proteins have all the ten essential amino acids in the required proportion by the human body to promote good growth, e.g. egg albumin, milk casein.

b. Partially incomplete proteins. These proteins are partially lacking one or more essential amino acids and hence can promote moderate growth, e.g. wheat and rice proteins (limiting Lys, Thr).

c. Incomplete proteins. These proteins completely lack one or more essential amino acids. Hence they do not promote growth at all e.g. gelatin (lacks Trp), zein (lack Trp, Lys).

AMINO ACIDS

Amino acids are the fundamental units of proteins. **1**. Amino acids are composed of an **amino group** (— NH_2), a **carboxyl group** (—COOH), a hydrogen atom, and a distinctive **side chain,** all bonded to a carbon atom (the **α-carbon)**. Side groups are what distinguish the **α-amino acids** from each other. One of the 20 amino acids, **proline,** is an **imino acid (—NH—), not** an **α amino acid** as are the other 19.

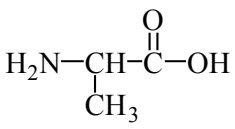
As many as 300 amino acids occur in nature. Of these, only **20** — known as **standard amino** acids are repeatedly found in the structure of proteins, isolated from different forms of life — animals, plant and microbial. This is because of the **universal nature** of the genetic code available for the incorporation of only 20 amino acids when the proteins are synthesized in the cells. After the synthesis of proteins, some of the incorporated amino acids undergo modifications to form their derivatives.

Classification of amino acids

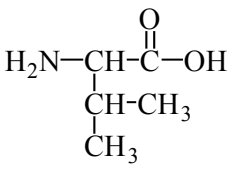
There are different ways of classifying the amino acids based on the tructure and chemical nature, nutritional requirement, metabolic fate etc.

Classification of amino acids based on polarity.

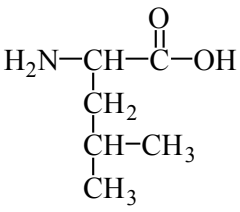
1. Non-polar amino acids (alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, praline). These amino acids are also referred to **hydrophobic**. They have no charge on the R group.



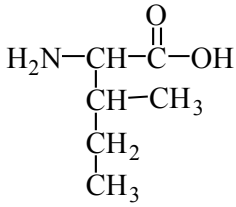
Alanine (Ala)



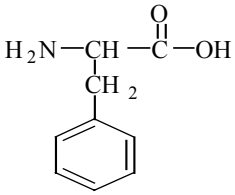
Valine (Val)



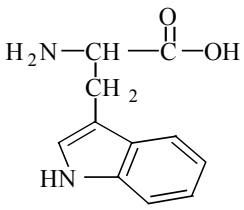
Leucine (Leu)



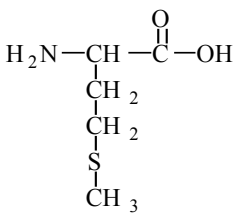
Isoleucine (Ile)



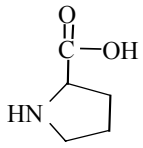
Phenylalanine (Phe)



Tryptophan (Trp)

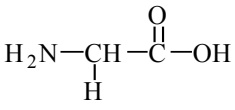


Methionine (Met)

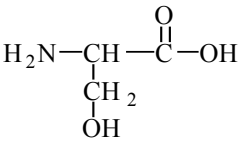


Proline (Pro)

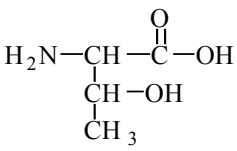
2. Polar hydrophilic non-charge amino acids (glycine, threonine, cysteine, tyrosine, serine, asparagine, glutamine). They possess groups such as hydroxyl, sulfhydryl and amine and participate in hydrogen bonding of protein structure.



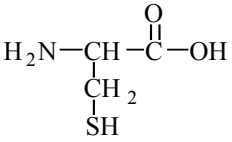
Glycine Gly)



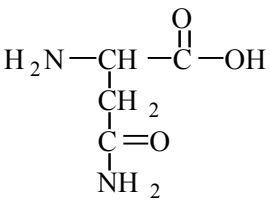
Serine (Ser)



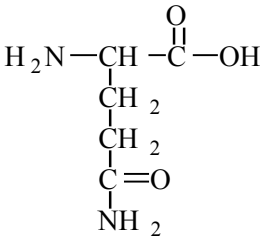
Threonine (Thr)



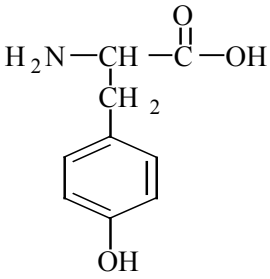
Cysteine (Cys)



Asparagine (Asn)

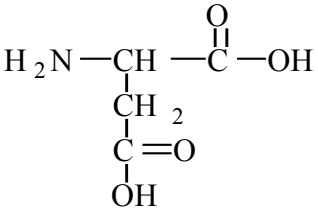


Glutamine (Gln)

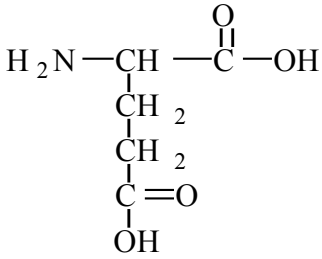


Tyrosine (Tyr)

3. **Acid amino acids have a negative charge** at pH 7.0 because they are strong proton donors (aspartate, glutamate):

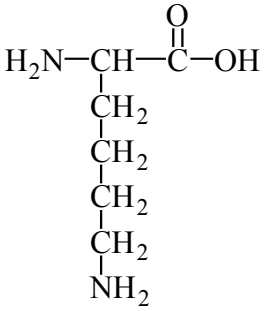


Aspartate (Asp)

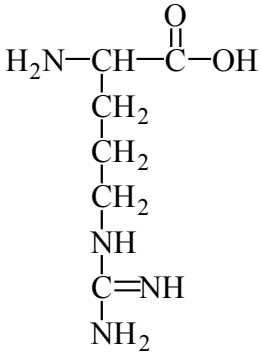


Glutamate (Glu)

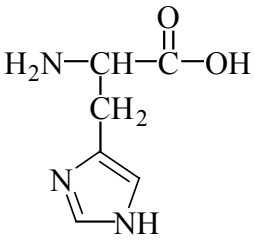
4. Basic acids (histidine, arginine and lysine) carry a positive charge at pH 7.0.



Lysine (Lys)



Arginine (Arg)



Histidine (His)

Nutritional classification of amino acids. Based on the nutritional requirements,
amino acids are grouped into two classed — **essential** or **non-essential**.

a. Essential or indispensable amino acids. The amino acids which cannot be synthesized by the body and, therefore, need to be supplied through the diet are called essential amino acids. They are required for proper growth and maintenance of the individual

Essential Amino Acids in Mammals	Non-Essential Amino Acids in Mammals
Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine	Alanine, Asparagine, Aspartic Acid, Cysteine, Glutamic Acid, Glutamine, Glycine, Proline, Serine, Tyrosine

Of the ten listed above, two amino acids — arginine and histidine — can be partly synthesized by adult human, hence these are considered as **semi-essential amino** acids. Thus, 8 amino acids are absolutely essential while 2 are semi-essential.

b. Non-essential or dispensable amino acids. The body can synthesize about 10 amino acids to meet the biological needs, hence they need not be consumed in the diet.

4. Amino acids classification based on their metabolic fate. The carbon skeleton of amino acids serves as a precursor for the synthesis of glucose (glycogenic) or fat (ketogenic) or both. From metabolic view point, amino acids are divided into three groups

a. Glycogenic amino acids. These amino acids can serve as precursors for the formation of glucose or glycogen (e.g. alanine, aspartate, glycine etc.)

b. Ketogenic amino acids. Fat can be synthesized from these amino acids. Two amino acids leucine and lysine are exclusively ketogenic.

c. **Glycogenic and ketogenic amino acids**. The four amino acids isoleucine, phenylalanine, tryptophan, tyrosine are precursors for synthesis of glucose as well as fat.

NON-STANDARD AMINO ACIDS

Besides the 20 standard amino acids (described above) present in the protein structure, there are several other amino acids which are biologically important. These include the **amino acid derivatives** found in proteins, **non-protein amino acids** performing specialized functions and the **D-amino acids**.

1. Amino acid derivatives in proteins. The 20 standard amino acids can be incorporated into proteins due to the presence of universal genetic code. Some of these amino acids **undergo specific modification** after the protein synthesis occurs. These derivatives of amino acids are very important for protein structure and functions. Selected examples: **collagen** — the most abundant protein in mammals — contains 4-hydroxyproline and 5-hydroxylysine**; histones** — the proteins found in association with DNA — contain many methylated, phosphorylated or acetylated amino acids; **cystine** is formed by combination of two cysteines.

2. Non-protein amino acids. These amino acids, although never found in proteins, perform several biologically important functions. They may be either α-or non-α-amino acids. Examples: Ornithine, citrullin and arginosuccinic acid are intermediates in the biosynthesis of urea; S-adenosylmethionine is methyl donor in biological system; homocysteine is intermediate in methionine metabolism etc.

3. D-Amino acids. The vast majority of amino acids isolated from animals and plants are of L-category. Certain D-amino acids are also found in the antibiotics (actinomycin-D, valinomycin, gramicidin-S). D-Glutamic acid and D alanine are present in bacterial cell walls.

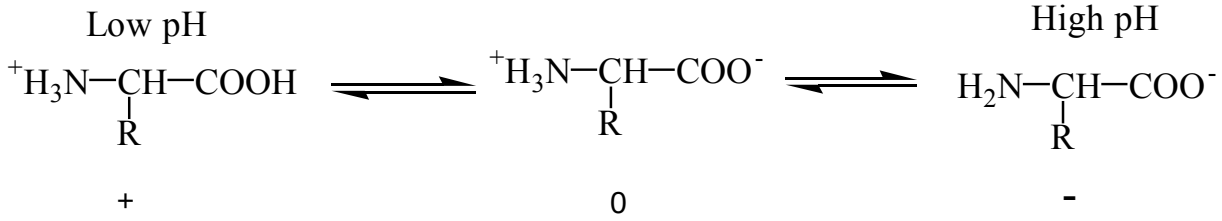
Properties of amino acids

1. Optical activity of amino acids. With the exception of glycine, all amino acids contain at least one **asymmetric carbon** atom and are, therefore, optically active. Amino acids exist as stereoisomeric pairs called **enantiomers**. These amino acid isomers are typically called L (levorotatory) or D (dextrorotatory) depending on the direction they rotate plane-polarized light. **L-Amino acids** are the only optically active amino acids that are incorporated into proteins. **D-Amino acids** are found in bacterial products (e.g., in cell walls) and in many peptide antibiotics, but they are not incorporated into proteins via the ribosomal protein synthesizing system.

2. Amphoteric properties. Amino acids are **amphoteric molecules;** that is, they have both basic and acidic groups. Monoamino-monocarboxylic acids exist in aqueous solution as **dipolar molecules (zwitterions),** which means that they have both positive and negative charges. **The α-carboxyl group** is dissociated and negatively charged. **The α-amino group** is protonated and positively charged. Thus, the overall molecule is **electrically neutral.**

At **low pH** (i.e., high concentrations of hydrogen ion), the carboxyl group accepts a proton and becomes uncharged, so that the overall charge on the molecule **is positive**.

At high pH (i.e., low concentrations of hydrogen ion), the amino group loses its proton and becomes uncharged; thus, the overall charge on the molecule **is negative.**



Some amino acids have **side chains** that contain **dissociating groups.** Those of aspartate and glutamate are acidic; those of histidine, lysine, and arginine are basic. Two others, cysteine and tyrosine, have a negative charge on the side chain when dissociated.

At the **pH of blood plasma** or the **intracellular space** (7.4 and 7.1, respectively), **carboxyl groups** exist almost entirely as **carboxylate ions**, R-COO^-. At these pH values, most **amino groups** are predominantly in the associated (**protonated**) form, R-NH_3^+. The **net charge** (the algebraic sum of all the positively and negatively charged groups present) of an amino acid depends upon the pH, or **proton concentration**, of the surrounding solution. The ability to alter the charge on amino acids or their derivatives by manipulating the pH facilitates the physical separation of amino acids, peptides, and proteins.

Isoelectric pH is defined as the pH at which a molecule exists as a zwitterions or dipolar ion and carries no net charge.

Properties of proteins

1. Solubility. Proteins form **colloidal solutions** instead of true solutions in water. This is due to huge size of protein molecules.

2. Shape. There is a wide variation in the protein shape. It may be globular (insulin), oval (albumin) fibrous or elongated (fibrinogen).

3. Isoelectric pH. The **nature of the amino acids** (particularly their ionizable groups) determines the pI of a protein. The acidic amino acids (Asp, Glu) and basic amino acids (His, Lys, Arg) strongly influence the pi. At isoelectric pH, the proteins exist as **zwitterions or dipolar ions.** They are electrically neutral (do not migrate in the electric field) with minimum solubility, maximum precipitability and least buffering capacity.

4. Acidic and basic proteins. Proteins in which the ratio (ε Lys + ε Arg)/(ε Clu + ε Asp) is greater than 1 are referred to as **basic proteins**. For **acidic proteins**, the ratio is less than 1. The acidic and basic properties of proteins are utilized for staining them. The acidic dye eosin (H^+-dye^-) combines with cationic form (-NH_3^+) of protein while the basic dye methylene blue (dye^+-OH^-) combines with anionic form (-COO^-) proteins.

5. Precipitation of proteins. Proteins exist in colloidal solution due to hydration of polar groups (-COO^-, -NH_3^+, -OH). Proteins can be precipitated by dehydration or neutralization of polar groups. Several methods are in use to achieve protein precipitation.

Precipitation at pI. The proteins in general are least soluble at isoelectric pH. Certain proteins (e.g. casein) get easily precipitated when the pH is adjusted to pI (4.6 for casein). Formation of curd from milk is a marvelous example of slow precipitation of milk protein, casein at pI. This occurs due to the lactic acid produced by fermentation of bacteria which lowers the pH to the pi of casein.

Precipitation by salting out. The process of protein precipitation by the additional of neutral salts such as **ammonium sulfate or sodium sulfate** is known as salting out. This phenomenon is explained on the basis of **dehydration of protein** molecules by salts. This causes increased protein-protein interaction, resulting in molecular aggregation and precipitation.

The amount of salt required for protein precipitation depends on the **size** (molecular weight) **of the protein molecule**. In general, the higher is the protein molecular weight, the lower is the salt required for precipitation. Thus, serum globulins are precipitated by half saturation with ammonium sulfate while albumin is precipitated by full saturation. Salting out procedure is conveniently used for separating serum albumins from globulins.

The addition of small quantities of neutral salts increases the solubility of proteins. This process called as **salting in** is due to the diminished protein-protein interaction at low salt concentration.

Precipitation by salts of heavy metals. Heavy metal ions like Pb^{2+}, Hg^{2+}, Fe^{2+}, Zn^{2+}, Cd^{2+} cause precipitation of proteins. These metals being positively charged, when added to protein solution (negatively charged) in alkaline medium results in precipitate formation.

Precipitation by anionic or alkaloid reagents. Proteins can be precipitated by trichloroacetic acid, sulphosalicylic acid, phosphotungstic acid, picric acid, tannic acid, phosphomolybdic acid etc. By the addition of these acids, the proteins existing as cations are precipitated by the anionic form of acids to produce protein-sulphosalicylate, protein-tungstate, protein-picrate etc.

Precipitation by organic solvents: Organic solvents such as alcohol are good protein precipitating agents. They dehydrate the protein molecule by removing the water envelope and cause precipitation.

LECTURE 2

STRUCTURE OF PROTEINS

Proteins are the polymers of L-α-amino acids. Every protein in its **native state** has a unique **three-dimensional structure,** which is referred to as its **conformation.** The function of a protein arises from its conformation. Protein structures can be classified into four levels of organization: **primary, secondary, tertiary, and quaternary.**

PRIMARY STRUCTURE OF PROTEIN

The primary structure is the covalent "backbone" of the polypeptide formed by the **specific amino acid sequence.**

The linking together of amino acids produces **peptide** chains, also called **polypeptides** if many amino acids are linked. The molecular weights of amino acid polymers, often referred to as polypeptides, range from several thousand to several million Dalton. Those with low molecular weight, typically consisting of less than 50 amino acids, are called peptides. The term protein is used to describe molecules with **more than 50 amino acids**.

1. The **peptide bond** is the bond formed between the **α-carboxyl group** of one amino acid and the **α-amino group of another**. In the process, water is removed.

2. Peptide bond formation is highly **endergonic** (i.e., energy-requiring) and requires the concomitant hydrolysis of high-energy phosphate bonds.

3. The peptide bond is a **planar** structure with the two adjacent α-carbons, a carbonyl oxygen, an α -amino nitrogen and its associated hydrogen atom, and the carbonyl carbon all lying in the same plane (Fig. 2—1).

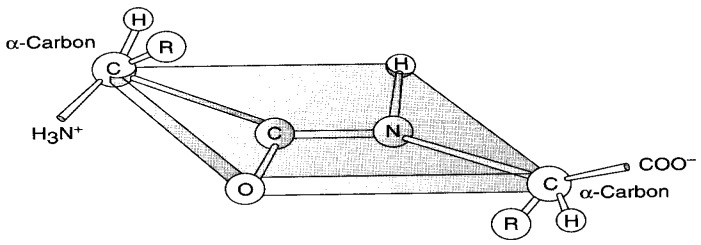
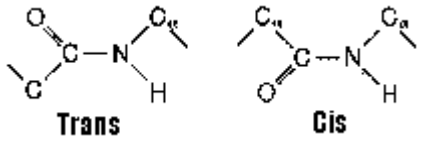


Figure 2—1. The planar nature of the peptide bond (by Davidson V.L. et al., 1999).

4. The –CN – bond has a partial double-bond character that prevents rotation around the bond axis.

5. The group of atoms about the peptide bond can exist in either the *trans* or *cis* configurations:



It turns out, however, that the ***trans*** configuration is usually favored in order to minimize the steric interaction between bulky R groups on adjacent α-carbon atoms. The major exception is bonds in the sequence X-Pro, where X is any amino acid and Pro is **proline**. In this case, the *cis* configuration may be favored at times.

6. In the absence of a catalyst, peptide bonds are fairly stable. That is, the uncatalyzed reaction is exceedingly slow at physiological pH and temperature. Thus, polypeptides are metastable. They hydrolyze rapidly only under extreme conditions or when suitable catalysts are present.

7. Amino acids, when in polypeptide chains, are customarily referred to as **residues.**

8. This sequence **is coded for by DNA** and **determines the final three-dimensional** form adopted by the protein in its native state.

9. By convention, peptide sequences are written from left to right, starting with the amino acid residue that has a free α-amino group (the so-called **N terminal amino acid)** and ending with the residue that has a free α-carboxyl group (the **C-terminal amino acid).** Either the three-letter abbreviations (e.g., Ala-Clu-Lys) or, for long peptides, the single-letter abbreviations are used. For naming peptides, the amino acid suffixes **–ine** (glycine), **-an** (tryptophan), —**ate** (glutamate) are changed to **–yl** with the exception of C-terminal amino acid.

Amphoteric properties

1. The formation of the peptide bond removes two dissociating groups, one from the α -amino and one from the α-carboxyl, per residue.

2. Although the N-terminal and C-terminal α-amino and α-carboxyl groups can play important roles in the formation of protein structures, and thus in protein function, the amphoteric properties of a polypeptide are mainly governed by the **dissociable groups** on the amino acid **side chains.**

3. Laboratory use. These properties of proteins are not only important in terms of protein structure and function but are also useful in a number of analytic procedures, such as ion exchange or high-performance liquid chromatography, for the purification and identification of proteins.

Biologically important peptides

Several peptides occur in the living organisms that display a wide spectrum of biological functions. Generally, the term **"peptide"** is applied when the number of constituent amino acids is less than 10.

1. Glutathione. It is tripeptide composed of 3 amino acids — γ-glytamate, cysteine and glycine. It is widely distributed in nature and exist in reduced or oxidized states.

Functions

a. Glutatione serves as coenzyme for certain enzymes.

b. It is prevents the oxidation of sulfhydryl (-SH) groups of several proteins to disulfide (-S-S) groups. This is essential for the protein function, including that of enzyme.

c. Glutatione (reduced) performs specialized functions in erythrocytes. It maintains red blood membrane structure and integrity. It protects hemoglobin from getting oxidized by agents such as H_2O_2

d. Glutathione is involved in the transport of amino acids in the intestine and kidney tubules via **γ-glutamyl cycle** or **Meister cycle**.

e. Toxic amounts of peroxides and free radicals produced in the cells are scavenged by glutathione peroxidase.

2. Thyrotropin releasing hormone. It is a tripeptide secreted by hypothalamus and stimulates pituitary gland to release thyrotropic hormones.

3. **Oxytocin**. It is a hormone secreted by posterior pituitary gland and contains 9 amino acids. Oxytocin causes contraction of uterus.

4. **Vasopressin (antidiuretic hormone, ADH)**. ADH is also a nonapeptide produced by posterior pituitary gland. It stimulated kidney to retain water and thus increases the blood pressure.

5. **Angiotensins.** Angiotensin I is a decapeptide (10 amino acids) which is converted to angiotensin II. The later has more hypertensive effect.

6. **Bradykinin and kallidin**. They are nona- and decapeptides, respectively. Both of them act as powerful vasodilators. They are produced from plasma proteins by snake venom enzymes.

SECONDARY STRUCTURE OF PROTEIN

The secondary structure is the spatial relation of neighboring amino acid residues.

1. Secondary structure is dictated by the primary structure. The secondary structure arises from interactions of **neighboring amino acids**. Because the DNA-coded primary sequence dictates which amino acids are near each other, secondary structure often forms as the peptide chain comes off the ribosome.

2. Hydrogen bonds. An important characteristic of secondary structure is the formation of **hydrogen bonds** (H bonds) between the –CO group of one peptide bond and the –NH group of another nearby peptide bond

a. If the H bonds form between peptide bonds in the same chain, either **helical** structures, such as the α-**helix,** develop or turns, such as β-**turns,** are formed.

b. If H bonds form between peptide bonds in different chains, **extended** structures form, such as the **β-pleated sheet.**

3. The α-helix is the most common spiral structure of protein. α-Helical structure was proposed by **Pauling** and **Corey** (1951) which is regarded as one of the milestones in the biochemistry research. The α-helix is a **rod-like structure** with the peptide bonds coiled tightly inside and the side chains of the residues protruding outward (Fig. 2—2).

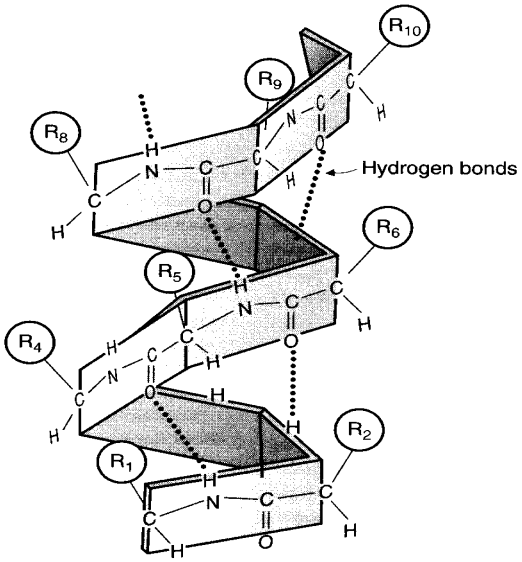


Figure 2—2. The right-handed α-helix (by Davidson V.L. et al., 1999).

a. Each –CO is hydrogen bonded to the –NH of a peptide bond that is **four residues away** from it along the **same chain.**

b. There are **3.6** amino acid residues per turn of the helix, and the helix is **right-handed** (i.e., the coils turn in a clockwise fashion around the axis). The right handed α-helix is more stable than left handed helix.

c. Distance traveled per turn, measured along the helix axis is **0.54 nm**.

d. Distance, measured along the helix axis, separating equivalent main chain atoms of adjacent residues is **0.15 nm.**

e. While α-helices tend to contain more of amino acids such as Ala, Glu, Leu and Met than of others such as Gly, Pro, Ser, or Tyr, this tendency is not useful for structural predictions.

4. β-Pleated sheet structures are found in many proteins, including some globular, soluble proteins, as well as some fibrous proteins (e.g., silk fibroin).

a. They are more extended structures than the α-helix and are "pleated" because the C–C bonds are tetrahedral and cannot exist in straight lines (Fig.2—3).

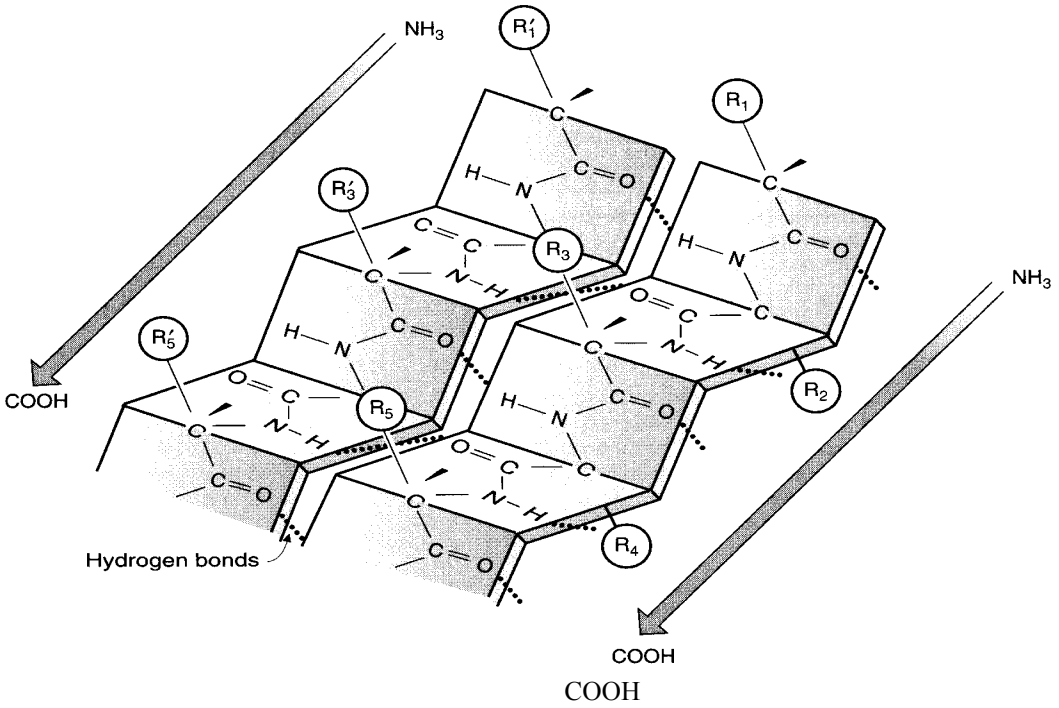


Figure 2—3. Parallel β-pleated sheet (by Davidson V.L. et al., 1999).

b. The chains lie side by side, with the **hydrogen bonds** forming between the –CO group of one peptide bond and the –NH group of another peptide bond in the **neighboring chain.**

c. The chains may run in the same direction, forming a **parallel β-sheet,** or they may run in opposite directions, as they do in a globular protein in which an extended chain is folded back on itself, forming an **antiparallel β-structure.**

5. A β-turn is the tightest turn a polypeptide chain can make, although there are many ways a polypeptide chain can turn. β-Turns result in a complete reversal in the direction of a polypeptide chain in just four amino acid residues.

Tertiary structure refers to the three dimensional spatial relations of more distant residues.

1. Folding. The secondarily ordered polypeptide chains of soluble proteins tend to fold into globular structures with the **hydrophobic side** chains (valine, leucine, phenylalanine, tryptophan, methionine) in the **interior of the structure** away from the water and the **hydrophilic side chains** on the **outside** in contact with water. This folding is due to associations between segments of α-helix, extended β-chains, or other secondary structures and represents a state of lowest energy (i.e., of **greatest stability)** for the protein in question.

2. The conformation results from:

a. Hydrogen-bonding within a chain or between chains.

b. The flexibility of the chain at **points of instability,** allowing water to obtain maximum entropy and thus govern the structure to some extent.

c. The formation of **other noncovalent bonds** between side-chain groups, such as salt linkages, hydrophobic bonds, electrostatic bonds, Van der Waals forces.

d. The sites and numbers of **disulfide bridges** between Cys residues within the chain (Cys residues linked by disulfide bonds are termed **cystine** residues).

3. A peptide chain free in solution will not achieve its biologically active tertiary structure as rapidly or properly as within the cell. Within the cell, some of the proteins that facilitate proper folding are:

a. Protein disulfide isomerase. This protein catalyzes the formation of proper disulfide bond formation between cysteine residues.

b. Chaperones. This family of proteins catalyzes the proper folding of proteins in part by inhibiting improper folding and interactions with other peptides.

Quaternary structure refers to the spatial relations between **individual polypeptide chains** in a **multichain protein**; that is, the characteristic noncovalent interactions between the chains (hydrogen bonds, salt bridges, hydrophobic interactions) that form the native conformation of the protein as well as occasional disulfide bonds between the chains.

1. Many proteins larger than 50 kdal have more than one chain and are said to contain **multiple subunits,** with individual chains known as **protomers.**

2. Many multisubunit proteins are composed of different kinds of **functional subunits** [e.g., the regulatory and catalytic subunits of regulatory proteins].

Denaturation

The protein structure is especially sensitive to a variety of environmental factors. Many physical and chemical agents can disrupt a protein's **native conformation.** The process of structure disruption is called **denaturation.** Denaturation results in the loss of secondary, tertiary and quaternary structure of proteins.

1. Depending on the degree of denaturation, the **loss** of the molecule's **biological activity** may be **partial** or **complete.**

2. Denaturation often results in easily observable changes in the **physical properties of proteins**.

3. The **primary structure** of a protein with peptide linkage **remains intact** i.e., peptide bonds are not hydrolyzed.

4. Denatured protein becomes insoluble in the solvent in which was originally soluble.

5. Denatured protein is more easily digested. This is due to increased exposure of peptide bonds to enzymes. Cooking causes protein denaturation and, therefore, cooked food (protein) is more easily digested.

Agents of denaturation

1. Chemical agents: strong acids or bases (changes in pH often result in the protonation or deprotonation of ionizable amino acid side chain); o**rganic solvents (**interfere with hydrophobic interactions); **detergents** (these amphipathic molecules disrupt hydrophobic interactions); **reducing agent** (convert disulfide bridges to sulfhydryl groups, e.g. β-mercaptoethanol); s**alt concentration** (the large number of salt ions can effectively complete with the protein for water molecules, that is, the solvation spheres surrounding the protein's molecules aggregate and then precipitate); **heavy metals Hg^{2+}, Pb^{2+}** (they may disrupt salt bridges and bound with sulfhydryl groups).

2. Physical agents: temperature changes (as the temperature increases, there is an increase in the rate of molecular vibrations and hydrogen bond are disrupted); **mechanical stress** (stirring and grinding actions disrupt the delicate balance of forces required to maintain protein structure); **X-rays, UV radiation.**

PROTEIN STRUCTURE-FUNCTION RELATIONSHIPS

Structure and changes in structure are crucial to the function of most protein. As an example of protein structure-function relationships, we discuss about myoglobin and hemoglobin, proteins that are of significance both in their own right and for the insight they provide into ways in which the structures of proteins conform to, or dictate, their biologic function.

Oxygen transport proteins. Oxygen enters cells by diffusion, and the availability of oxygen is limited by the distance over which diffusion must occur. As the size of cells and organisms has increased, circulatory systems with oxygen-binding molecules have evolved to carry oxygen to cells and subcellular areas that cannot get oxygen by direct diffusion. In higher animals, oxygen transport is mediated by two oxygen-binding globular proteins: **myoglobin** and **hemoglobin**.

Myoglobin

1. Function.

a. Myoglobin is found in **muscle cells**, where it **stores** oxygen and **transports oxygen** to the **mitochondria**. To perform its function, myoglobin must be able to bind oxygen well at the relatively low oxygen tension in the tissues where hemoglobin releases oxygen. This ability to bind oxygen at low oxygen tension is seen by looking at its oxygen-binding curve.

b. The oxygen-binding curve (also called an oxygen-dissociation curve) for myoglobin is a rectangular hyperbola (Fig. 2—4) with the binding sites 90% saturated at 20 mm Hg (torr), which is the partial pressure of oxygen (PO_2) in muscle.

2. Structure

a. Myoglobin is a compactly folded, single peptide chain (MW 17,000) that is 153 residues in length (Fig. 2—5).

b. Approximately 75% of the structure of myoglobin consists of **eight** α-helical segments (lettered A through H in Fig. 2—5). The polar (hydrophilic) residues tend to be on the outside of the molecule, whereas almost all of the nonpolar (hydrophobic) residues are on the inside of the molecule.

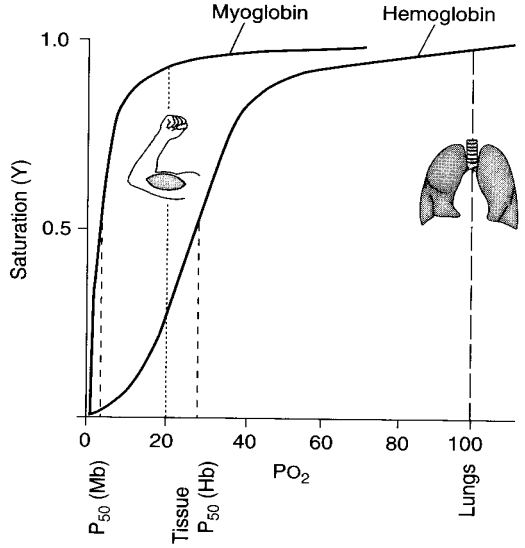


Figure 2—4. Oxygen-binding curves for myoglobin (Mb) and hemoglobin (Hb). P_{so}(Hb) = 50% saturation of hemoglobin; P_{50}(Mb) = 50% saturation of myoglobin; Po_2 = partial pressure of oxygen (by Davidson V.L. et al., 1999).

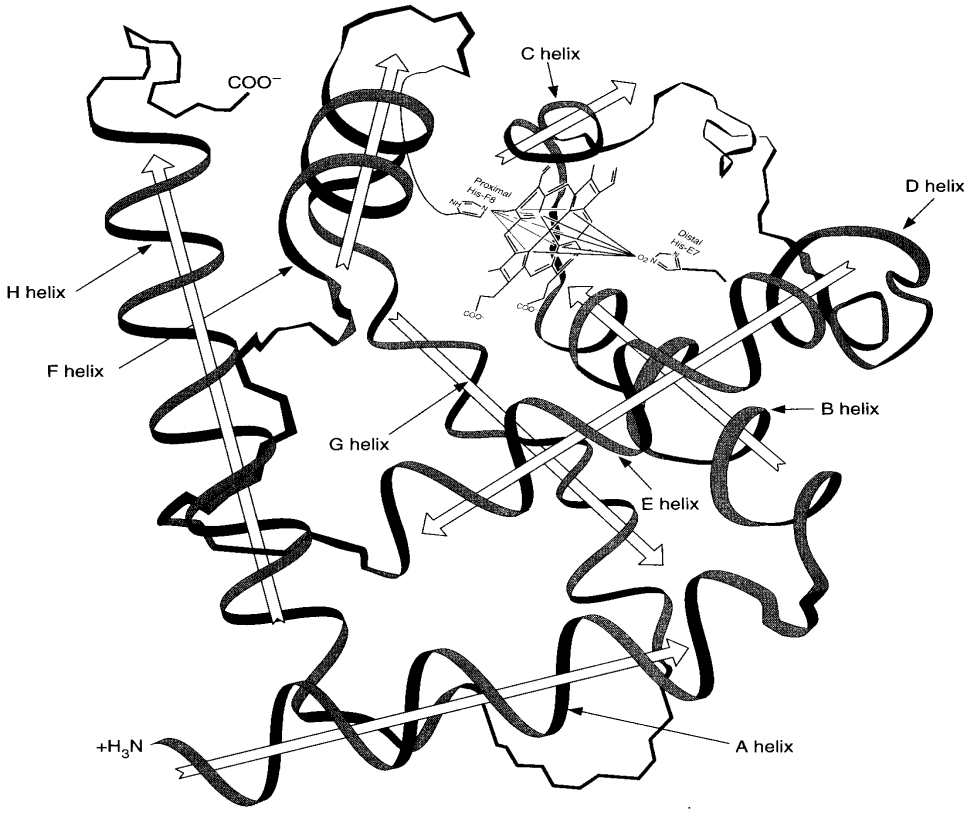


Figure 2—5. Model of myoglobin. The heme molecule shows bound oxygen between the seventh amino acid (histidine) in the E-helix and the eighth amino acid (histidine) in the F-helix (by Davidson V.L. et al., 1999).

c. Heme. Heme is **a prosthetic group** that **enables the binding of oxygen to myoglobin and hemoglobin**. Without the heme group **(apoprotein** form), myoglobin will not bind oxygen. Functional heme is made **of ferrous iron** (Fe^{2+}) and a protoporphyrin group called **protoporphyrin IX,** which is composed of four linked pyrrole groups. The iron is bound to the center of protoporphyrin IX by four nitrogen groups. Heme fits into a **hydrophobic pocket** of the myoglobin molecule.

d. Oxygen-binding site. The ferrous iron in heme can form **six ligand bonds**, four of which bind the iron to the protoporphyrin ring. The **fifth bond** is to the **histidine residue** in the **F** α-helix (His-F8), on the proximal side of the protoporphyrin plane. The **sixth bond forms** between an **oxygen molecule** interposed between the iron and a histidine residue in the **E** α-helix (His—E7) on the distal side of the protoporphyrin ring.

Hemoglobin

Hemoglobin**,** found in **red blood cells (RBCs),** carries oxygen from the lungs to the tissues and carries carbon dioxide (CO_2) from the tissues to the lungs. The packaging of hemoglobin into RBCs allows it to be present at high concentrations without osmotic pressure or viscosity problems. In humans, there are approximately five billion RBCs per milliliter of blood. Each RBC contains 280 million hemoglobin molecules.

1. Function. As the oxygen carrier in blood, hemoglobin must be able to bind **oxygen at** the relatively **high PO_2** in the lungs and release oxygen at the relatively low Po_2 in the tissues. Likewise, hemoglobin must also carry CO_2 from the tissues and release it in the lungs.

2. Structure. Hemoglobin is a **tetramer** of four noncovalently linked subunits, each of which is structurally and evolutionary similar to myoglobin. Like myoglobin, each hemoglobin subunit has a hydrophobic pocket that contains heme and serves as the site of oxygen binding. The heme prosthetic groups of myoglobin and hemoglobin are identical.

3. Oxygen-binding curve (see Fig. 2—5). Unlike the oxygen-binding curve for myoglobin, the oxygen-binding curve for hemoglobin is **sigmoidal.**

a. A comparison of the myoglobin and hemoglobin oxygen-binding curves shows that hemoglobin very efficiently binds oxygen at the PO_2 levels found in lungs, and it releases the oxygen at the PO_2 levels found in tissues (e.g., muscle).

b. In contrast, myoglobin binds oxygen very efficiently at the low PO_2 levels at which hemoglobin releases oxygen.

c. Cooperative oxygen binding. The shape of the oxygen-binding curve of hemoglobin is sigmoidal because oxygen binding is cooperative. That is, as oxygen binds to some sites on hemoglobin, the binding of more oxygen to hemoglobin becomes.

3. Cooperative oxygen binding

a. In **deoxyhemoglobin** (i.e., hemoglobin with **no bound oxygen),** the iron is slightly (0.03 nm) out of the heme plane. This is due in part to steric repulsion between the proximal histidine and the nitrogen atoms of the porphyrin ring. On oxygenation, the iron atom moves into the plane of the porphyrin ring so that it can form a strong bond with oxygen.

b. Structural change increases the oxygen-binding affinity constants.

c. The movement of the iron into the plane of the porphyrin ring results in the pulling of the His-F8 residue toward the heme. This in turn significantly changes the interaction of the F-helix with the C-helix of the adjacent subunit.

d. This change in structure, which is transmitted to the adjacent subunit, increases the affinity constant for the binding of oxygen of the adjacent subunit. Therefore, the binding of the first oxygen cooperatively enhances the binding of subsequent oxygens to the other heme groups in hemoglobin.

Biomedical implication

Myoglobinuria. Following massive crush injury, myoglobin released from ruptured muscle fibers appears in the urine, coloring it dark red. While myoglobin can be detected in plasma following a myocardial infarct, assay of serum enzymes.

Anemias. Common anemias (reduction, in the amount of red cells or of hemoglobin in the blood) result from impaired synthesis of hemoglobin or impaired production of erythrocytes.

Hemoglobinopathies. While mutation of certain critical residues of hemoglobin has serious consequences, mutation of many surface residues far removed from the heme-binding site may present no clinical abnormalities.

LECTURE 3

PURIFICATION OF PROTEINS

A protein **in biologic fluids** may require some degree of purification before it can be specifically measured, studied, or used.

1. Separation of a protein from other proteins and molecules is achieved by applying a **combination of several methods** based on properties such as **solubility, molecular size, molecular charge**, and **specific binding of the protein to a specific substance**.

2. Diagnostic and therapeutic purposes. Clinical laboratories routinely separate proteins for diagnostic purposes. Plasma proteins, for example, are routinely examined by gel electrophoresis. Similar techniques are used to purify proteins for therapeutic purposes.

SEPARATION PROCEDURES

Protein solubility is influenced by the **salt concentration** of the solution.

1. Salting out. Adding salts, such as ammonium sulfate, to a solution of a protein mixture precipitates some proteins at a given salt concentration but not

others.This type of separation is performed to increase the amount of a given protein in a fraction of a highly complex mixture, such as a tissue homogenate or a sample of blood plasma.

b. **Salting in.** Some proteins require inorganic ions for water solubility. Extensive **dialysis** against a solution with a low salt concentration may, therefore, cause certain proteins from a mixture to precipitate out of solution.

Separation on the basis of molecular size

1. Dialysis. A mixture of proteins and small solutes can be separated by dialysis through a **semipermeable membrane.** The point at which molecules are excluded or prevented from passing through the membrane depends on the **pore size** of the dialysis membrane.

2. Gel filtration (molecular exclusion chromatography, molecular sieving) uses a column of insoluble carbohydrate polymer in the form of porous beads (Fig. 3—1). Small molecules can enter the pores, but large molecules cannot. Therefore, the volume of the solvent available for the small molecules is greater than that for the large molecules, so the small molecules flow through the column more slowly. Accordingly, the rate at which a molecule flows through the column depends on its size and shape. Gel filtration is used to estimate the molecular weight of a protein as well as to separate proteins.

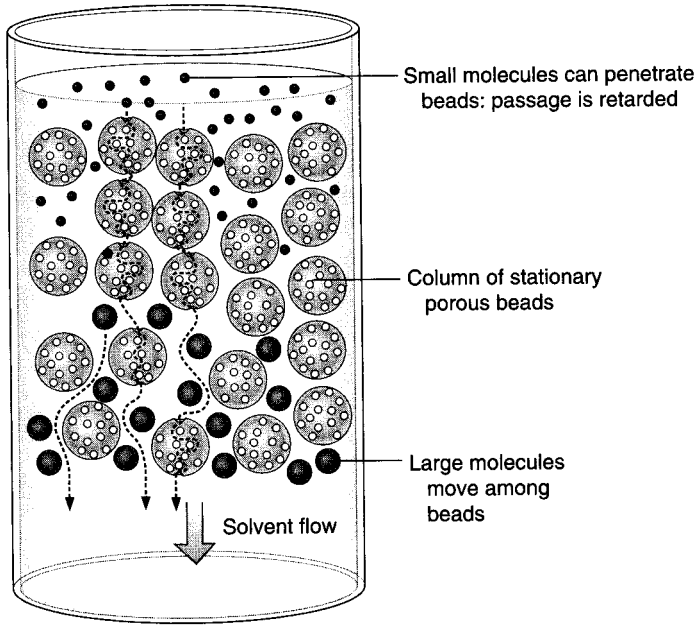


Figure 3—1. Gel filtration chromatography (by Davidson V.L. et al., 1999).

3. Ultracentrifugation. High-speed centrifugation can separate a protein solution into multiple components.

a. The rate at which protein sediments in a centrifugal field depends on its size and shape. For proteins of similar shape, the larger the molecular weight, the faster it sediments.

b. The data from an ultracentrifugation study are often expressed in terms of **Svedburg units** (S), which are related to the rate of sedimentation of the protein in the centrifugal field.

4. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, done on a cross-linked polyacrylamide gel in the presence of SDS and a reducing agent, such as 5-mercaptoethanol, separates proteins on the basis of their molecular weight (Fig. 3—2).

a. SDS, a 12-carbon-chain anionic detergent, denatures the protein, while the reducing agent breaks the disulfide bonds, thereby minimizing the effects of the protein's shape on the molecular weight determination. The SDS dissociates quaternary structures into protomers.

b. Because the SDS forms negatively charged micelles with the protein, the effect of the protein charge is lost, and the **shape** of all molecules becomes **rod-like.**

c. The SDS-protein micelles are separated according to size by gel electrophoresis. The SDS-protein micelles migrate to the positive pole with the cross-linked polyacrylamide acting as a molecular sieve. Smaller molecules migrate through the gel faster than larger molecules.

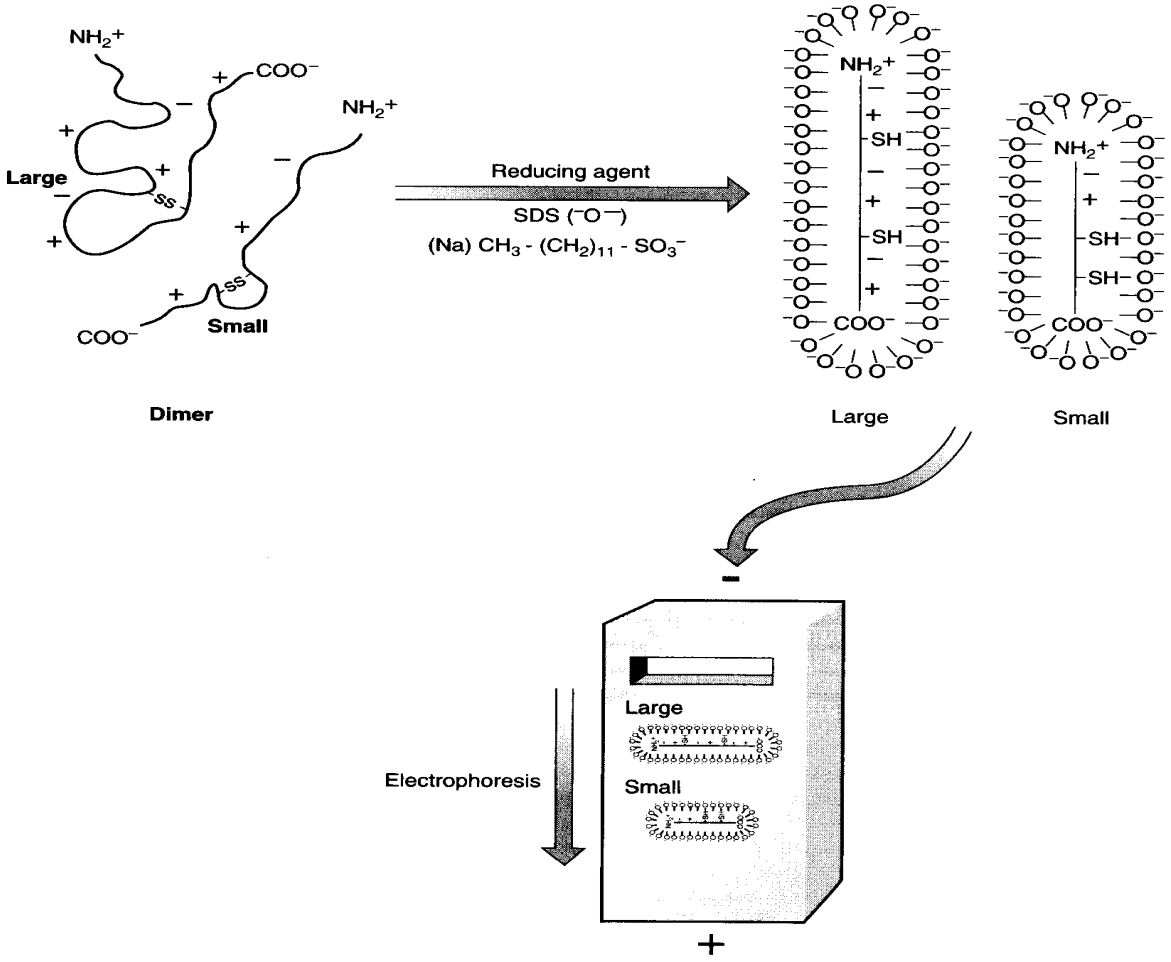


Figure 3—2. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (by Davidson V.L. et al., 1999).

Separation on the basis of molecular charge

1. Ion-exchange chromatography (Fig. 3—3)

a. Proteins are bound to the ion-exchange resin in the column. The tightness of binding of particular proteins depends on how many residues are available to interact with the ion-exchange resin.

b. A column of insoluble ion-exchange material carrying either polyanionic or polycationic groups is used (phosphocellulose $–PO_3^-$, carboxymethyl cellulose $–CH_3\text{-}COO^-$, diethylaminoethyl cellulose $–(CH_2)_2\text{-}N(C_2H_5)_2$). At the appropriate pH, these groups bind oppositely charged groups by ionic interactions.

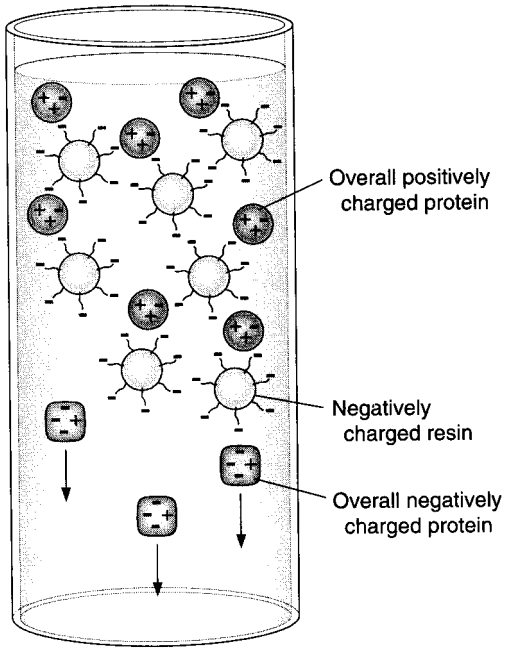


Figure 3—3. Ion-exchange chromatography (by Davidson V.L. et al., 1999).

c. Proteins may be eluted from the exchanger by washing with a **solution containing salts** that disrupt the electrostatic interactions of the proteins and ion-exchange resin.

d. If a gradually **increasing salt concentration** (i.e., a salt gradient) is applied to the column, weakly bound proteins are eluted before tightly bound proteins.

2. High-performance liquid chromatography (HPLC)

a. HPLC is similar to ion-exchange chromatography and other chromatographic methods in that solutions of proteins are passed through special resins that have attached side groups, which can interact either ionically or hydrophobically with proteins, depending on the type of resin.

b. HPLC differs from conventional chromatography in that it is done under high pressure (typically 5000—10,000 pounds per square inch). This high-pressure chromatography is faster and results in better resolution than conventional low-pressure chromatography.

3. Electrophoresis. Methods that use an electric field to drive the movement of any molecule with a net charge are termed electrophoresis. Charged molecules move in an electric field at a rate determined by their charge-to-mass ratio and their shape.

a. In an electric field, proteins migrate in a direction determined by the net charge on the molecule. The **net charge on a protein** is determined by the nature of the ionizing groups on the protein and the prevailing pH

b. For each protein, there is a pH, called the **isoelectric point** (pI), at which the molecule has no net charge and does not move in an electric field.

c. At pH values **more acidic** than the pI, the protein bears a net positive charge, behaves as a cation, and moves toward the negatively charged pole.

d. At pH values higher than the pi, the protein has a **net negative charge and** behaves as an **anion,** moving toward the positively charged pole.

e. The migration of a protein in an electric field is defined by its **electrophoretic mobility** (μ), which is a ratio of the velocity of migration (V) to the electric field strength (E), or ($x = V/E$, measured in cm^2 per volt-second. Proteins migrate much more slowly than simple ions because they have a much smaller **ratio of charge-to-mass.**

Electrophoretic procedures

1. Gel electrophoresis is often used to separate plasma proteins for diagnostic purposes. The sample is layered on a matrix as a thin zone and is then electrophoresed through the matrix, which is usually made of polyacrylamide or agarose gels. After electrophoresis, the proteins are stained. Different proteins appear in different zones (bands) depending on their overall charge, size, and shape.

a. The matrix tends to stabilize the sample against convection during electrophoresis.

b. The matrix also provides a sieving component that plays a role in separating proteins according to their size and shape.

2. In isoelectric focusing, polyamino-polycarboxylic acids (i.e., amphoteric molecules called **ampholines)** with known pi values are used to set up a pH gradient in an electric field. A protein migrates to the part of the gradient that has the same pi as the protein.

3. SDS polyacrylamide gel electrophoresis separates proteins on the basis of size.

4. Free zone capillary electrophoresis is a technique in which electrophoretic separation of charged molecules is achieved free in solution in very small-bore (0.05-0.3 mm) capillary tubes. A matrix is not needed to stabilize against convection forces because the heat of electrophoresis is rapidly dissipated in these capillary tubes.

a. Because of the efficient dissipation of heat, very high voltages (10 kV) can be used to quickly separate charged biomolecules.

b. This relatively new technology is being increasingly used for diagnostic purposes because of its speed and the need for very small amounts (i.e., typically nanoliters) of starting material.

Separation by specific affinity binding

1. Affinity (absorption) chromatography

a. This technique is based on a property that some proteins possess, that of binding strongly to another molecule (called the **ligand)** by specific, noncovalent bonding.

b. The ligand is covalently attached to the surface of large, hydrated particles of porous material to make a chromatographic column. If a solution containing a mixture of proteins is passed through the column, the protein to be selectively absorbed binds tightly to the ligand molecules, whereas the other proteins flow through the column unhindered. After traces of the other proteins are washed out, the absorbed protein can be eluted by a solution with a high concentration of pure ligand, which competes for the protein with the bound ligand.

2. Precipitation by antibodies

a. Antibodies to specific proteins can be prepared and used to react with the desired protein in a mixture of proteins (e.g., a tissue extract or body fluid). The interaction of protein and antibody may produce an antigen-antibody complex large enough to be centrifuged out of solution, allowing recovery of the protein.

b. It is often necessary to create a larger complex than the antigen-antibody complex by adding rabbit anti-gamma globulin (anti-IgG) to the antibody-protein mixture and then recovering the triple complex by centrifugation.

c. Like ligands, antibodies can be attached to hydrated matrices and made into affinity chromatography columns.

LECTURE 4

GENERAL CHARACTERISTICS OF ENZYMES

Enzymes are biochemical catalysts. By a "**catalyst**" we mean a substance that accelerates a chemical reaction without itself undergoing any change in the overall process. Enzymes may be **defined** as biocatalysts synthesized by living cells. They are protein in nature, colloidal and thermolabile in character, and specific in their action.

Historical background

In 1835 **Jon Berzelius** discovered several elements, introduced the way of writing chemical symbols, and coined the term "catalysis". In the period from 1850 to 1860, **Louis Pasteur** demonstrated that fermentation, the anaerobic breakdown of sugar to CO_2 and ethanol, occurred in the presence of living cells, and did not occur in a flask that was capped after any cells that it contained had been killed by heat. Then, in 1897, **Eduard Buchner** quite by accident that fermentation was catalyzed by clear juice that he had prepared by grinding yeast with sand and filtering out the unbroken cells. Looking for a way to preserve the juice, Buchner had added sugar. His reasoning was that cooks used sugar to preserve jam. It probably was a disappointment to him that the sugar was broken down rapidly and the mixture frothed with CO_2. In spite of his disappointment, Buvhner's discovery made it possible to explore metabolic processes such as fermentation in a greatly simplified system, without having to deal with the complexities of cell growth and multiplication, and without the barriers imposed by cell walls or

membranes. **Arthur Harden** and **William Young** soon showed that yeast extracts contained two different types of molecules, both of which were necessary in order for fermentation to occur. Some were small, dialyzable, heat-stable molecules such as inorganic phosphate; other were much larger, nondialyzable molecules, the enzyme, which were destroyed easily by heat.

Although early investigators surmised that enzymes might be proteins, the point remained in dispute until 1927, when **James Sumner** succeeded in purifying and crystallizing the enzyme urease from beans. In the 1930s, **John Northrup** isolated and characterized a series of digestive enzymes, generalizing Summer's conclusion that enzymes are proteins. Since then, thousands of different enzymes have been purified, and the structures of many of them have been solved to atomic resolution; almost all of these molecules have proved to be proteins.

Shared properties with chemical catalysts

1. Enzymes **are neither consumed** nor **produced** during the course of a reaction.

2. Enzymes **do not cause reactions to take place**, but they greatly enhance the rate of reactions that would proceed much slower in their absence.

3. Enzymes **do not alter the free energies** of the substrates or the products of the reaction.

4. Enzymes **alter the rate** but **not the equilibrium** constants of reactions that they catalyze.

Differences between enzymes and chemical catalysts

1. Enzymes **are proteins**.

2. Enzymes are **more effective** than chemical catalysts. For example, activation energy needed for decomposition of hydrogen peroxide (H_2O_2) is 765 kJ/mol. In the presence of platinum, the activation energy is 49 kJ/mol. In the presence of the enzyme catalase, the activation energy is < 8 kJ/mol.

3. Enzymes are **highly specific** and produce only the expected products from the given reactants, or **substrates** (i.e., there are no side reactions).

4. Enzymes usually function within a **moderate pH** and **temperature** range.

5. Enzymes **are capable of being regulated**.

Enzyme specificity

Enzymes are highly specific in their action when compared with the chemical catalysts. Specificity is a characteristic property of the active site.

Three types of enzyme specificity are well-recognized.

1. Stereospecificity or **optical** specificity. Stereoisomers are the compounds which have the same molecular formula, but differ in their structural configuration. The enzymes act only on one isomer and, therefore, exhibit stereospecificity. E.g. L-amino acid oxidase and D-amino acid oxidase act on L- and D-amino acids respectively.

2. Reaction specificity. The same substrate can undergo different types of reactions, each catalyzed by a separate enzyme and this is referred to as reaction specificity. An amino acid can undergo transamination, oxidative deamination, decarboxylation etc. The enzymes however, are different for each of these reactions.

3. Substrate specificity. The substrate specificity varies from enzyme to enzyme. It may be absolute, relative or broad.

a. Absolute substrate specificity. Certain enzymes act only on one substrate e.g. glucokinase acts on glucose to give glucose-6-phosphate, urease cleaves urea to ammonia and carbon dioxide.

b. Relative substrate specificity. Some enzymes act on structurally related substances. This, in turn, may be dependent on the specific group or a bond present. The action of trypsin and chymotrypsin is a good example for group specificity. Trypsin hydrolyses peptide linkage involving arginine or lysine. Examples of bond specificity-glycosidases acting on glycosidic bonds of carbohydrates, lipases cleaving ester bonds of lipids etc.

c. Broad specificity. Some enzymes act on closely related substrates which are commonly known as broad substrate specificity, e.g. hexokinase acts on glucose, fructose, mannose and glucosamine and not on galactose. It is possible that some structural similarity among the first four compounds makes them a common substrate for the enzyme hexokinase.

Measures of enzyme activity

1. Specific activity is usually expressed as μmol of substrate transformed to product per minute per milligram of enzyme under optimal conditions of measurement.

2. Turnover number, or k_{cat}, is the number of substrate molecules metabolized per enzyme molecule per unit time with units of min^{-1} or s^{-1}

Enzyme nomenclature

1. Enzymes often are known by common names obtained by adding the suffix *-ase* to the name of the substrate or to the reaction that they catalyze. Thus, urease catalyzes the hydrolysis of urea; glucose-6-phosphatase catalyzes the hydrolysis of phosphate from glucose-6-phosphate.

2. Common names also are used for some group of enzymes. For example, an enzyme that transfers a phosphate group from ATP to other molecule is usually called a "kinase", instead of the more formal "phosphotransferase".

3. Trivial names (e.g., trypsin, pepsin), which give no indication of the function of the enzyme, are commonly used.

4. International Union of Biochemistry (IUB) instituted a systematic naming scheme for enzymes. Each enzyme is now classified and named according to the type of chemical reaction it catalyzed.

a. Enzymes are divided into **six major classes** with several subclasses:

(1). Oxidoreductases catalyze oxidation-reduction reactions.

(2). Transferases catalyze the transfer of a functional group from one molecule to another (e.g., amino or phosphate groups).

(3) Hydrolases catalyze bond cleavage by the introduction of water.

(4) Lyases catalyze the removal of a group to form a double bond, or the addition of a group to double bond.

(5) Isomerases catalyze rearrangements of atoms within a molecule.

(6) Ligases catalyze reactions that join two molecules and ATP is used.

b. Each enzyme has a code number (EC) that characterizes the reaction type as to class (first digit), subclass (second digit), and subsubclass (third digit). The fourth digit is for the specific enzyme.

e. Each enzyme is given a specific name indicating the substrate, coenzyme (if any) and the type of the reaction catalyzed by the enzyme. Although the IUB names for the enzymes are specific and unambiguous, they have nor been accepted for general use as they are complex and cumbersome to remember.

Structure of enzyme

1. According to a structure of enzymes they are classified in 2 groups: a **simple** and **conjugated protein.**

2. A simple enzyme consists of amino acids only.

3. Many enzymes require certain non-protein small organic or inorganic molecule known as a **cofactor**. In this case the enzyme is called **conjugated.**

4. If the cofactor is linked to an enzyme by noncovalent forces it is named **coenzymes**.

5. The term **prosthetic group** is used when the non-protein moiety tightly (covalently) bind with the apoenzyme. The coenzyme can be separated by dialysis from enzyme while the prosthetic group cannot be.

6. An enzyme lacking an essential cofactor is called an **apoenzyme**, and the intact enzyme with the bound cofactor is called a **holoenzyme**.

7. Cofactors can be simple inorganic ions such as Mg^{2+}, or complex organic molecules (vitamins, nucleotides etc.).

8. The word **monomeric** enzyme is used if it is made up of a single polypeptide. Some of the enzymes that possess more than one polypeptide (subunit) chain are known as **oligomeric** enzymes. There are certain **multienzyme** complexes possessing specific sites to catalyze different reactions in a sequence.

9. The specificity of the enzyme is mostly dependent on the apoenzyme and not on the coenzyme.

Active site

Enzymes are big in size compared to substrates which are relatively smaller. Evidently, a small portion of the huge enzyme molecule is directly involved in the substrate binding and catalysis.

The **active site** (or active centre) of an enzyme is defined as the small region at which the substrate binds and participates in the catalysis.

Salient features of active site

1. The existence of active site is due to the tertiary structure of protein resulting in three-dimensional native conformation. Loss of native enzyme structure (say by denaturation) will results in derangement of active site.

2. The active site is made up of amino acids (known as catalytic residues) which are far from each other in the linear sequence of amino acids (primary structure of protein).

3. Active sites are regarded as cleft or crevices or pockets occupying a small region in a big enzyme molecule.

4. The active site is not rigid in structure and shape. It is rather flexible to promote the specific substrate binding.

5. Generally, the active site possesses a substrate binding site and a catalytic site. The latter is for the catalysis of the specific reaction.

6. The coenzymes or cofactors on which some enzymes depend are present as a part of the catalytic site.

7. The substrate(s) binds at the active site by weak noncovalent bonds.

8. Enzymes are specific in their function due to the existence of active sites.

9. Of the 20 amino acids that could be present in enzyme structure, only some of them are repeatedly found at the active sites of various enzymes. These amino acids are serine, aspartate, histidine, cysteine, lysine, arginine, glutamate, tyrosine etc.

10. The substrate binds the enzyme at the active site to form enzyme-substrate complex. The product is released after the catalysis and the enzyme is available for reuse.

Allosteric site

1. Some enzymes have a regulatory site, which differs from its catalyc site and called **allosteric** (the word allosteric means “another site”).

2. These enzymes are called **allosteric enzymes**.

3. Activity of allosteric enzymes is affected by effector molecules (modulators or modifier) that bind to allosteric side. Positive or stimulatory effectors enhance substrate binding, whereas negative effectors reduce substrate binding. Allosteric enzymes generally catalyze key regulatory steps in biochemical pathways.

MECHANISM OF ENZYME ACTION

Chemical reactions

1. Free-energy changes that occur during a chemical reaction when the reaction is catalyzed (lower curve) and uncatalyzed (upper curve) are illustrated in Fig. 4—1.

2. Energy of activation is required to sufficiently energize a substrate molecule to reach a **transition state** in which there is a high probability that a chemical bond will be made or broken to form the product. Enzymes increase the rate of reaction by decreasing the energy of activation.

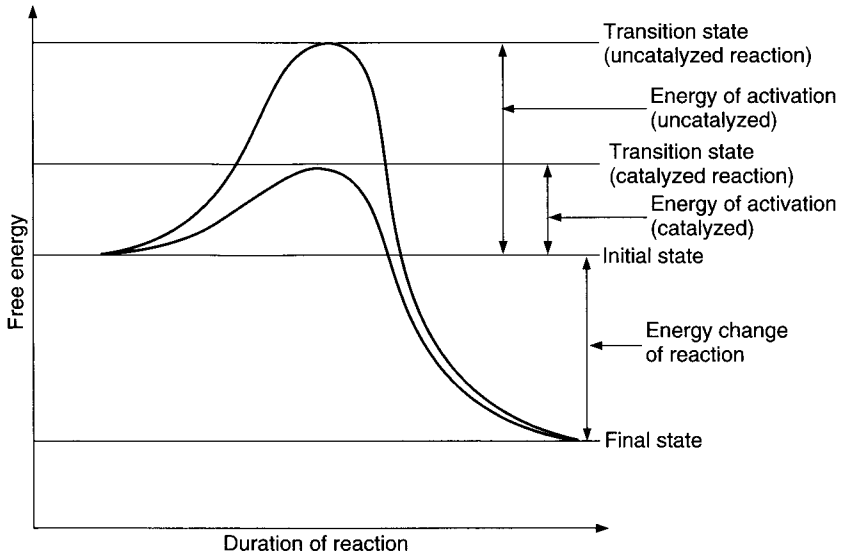


Figure 4—1. Diagrammatic representation of the free energy of activation of a chemical reaction.

Enzyme-substrate complex formation

1. The prime requisite for enzyme catalysts is that the substrate (S) must combine with the enzyme (E) at the active site to form enzyme-substrate complex (ES) which ultimately results in the product formation (P).

$$E+S \leftrightarrow ES \rightarrow E + P$$

2. Two theories have been proposed to explain the mechanism of enzyme-substrate complex formation.

a. Lock and key theory (E.Fischer). This is in fact the very first model proposed to explain an enzyme-catalyzed reaction. The enzyme active site is complementary in conformation to the substrate, so that enzyme and substrate recognize one another. The substrate fits to the binding site (now active site) just as a key fits into the proper lock or a hand into the proper glove. Thus the active site of an enzyme is a rigid and pre-shaped template where only a specific substrate can bind. This model does not give any scope for the flexible nature of enzymes; hence the model totally fails to explain many facts of enzymatic reactions, the most important being the effect of allosteric modulators.

b. Induced-fit theory (D.Koschland, 1958). It is more acceptable and realistic model for enzyme-substrate complex formation. As per this model, the active site is not rigid and pre-shaped. The essential features of the substrate-binding site are present at the nascent active site. The interaction of the substrate with the enzyme induces a fit or a conformation change in the enzyme, resulting in the formation of a strong substrate-binding site. Further, due to induced fit, the appropriate amino acids of the enzyme are repositioned to form the active site and bring about the catalysts.

Mechanism of enzyme catalysis

1. Proximity and strain effects

a. For a biochemical reaction to occur, the substrate must come into close proximity to catalytic functional groups within the active site.

b. The substrate must be precisely oriented in relation to the catalytic groups. Once the substrate is correctly positioned, a change in the enzyme's conformation may results in a strained enzyme-substrate complex.

c. The substrate is strained due to the induced conformation change in the enzyme. It is also possible that when a substrate binds to the preformed active site, the enzyme induces a strain to the substrate. The strained substrate leads to the formation of product. The concept of substrate strain explains the role of enzyme in increasing the rate of reaction.

2. Electrostatic effects

a. The strength of electrostatic interactions is related to the capacity of surrounding solvent molecules to reduce the attractive forces between chemical groups.

b. Weak electrostatic interaction, such as those between permanent and induced dipoles in both the active site and the substrate, are believed to contribute to catalysis.

c. A more efficient **binding of substrate** causes a lowering in the free energy of the transition state, which results in an acceleration of the reaction.

3. Acid-Base catalysis

a. Chemical groups can often be made more reactive by the addition or removal of a proton.

b. Enzyme active sites contain side groups that act as proton donors or acceptors. Such groups can be precisely positioned in relation to the substrate.

4. Covalent catalysis

a. In the covalent catalysis, the negatively charged (nucleophilic) or positively charged (electrophilic) group is present at the active site of the enzyme.

b. This group attacks the substrate that results in the covalent binding of the substrate to the enzyme.

In the actual catalysis of the enzymes, more than one of the processes — acid-base catalysis, substrate strain, covalent catalysis, electrostatic effect are simultaneously operate. This will help the substrate(s) to attain a transition state leading to the formation of products.

Factors Affecting Enzyme Activity

1. Effect of enzyme concentration on reaction velocity. If the substrate concentration is held constant, the velocity of the reaction is proportional to the enzyme concentration (Fig. 4-2).

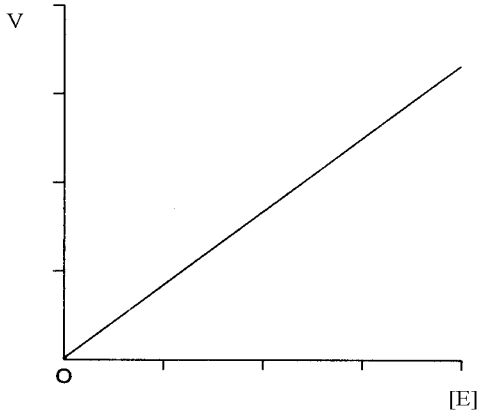


Figure 4—2. Effect of enzyme concentration on enzyme velocity

2. Effect of substrate concentration on reaction velocity (Fig. 4—3 A).

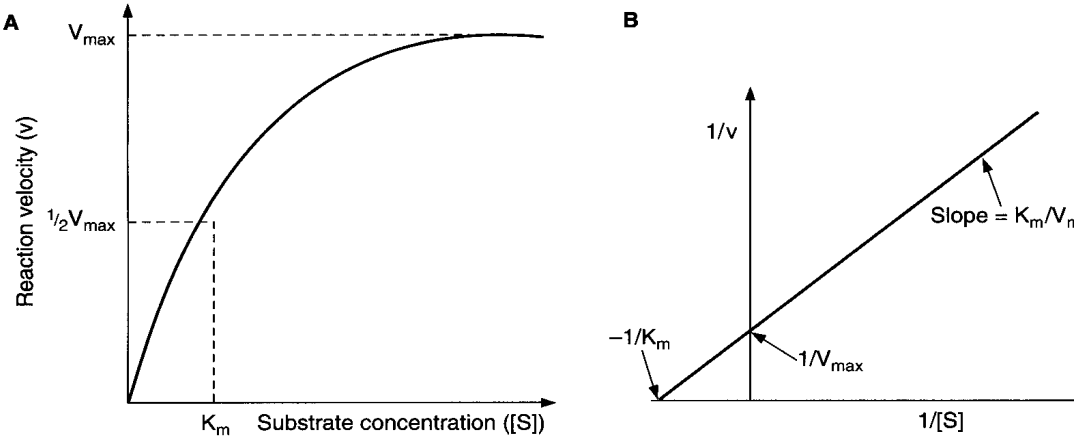


Figure 4—3. (A) The hyperbolic dependence of velocity (v) on substrate concentration ([S]) for a typical enzyme-catalyzed reaction. Km is the substrate concentration at ½ V_{max} . V_{max} is the maximum rate at which the enzyme can catalyze the reaction. (B) A Lineweaver-Burk plot of 1/V against 1/[S] for an enzyme-catalyzed reaction.

Increase in the substrate concentration gradually **increases the velocity of the enzyme reaction** within the limited range of substrate levels. A rectangular hyperbola is obtained when velocity is plotted against the substrate concentration.

a. Three distinct phases of the reaction are observed in the graph. When the substrate concentration ([S]) is low, the reaction velocity (v) is **first-order** with respect to substrate (i.e., v is directly proportional to [S]). At high substrate concentration, the reaction is **zero-order** (i.e., v is independent of [S]). At mid-[S], the reaction is mixed-order (i.e., the proportionality is changing).

b. Michaelis-Menten kinetic model. An enzyme-catalyzed reaction involves the reversible formation of an enzyme-substrate complex [ES], which breaks down to form free enzyme [E] and product [P].

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E\,S \xrightarrow{k_3} E + P \qquad (1)$$

In equation (1), k_1 is the rate constant for ES formation, k_2 is the rate constant for the dissociation of ES back to E + S, and k_3 is the rate constant for the dissociation of ES to E + P.

The relationship of substrate concentration to velocity for many enzymes may be described by equation (2), where v is the initial velocity of the reaction, $V_{max} = k_3[E]_T$, and $K_m = (k_2 + k_3)/k$,. E_T is the total [E] present.

$$V=V_{max}[S]/([S]+K_m) \quad (2)$$

The Michaelis-Menten equation is based on three key assumptions.

(1) [S] is very large compared with [E], so that when all E is bound in the form ES, there is still an excess of S.

(2) Only initial velocity conditions are considered. Thus, there is very little accumulation of P, and the formation of ES from E+P is negligible.

(3) Steady-state assumption. The rate of breakdown of ES equals the rate of formation of ES.

c. Using the Michaelis-Menten equation

(1). Significance of the Michaelis constant (Km). If K_m is set equal to [S] and substituted into equation (2), then $v = ½V_{max}$. Therefore, **K_m is equal to the substrate concentration at which the velocity is half-maximal.** K_m is not a true dissociation constant, but it does provide a measure of the affinity of an enzyme for its substrate. The lower the value of K_m, the greater the affinity of the enzyme for enzyme-substrate complex formation.

(2). Lineweaver-Burk linear transform. Because it is difficult to estimate V_{max} from the position of an asymptote, as in the plot of a rectangular hyperbola (see Fig. 4-3B), this linear transform of the Michaelis-Menten equation is often used

$$1/V=1/V_{max} + K_m \cdot 1/[S]$$

(3). Graphical analysis. Figure 4-3B shows the straight-line graph obtained by plotting 1/v against 1/[S], where the y-intercept = $1/V_{max}$, the x-intercept = $-1/K_m$, and the slope = K_m/V_{max}.

3. Effect of pH on reaction velocity. A change in pH can alter the rates of enzyme-catalyzed reactions, with many enzymes exhibiting a bell-shaped curve when enzyme activity is plotted against pH (Fig. 4—4A).

Changes in pH can alter the following:

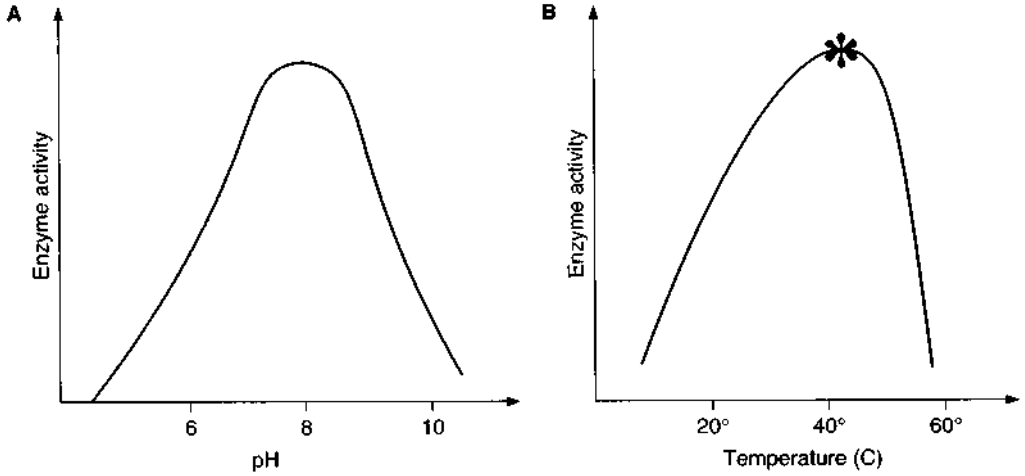
a. The ionization state of the substrate or the enzyme-binding site for substrate.

b. The ionization state at the catalytic site on the enzyme.

c. Protein molecules so that their conformation and catalytic activity change

Although a few enzymes tolerate large changes in pH, most enzymes are active only within a narrow pH range. For this reason, living organisms employ buffers that closely regulate pH. The **pH at which an enzyme's activity is maximal is called the pH optimum**. The **optimum pH** of majority human enzymes is **approximately 7**. The pH optima of enzymes vary considerably. For

example, the optimum pH of pepsin, a proteolic enzyme produced in the stomach is approximately 2; chymotrypsin, which digests protein in the small intestine, has an optimum pH of approximately 8.



AB

Figure 4—4. *(A)* A typical pH-activity plot of an enzyme-catalyzed reaction. *(B)* Effect of temperature on the activity of an enzyme.

4. Effect of temperature on reaction velocity. Temperature coefficient or Q_{10} is defined as increase in enzyme velocity when the temperature is increased by 10°. For a majority of enzymes, Q_{10} is 2 between 0°C and 40°C. Increase in temperature results in higher activation energy of the molecules and more molecular (enzyme and substrate) collision and interaction for the reaction to proceed faster. In general, when the enzymes are exposed to a temperature above 50°C, denaturation leading to derangement in the native (tertiary) structure of the protein and active site are seen. Majority of the enzymes become inactive at higher temperature (above 70°). The rate of an enzyme-catalyzed reaction usually increases with increasing temperature up to an **optimum.**

5. Effect of product concentration. The accumulation of reaction products generally decreases the enzyme velocity. For certain enzymes, the products combine with the active site of enzyme and form a loose complex and, thus, inhibit the enzyme activity. In the living system, this type of inhibition is generally prevented by a quick removal of products formed.

LECTURE 5

ACTIVATORS OF ENZYMES. ENZYME INHIBITION. ENZYME REGULATION

ACTIVATORS OF ENZYMES

Some of the enzymes require certain inorganic metallic cations like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Na^{+}, K^{+} etc. for their optimum activity. Rarely, anions are also needed for enzyme activity, e.g. chloride ion (Cl^{-}) for amylase.

Metals function as activators of enzyme velocity through various mechanisms — combining with the substrate, formation of ES-metal complex, direct participation in the reaction and bringing a conformational change in the enzyme.

Two categories of enzymes requiring metals for their activity are distinguished

1. Metal-activated enzymes: the metal is not tightly held by the enzyme and can be exchanged easily with other ions e.g. ATPase.

2. Metalloenzymes: these enzymes hold the metals rather tightly which are not readily exchanged. e.g. alcohol dehydrogenase, carbonic anhydrase, alkaline phosphatase.

ENZYME INHIBITION

The activity of enzymes can be inhibited. Study of the methods by which enzymes are inhibited have practical applications. For example, many clinical therapies and biochemical research tools are based on enzyme inhibition.

A variety of substance have the ability to **reduce** or **eliminate** the catalytic activity of specific enzymes.

Inhibition may be **irreversible** or **reversible**.

Irreversible inhibitors

1. Irreversible inhibitors bind **covalently** or so **tightly** to the active site that the enzyme is inactivated irreversibly. They react with enzymes by formation of a covalent bond to the functional group of an amino acid side chain or to a bound coenzyme. Some examples of such inhibitors are given in Table 5-1.

Table 5—1.

Some inhibitors of enzymes that form covalent linkages with functional groups of the enzyme

Inhibitor	**Enzyme group that combines with inhibitor**
Cyanide	Fe, Cu, Zn, other transition metals
β-Mercuribenzoate	Sulfhydryl
Diisopropylfluorophosphate	Serine hydroxyl
Iodoacetate	Sulfhydryl, imidazole, carboxyl, thioether

Irreversible inhibitors often provide **clues** to the nature of the active site on an enzyme. For example, enzymes containing free sulfhydryl groups can react with alkilating agents such as iodoacetate. Enzymes that use sulfhydryl groups to form covalent bonds with metal cofactors are often irreversibly inhibited by heavy metals (e.g., **mercury** and **lead**). The **anemia** that occurs in lead poisoning is caused in part because of lead binding to a sulfhydryl group of ferrochelatase. Ferrochelatase catalyzes the insertion of Fe^{2+} into heme.

Diisopropylfluorophosphate reacts irreversibly with a critical serine residue in many proteolytic enzymes, including trypsin and chymotrypsin. The reaction of the serine group destroys the catalytic activity. Toxic **organophosphorous** compounds such as certain **insecticides** act as irreversible inhibitors by binding to the active site of human **acetylcholinesterase**, which leads to a toxic accumulation of acetylcholine.

2. The chemical modification of an amino acid side chain generally causes some perturbation of the secondary or tertiary structure of a protein. A reaction involving an amino acid residue well outside the active site thus could have a long-range disruptive effect that alters the structure of the active site sufficiently to inhibit the enzyme.

3. Irreversible inhibition **does not obey** Michaelis-Menten kinetics.

4. An irreversible inhibitor often can be designed for the active site of a particular enzyme by incorporating a reactive group in a molecule that resembles a substrate.

a. Affinity labels. These are **substrate analogs** that possess a **highly reactive group** that is **not present on the natural substrate**. The active site is permanently blocked from the substrate because the **group reacts covalently with an amino acid residue**. The residue that is modified is not necessarily involved in catalysis.

b. Mechanism-based or suicide inhibitors. These are **substrate analogs** that are **transformed by the catalytic action of the enzyme**. Their structures are such that the **product of this reaction is highly reactive** and **subsequently combines covalently with an amino acid residue** in the **active site**, thus inactivating the enzyme.

c. Transition-state analogs. These are **substrate analogs** whose structures **closely resemble the transition state of the natural substrate**. Transition-state analogs do not covalently modify the enzyme but **bind the active site** so **tightly** that they irreversibly inactivate it.

Reversible inhibition

In **reversible inhibition** the inhibitor can dissociate from the enzyme because it binds through noncovalent bond. The most common forms of reversible inhibition are **competitive**, **noncompetitive** and **uncompetitive**

1. Competitive inhibition

a. The **structure of a competitive inhibitor closely resembles** that of the enzyme's **normal substrate**. Inhibitors compete directly with substrate for binding to the active site. Because of its structure, a competitive inhibitor binds reversibly to the enzyme's active site. In so doing, the inhibitor forms an enzyme-inhibitor complex (EI) that is equivalent to the ES complex. EI complex readily dissociated and the empty active site is then available for substrate binding.

b. The effect of a competitive inhibitor on activity is reversed by **increasing** the concentration of substrate. At high [S], all the active sites are filled with substrate, and reaction velocity reaches the value observed in the absence of inhibitor.

c. In competitive inhibition, the **Km value increases** whereas **Vmax** remains **uncharged** (Fig.5—1).

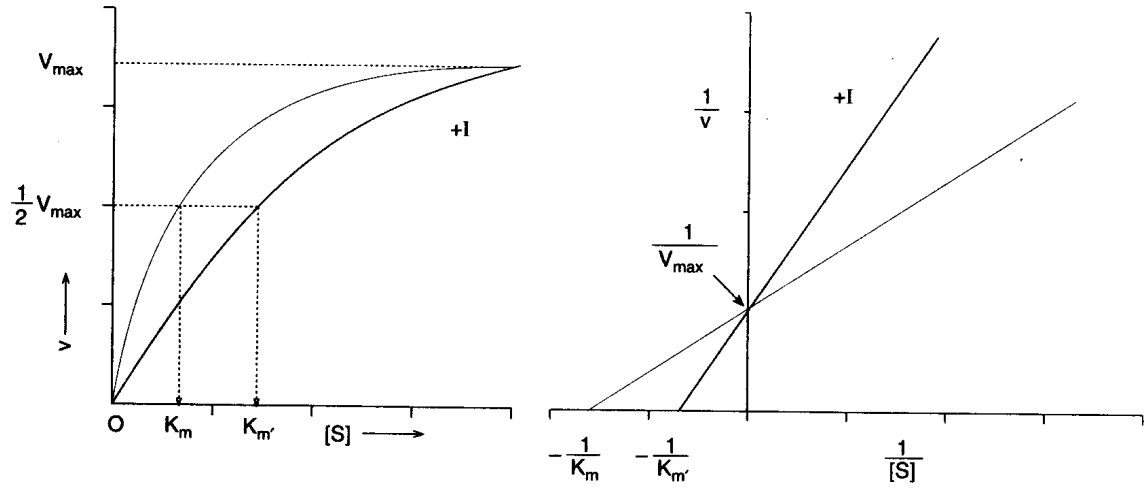
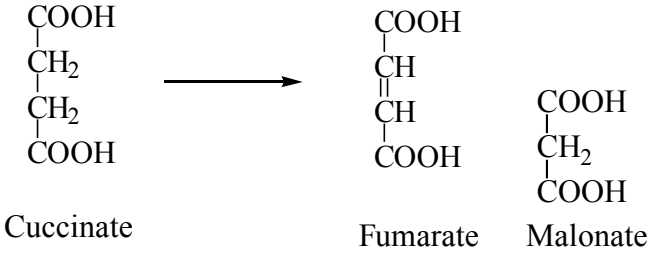


Figure 5—1. Effect of competitive inhibitor (I) on enzyme velocity

For example, succinate dehydrogenase, an enzyme in the Krebs citric acid cycle, catalyzes formation of fumarate by removal of one hydrogen atom from each α-carbon atom of succinate:



Cuccinate Fumarate Malonate

This reaction is inhibited by malonate. Malonate binds to the enzyme's active site but cannot be converted to product.

2. Uncompetitive inhibition

a. Inhibitors bind only to the ES complex at a site distinct from the active site (i.e., the allosteric site).

b. Uncompetitive inhibitor **decreases both K_m** and **V_{max}** values of the enzyme.

3. Noncompetitive inhibition

a. Inhibitors bind both to the free enzyme and to the ES at the allosteric site, which is distinct from the active site. Both EI and EIS complexes form. Inhibitor binding causes an alteration in the enzyme's three-dimensional configuration that prevents the reaction from occurring.

b. Noncompetitive inhibition is not reversed by increasing the concentration of substrate.

c. For non-competitive inhibition the **Km** value is **unchanged** while **Vmax** is **lowered** (Fig. 5—2).

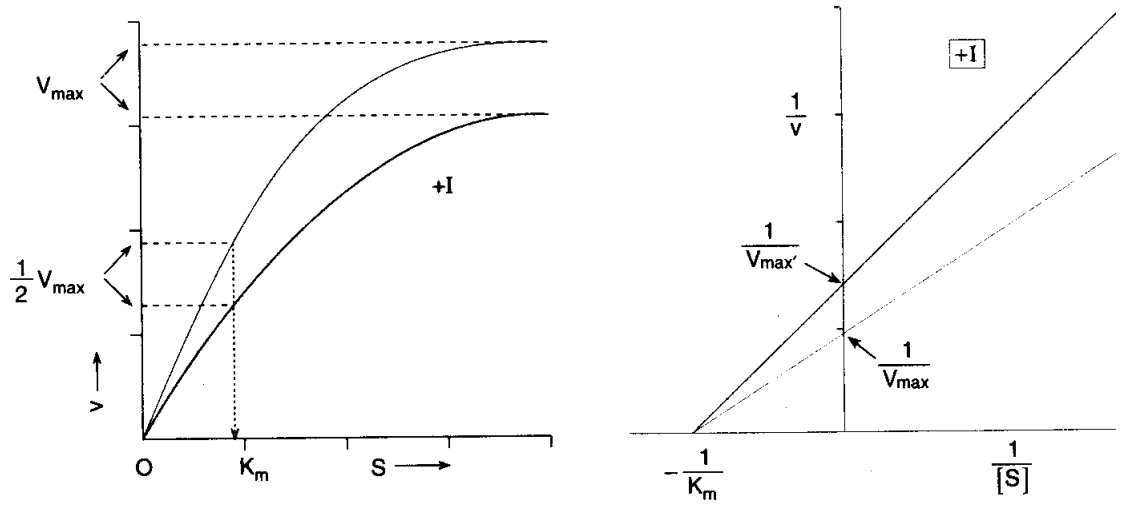


Figure 5—2. Effect of non-competitive inhibitor (I) on enzyme velocity

Medical relevance of enzyme inhibitors

1. Toxicity. Many highly toxic, naturally occurring, and man-made compounds are irreversible enzyme inhibitors. For example, the nerve gas, sarin, is an acetylcholinesterase inhibitor.

2. Therapeutic applications (Table 5—2)

a. **Antimetabolites:** these are the chemical compounds that block the metabolic reactions by their inhibitory action on enzymes. Antimetabolites are usually structural analogues of substrate and thus are competitive inhibitors. They are in use for cancer therapy, gout etc.

b. Synthetic compounds. The rational design of therapeutic drugs often involves the synthesis of inhibitors of certain enzymes (e.g., fluorouracil).

c. Natural compounds used as drugs can also inhibit enzymes (e.g., penicillin).

Table 5—2.

Examples of enzyme inhibitors with therapeutic applications

Inhibitor	Target Enzyme	Effect or Application
Allopurinol	Xanthine oxidase	Treatment of gout
Aspirin	Cyclooxygenase	Anti-inflammatory agent
5-Fluorouracil	Thymidylate synthetase	Antineoplastic agent
Lovastatin	HMG-CoA reductase	Cholesterol-lowering agent
Pargyline	Monoamine oxidase	Antihypertensive agent
Penicillin	Transpeptidase	Antibacterial agent

HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A.

ENZYME REGULATION

The thousands of enzyme-catalyzed chemical reactions that occur in living cells are organized into a series of **biochemical** (or **metabolic**) **pathways**. Each pathways consists of a sequence of catalytic steps in which the product of the first reaction becomes the substrate of the next and so on. The number of reac-

tions varies from one pathway to another. Frequently, biochemical pathways have branch points.

There are following mechanisms of the enzyme regulation.

1. Covalent modification. Some enzymes are regulated by the **reversible** interconvention between their active and inactive forms. Several types of covalent modifications of enzyme structure are responsible for these changes in function.

a. Phosphorylation. In certain enzymes, the addition of a phosphate group to a specific amino acid residue [usually serine (Ser), tyrosine (Tyr), or threonine (Thr)] by specific protein kinases dramatically enhances or depresses activity. **This modification is reversible.** The phosphorylated enzyme may be dephosphorylated by specific phosphatases. For example, glycogen phosphorylase catalyzes the first reaction in the degradation of glycogen, a carbohydrate energy storage molecule. In a process that is under hormonal control the inactive form of the enzyme (glycogen phosphorylase b) is converted to the active form (glycogen phosphorylase a) with the addition to a phosphate group to a specific serine residue.

b. Nucleotidylation. The activities of certain enzymes are regulated by the reversible addition of a nucleotide (e.g., adenosine) to a specific aminoacid. **This modification is reversible.** For example, an adenylated enzyme may be deadenylated by a specific enzyme. Other types of reversible covalent modification include methylation and acetylation.

2. Proteolytic cleavage. Several enzymes are produced and stored as inactive precursors called **proenzymes or zymogens** that become active only after being cleaved at a specific site in their polypeptide chain by specific **proteases.**

a. Many **digestive enzymes that hydrolyze proteins** are synthesized as zymogens in the stomach and pancreas. For example, chymotrypsinogen is produced in the pancreas. After chymotrypsinogen is secreted into the small intestine, trypsine (another proteolytic enzyme) cleaves the peptide bond between Arg 15 and Ile 16. The catalytically active product chymotrypsin is then avaible to assist in the digestion of dietary protein. Other enzymes that are activated by partial proteolysis include pepsine, trypsin, elastase, collagenase.

b. Blood clotting is mediated by a series of proteolytic zymogen activities of several serum enzymes.

3. Allosteric regulation

a. In each biochemical pathway there is at least one enzyme that act as a pacemarker, that is, it sets the rate for the entire pathway. **Pacemaker enzymes** (or **regulatory enzymes**) usually catalyze the first unique (or **"committed")** step in a pathways. Another typical control points is the first step of a branch in a pathway that leads to an alternate product. There are two major strategies for regulating pacemaker enzymes: covalent modification (discussed above) and allosteric regulation.

b. Cells use allosteric regulation to respond effectively to **change** in **intracellular conditions**. Allosteric enzymes are usually composed of **several pro-**

tomers whose properties are affected by effectors molecules. Although many effectors are relatively small molecules, the activity of some enzymes is modulated when they bind to another protein. For example, the pancreas produces trypsin inhitor, which binds to the active site of trypsin. Trypsin inhibitor prevents the premature activation of trypsin.

c. The binding of an effector to one protomer of an allosteric enzyme can affect the binding of ligands by other protomers.

(1) If the substrate influences the substrate binding through allosteric mechanism the term **homotropic effect** is used and their effect always positive.

(2)Heterotropic effect is used when an allosteric modulator effects the binding of substrate to the enzyme. Heterotropic interactions are either positive or negative. Both homotropic and heterotropic interactions results from **cooperativity** between protomers.

d. The activity of allosteric enzymes can be **modulated by the binding of allosteric effectors** to the allosteric site on the enzyme. The biding curves for allosteric enzymes are sigmoidal.

(1) Effectors are positive if they enhance the rate of a reaction (i.e., activators) and negative if they decrease the rate of reaction (i.e., inhibitors).

(2) Feedback inhibition (or end product inhibition) is negative modulation of the committed step of a metabolic pathway by its end product. This prevents unnecessary production of an excess of end product by shutting down the pathway until more is needed.

e. There are two theoretical models that attempt to explain the behavior of allosteric enzymes: the concerted model and the sequential model

(1) The **concerted** (or **symmetry**) **model** assumes that the enzyme binds substrate as indicate in the lock-and-key model. It proposes that enzyme exists in only two state: T(aut) and R(elaxed). Substrates and activators bind more easily to the R conformation, while inhibitors favor the T conformation. The term "concerted" is applied to this model because the conformations of all of the protein's protomers are believed to change simultaneously when the first effector bind. The binding of an activator shifts the equilibrium between the T and R conformations in favor of the R form. An inhibitor shifts the equilibrium toward the T conformation.

The concerted model is too simple to account for the complex behavior of many enzymes. It cannot account for **negative cooperativity**, a phenomenon that is observed in a few enzymes in which the binding of the first ligand reduced the affinity of the enzyme for subsequent ligand binding. The concerted model accounts only **positive cooperativity**, a process in which the first ligand increases subsequent ligand binding.

(2) In the **sequential model,** the binding of a ligand to one protomer results in a conformational change that is sequentially transmitted to the other protomers in the protein. The sequential model allows for the intermediate conformations, which are believed to be a closer approximation to reality than the simpler

concerted model. Negative cooperativity is also accounted for. A ligand binding might make ligand binding less likely.

Enzymes in clinical diagnosis

Enzymes are useful in modern medical practice for several reasons:

1. Enzyme assays provide important information concerning the presence and severity of a variety of disease.

2. Enzymes often provide a means of monitoring a patient's response to therapy.

3. Genetic predispositions to certain diseases may also be determined by measuring specific enzyme activities.

In the clinical laboratory, enzymes are used in two ways:

1. The activity of certain enzymes may be measured directly. Many enzymes are present **in serum**, and their activity can be easily assayed without purification. **Elevation** or **depression** of the levels of activity of specific enzymes may indicate either the presence of a disease or damage to a specific tissue (Table 5—3).

Table 5—3.

Enzyme Activities Useful in Clinical Diagnosis

Assayed Enzyme	Diagnostic Uses
Acid phosphatase	Prostate cancer
Alanine aminotransferase	Viral hepatitis, liver damage
Alkaline phosphatase	Liver disease, bone disorders
Amylase	Acute pancreatitis
Creatine kinase	Muscle disorders, heart attack
Lactate dehydrogenase	Heart attack

Enzymes are measured by using blood **plasma** (the liquid remaining after the blood cells have been removed) or blood **serum** (the straw-colored liquid that results when blood has been allowed to clot).

Blood plasma contains two types of enzymes:

a. The enzymes that generally are synthesized in the liver but are present in blood in equivalent or higher concentrations. They are **specific** to plasma and include the enzymes involved in the blood-clotting process (e.g. thrombin and plasmin) and lipoprotein metabolism.

b. The **nonspecific** plasma enzymes have no physiological role in plasma and are normally present in low concentration. Their presence usually reflects a normal turnover of cells in which there is a release of intracellular enzymes. Damage to an organ that occurs because of disease or trauma may cause an elevation of nonspecific plasma enzyme. Because there are few enzymes that are specific to one organ, the activities of several enzymes must often be measured.

Nonspecific plasma enzymes include those in **exocrine** secretions and true **intracellular** enzymes. Exocrine enzymes — pancreatic amylase, lipase, bile alkaline phosphatase — diffuse into plasma. True intracellular enzymes normally are absent from the circulation.

2. Several enzymes are used **as reagent**. Many purified enzymes are now commercially available for use in the determination of components in blood and tissues. Such enzymatic assays are usually more **specific**, **sensitive**, **cost-effective** and **faster** than chemical determinations. Examples of clinically relevant compounds that can be determined enzymatically include glucose, urea, ethanol, and triglycerides.

Isozymes

The **multiple forms of an enzyme** catalyzing the same reaction are **isoenzyme** or **isozyme**. They, however, differ in their physical and chemical properties which include the structure, electrophoretic and immunological properties. Analysis of the distribution of isozymes of particular enzymes is sometimes a useful tool in clinical diagnosis. Example, lactate dehydrogenase (LDH) is a tetramer composed of two types of protomers: heart type (H) and muscle type (M). There are five different LDH isozymes. LDH 1 (H_4) and LDH 2 (H_3M) are found only in heart muscle and red blood cells. LDL 5 (M_4) occurs in both liver and skeletal muscle. LDH_3 and LDH_4 are found in other organs. The use of information generated from measuring the blood level and migration pattern of LDH virtually guarantied that a correct diagnosis will be made.

Therapeutic uses of enzymes

The use of enzymes in medical therapy has been limited. When administered to patients, enzymes are often rapidly inactivated or degraded. The large amounts of enzyme that are often required to sustain a therapy may provoke allergic reactions. There are, however, several examples of successful enzyme therapies:

1. Streptokinase is currently used with significant success in the treatment of myocardial infarction, which results from the occlusion of the coronary arteries. If administered soon after the beginning of a heart attack, streptokinase can offen prevent or significantly reduce further damage to the heart. Streptokinase catalyzes the conversion of plasminogen to plasmin, the trypsinlike enzyme that digests fibrin.

2. **Asparaginase** is used in the treatment of several types of cancer. Presumably, the lack of asparagine results in an inhibition of protein synthesis, a condition that results in cell death.

LECTURE 6

MEMBRANES. TRANSPORT SYSTEMS

Most of the properties that are attributed to living organisms (e.g., movement, growth, reproduction, and metabolism) are dependent, either directly or indirectly, on membranes.

1. Plasma membranes form closed compartments around cellular protoplasm to separate one cell from another and thus permit cellular individuality.

2. The plasma membrane has selective permeability and acts as a barrier, there by maintaining differences in composition between the inside and the outside of the cell.

3. Membranes also form specialized compartments within the cell. Such intracellular membranes form many of the morphologically distinguishable structures (organelles), e.g., mitochondria, endoplasmic reticulum, sarcoplasmic reticulum, Golgi complexes, secretory granules, lysosomes, and the nuclear membrane.

4. Membranes localize enzymes, function as integral elements in excitation-response coupling, and provide sites of energy transduction, such as in photosynthesis and oxidative phosphorylation.

Membrane structure

All biological membranes have the same **general structure**. Because each type of living cell has its own specific set of functions, it follows that the structure of its membranes is also unique.

1.Membranes are complex structures composed of **lipids**, **proteins** and **carbohydrates.**

2.The proportion of lipid and protein varies considerably among different cell types and among organelles within each cell. The types of lipid and protein found in each membrane also vary.

3.Membranes **are asymmetric** sheetlike enclosed structures with an inside and an outside surface.

4.These **sheetlike** structures are **noncovalent** assemblies that are thermodynamically stable and metabolically active.

5.Specific protein molecules are anchored in membranes, where they carry out specific functions of the organelle, the cell, or the organism.

Membrane lipids

1. The major lipids in mammalian membranes are **phospholipids, glycosphingolipids and cholesterol.**

a. The 2 major **phospholipids** classes present in membranes:

(1) Phosphoglycerides are the more common and consist of a glycerol backbone to which are attached 2 fatty acids in ester linkage and a phosphorylated alcohol (Fig. 6—1).

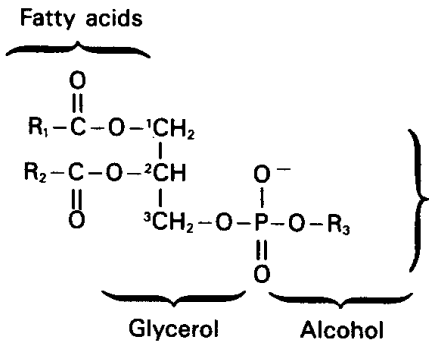


Figure 6—1. A phosphoglyceride showing the fatty acids (R_1 and R_2), glycerol, and phosphorylated alcohol components. In phosphatidic acid, R_3 is hydrogen

The fatty acid constituents are usually even-numbered carbon molecules most commonly containing 16 or 18 carbons. They are unbranched and can be **saturated** or **unsaturated.**

(2) Sphingomyelins contain a sphingosine backbone rather than glycerol. A fatty acid is attached by an amide linkage to the amino group of sphingosine. The primary hydroxyl group of sphingosine is esterified to phosphorylcholine. Sphingomyelins, as the name implies, are prominent in myelin sheaths.

b. Glycosphingolipids. The glycolipids are sugar-containing lipids such as cerebrosides and gangliosides. The **cerebrosides** and **gangliosides** differ from sphingomyelin in the moiety attached to the primary hydroxyl group of sphingosine. In sphingomyelin, a phosphorylcholine is attached to the alcohol group. A cerebroside contains a single hexose moiety, glucose or galactose, at that site. A ganglioside contains a chain of 3 or more sugar — at least one of which is a sialic acids — attached to the primary alcohol of sphingosine.

c. Steroids.

(1) The most common sterol in membranes is **cholesterol** (Fig. 6—2), which exist almost exclusively in the **plasma membrane** of mammalian cells but can also be found in lesser quantity in mitochondria, Golgi complex, and nuclear membranes.

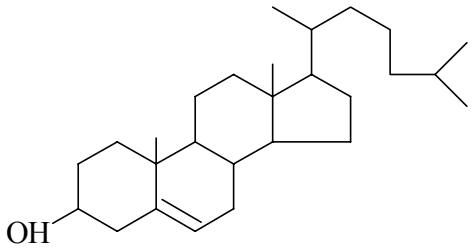


Figure 6—2. Cholesterol.

(2) Cholesterol is generally more abundant toward the outside of the plasma membrane.

(3) Cholesterol intercalates among the phospholipids of the membrane, with its hydroxyl group at the aqueous interface and the remainder of the molecule within the leaflet.

2. Membranes are amphipathic structure

a. All major lipids in membranes contain both hydrophobic and hydrophilic regions and are therefore termed **amphipathic.** Membranes themselves are thus amphipathic.

b. The amphipathic membrane lipids have a polar head group and nonpolar tails, as represented in Figure 6—3.

c. Saturated fatty acids make **straight tails**, whereas unsaturated fatty acids, which generally exist in the *cis* form in membranes, make **kinked tails**. As more kinks are inserted in the tails, the membrane becomes less tightly packed and therefore **more fluid.**

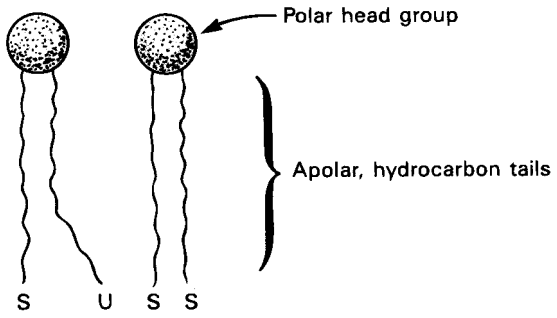


Figure 6—3. Diagrammatic representation of a phospholipids or other membrane lipids. The polar head group is hydrophilic, and the hydrocarbon tails are hydrophobic or lipophilic. The fatty acids in the tails are saturated (S) or unsaturated (U) (by Murray R.K. et al., 1996).

e. A bilayer exists as a sheet in which the **hydrophobic regions of the phospholipids are protected from the aqueous environment, while the hydrophilic regions are immersed in water** (Fig. 6—4).

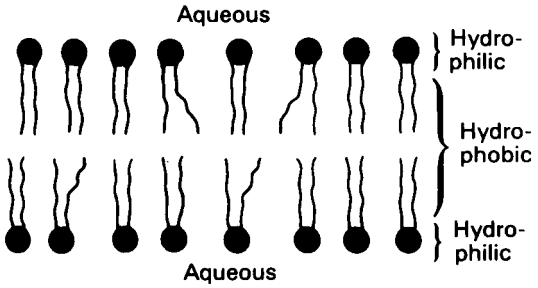


Figure 6—4. Diagram of a section of a bilayer membrane formed from phospholipid molecules (by Murray R.K. et al., 1996)

Membrane Proteins

As was previously mentioned, most of the functions associated with biological membranes require protein molecules. Membrane proteins are often classified by **the type of function** they perform. Most of these molecules are **structural components**, **enzymes, hormone receptors**, or **transport mechanisms**.

1. Membrane proteins are also classified according to their structural relationship to membrane.

a. Proteins that are embedded in and/or extend through a membrane are referred to as **integral proteins**. Such molecules can be extracted only by disrupting the membrane with organic solvents or detergents. They interact with the phospholipids. Integral proteins are asymmetrically distributed across the membrane bilayer.

b. Peripheral proteins do not interact directly with the phospholipids in the bilayer and they are weakly bound to the hydrophilic regions of specific integral membrane proteins. Some peripheral proteins interact directly with the

lipid bilayer. Typically, peripheral proteins hence do not require use of detergents for their release and can be released from membrane with relatively gentle methods (e.g., concentrated salt solutions or pH changes). Many hormone receptor molecules are integral proteins, and the specific polypeptide hormones that bind to these receptor molecules may therefore be considered peripheral proteins.

2. Membrane phospholipids act as a solvent for membrane proteins, creating an environment in which the latter can function.

3. Proteins are **amphipathic** and form an integral part of the membrane by having hydrophilic regions protruding at the inside and outside faces of the membrane but connected by a hydrophobic region traversing the hydrophobic core of the bilayer. In fact, those portions of membrane proteins that verse membranes do contain substantial numbers hydrophobic amino acids and a high α-helical or β-pleated sheet content.

Properties of membranes

1. Membrane fluidity. The term "fluidity" describes the resistance of membrane components to movement. Rapid **lateral movement** is apparently responsible for the proper functioning of many membrane proteins. A membrane's fluidity is largely determined by **the lipid composition** and the percentage of **unsaturated fatty acids** in its phospholipid molecules. (Recall that unsaturated hydrocarbon chains pack less densely than saturated ones.)

Cholesterol also acts as a moderator molecule in membranes, producing intermediate states of fluidity. If the acyl side chains exist in a disordered phase, cholesterol will have a condensing effect; if the acyl side chains are ordered or in a crystalline phase, cholesterol will induce disorder. At high cholesterol: phospholipid ratios, transition temperatures are abolished altogether.

The fluidity of a membrane significantly affects **its functions**. As membrane fluidity increases, so does its permeability to water and other small hydrophilic molecules. The lateral mobility of integral proteins increases as the fluidity of the membrane increases. If the active site of an integral protein involved in some given function resides exclusively in its hydrophilic regions, changing lipid fluidity will probably have little effect on the activity of the protein; however, if the protein is involved in a transport function in which transport components span the membrane, lipid phase effects may significantly alter the transport rate.

Cholesterol moderates membrane fluidity because its rigid structure serves to interrupt the lateral diffusion of nearby phospholipids.

2. Selective permeability. Because of their hydrophobic nature, the hydrocarbon chains in lipid bilayers provide a virtually impenetrable barrier to ionic and polar substances. (Specific membrane proteins are responsible for the regulated movement of such substances into and out of cells.) To diffuse across a lipid bilayer, a hydrophilic molecule must shed its solvation sphere, an unlikely

possibility. Water molecules are an exception to this rule, since small amounts of water have been observed to penetrate artificial bilayers. However, most water movement through biological membranes appears to be through protein channels.

3. Self-sealing capability. When lipid bilayers are disrupted, they immediately and spontaneously reseal. This process occurs because a break in a lipid bilayer exposes the hydrophobic hydrocarbon chains to water. Because breaches in cell membranes are potentially lethal, this resealing property is obviously critical. (In living cells, certain protein components of membrane and the cytoskeleton, as well as calcium ions, also assist in membrane resealing.)

4. Asymmetry. Biological membranes are asymmetric:

a. The lipid composition of each half of a bilayer is different. There is inside-outside (transverse) asymmetry of the phospholipids. The choline-containing phospholipids (phosphatidylcholine and sphingomyelin) are located mainly in the outer molecular layer; the aminophospholipids (phosphatidylserine and phosphatidylethanolamine) are preferentially located in the inner layer. Cholesterol is generally present in larger amounts on the outside than on the inside. Obviously, if this asymmetry is to exist al all, there must be limited transverse mobility (flip-flop) of the membrane phospholipids.

b. This asymmetry can be partially attributed to the irregular distribution of proteins within the membranes.

c. An **inside-outside asymmetry** is also provided by the external location of the carbohydrates attached to membrane proteins.

d. Specific enzymes are located exclusively on the outside or inside of membranes, as in the mitochondrial and plasma membranes.

e. There are **regional asymmetries** in membranes. Some, such as occur at the villous border of mucosal cells, are almost macroscopically visible. Others, such as those at gap junctions, tight junctions, and synapses, occupy much smaller regions of the membrane and generate correspondingly smaller local asymmetries.

5. Membranes and their components are dynamic structure. The lipids and proteins in membranes turn over, just as they do in other compartments of the cell. Different lipids have different turnover rates, and the turnover rates of individual species of membrane proteins may vary widely. Membrane proteins and lipids diffuse **laterally** and of rotate about an axis perpendicular to the plane of the bilayer. The specific orientations maintained by integral membrane proteins with respect to the bilayers suggest that rotation of these molecules through the plane of the bilayer (**flip-flop or transverse motion**) does not occur.

Model of membrane structure

The **fluid mosaic model** of membrane structure was proposed in 1972 by S.J. Singer and G.L.Nicolson (Fig. 6—5).

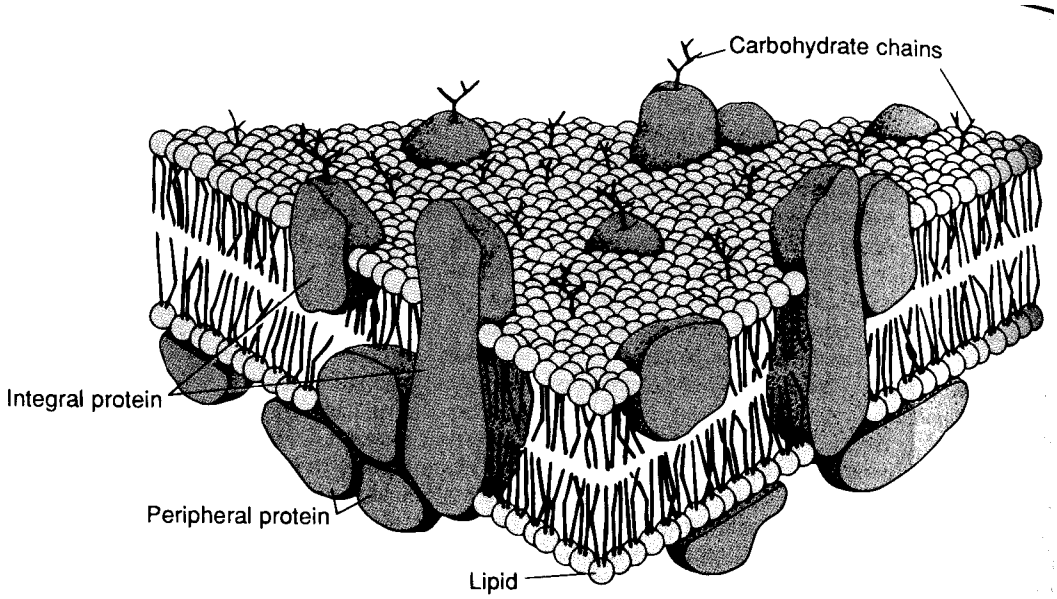


Figure 6—6. The fluid mosaic model of membrane structure (by Murray R.K. et al., 1996).

The membrane consists of a bimolecular lipid layer with proteins inserted in it or bound to the cytoplasmic surface. Integral membrane proteins are firmly embedded in the lipid layers. Some of these proteins completely span the bilayer and are called transmembrane proteins, while other are embedded in either the outer or inner leaflet of the lipid bilayer. Loosely bound to the outer or inner surface of the membrane are the peripheral proteins. Many of the proteins and lipids have externally exposed oligosaccharide chains.

MEMBRANE SELECTIVITY

Membrane transport mechanisms are vital to living organisms. There is a constant movement of ions and molecules across cell plasma membranes as well as across the membranes of organelles. This flux must be carefully regulated so that each cell's metabolic needs are met. For example, a cell's plasma membrane regulates the entrance of nutrient molecules and the exit of waste products. Additionally, it regulates intracellular ion concentrations. Because of the generally impenetrable nature of lipid bilayers to ions and polar substances, specific transport components must be inserted into cellular membranes.

Mechanisms move molecules across membranes

Molecules can passively traverse the bilayer down electrochemical gradients **by simple** or **facilitated** diffusion (Fig. 6—6).

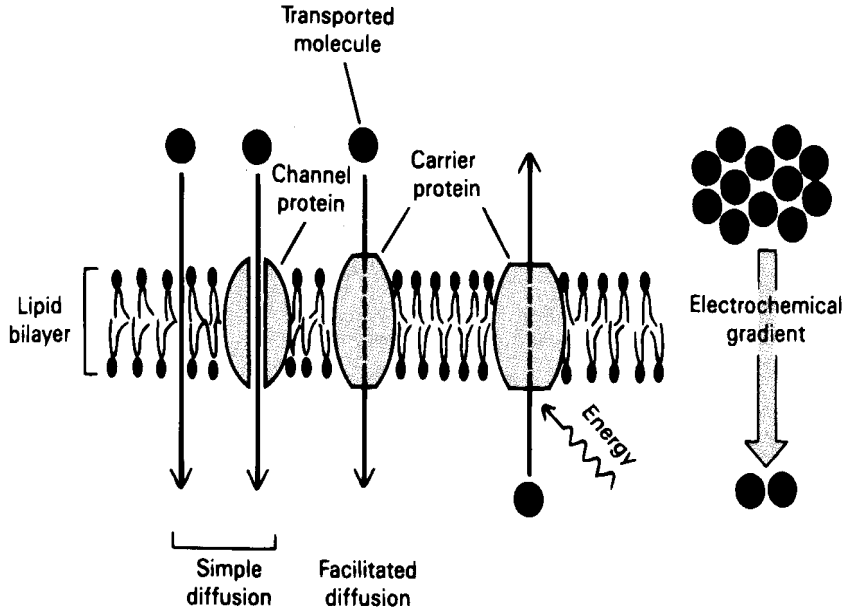


Figure 6—6. Mechanisms move molecules across membrane (by Murray R.K. et al., 1996).

1. Passive Diffusion. Some solutes such as gases can enter the cell by diffusing down an **electrochemical gradient** across the membrane and **do not require metabolic energy.** The simple diffusion of a solute across the membrane is limited by the **thermal agitation of that specific molecule**, by the **concentration gradient** across the membrane, and the **solubility** of that solute (the permeability coefficient, Fig. 6—7) in the hydrophobic core of the membrane bilayer.

In natural membranes there are **transmembrane** channels, porelike structures composed of proteins that constitute selective ion-conductive pathways. The permeability of a channel depends upon the size, extent of hydration, and extent of charge density on the ion. Specific channels for Na^+, K^+, and Ca^{2+} have been identified. Channels are open transiently and thus are **gated.** Gates can be controlled by opening or closing. In **ligand-gated channels,** a specific molecule binds to a receptor and opens the channel. **Voltage-gated channels** open (or close) in response to a change in membrane potential.

Some microbes synthesize small organic molecules, **ionophores** that function as shuttle for the movement of ions across membranes. These ionophores contain hydrophilic centers that bind specific ions and are surrounded by peripheral hydrophobic regions$ this arrangement allows the molecules to dissolve effectively in the membrane and diffuse transversely therein.

2. Facilitated diffusion & Active transport

Molecules that cannot pass freely through the bilayer membrane by themselves do so in association with **carrier proteins**. This involves 2 processes — facilitated diffusion and active transport — and specific transport systems.

Transport systems can be described in a functional sense according to the number of molecules moved and the direction of movement (Fig. 6—7) or according to whether movement is toward or away from equilibrium.

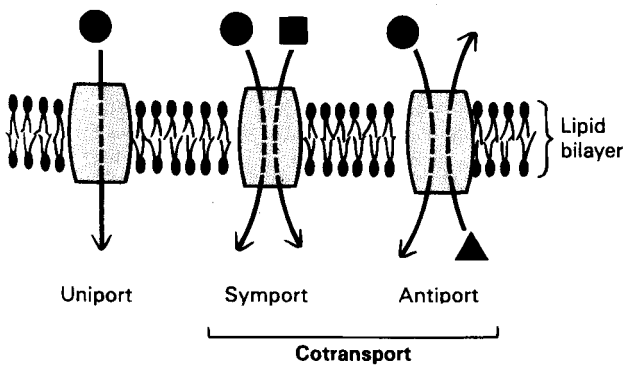


Figure 6—7. Schematic representation of types of transport systems (by Murray R.K. et al., 1996).

A **uniport** system moves one type of molecule bidirectionally. In **cotransport** systems, the transfer of one solute depends upon the stoichiometric simultaneous or sequential transfer of another solute. A **symport** moves these solutes in the same direction. Examples are the proton-sugar transporter in bacteria and the Na^+-sugar transporters (glucose, mannose, galactose, xylose, and arabinose) and the Na^+-amino acid transporters in mammalian **Antiport** systems move 2 molecules in opposite directions (e.g., Na^+ in and Ca^{2+} out).

Molecules that cannot pass freely through the bilayer membrane by themselves do so in association with **carrier proteins**. This involves 2 processes — **facilitated diffusion and active transport** — and specific transport systems.

Facilitated diffusion and active transport **share** many features:

a.Both appear to involve **carrie proteins**

b. Both show specificity for ions, sugars, and amino acids.

c.Facilitated diffusion and active transport resemble a substrate-enzyme reaction except that no covalent interacti curs.

Major **differences** are the following:

a.Facilitated diffusion can operate **bidirectionally**, whereas active transport is usually **unidirectional.**

b. Active transport always occurs against an electrical or chemical gradient, and so it **requires energy**.

Facilitated diffusion

1. The rate of facilitated diffusion, a uniport system, can be **saturated**; ie, the number of sites involved in diffusion of the specific solutes appears finite.

2. Many facilitated diffusion systen stereospecific but, like simple diffusion, require no metabolic energy.

3. The transport of certain large or charged molecules occurs through special **carriers**. In carrier-mediated transport, a specific solute binds to the carrier on one side of a membrane. As the result of binding, a conformational change in the carrier occurs. The solute is then translocated across the membrane and released.

A "ping pong" mechanism (Fig. 6—8) **explains facilitated diffusion.**

In this model, the carrier protein exists in 2 principal conformations. In the "pong" state it is exposed to high concentrations of solute, and molecules of the solute bind to specific sites on the carrier protein. Transport occurs when a conformational change exposes the carrier to a lower concentration of solute ("ping" state). This process is completely reversible, and net flux across the membrane depends upon the concentration gradient.

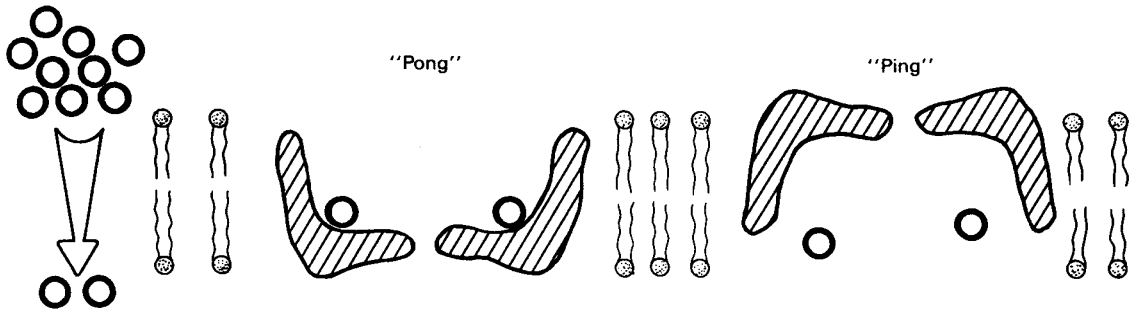


Figure 6—8. The "ping-pong" model of facilitated diffusion (by Murray R.K. et al., 1996).

The rate at which solutes enter a cell by facilitated diffusion is determined by the following factors:

1. The concentration gradient across the membrane.

2. The amount of carrier available (**this is a key control step**).

3. The rapidity of the solute-carrier interaction.

4. The rapidity of the conformational change for both the loaded and the unloaded carrier.

Active Transport

1. The process of active transport differs from diffusion in that molecules are transported away from thermodynamic equilibrium; hence, **energy is required**.

2. This energy can come from the **hydrolysis of ATP**, from **electron movement**, or **from light**. The maintenance of electrochemical gradients in biologic systems is so important that it consumes perhaps 30-40% of the total energy expenditure in a cell.

3. There are two forms of active transport: primary and secondary. In **primary active transport,** ATP provides energy. Transmembrane ATP-hydrolyzing enzymes use the energy derived from ATP to drive the transport of ions or molecules. The Na^+-K^+ pump (also referred to as the Na^+-K^+ ATPase) is a prominent example of a primary transporter. In **secondary active transport,** concentration gradients generated by primary active transport are harnessed to move various substances across membranes. For example, the Na^+ gradient created by the Na^+-K^+ ATPase is used in kidney tubule cells and intestinal cells to transport of glucose.

In general, cells maintain a low intracellular Na^+ concentration and a high intracellular K^+ concentration, along with a net negative electrical potential inside. The pump that maintains these gradients is an ATPase that is activated by Na^+ and K^+ (Fig. 6—9).

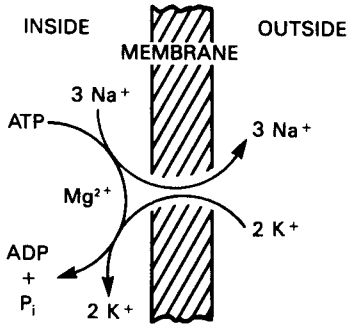


Figure 6—9. Stoichiometry of the Na^+/K^+-ATPase pump (by Murray R.K. et al., 1996).

The ATPase is an **integral membrane** protein (M.W. 250 000) and requires phospholipids for activity. It consists of two α and two subunits β. The ATPase has catalytic centers for both ATP and Na^+ on the cytoplasmic side of the membrane, but the K^+ binding site is located on the extracellular side of the membrane. Ouabain (or digitalis) inhibits this ATP-ase by binding to the extracellular domain. Inhibition of the ATP-ase by ouabain can be antagonized by extracellular K^+.

Transport of macromolecules

The transport of macromolecules such as proteins, polysaccharides and polynucleotides across the membrane is equally important. This brought about by two independent mechanisms namely **endocytosis** — intake of macromolecules by the cells and **exocytosis** — release of macromolecules from the cells to the outside.

1. Endocytosis. It is estimated that approximately 2% of the exterior surface of plasma membrane possesses characteristic coated pits. These pits can be internalized to form coated vesicle which contain an unusual protein called **clathrin.** The uptake of low density lipoprotein (LDL) molecules by the cells is a good example of endocytosis.

There are 2 general types of endocytosis:

a. Phagocytosis occurs only in specialized cells such as macrophages and granulocytes. Phagocytosis involves the ingestion of **large particles** such as viruses, bacteria, cells, or debris.

b. Pinocytosis is a property of all cells and leads to the cellular uptake of fluid and fluid contents.

2. Exocytosis. The release of macromolecules to the outside of the cells mostly occurs via the participation of Golgi apparatus. The macromolecules are transported to the plasma membrane in vesicles and let out. The secretion of hormones (e.g. insulin, parathyroid hormone) usually occurs by exocytosis.

LECTURE 7

INTRODUCTION TO METABOLISM. NUTRITION

Metabolism involves the transformation of both **matter and energy.** These transformations often involve a number of reactions that are catalyzed by a sequence of enzymes. The **sequence of enzymatic reactions** collectively con-

stitutes a **biochemical (metabolic) pathway.** The product of one enzyme reaction becomes the substrate for the next reaction in the sequence. The successive products of the reactions are known as **metabolites, or metabolic intermediates.** Frequently, biochemical pathways have branch points.

Living organisms have evolved sophisticated mechanisms for regulating biochemical pathways. Regulation is essential for several reason:

1. Maintenance of an ordered state. Regulation of each pathway results in the production of the substances required for the maintenance of cell structure and function in a timely fashion and without huge excesses.

2. Conversation of energy. Cell constantly control energy-generating so that consume just enough nutrients to meet their energy requirements.

3. Responsiveness to environmental changes. Cells can make relatively rapid adjustments to changes in temperature, pH, ionic strength, and nutrient concentrations because of their capacity to increase or decrease the rates of specific reactions.

The regulation of biochemical pathways is complex. It is achieved primarily by adjustments in the concentrations and activities of certain enzymes. Control is accomplished by utilizing various combinations of the following mechanisms: **(1) genetic control, (2) covalent modification** (discussed above)**, (3) allosteric regulation** (discussed above)**, and (4) compartmentalization.**

2. Genetic control. There are two types of enzymes. a) **Constitutive enzymes** (house-keeping enzymes) — the levels of which are **not controlled** and **remain fairly constant**. b) **Adaptive enzymes —** their concentrations increase or decrease as per body needs and are **well-regulated**. The synthesis of enzymes (proteins) is regulated by the genes. The term **induction** is used to represent **increased synthesis** of **enzyme** while **repression** indicates its **decreased synthesis**. Induction or repression which ultimately determines the enzyme concentration at the gene level through the mediation of hormones or other substrate.

The regulatory enzymes are most **rapidly degraded**. If not needed, they immediately disappear and, as and when required, they are quickly synthesized.

3. Compartmentalization of metabolic pathways in cells. The set of enzymes that catalyze a particular metabolic pathway are often localized in a specific intracellular compartment.

a. The cytosol is the liquid portion of the cytoplasm that includes macromolecules but not subcellular organelles.

b. Mitochondria are called the **"power plants"** of the cell because they convert energy to forms that can be used by the cell. A mitochondrion is a double-membrane organelle. The internal membrane is highly folded, with many **cristae** protruding into the large internal compartment called the **matrix.**

c. In some instances, certain enzymes for a metabolic pathway are contained in the **cytosol** (glycolysis, pentose phosphate pathways, fatty acids synthesis)**,** and others are contained in the **mitochondrial matrix** (citric acid cycle, oxidative phosphorylation, β-oxidation of fatty acids, ketone body formation)**.**

d. The biologic advantage of compartmentalization of metabolic pathways is that it allows the separation of processes that proceed in opposite directions and may otherwise interfere with one another. For example:

(1) The anabolic process of fatty acid **biosynthesis from acetyl coenzyme A** (CoA) is confined to the **cytosol.**

(2) The catabolic process of fatty acid **oxidation to acetyl CoA** occurs in the **mitochoidrial matrix.**

METABOLISM consists of catabolism and anabolism.

Catabolism (Fig. 7-1) encompasses the **degradative processes,** whereby complex molecules are broken down into simpler ones.

1. Stage 1. Complex macromolecules (e.g., starch, protein, triacylglycerols) are broken down into smaller units, such as monosaccharides, amino acids, glycerol, and fatty acids. In stage 1 the major nutrient molecules (proteins, fats, and polysaccharides) are degradated to building block molecules in a process called **digestion.** During this stage, little or no free energy is trapped.

2. Stage 2. These smaller molecules are further degraded to a few molecules. The most important end product of stage 2 is **acetyl CoA** that can be oxidized to carbon dioxide (CO_2) and water along a common pathway. In this stage, some free energy is trapped as adenosine triphosphate

3. Stage 3 is the final common pathway by which these molecules are oxidized to CO_2 and water and trap the available free energy as ATP. It consists of:

a. The citric acid cycle

b. The electron transport chain

c. Oxidative phosphorylation

Anabolism (Fig. 7—2) encompasses **biosynthetic pathways** in which small precursors are converted into complex macromolecules. Once the building block molecules (e.g., amino acids, sugars, and fatty acids) are produced or acquired in the diet, they are incorporated into larger, more complex molecules. Anabolic processes require energy inputs, which can be supplied in two ways:

1. By ATP generated during catabolism

2. In some cases, by high-energy electrons in the form of reduced nicotinamide adenine dinucleotide phosphate (NADPH)

Amphibolic pathways have more than one function and occur at the "crossroads" of metabolism, acting as links between the anabolic and catabolic pathways, e.g. the citric acid cycle. Most reaction of amphibolic pathways are reversible. The irreversible reactions in amphibolic pathways are often highly regulated metabolic control points that are catalyzed by allosteric enzymes.

In human the **nervous** and **endocrine** systems are primarily responsible for the coordination of metabolism.

1. The nervous system provides a rapid and efficient mechanism for acquiring and processing environmental information. Nerves cells, called neurons, release **neurotransmitters** and the end of long cell extensions called **axons** into

tiny intracellular spaces called **synapses**. The subsequent binding of neurotransmitter molecules to nearby cells results in specific responses from those cells.

2. Metabolic regulation by the endocrine system is achieved by secretion of chemical signals called **hormones** directly into the blood. Most hormone-induced changes in cell function results alteractions in the activity or concentrations of enzymes.

Classification of organisms

In order to maintain living processes, all organisms must obtain supplies of free energy from their environment:

1. Autotrophic organisms couple their metabolism to some simple exergonic process in their surrounding; e.g. green plants utilize the energy of sunlight, and some autotrophic bacteria utilize the reaction $Fe^{2+} \rightarrow Fe^{3+}$.

2. Heterotrophic organisms obtain free energy by coupling their metabolism to the breakdown of complex organic molecules in their environment.

According to the **capacity to use oxygen** in energy generation all organisms may be classified:

1. Obligate anaerobes, organisms that grow only in the absence of oxygen, avoid the gas by living in highly reduced environments such as soil. They use fermentative processes to satisfy their energy requirements.

2. Aerotolerant anaerobes, which also depend on fermentation for their energy needs, possess detoxifying enzymes and antioxidant molecules that protect against oxygen's toxic products.

3. Facultative anaerobes not only possess the mechanisms needed for detoxifying oxygen metabolites, they can also utilize oxygen to generate energy when the gas is present.

4. Obligate aerobes are highly dependent on oxygen for energy production. They protect themselves from its toxic effects with an impressive array of enzymes and antioxidant molecules.

NUTRITION

The science of nutrition examines the qualitative and quantitative requirements of the diet necessary to maintain good health.

Energy requirement

The mammalian body requires nutrients sufficient to provide free energy to manufacture the daily requirement of high-energy phosphate (mainly ATP) and reducing equivalents (2H) needed to power all body functions.

Energy-yielding nutrients are provided by dietary **carbohydrate**, **fat**, and, to lesser extent, protein in widely varying proportions among different human and animal populations. Consumption of alcohol can also provide a significant proportion of energy intake.

The amount of energy available in the major food sources is follows: protein 4.1 kcal/g (17.2 kJ/g), fat 9.3 kcal/g (38.9 kJ/g), carbohydrate 4.1 kcal/g (17.2 kJ/g) and ethanol 7.1 kcal/g (29.7 kJ/g). The large energy content per gram of fat compared to that of protein or carbohydrate and the relatively high energy content of alcohol are two notable fact.

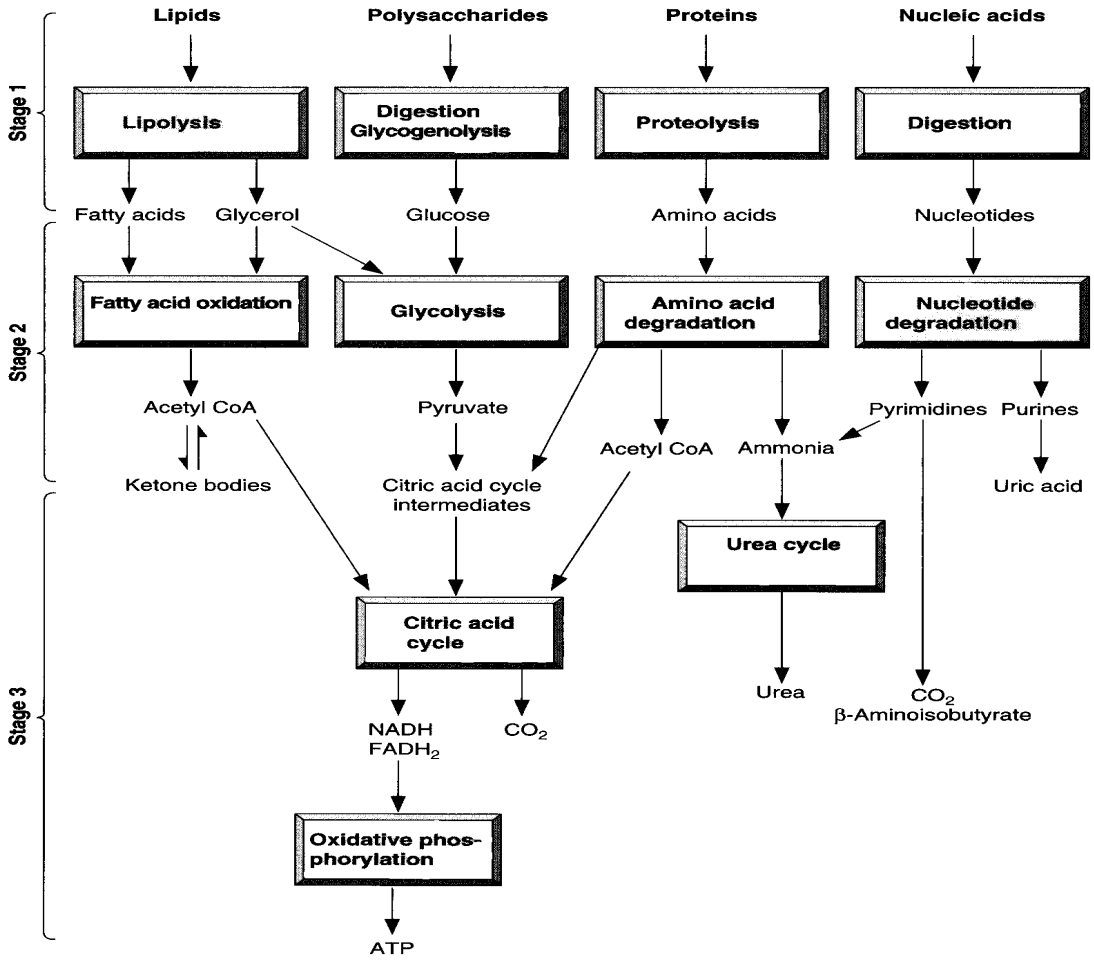


Figure 7—1. A summary of catabolism. *Acetyl CoA* = acetyl coenzyme A; *ATP* = adenosine triphosphate; CO_2 = carbon dioxide; $FADH_2$ = reduced flavin adenine dinucleotide; *NADH* = reduced nicotinamide adenine dinucleotide (by Davidson V.L. et al., 1999).

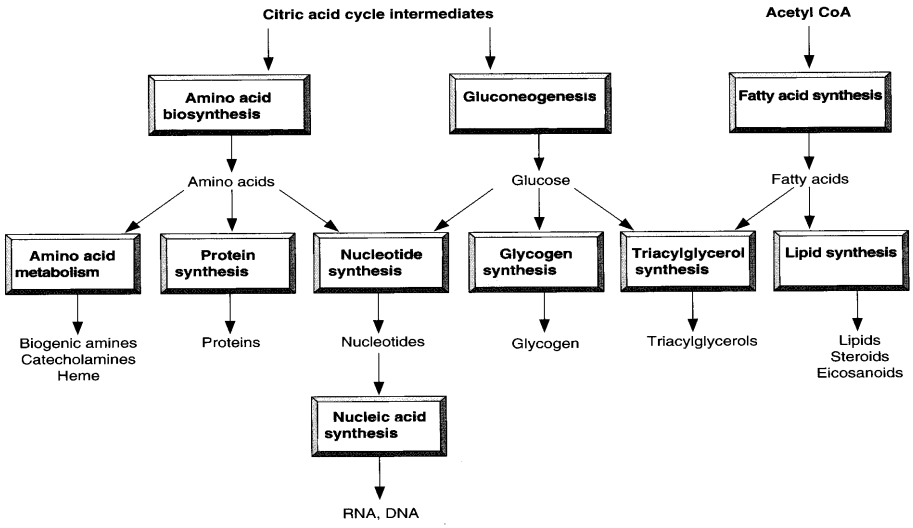


Figure 7-2. A summary of anabolism. *Acetyl CoA* = acetyl coenzyme A; *DNA* = deoxyribonucleic acids; *RNA* = ribonucleic acid (by Davidson V.L. et al., 1999).

Under conditions of **energy equilibrium** (calorie balance), energy intake must equal **energy expenditure**. Energy expenditure varies widely in different conditions and may be measured by placing an animal inside an insulated chamber and measuring the energy output represented by heat loss and excretory products. It is more convenient to measure **oxygen consumption,** since under most conditions 1 L of O_2 consumed accounts for approximately 20 kJ (4.83 kcal) of energy expended.

The energy expended by an individual depends on four main factors:

1. The **basal metabolic rate** is the energy expenditure necessary to maintain basic physiologic functions under standardized conditions. The basal metabolic rate is proportionate to lean body weight and to surface area. It is higher in males than females, in young children, and in people with **fever** and **hyperthyroidism**. It is lower in **hypothyroidism** and in **starvation**.

2. The **thermogenic effect** (specific dynamic action) of food is equivalent to about 5—10% of total energy expenditure and is attributed to the energy expenditure due to digestion and to any stimulation of metabolism caused by the influx of new substrate.

3. Physical activity is the largest variable affecting energy expenditure; the range is over tenfold between resting and maximum athletic activity.

4. When **environmental temperature** is low, it causes increased energy expenditure owing to shivering, and to nonshivering thermogenesis in animals having brown fat. At temperatures above blood heat, extra energy is expended in cooling.

NUTRITIONAL IMPORTANCE OF CARBOHYDRATES

Dietary carbohydrates are the chief source of energy. They contribute to 60—70% of total caloric requirement of the body. Incidentally, carbohydrate rich foods cost less.

Carbohydrates are the most abundant dietary constituents, despite the fact that they are not essential nutrients to the body. From the nutritional point of view, carbohydrates are grouped into 2 categories.

1. Carbohydrates **utilized** by the body — starch, glycogen, sucrose, lactose, glucose, fructose etc.

2. Carbohydrates **not utilized** (not digested) by the body — cellulose, hemicellulose, pectin, gums, etc.

Among the carbohydrates utilized by the body, starch is the most abundant. The consumption of starch has distinct advantages due to its bland taste, satiety value and slow digestion and absorption. Sucrose (the table sugar), due to its sweetness, can be consumed to a limited extent. Excessive intake of sucrose causes dental caries and an increase in plasma lipid levels and is associated health complications.

Functions of carbohydrates

1. Major sources of energy. Carbohydrates are the principal source of energy, supplying 60—80% of the caloric requirements of the body. The actual intake of carbohydrate is dependent on the food habits and economic status of the individual.

2. Protein sparing action. Proteins perform a specialized function of body building and growth. The wasteful expenditure of proteins to meet the energy needs of the body should be curtailed. Carbohydrates come to the rescue and spare the proteins from being misused for caloric purpose.

3. Absolute requirement by brain. The brain and other parts of central nervous system are dependent on glucose for energy. Prolonged hypoglycemia may lead to irreversible brain damage.

4. Required for the oxidation of fat. Acetyl CoA is the product formed in fatty acid oxidation. For its further oxidation via citric acid cycle, acetyl CoA combines with oxaloacetate, the latter is predominantly derived from carbohydrates. It may therefore be stated **"Fat burns in a fuel of carbohydrate"**. Excess utilization of fats coupled with deficiency of carbohydrates leads to ketosis.

5. Energy supply for muscle work. The muscle glycogen is broken down to lactic acid (glycolysis) to provide energy for muscle contraction.

6. Synthesis of pentoses. Pentoses (e.g. ribose) are the constituents of several compounds in the body e.g. nucleic acids (DNA, RNA), coenzymes. These pentoses are produced in carbohydrate metabolism.

7. Synthesis of fat. Excess consumption of carbohydrates leads to the formation of fat which is stored in the adipose tissue.

8. Special functions in liver. Liver is the central organ that integrates the body metabolisms. Carbohydrates play an active role in this metabolic integration. The liver also utilizes certain products of carbohydrate metabolism for detoxication.

9. Importance of non-digestible carbohydrates. These are the carbohydrates not utilized by the body. Yet, they are important since they improve bowel motility, prevent constipation, lower cholesterol absorption and improve glucose tolerance.

Sources of carbohydrates

Carbohydrates are abundant in several naturally occurring foods. These include table sugar (99%), cereals (60—80%), pulses (50—60%), roots and tubers (20—40%) and bread (50—60%).

NUTRITIONAL IMPORTANCE OF LIPIDS

Triacylglycerols (fats and oils) are the concentrated dietary source of fuel, contributing 15—50% of the body energy requirements. Phospholipids and cholesterol (from animal sources) are also important in nutrition.

Major nutritional functions of lipids

Dietary lipids have two major nutritive functions:

1. Supply triacylglycerols that normally constitute about 90% of dietary lipids which is a concentrated source of fuel to the body.

2. Provide essential fatty acids and fat-soluble vitamins (A, D, E and K).

Essential fatty acids

The unsaturated fatty acids which the body cannot synthesize and, therefore, must be consumed in the diet are referred to as essential fatty acids (EFA).

The fatty acids — **linoleic** and **linolenic** acid — cannot be synthesized by humans. In a strict sense, only these two are essential fatty acids. Arachidonic acid can be synthesized from linoleic acid in some animal species, including man. However, the conversion efficiency of linoleic acid to arachidonic acid is not clearly known in man. And for this reason, some nutritionists recommend that it is better to include **some amount of arachidonic acid** also in the diet.

Functions of EFA

1. Essential fatty acids are the **structural components of biological membranes**.

2. Participate in the transport and utilization of cholesterol.

3. Prevent fat accumulation in the liver.

4. Required for the synthesis of prostaglandins.

5. Maintain proper growth and reproduction of the organisms.

Deficiency of EFA

Essential fatty acid deficiency is associated with several complications. These include impairment in growth and reproduction, increased basal metabolic rate and high turnover of phospholipids. The EFA deficiency in humans is characterized by a scaly dermatitis on the posterior and lateral parts of limbs and buttocks. This condition is referred to as **phrynoderma** or **toad skin**. Poor wound healing and hair loss is also observed in EFA deficiency.

EFA content of foods

The essential fatty acids, more frequently called polyunsaturated fatty acids (PUFA), are predominantly present in vegetable oils and fish oils. The rich vegetable sources include sunflower oil, cotton seed oil, corn oil, soybean oil etc. The fat of animal origin (exception — fish), contain less PUFA e.g. butter, fat of meat, pork and chicken.

Dietary intake of EFA

Nutritionists recommend that at least 30% of the dietary fat should contain PUFA. Very high intake of PUFA (i.e. totally replacing saturated fatty acids) may not be advisable. This is due to the fact that excess PUFA, unless accompanied by antioxidants (vitamin E, carotenes), is believed to be injurious to the cells due to the overproduction of free radicals.

NUTRITIONAL IMPORTANCE OF PROTEINS

Functions of proteins

1. Proteins are the fundamental basis of cell structure and its function.

2. All the enzymes, several hormones, immunoglobulins, transport carriers etc., are proteins.

3. Proteins are involved in the maintenance of osmotic pressure, clotting of blood, muscle contraction etc.

4. During starvation, proteins (amino acids) serve as the major suppliers of energy. It may be noted that the structural proteins themselves serve as “storage proteins” to meet the emergency energy needs of the body. This is in contrast to

lipids and carbohydrates which have the respective storage forms triacylglycerols (in adipose tissue) and glycogen (in liver and muscle).

Protein normally provides the body's requirement for amino acid nitrogen and for specific amino acids. Dietary protein is digested and enters the circulation as individual amino acids. The tissues require 20 amino acids to synthesize specific proteins and other nitrogen-containing compounds such as purines, pyrimidines, and heme.

There are **nine essential amino acids in humans**: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Two other amino acids, cysteine and tyrosine, may be formed from essential amino acids methionine and phenylalanine, respectively. If sufficient cysteine and tyrosine are present in the diet, they spare the requirement for methionine and phenylalanine.

As long as sufficient amount of essential amino acids are present in the diet, the remaining amino acids required for the protein synthesis and other purposes can be formed through transamination and other reactions.

An adult animal in a state of **metabolic equilibrium** requires dietary protein to replace the essential amino acids and amino acid nitrogen lost during metabolic turnover. Nitrogen is lost in the urine, feces, saliva, desquamated skin, hair, and nails. Pregnancy, lactation, tissue repair after injury, recovery from illness, and increased physical activity all require more dietary protein. For moat situations, a diet in which 12% of the energy is supplied as protein is adequate in humans.

The quantity of protein required is affected by three major factors: **protein quality, energy intake, and physical activity**.

1. Protein quality. The quality of protein is measured by comparing the proportions of essential amino acids in a food with the proportions required for the nutrition. The closer the proportions are, the higher the protein quality. **Egg** and **milk** proteins are **high-quality proteins** that are efficiently utilized by the body and are used as reference standards against which other proteins can be compared. Meat protein is of high protein quality, whereas several proteins from plants used as major food sources are relatively deficient in certain essential amino acids, e.g. tryptophan and lysine in maize (corn), lysine in wheat, and methionine in some beans. In a mixed diet, a deficiency of an amino acid in one protein is made up by its abundance in another; such proteins are described as **complementary**; e.g. the protein of wheat and bans combined provides a satisfactory amino acid intake. Under such circumstances, a greater total amount of protein must be consumed to satisfy requirements. Amino acids that are not incorporated into new protein and are unnecessary for immediate requirements cannot be stored and are rapidly degraded, and the nitrogen is excreted as urea and other products.

2. Energy intake. The energy derived from carbohydrate and fat affects protein requirements because it spares the use of protein as an energy source. To

use expensive (high-quality) dietary protein efficiently and to reduce requirements for it to a minimum, it is necessary to ensure adequate provision of energy from nonprotein sources, some of which should be carbohydrate in order to spare protein from gluconeogenesis.

3. Physical activity. Physical activity increases nitrogen retention from dietary protein.

Recommended dietary allowances

The recommended dietary allowances (RDA) represent the **quantities of the nutrients to be provided in the diet daily** for maintaining good health and physical efficiency of the body. It must be remembered that RDA is not the minimum amount to just meet the body needs, but allowance is given for a safe margin.

Factors affecting RDA

1. Sex. The RDA for men is about 20% higher than that for women. Iron is an exception as the requirement is greater in menstruating women. Additional requirements (20—30% above normal) are needed for pregnant and lactating women.

2. Age. In general, the nutrient requirement is much higher in the growing age. For instance, the protein requirement for a growing child is about 2 g/kg body wt/day compared to 1 g/kg body wt/day for adults.

BALANCED DIET

A **balanced diet** is defined as the **diet which contains different types of foods, possessing the nutrients** — carbohydrates, fats, proteins, vitamins and minerals — **in a proportion to meet the requirements of the body**. A balanced diet invariably supplies a little more of each nutrient than the minimum requirement to withstand the short duration of leanness and keep the body in a state of good health.

The basic composition of balanced diet is highly variable, as it differs from country to country, depending on the availability of foods. Social and cultural habits, besides the economic status, age, sex and physical activity of the individual largely influence the intake of diet.

PROTEIN-ENERGY MALNUTRITION

Kwashiorkor

The term kwashiorkor was introduced by Cicely Williams (1933) to a nutritional disease affecting the people of Gold Coast (modern Ghane) in Africa. Kwashiorkor literally means **sickness of the deposed child** i.e. a disease the child gets when the next baby is born.

Occurrence and causes. Kwashiorkor is predominantly found in children between 1—5 years of age. This is primarily due to **insufficient intake** of **proteins**, as the diet of a weaning child mainly consists of carbohydrates.

Clinical symptoms. The major clinical manifestations of kwashiorkor include stunted growth, edema (particularly on legs and hands), diarrhea, discoloration of hair and skin, anemia, apathy and moonface.

Biochemical manifestations. Kwashiorkor is associated with a decreased plasma albumin concentration (<2 g/dl against normal 304.5 g/dl), fatty liver, deficiency of K^+ due to diarrhea. Edema occurs due to lack of adequate plasma proteins to maintain water distribution between blood and tissues. Disturbance in the metabolism of carbohydrate, protein and fat is also observed. Several vitamin deficiencies occur. The immunological response of the child to infection is very low.

Treatment. Ingestion of protein-rich foods or the dietary combinations to provide about 3-4 g of protein/kg body weight/day will control kwashiorkor. The treatment can be monitored by measuring plasma albumin concentration, disappearance of edema and gain in body weight.

Marasmus

Marasmus literally means "to waste". It mainly occurs in children under one year age. Marasmus is predominantly due to the deficiency of calories. This is usually observed in children given watery gruels (of cereals) to supplement the mother's breast milk.

The symptoms of marasmus include growth retardation, muscle wasting (emaciation), anemia and weakness. A marasmus child does not show edema or decreased concentration of plasma albumin. This is major difference to distinguish marasmus from kwashiorkor.

LECTURE 8

BIOENERGETICS. BIOLOGIC OXIDATION. RESPIRATORY CHAIN

BIOENERGETICS

Bioenergetics, or **biochemical thermodynamics**, is the study of the energy changes accompanying biochemical reactions. Nonbiologic systems may utilize heat energy to perform work, but biologic systems are essentially **isothermic** and use chemical energy to power the living processes. Death from **starvation** occurs when available energy reserves are depleted, and certain forms of malnutrition are associated with energy imbalance (**marasmus**). The rate of energy release, measured by the metabolic rate, is controlled by the thyroid hormones, whose malfunction is a cause of disease. Excess storage of surplus energy results in **obesity**.

General laws of thermodynamics

1. The first law of thermodynamics states that the **total energy of a system, including its surrounding, remains constant.** It implies that within the total system energy is neither lost nor gained during any change. However, within that total system, energy may be transferred from one part to another or may be transformed into another form energy. For example, in living systems, chemical energy may be transformed into heat, electrical energy, radiant energy, or mechanical energy.

2. The second law of thermodynamics states that **the total entropy of a system must increase if a process is to occur spontaneously**.

Entropy (S) is the **degree of randomness,** or disorder, of the system and becomes maximum in a system as it approaches true equilibrium.

3. Free energy (G) is the **amount of useful work** that can be obtained from a system.

4. Under conditions of constant temperature and pressure, the relationship between the free energy change (ΔG) of a reacting system and the change in entropy (ΔS) is given by the following equation

$\Delta G = \Delta H - T\,\Delta S$, where Δ H is the change in **enthalpy** (heat) and T is the absolute temperature.

5. Spontaneity of a reaction is measured by the value of ΔG.

a. Exergonic reaction. If the change in free energy is less than zero ($\Delta G < 0$)**, the reaction can proceed spontaneously with the release of energy.**

b. Endergonic reaction. If the change in free energy is more than zero ($\Delta G > 0$), **the reaction cannot proceed spontaneously unless there is an input of energy** to drive the reaction forward.

c. If Δ G is zero, the system is at equilibrium and no net change takes place.

Coupled reactions

The vital processes — e.g. synthetic reactions, muscular contraction, nerve impulse conduction, and active transport — obtain energy by chemical linkage, or **coupling**, to oxidative reactions. In its simplest form, this type of coupling may be represented as shown in Figure 8—1. The conversion of metabolite A to metabolite B occurs with release of free energy. It is coupled to another reaction, in which free energy is required to convert metabolite C to metabolite D.

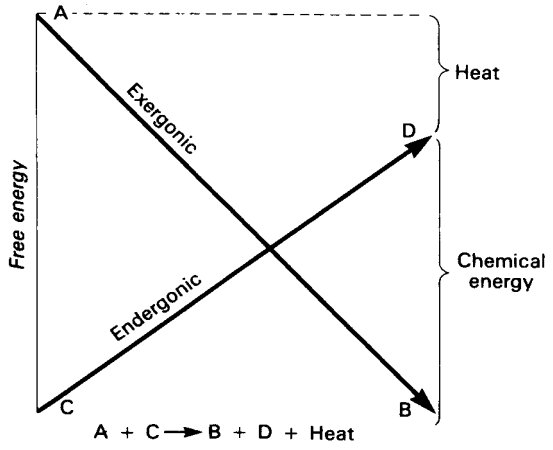


Figure 8—1. Coupling of an exergonic to an endergonic reaction (by Murray R.K. et al., 1996)

A method of coupling an exergonic to an endergonic process is to **synthesize** a compound of **high-energy potential** (~E) in the **exergonic reaction** and to incorporate this new compound into the endergonic pathways. This would allow (~E) to serve as a transducer of energy from a wide range of exergonic reactions to an equally wide range of endergonic reactions or processes (Fig. 8—2).

In the living cell, the principal high-energy intermediate or carrier compound is **adenosine triphosphate** (**ATP**).

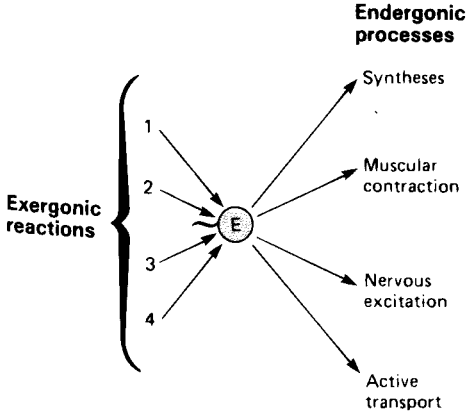


Figure 8—2. Transduction of energy through a common high-energy compound to energy-requiring (endergonic) biologic processes (by Murray R.K. et al., 1996).

ATP

ATP plays a central role in the transference of free energy from the exergonic to the endergonic processes. ATP is a nucleoside triphosphate containing adenine, ribose, and three phosphate group (Fig. 8—3). In its reactions in the cell, it functions as the Mg^{2+} complex.

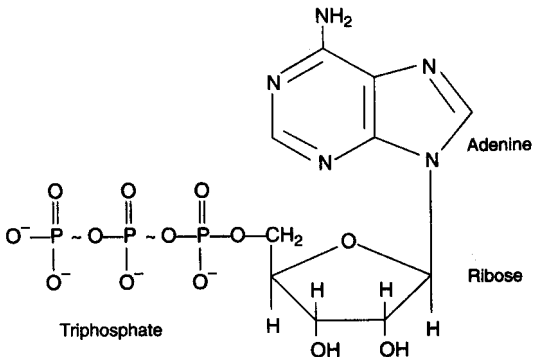


Figure 8—3. Adenosine triphosphate (ATP) shown as the magnesium complex.

The standard free energy of hydrolysis of a number of biochemically important phosphate is shown in Table 8—1.

Table 8—1.

Standard free energy of hydrolysis of some organophosphates of biochemical importance

Compound	ΔG, kJ/mol
Phosphoenolpyruvate	-61.9
Carbamoyl phosphate	-51.4
1,3-Bisphosphoglycerate	-49.3
Creatine phosphate	-43.1
ATP → ADP + Pi	-30.5
ADP → AMP + Pi	-27.6
Pyrophosphate	-27.6
Glucose-1-phosphate	-20.9
Fructose-6-phosphate	-15.9

It may be seen from the table that the value for the hydrolysis of the terminal phosphate of ATP divided the list into two groups. One group of **low-energy phosphates** has ΔG values smaller than ATP, while in the other group, designated **high-energy phosphates**, the value is higher than that of ATP. The components of this latter group, including ATP, are usually **anhydrides** (e.g. 1,3-bisphosphoglycerate), **enolphosphates** (e.g. phosphoenolpyruvate), and **phosphoguanidines** (e.g. creatine phosphate). The intermediate position of ATP allows it to play an important role in energy transfer. The high free energy on hydrolysis of ATP is due to charge repulsion of adjacent negatively charged oxygen atoms and to stabilization of the reaction products, especially phosphate, as resonance hybrids.

The symbol **~P** is introduced to indicate the presence of the high-energy phosphate group.

ATP/ADP cycle

As a result of its position midway down the list of standard free energies of hydrolysis, **ATP** is able to act as a **donor** of high-energy phosphate to form those compounds below it in the table. Likewise, provided the necessary enzymatic machinery is available, ADP can **accept** high-energy phosphate to form ATP from those compounds above ATP in the table. Thus, ATP is continuously **consumed** and **regenerated**. This occurs at a vary rapid rate, since the total ATP/ADP pool is extremely small and sufficient to maintain an active tissue only for a few seconds. It is estimated that a resting man consumes about 40 kg ATP per day. As much as 0.5 kg ATP/minute is believed to be spent during strenuous exercise. 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 synthesis in two ways:

1. Oxidative phosphorylation. Oxidative phosphorylation is the greatest quantitative source of ATP in aerobic organisms. The free energy to drive this process comes from respiratory chain oxidation using molecular O_2 within mitochondria.

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

Nucleoside triphosphates other than adenine are also required in some metabolic processes. These are formed in reactions that depend on ATP for their resynthesis and are catalyzed by **nucleoside kinases.**

1. Guanosine triphosphate (GTP) is used to supply energy in protein synthesis (as well as act as a modulator of protein conformation).

2. Cytidine triphosphate (CTP) supplies energy in lipid synthesis.

3. **Uridine triphosphate (DTP)** supplies energy in polysaccharide synthesis.

ATP is the **universal accumulator** of energy since for its formation energy generated during oxidation of various substances is used. ATP is a **universal energy source** as its energy is used for various processes (synthesis, transport through membranes, muscular construction, etc.) is allocated.

BIOLOGIC OXIDATION

Chemically, **oxidation** is defined as the removal of electrons (or electrons and protons) and **reduction** as the gain of electrons. Thus, oxidation is always accompained by reduction of an electron acceptor. This principle of oxidation-reduction applies equally to biochemical systems and is an important concept underlying understanding of the nature of biologic oxidation. Many biologic oxidations can take place without the participation of molecular oxygen, e.g. dehydrogenase.

Enzymes of oxidation-reduction

Enzymes involved in oxidation and reduction are called **oxidoreductases** and are classified into four groups: **oxidases**, **dehydrogenases**, **hydroperoxidases** and **oxygenases**.

1. Oxydases use oxygen as hydrogen acceptors. Oxidases catalyze the removal of hydrogen from a substrate using oxygen as hydrogen acceptors. They form water or hydrogen peroxide as a reaction product.

2. Dehydrogenases cannot use oxygen as hydrogen acceptors. There are a large number of enzymes in this class. They perform two main functions:

a. Transfer of hydrogen from one substrate to other in a coupled oxidation-reduction reaction. These dehydrogenases are specific for their substrates but often utilize common coenzyme or hydrogen carriers, e.g. **NAD^+**. This type of reaction is particulary useful in anabling oxidative processes to occur in the absence of oxygen.

b. As a components in the **respiratory chain** of electron transport from substrate to oxygen.

There are two groups of dehydrogenases depending on their cofactors:

(1) Many dehydrogenases are specific for either pyridine nucleotides (nicotinamide adenine dinucleotide (**NAD^+**) and nicotinamide adenine dinucleotide phosphate (**$NADP^+$**)) as coenzymes (Fig. 8—4). They are named as: **NAD-linked** (NAD-dependent) **dehydrogenases** and **NADP-linked** (NADP-dependent) **dehydrogenases**.

(a) The active portion of NAD^+ and $NADP^+$ is the **nicotinamide ring**, which accepts a **proton** and **two electrons** (equivalent to a hydride ion, H^-) from the substrate to yield the reduced forms, NADH and NADPH (Fig. 8—1).

(b) NAD^+ and $NADP^+$ are identical except that in $NADP^+$ one of the hydroxyl groups on the ribose (2') bound to adenine is phosphorylated.

(c) NAD^+ and $NADP^+$ are formed in the body from the vitamin **niacin**.

(d) NAD^+ and $NADP^+$ are usually **freely dissociable** from enzymes.

(e) The energy stored in **NADH** is primarily used to drive the **synthesis** of **ATP** (oxidative phosphorylation). **NADPH** serves as an energy source for reductive **biosyntheses**, in which the precursors are more oxidized than the products.

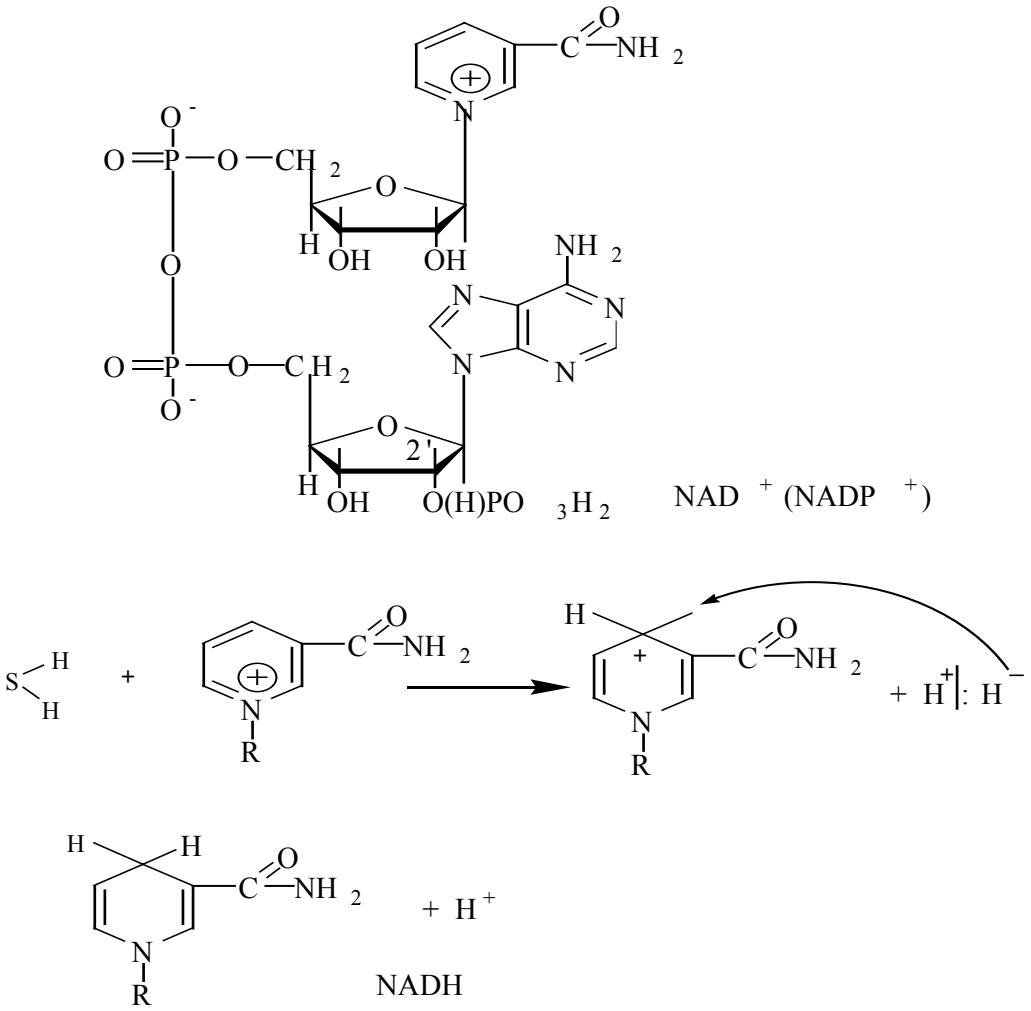
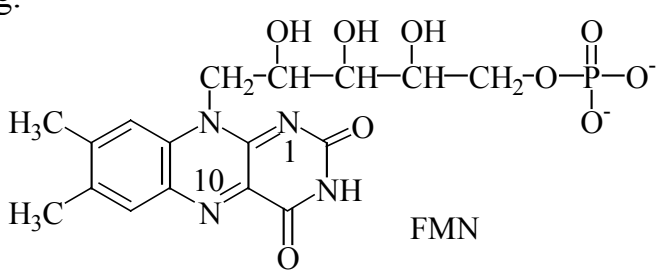


Figure 8—4. Structure of NAD^+ and $NADP^+$. Conversions of the oxidized form of NAD^+ to the reduced form NADH

(2) Flavoprotein enzymes contain flavin adenine dinucleotide **(FAD)** and flavin mononucleotide **(FMN) as prosthetic groups** (Fig. 8-5)**.** They are formed in the body from the vitamin **riboflavin**. These enzymes are named **FMN-dependent dehydrogenases** and **FAD-dependent dehydrogenases**

(a) The active portion of FAD and FMN is the **isoalloxazine ring**, which accepts **two protons** and **two electrons** (equivalent to 2H) from the substrate to yield the reduced forms, $FADH_2$ and $FMNH_2$ (Fig. 8-5).

(b) FMN differs from FAD in that it has only ribitol phosphate bound to the isoalloxazine ring.



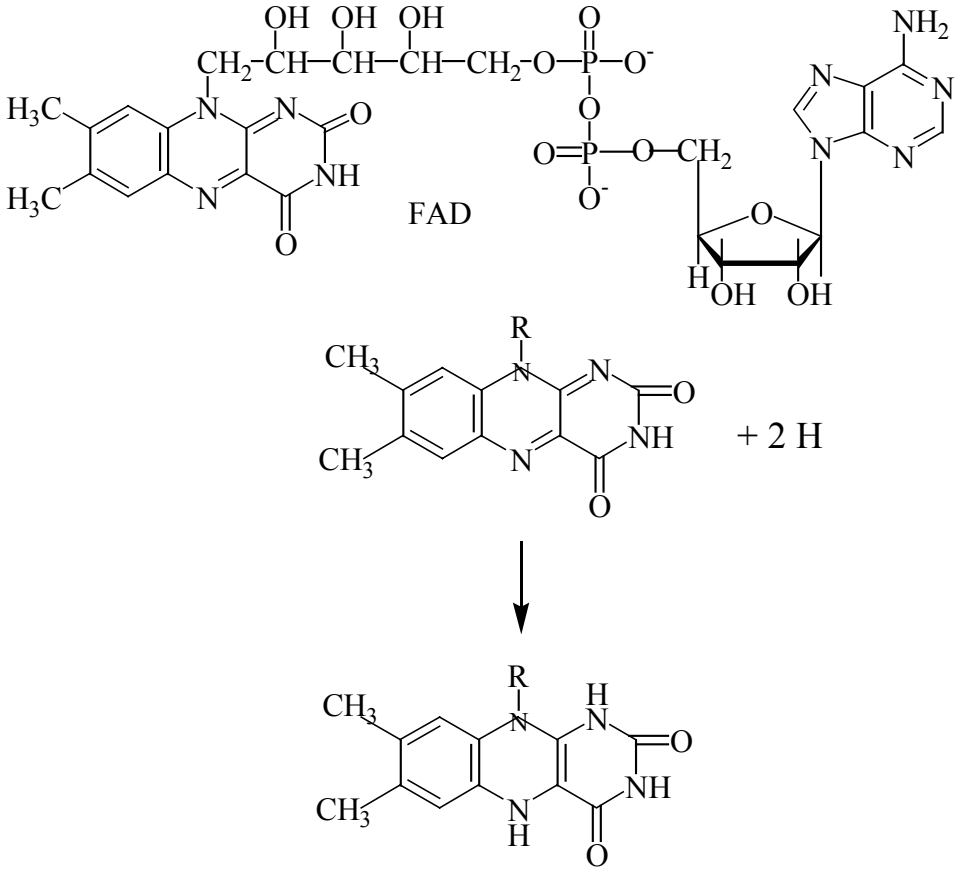


Figure 8—5. Structure of FMN and FAD. Conversions of the oxidized form of FMN and FAD

(c) In enzymes, using FAD or FMN as an electron acceptor, the **cofactor is tightly bound** (but not covalently) to the enzyme and does not dissociate.

(d) Many flavoprotein enzymes contain one or more metals as essential cofactors.

3. Hydroperoxidases use hydrogen peroxide or an organic peroxide as substrate

4. Oxygenases catalyze the direct transfer and incorporation of oxigen into a substrate.

Pathways of biologic oxidation

The substrate may be oxidized by dehydrogenation or oxygen incorporation. Therefore, the following pathways of biologic oxidation have been proposed.

1. The substrate is oxidized by removal the hydrogen atoms and they are transferred by carries of respiratory chain to oxygen. The energy of oxidation is used for ATP synthesis. This process is localized in mitochondria. ADP+Pi ATP

$$RH_2 - 2H + \tfrac{1}{2}\,O_2 \longrightarrow R + H_2O$$

2. The substrate is oxidized by removal the hydrogen atoms and these atoms are bound with oxygen molecules. The peroxide is formed. The energy liberates as heat. The responsible enzymes are named oxidases and process is localized in peroxysomes. $RH_2 - 2H + O_2 \rightarrow R + H_2O_2 + Q$

3. The substrate is oxidized by incorporation of one atom of oxygen into the substrate. The responsible enzymes are called monooxygenases and this process is localized in endoplasmic reticulum. $RH_2 + O_2 \rightarrow R\text{-}OH + H_2O$

4. The substrate is oxidized by incorporation of both oxygen atoms into the substrate. The responsible enzymes are called dioxygenases and this process is localized in endoplasmic reticulum. $R + O_2 \rightarrow RO_2$

5. Free radical oxidation.

RESPIRATORY CHAIN

Respiration — the process by which cells derive energy in the form of ATP from the controlled reaction of hydrogen with oxygen to form water. Most of the energy liberated during the oxidation of carbohydrate, fatty acids, and amino acids is made available within mitochondria as reducing equivalents (-H or electrons).

Structure of mitochondria

Most vertebrate cells contain several hundred mitochondria, but the number can be as low as 1 and as high as 100,000. Mitochondria have an **outer** membrane, an **inner** membrane, an **intermembrane space**, and a **matrix**, located within the inner membrane. The **outer** membrane is **porous**, whereas the **inner** membrane is much **tighter**, serving as a barrier to many biological metabolites. The inner membrane is highly folded into **cristae**, which project into, and often nearly through the interior of the mitochondrion.

The mitochondria contain the series of catalysts known as the **respiratory chain** that **collect** and **transport** reducing equivalents and direct them to their final reaction with oxygen to form **water**. The enzymes of the electron transport chain are embedded in the **inner mitochondrial membrane** in association with the enzymes of oxidative phosphorylation.

Mitochondria also contain the enzyme systems responsible for producing most of the reducing equivalents. Processes occurring inside the mitochondrial matrix include **pyruvate oxidation**, **fatty acid oxidation**, **amino acid metabolism**, and the **citric acid cycle**.

Function of respiratory chain

1. It is the **final common pathway in aerobic cells** by which electrons derived from various substrates are transferred to oxygen.

2. Different substrates may use this common pathway because they are oxidized by enzymes that use nicotinamide adenine dinucleotide (**NAD^+**) or flavin adenine dinucleotide (**FAD**) as electron acceptor cofactors. The reduced **NADH** and **$FADH_2$** then donate the electrons to the electron transport chain. Reduced electron carriers donate their electrons to acceptor molecules and become reoxidized in the process. The acceptor molecules are reduced because the oxidation of one species (e.g., the reduced electron carrier) cannot occur without the simultaneous reduction of another species (e.g., the acceptor molecule).

ORGANIZATION OF THE ELECTRON TRANSPORT CHAIN

The respiratory chain in mitochondria consists of a number of redox carriers that proceed from the **NAD-linked dehydrogenase systems,** through **flavoproteins and cytochromes, to molecular oxygen** (Fig. 8—6)**.** Not all substrates are linked to the respiratory chain through NAD-specific dehydrogenase; some, because their redox potentials are more positive are linked directly to **flavoprotein dehydrogenases**, which in turn are linked to the cytochromes of the respiratory chain.

Respiratory chain contains 4 multiprotein complexes.

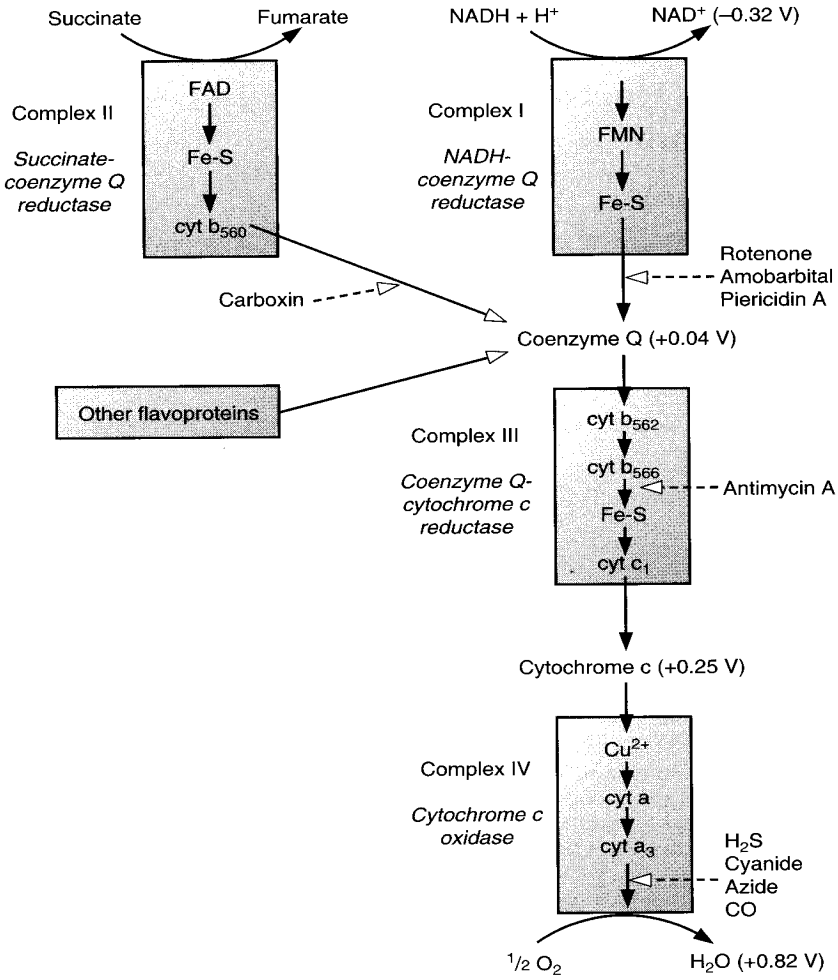


Figure 8—6. The mitochondrial electron transport chain (by Davidson V.L. et al., 1999).

1. Complex I is the point of entry into the electron transport chain for electrons from NADH.

a. This **enzyme complex** is called **NADH-coenzyme Q reductase or NADH dehydrogenase.**

b. Prosthetic groups

(1) Flavin mononucleotide (FMN)

(2) Iron-sulfur (Fe-S) centers. The complex contains seven iron-sulfur cen-

ters. Iron-sulfur centers, which may consist of **two** or **four iron atoms** complexed with an equal number of sulfide ions, mediate one-electron transfer reactions. Proteins that contain iron-sulfur centers are often referred as **nonheme iron proteins**.

c. The electron acceptor for complex I is **coenzyme Q** (also called ubiquinone or simply Q).

d. Path of electron transfer. Electrons from NADH are transferred to FMN, then to the Fe-S center, then to coenzyme Q. The iron atom undergoes oxidoreduction between Fe^{2+} and Fe^{3+}.

e. Inhibitors of electron transfer from NADH to coenzyme Q include:

(1) Rotenone, an insecticide

(2) Barbiturates, such as amobarbital and secobarbital

(3) Piericidin A, an antibiotic

2. Complex II is the point of entry into the electron transport chain for electrons from succinate.

a. This **enzyme complex** is called **succinate-coenzyme Q reductase** and includes succinate dehydrogenase, which is the same enzyme that participates in the citric acid cycle.

b. Prosthetic groups include:

(1) FAD

(2) Fe-S centers

(3) Heme (the prosthetic group of cytochrome b_{560})

c. The electron acceptor for complex II is coenzyme Q.

d. Path of electron transfer. Electrons from succinate are transferred to FAD, then to the Fe-S centers, then to cytochrome b_{560}, and then to coenzyme Q.

e. Carboxin inhibits complex II.

3. Coenzyme Q is a highly lipid soluble molecule that is **firmly embedded in the membrane.** It accepts electrons from both complex I and complex II and donates electrons to complex III (Fig. 8—7).

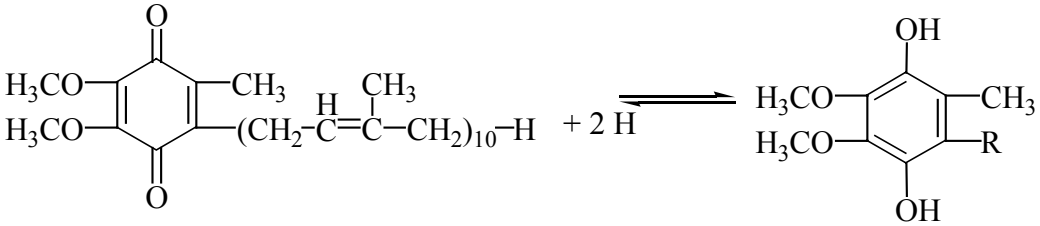


Figure 8—7. Structure of CoQ

4. Complex III is the electron acceptor for coenzyme Q.

a. This **enzyme complex** is called **coenzyme Q-cytochrome c reductase.**

b. Prosthetic groups in complex III include:

(1) Heme (b-type cytochromes and cytochrome c_1)

(2) Fe-S centers

c. The electron acceptor for complex III is cytochrome c.

d. Path of electron transfer. Electrons from coenzyme Q are transferred to the b-type cytochromes, then to the Fe-S center, then to cytochrome c_1, and then to cytochrome c. The cytochromes are iron-containing proteins in which the iron atom oscillates between Fe^{3+} and Fe^{2+} during oxidation and reduction.

e. Inhibitors of electron transfer from coenzyme Q to cytochrome c include **antimycin A**, an antibiotic.

5. Cytochrome c

a. Unlike the other components of the respiratory chain, cytochrome c is a **soluble protein** that binds to the membrane to perform its electron transfer role.

b. Function. It mediates the transfer of electrons from complex III to complex IV.

c. The prosthetic group of cytochrome c_1 as well as the other cytochromes, is heme.

6. Complex IV is the electron acceptor for cytochrome c.

a. This **enzyme complex** is called **cytochrome c oxidase.**

b. Prosthetic groups in complex IV include:

(1) Copper (Cu)

(2) Heme (cytochrome a and cytochrome a_3). These cytochromes are combined with a single protein, and the complex is known as cytochromes aa_3 or **cytochrome oxidase**.

c. The electron acceptor for complex IV is molecular oxygen (O_2), and the **product** of this reaction is water (H_2O).

d. Path of electron transfer. Electrons from cytochrome c are transferred to Cu^{2+}, then to the a-type cytochromes, and then to O_2.

e. Inhibitors of electron transfer from cytochrome c to O_2 include:

(1) Carbon monoxide (CO), which competes with O_2 for its binding site on cytochrome oxidase

(2) Hydrogen sulfide (H_2S)

(3) Azide (an N_3-containing compound)

(4) Cyanide (CN^-)

The respiratory chain is named **completely** if the electrons enter the respiratory chain through complex I. The respiratory chain is named **incompletely** if the electrons enter the respiratory chain through complex II.

LECTURE 9

OXIDATIVE PHOSPHORYLATION

The process of **synthesizing ATP from ADP and Pi coupled with the electron transport chain** is known as oxidative phosphorylation.

Function

1. It is the **main source of energy in aerobic cells.**

2. It is the process whereby the free energy that is released when electrons are transferred along the electron transport chain is coupled to the **formation of**

adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (Pi).

Oxidation-reduction potentials

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 pair are assigned specific standard redox potential (E_0 volts) at pH 7.0 and 25ºC.

The standard oxidation-reduction potentials of the electron carriers in the respiratory chain become more positive going from the oxidation of NADH to the reduction of molecular oxygen.

1. **Release of energy.** From the difference in potentials between the NAD/NADH and O_2/H_2O couples, which is 1.14 volts, it can be calculated that for each electron pair that passes through the chain, approximately 52 kcal of energy are released.
2. **Coupling of free energy.** The energy released for each electron pair passing through the respiratory chain is coupled to the formation of ATP.

Coupling sites for ATP synthesis. The energy released by the electron transfers catalyzed by three coupling sites—complex I, complex III, and complex IV — is sufficient for each to support the formation of approximately 1 mole of ATP.

1. **Electrons that enter the chain from NADH** supports the synthesis of approximately 3 moles of ATP.
2. **Electrons that enter the chain from $FADH_2$** bypass complex I and support the synthesis of approximately 2 moles of ATP.
3. **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 electron transport chain. For NAD^+ the P:O ratio is approximately 3:1. For FAD the P:O ratio is approximately 2:1. (some worker suggest a P:O ratio of 2.5 for NAD^+, and 1.5 for FAD, based on the proton translocation).

Hypothesis of oxidative phosphorylation

1. 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 intermediated are first produced which are utilized for the synthesis 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.

2. Conformational coupling hypothesis

Paul Boyer proposed this hypothesis in 1964. As a consequence of electron transport, a **conformational change in the membrane protein** is induced. This is believed to be responsible for the synthesis ATP. The protein attains a high-energy conformation due to electron transfer which, when reverted back to random state (low-energy), results in the production of ATP from ADP + Pi. There is some evidence demonstrating conformational changes in mitochondrial mem-

brane proteins. However, there is no conclusive proof that such changes may be actually responsible for ATP synthesis.

3. Chemiosmotic theory (Mitchel, 1961)

An electrochemical gradient of protons (H^+) across the mitochondrial inner membrane serves to couple the energy flow of electron transport to the formation of ATP.

1. The respiratory chain complexes I, III and IV acts as proton pumps. which cause vectorial (directional) **pumping of H^+ across the membrane** (Fig. 9—1).

2. Because H^+ is a charged particle, the flow of free energy across the inner membrane is due to the combination of a **concentration gradient and a charge gradient.**

3. In the electron transport chain, H^+ is separated from the electron. As **electrons move down the chain, H^+ is transferred from the mitochondrial matrix to the intermembrane space.**

4. The protons in the intermembrane space pass through the inner membrane and back into the matrix via the **ATP synthase** (see Fig. 9—1). The dissipation of energy that occurs as the protons pass down the concentration gradient to the matrix drives the phosphorylation of ADP to ATP by the synthase.

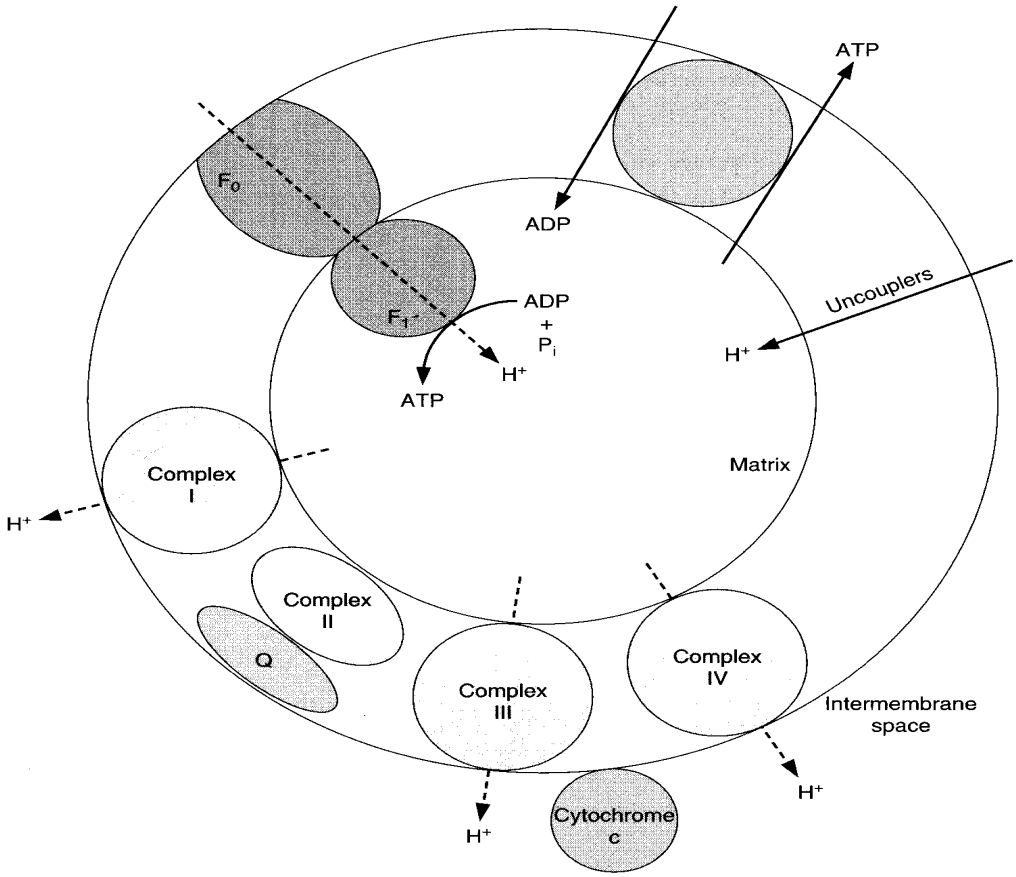


Figure 9—1. Schematic diagram of the coupling of electron transport to oxidative phosphorylation (by Davidson V.L. et al., 1999).

The enzyme complex that synthesizes ATP (see Fig. 9—1) is called **ATP synthase** and is also known as **H^+-ATPase** or **F_0F_1-ATPase.**

1. Composition. ATP synthase is composed of two units.

a. F_0 spans the membrane and is composed of four subunits. It forms a **channel** or path through which protons cross the membrane.

b. F_1 is tightly bound to F_0 and sits on the matrix side of the mitochondrial membrane. The F_1 unit contains three αβ dimers (Fig. 9—2) and the **catalytic site** for ATP synthesis.

2. Inhibitors of ATP synthase include:

a. Oligomycin, an antibiotic. It binds with the enzyme ATP 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.

b. Dicyclohexylcarbodiimide (DCCD)

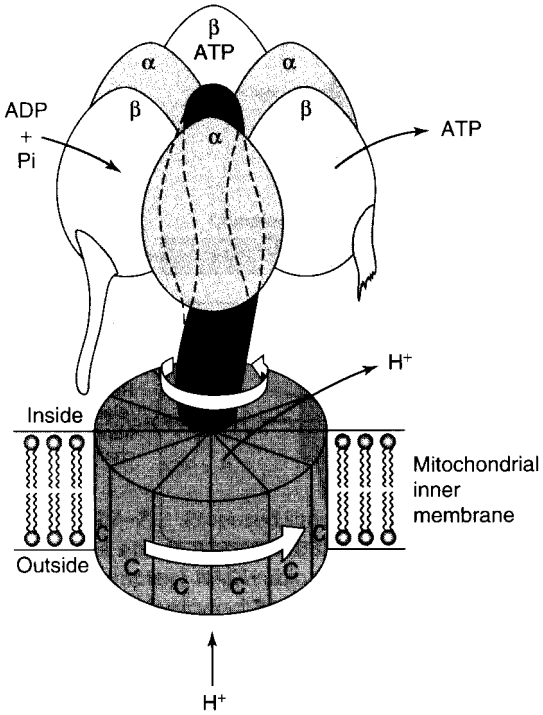


Figure 9—2. Structure ATP synthase (by Murray R.K. et al., 1996).

3. Other enzymes that can couple ATP synthesis to the transport of ions other than hydrogen down a concentration gradient are found in other parts of the cell.

a. The Ca^{2t}-ATPase of the sarcoplasmic reticulum

b. The Na^+K^+-ATPase of the plasma membrane

Coupling of phosphorylation to respiration

The rate of respiration of mitochondria can be controlled by the concentration of ADP because **oxidation and phosphorylation are tightly coupled.** That is, as energy is released from oxidation reactions, the energy is used to phosphorylate ADP to create ATP.

Five states of respiratory control

1. State 1 is limited by the availability of ADP and substrate (source of electrons).

2. State 2 is limited by the availability of substrate.

3. State 3 is limited by the capacity of the electron chain itself, when ADP, oxygen, and substrate are saturating.

4. State 4 is limited by the availability of ADP only. This is typically the resting state in cells.

5. State 5 is limited by the availability of oxygen only.

ADP/ATP transport

ADP must be transported into the mitochondrial matrix to be used for ATP synthesis, and ATP produced in the mitochondria must be transported out for use by the cell.

1. A membrane-bound transporter system catalyzes the exchange of ADP and ATP across the membrane (see Figure 9-1).

2. Inhibitors of the ADP/ATP transporter include:

a. Atractyloside

b. Bongkrekic acid

Uncouplers of oxidative phosphorylation are compounds that allow normal function of the electron transport chain without production of ATP. The action of uncouplers is to dissociate oxidation in the respiratory chain from phosphorylation, and this action can explain the toxic action of these compounds in vivo. Uncouplers **cause leakage or transport of H^+** across the membrane that collapses the proton gradient before it can be used for ATP synthesis (see Figure 9—1). Energy still may be released as the electrons are transferred down the transport chain; however, this energy is not trapped as ATP but appears instead as heat. Oxidative phosphorylation uncouplers include:

1. 2,4-DinitrophenoI, which was once used as a weight-loss drug but was discontinued because of its toxicity

2. Dicumarol, which is an anticoagulant

3. Chlorcarbonylcyanide phenylhydrazone (CCCP), which is a compound that carries protons across the membrane

4. Certain **physiological** substances which act as uncouplers at higher concentration have been identified. These include the hormone **thyroxine** and **long chain free fatty acids**.

5. Bilirubin, which is a metabolite of heme degradation but is not normally present in mitochondria in concentrations high enough to affect normal function

Ionophores permit specific cations to penetrate membranes.

1. Ionophores are so termed because of their ability to complex specific cations and facilitate their transport through biologic membranes.

2. This property of ionophores is due to their lipophilic character, which allows penetration of lipoid membranes such as mitochondrial membrane.

3. Valinomycin allows penetration of K^+ through the mitochondrial membrane and then discharges the membrane potential between the inside and the outside of the mitochondrion.

4. Nigericin acts as an ionophore for K^+ but in exchange for H^+. In the presence of both valinomycin and nigericin, both the membrane potential and the pH gradient are eliminated, and phosphorilation is therefore completely inhibited.

Disorders of mitochondrial oxidative phosphorylation

Genetics

1. Mitochondria are the only organelles outside the nucleus that contain their own DNA.

2. Some of the proteins that comprise the respiratory chain and oxidative phosphorylation system are encoded by mitochondrial DNA, whereas others are encoded by nuclear DNA.

3. Several disorders of oxidative phosphorylation have been identified that are the result of mutations in either nuclear or mitochondria] DNA that is involved in proper biosynthesis of the oxidative phosphorylation system.

4. Clinical manifestations include muscle cramping and weakness, fatigue, lactic acidosis, central nervous system (CNS) dysfunction, and vision problems.

5. Treatment of patients with respiratory chain disorders is difficult and often unsuccessful. In some cases improvement was reported after therapeutic doses of substances that may mediate electron transfer, such as ubiquinone, vitamin C, and menadione.

LECTURE 10

PEROXYDASE PATHWAY. MONOOXYGENASE SYSTEMS. DIOXYGENASE SYSTEM. FREE RADICALS AND ANTIOXYDANTS. COMMON PATHWAY OF METABOLISM (CITRIC ACID CYCLE)

Peroxydase pathway

The substrate is oxidized by removal the hydrogen atoms and these atoms are bound with oxygen molecules. The **peroxide** is formed. The energy liberates as **heat**. $RH_2 - 2H + O_2 \rightarrow R + H_2O_2 + Q$. The responsible enzymes are named **oxidases**. These are **simple oxidation systems**. Oxidases contain FAD or FMN as a prosthetic group. This pathway takes part in metabolism of aldehyde, amines, L- and D-amino acids, purines etc.

Hydrogen peroxide is metabolized by **catalase** or **peroxidase**.

In the reaction catalyzed by **peroxidase**, hydrogen peroxide is reduced at the expense of several substances that will act as electron acceptors, such as ascorbate, quinines, cytochrome c, and glutathione. The reaction catalyzed by peroxidase is complex, but the overall reaction is a follows:

$$H_2O_2 + AH_2 \rightarrow 2\ H_2O + A.$$

In **erythrocytes** and other tissues selenium-containing **glutathione peroxidase** is of significance. Reduced glutathione is converted to oxidized glutathione and H_2O_2 is detoxified to H_2O. The reduced glutathione can be regenerated by the enzyme **glutathione reductase** utilizing NADPH. Glutathione peroxidase protects membrane lipids and hemoglobin against oxidation by peroxide.

Catalase is a hemoprotein containing four heme groups. In addition to possessing peroxidase activity, it is able to use one molecule of H_2O_2 as a substrate electron donor and another molecule of H_2O_2 as oxidant or electron acceptor: $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$. Catalase is found in **blood, bone marrow, mucous membranes, kidney, and liver**. Its function is assumed to be the destruction of hydrogen peroxide formed by the action of oxidase. **Microbodies** or **peroxisomes** are found in many tissues, including liver. They are rich in oxidases and in catalase, which suggests that there may be a biologic advantage in grouping the enzymes that produce H_2O_2 with the enzyme that destroys it.

Monooxygenase systems (microsomal oxidation)

The substrate may be oxidized by incorporation of one atom of oxygen into the substrate. The responsible enzymes are called **monooxygenases** or cytochrome P_{450}. These enzymes are also called **mixed function oxidase.** They are so named because in a typical reaction, one molecule of oxygen is reduced per substrate molecule, one oxygen appearing in the product and the other in a molecule of water. These enzymes are associated with the **endoplasmic reticulum** (during the process of cell fractionation, rough endoplasmic reticulum is disrupted to form small vesicles known as **microsomes**; it may be noted that microsomes as such do not occur in the cell) and **inner mitochondrial membranes**. The hemoproteins that are referred to as cytochrome P_{450} are so named because of the complexes that they form with carbon monoxide. In the presence of the gas, light is strongly absorbed at wavelength of 450 nm.

All of the oxidative reactions that are catalyzed by cytochrome P_{450} may be viewed as **hydroxylation** reactions (i.e., an OH group appears in each reaction). The general reaction is as following $RH + O_2 + NADPH+H^+ \rightarrow R\text{-}OH + H_2O + + NADP^+$, where RH is the substrate.

1. The substrate (RH) binds to oxidized form P_{450} (Fe^{3+}) and enzyme-substrate complex is formed $RH — P_{450}$ (Fe^{3+}).

2. NADH transfers 2 hydrogen atoms on FAD. FAD is reduced and $FADH_2$ is formed.

3. $FADH_2$ then transfers its electron to the FeS prosthetic group, reducing iron from Fe^{3+} to Fe^{2+}.

4. One electron binds to complex $RH — P_{450}$ (Fe^{3+}) reducing Fe^{3+} to Fe^{2+}.

5. After reduction, cytochrome P_{450} is capable of binding O_2.

6. A second electron is transferred from FeS to the bound O_2, thus forming a transient Fe^{2+}-O_2^--substrate species.

7. The brief association ends when the oxygen-oxygen bond is broken.

8. One atom is released as water, while the other is transferred to the substrate.

9. The cycle ends with the release of the product from the active site.

Function of monooxygenase systems

1. Monooxygenases systems convert substrates to more polar forms by introducing OH-group. It is important for **metabolism** of **xenobiotics**. Some of these reactions results in the formation of highly unstable (and therefore potentially toxic) intermediates.

2. These systems play role in **synthesis** of some substances. These systems are found in steroidogenesis tissues such as adrenal cortex, testis, ovary, and placenta are concerned with the biosynthesis of steroid hormones from cholesterol. Renal systems catalyze hydroxylation in calcitriol synthesis, and the liver catalyzes hydroxylation in bile acid synthesis. In the adrenal cortex, mitochondrial cyrochrome P_{450} is six time more abundant than cytochromes of the respiratory chain.

Dioxygenase systems

The substrate may be oxidized by incorporation of both oxygen atoms into the substrate. The responsible enzymes are called **dioxygenases** and this process is localized in **endoplasmic reticulum**. $R + O_2 \rightarrow RO_2$. These enzymes disrupt the **aromatic** rings. Examples of this type include enzymes that contain iron, such as homogentisate dioxygenase (oxidase).

Free radicals and antioxidants

In general, biological oxidations ensure that molecular oxygen is completely reduced to water. However, partial reduction of O_2 generates **reactive oxygen species (ROS)** which are more commonly referred to as free radicals.

Free radicals are the **chemical species** (molecules or molecular fragments) that **possess one or more unpaired electrons** and have an independent existence. Conventionally, free radicals are represented by superscript a dot or a dash.

The free radicals of biological importance are — **superoxide radical** ($O_2^{\bullet -}$), **hydrogen peroxide** (H_2O_2), **hydroxyl peroxide** (OH^-)**, lipid peroxide radical** (ROO^-), **nitric oxide** (NO^-), **peroxynitrite** ($ONOO^-$) and **hypochlorous acid** (HOCI).

The reaction steps for the formation of some free radicals during the course of conversion of molecular oxygen to H_2O are shown hereunder.

$O_2 + e^- \rightarrow O_2^{\bullet -} + e^-$, $2H^+ \rightarrow H_2O_2 + e^-$, $H^+ \rightarrow OH^- + H_2O + e^- \rightarrow H_2O$

Sources and generation of free radicals

The production of free radicals is mostly associated with the normal biological processes operative in the living cells.

1. Normal metabolism. In the oxidation-reduction reactions involving O_2, generation of free radicals is a common occurrence e.g., improper operation of electron transport chain; action of xanthine oxidase to produce uric acid; synthesis of prostaglandins from arachidonic acid (lipid peroxides formed); synthesis of nitric oxide.

2. Respiratory burst. Phagocytosis is the process of engulfment and killing of bacteria by macrophages. It is accompanied by rapid consumption of O_2

and generation of free radicals ($O_2^{\bullet -}$, H_2O_2, HOCI), a process referred to as respiratory burst. In fact, the free radicals generated during respiratory burst **kill bacteria.** This is truly a **beneficial affect of free radicals.**

3. Exogenous causative agents. These include exposure to ionizing radiations, ultraviolet light, cigarette smoke, environmental pollution, hyperoxia etc. All these factors are directly or indirectly linked with the generation of free radicals.

Harmful effects of free radicals

Free radicals are highly reactive and are capable of damaging almost all types of biomolecules (proteins, lipids, polysaccharides, nucleic acids). The fact is that **free radicals beget free radicals** i.e., generate free radicals from normal compounds which continues as a chain reaction.

1. Proteins. Free radicals cause oxidation of sulfhydryl groups, modification of certain amino acids (such as methionine, arginine, histidine and proline). The net result is that proteins lose their biological activity, which may be often associated with cleavage of proteins (into fragments).

2. Lipids. Polyunsaturated fatty acids (PUFA) are highly susceptible to damage by free radicals. PUFA undergo **peroxidation** resulting in the formation of **malondialdehyde** (a compound that is commonly estimated in the biological fluids to assess oxidative damage). Lipid peroxidation is associated with loss of membrane function.

3. Nucleic acids. Free radicals can break DNA strands, besides alterations in nitrogen bases, which may lead to cell death or carcinogenesis.

Free radicals and diseases

As already stated, extensive damage is caused by free radicals to biomolecules, and in turn to cells. Free radicals have been implicated in the causation and progress of several diseases.

1. Cancer. Free radicals act as promoting agents of cancer. This is attributed to gross chromosomal damage, altered gene expression, inhibition of DNA repair process etc.

2. Cardiovascular diseases (CHD). Oxidised low density lipoproteins (LDL), formed by the action of free radicals, have been implicated in the onset of CHD.

3. Diabetes mellitus. Destruction of islets of pancreas due to accumulation of free radicals is one of the causes in the pathogenesis of insulin dependent diabetes.

4. Inflammatory disease. Rheumatoid arthritis is a chronic inflammatory disease. Its occurrence is linked with respiratory burst (caused by free radicals), destroying invading microorganisms.

5. **Cataract**. Increased exposure to oxidative stress contributes to cataract formation.

6. **Male infertility**. Free radicals have been shown to reduce sperm motility and viability and thus may contribute to male infertility.

7. Free radicals play a significant role in the diseases of liver, lung, kidney, besides their involvement in the ageing process.

The antioxidant system

The body possesses several defense mechanisms (collectively referred to as antioxidant system) to control/destroy the free radicals generated, besides bringing out repair to the damages biomolecules.

1. Preventive antioxidants. The body has certain proteins which try to prevent the formation of free radicals e.g., ceruloplasmin, transferring, ferritin, albumin, myoglobin.

2. Antioxidant system. These are truly the scavengers of free radicals. The most important antioxidant enzymes are superoxide dismutase, catalase and glutathione peroxidase.

a. Superoxide dismutase is a metalloenzyme that convert superoxide to hydrogen peroxide and oxygen. This enzyme acts as a first line of defense to protect from the injurious effects of superoxide.

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

Superoxide dismutase found in the cytosol of eukaryotes contains copper and zinc. Mitochondrial enzyme contains manganese.

Oxygen can be extremely toxic to premature infants, who are often ventilated with high concentrations of oxygen to compensate for their immature lung development. Breathing high concentrations of oxygen over a prolonged period of time may to **increased production of superoxide** and, in premature infants; their **capacity produce superoxide dismutase is not fully developed.** One possible consequence of oxygen toxicity to premature infants is **blindness.**

b. Hydrogen peroxide is metabolized by **catalase** or **peroxidase** (see above).

3. Antioxidant compounds. Several small molecules can act as antioxidant. These include antioxidant vitamins — vitamin C (ascorbic acid), vitamin E (tocopherol), and β-carotine; others — uric acid, glutathione and flavonoids.

4. Repair enzyme. Certain enzymes (e.g. DNA repair enzymes, methionine sulfoxide reductase) try to repair the damage caused to macromolecules by free radicals.

Citric acid cycle

The citric acid cycle was proposed by Hans Adolf Krebs in 1937, based on the studies of oxygen consumption in pigeon breast muscle. The cycle is named in his honour and he was awarded the Nobel Prize for Physiology and Medicine in 1953.

The citric acid cycle (also known as the tricarboxylic acid cycle (TCA) and the Krebs cycle) is a series of enzymatically catalyzed reactions that form a common pathway for the **final oxidation of all metabolic fuels** (i.e., carbohydrates, free fatty acids, ketone bodies, amino acids), which are catabolized to the substrate of the citric acid cycle (acetyl CoA). These reactions occur within the **mitochondrial matrix.**

Functions

1. The citric acid cycle is involved in both anabolic and catabolic processes.

a. Anabolic reactions. The intermediates of the citric acid cycle are used as precursors in the **biosynthesis** of many compounds.

b. Catabolic reactions. The cycle provides a means for the **degradation** of two-carbon acetyl residues, which are derived from carbohydrates, fatty acids, and amino acids.

2. The citric acid cycle **provides much of the energy for respiration.** Electrons that are generated from the action of this cycle are transferred to the electron transport chain and used in the process of oxidative phosphorylation to generate ATP.

Summary of citric acid cycle

Krebs cycle basically involved the combination of a two carbon acetyl CoA with a four carbon oxaloacetate to produce a six carbon tricarboxylic acid, citrate. In the reactions that follow, the two carbons are oxidized to CO_2 and oxaloacetate is regenerated and recycled.

$$\text{Acetyl CoA} + 3\ NAD^+ + FAD + GDP + Pi + 2\ H_2O \rightarrow$$
$$2\ CO_2 + 3\ NADH + FADH_2 + GTP + 3\ H^+ + CoA$$

Reactions of citric acid cycle

1. Krebs cycle proper starts with the condensation of acetyl CoA and oxaloacetate, catalyzed by the enzyme **citrate synthase**. Citrate is freely permeable across the mitochondrial membrane. It serves as a good source of cytosolic acetyl CoA which is used for the synthesis of fatty acids.

2. Citrate is isomerized to isocitrate by the enzyme **aconitase.** This is achieved in a two stage reaction of dehydration followed by hydration through the formation of an intermediate — *cis*-aconitate.

3. Formation of α-ketoglutarate. The enzyme isocitrate dehydrogenase (ICD) catalyses the conversion (oxidative decarboxylation) of isocitrate to oxalosuccinate and then to α-ketoglutarate. The formation of **NADH** and the liberation of **CO_2** occur at this stage.

4. Conversion of α-ketoglutarate to succinyl CoA occurs through oxidative decarboxylation, catalysed by **α-ketoglutarate dehydrogenase** complex. This enzyme is dependent on five cofactors — thiamine pyrophosphate (TPP), lipoamide, NAD^+, FAD and CoA. At this stage of the TCA cycle, second **NADH** is produced and the second **CO_2** is liberated.

5. Formation of succinate. Succinyl CoA is converted to succinate by succinyl thiokinase. This reaction is coupled with the phosphorylation of GDP to GTP. This is a substrate level phosphorylation. GTP is converted to ATP by the enzyme nucleoside diphosphate kinase.

6. Conversion of succinate to fumarate. Succinate is oxidized by **succinate dehydrogenase** to fumarate. This reaction results in the production of $FADH_2$ and not NADH. This is due to the fact that the reducing power of succinate is not adequate to reduce NAD^+, hence FAD is utilized.

7. Formation of malate. The enzyme fumarase catalyses the conversion of fumarate to malate with the addition of H_2O.

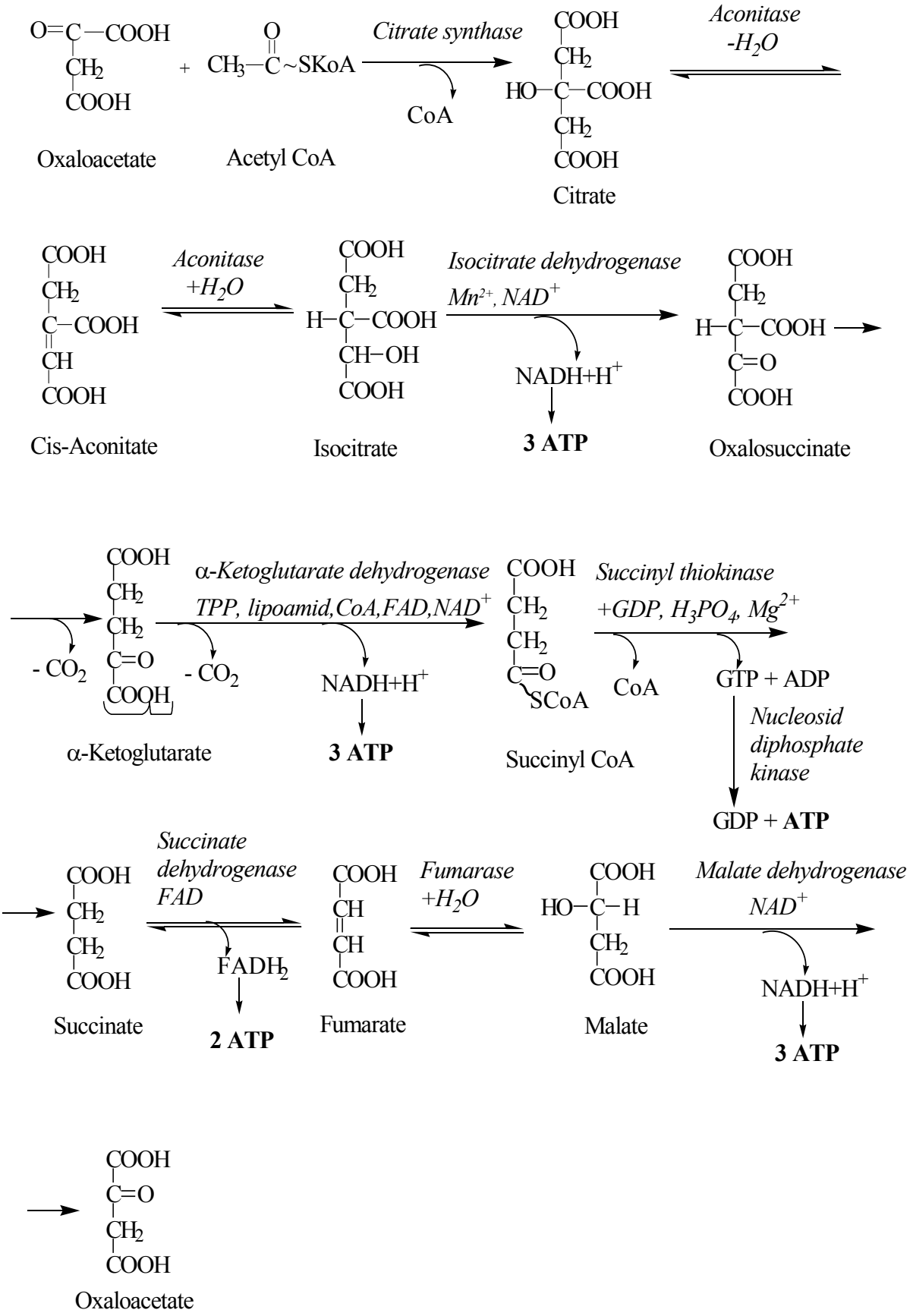


Figure 10—1. The citric acid (Krebs) cycle.

8. Conversion of malate to oxaloacetate. Malate is then oxidized to oxaloacetate by **malate dehydrogenase**. The third and final synthesis of **NADH** occurs at this stage. The **oxaloacetate** is regenerated which can combine with another molecule of acetyl CoA and continue the cycle.

Requirement of O_2 by TCA cycle

There is no direct participation of oxygen in Krebs cycle. However, the cycle operates only under **aerobic conditions.** This is due to the fact that NAD^+ and FAD (from NADH and $FADH_2$, respectively) required for the operation of the cycle can be regenerated in the respiratory chain only in the presence of O_2.

Energetics of citric acid cycle

During the process of oxidation of acetyl CoA via citric acid cycle, 4 reducing equivalents (3 as NADH and one as $FADH_2$) are produced. Oxidation of 1 NADH by electron transport chain coupled with oxidative phosphorylation results in the synthesis of 3 ATP, whereas $FADH_2$ leads to the formation of 2 ATP. Besides, there is one substrate level phosphorylation. A total of **twelve ATP** are produced from one acetyl CoA.

Inhibitors of Krebs cycle

The important enzymes of TCA cycle inhibited by the respective inhibitors are listed

Enzyme	Inhibitor
Aconitase	Fluoroacetate (non-competitive)
α-Ketoglutarate dehydrogenase	Arserine (non-competitive)
Succinate dehydrogenase	Malonate (competitive)

Regulation of citric acid cycle

The cellular demands of ATP are crucial in controlling the rate of citric acid cycle. The regulation is brought about either by enzymes or the levels of ADP. Three enzymes — **citrate synthase, isocitrate dehydrogenase and α-keto-glutarate dehydrogenase —** regulate citric acid cycle.

Citrate synthase is inhibited by ATP, NADH, acyl CoA and succinyl CoA.

1. Isocitrate dehydrogenase is activated by ADP and inhibited by ATP and NADH.

2. α-Ketoglutarate dehydrogenase is inhibited by succinyl CoA and NADH.

3. Availability of ADP is very important for the citric acid cycle to proceed. This is due to the fact that unless sufficient levels of ADP are available, oxidation (coupled with phosphorylation of ADP to ATP) of NADH and $FADH_2$ through electron transport chain stops. The accumulation of NADH and $FADH_2$ will lead to inhibition of the enzymes and also limits the supply of NAD^+ and FAD which are essential for citric acid cycle to proceed.

Anaplerotic reactions

The synthetic reactions deplete the intermediates of citric acid cycle. Anaplerotic reactions can increase the concentration of citric acid cycle intermediates, allowing an increased rate of oxidation of two-carbon units. As more intermediates are available, more moles of acetyl CoA can be processed. The intermediates also may be used for other biosynthetic reactions and need to be replaced.

LECTURE 11

METABOLISM OF CARBOHYDRATES. METABOLISM OF GLUCOSE UNDER AEROBIC CONDITIONS

Functions of carbohydrates

1. They are the most abundant dietary **source of energy** (4 kCal/g) for all organisms. Carbohydrates are the **major source of energy** for the living cells.

2. Carbohydrates are **precursors for many organic compounds** (fats, amino acids).

3. Carbohydrates (as glycoproteins and glycolipids) participate in the **structure of cell membrane** and **cellular functions** such as growth, adhesion and fertilization.

4. They are **structural components** of many organisms. These include the fiber (cellulose) of plants, exoskeleton of some insects and the cell wall of microorganisms.

5. Carbohydrates also serve as the **storage form of energy** (glycogen) to meet the intermediate energy demands of the body.

The **monosaccharide glucose** is the central molecule in carbohydrate metabolism **since all the major pathways of carbohydrate metabolism are connected with it**. The other monosaccharides important in carbohydrate metabolism are **fructose, galactose and mannose.**

The fasting blood glucose level in normal individuals is **60—100 mg/dl** (**4.5—5.5 mmol/l**) and it is very efficiently maintained at this level.

Sources of blood glucose

1. Dietary sources. The dietary carbohydrates are digested and absorbed as monosaccharides (glucose, fructose, galactose etc.). The liver is capable of converting fructose and galactose into glucose, which can readily enter blood.

2. Glycogenolysis. Degradation of glycogen in liver produces free glucose. This is in contrast to muscle glycogenolysis where glucose is not formed in sufficient amount due to lack of the enzyme glucose 6-phosphatase. However, the contribution of liver glycogenolysis to blood glucose is rather limited and can meet only the short intervals of emergency.

3. Gluconeogenesis. Lactate, glycerol, propionate and some amino acids are good precursors for glucose synthesis (gluconeogenesis) that actively occurs in liver and kidney. Gluconeogenesis continuously adds glucose to the blood.

Major pathways of glucose metabolism

1. Glycolysis (Embden-Meyerhof pathway). The oxidation of glucose to pyruvate and lactate.

2. Glycogenesis. The formation of glycogen from glucose.

3. Pentose phosphate pathway (hexose monophosphate shunt or direct oxidative pathway). This pathway is an alternative to glycolysis for the oxidation of glucose. Here, glucose is directly oxidized to carbon dioxide and water.

4. Uronic acid pathway. Glucose is converted to glucuronic acid, pentoses and, in some animals, to ascorbic acid (not in man). This pathway is also an alternative oxidative pathway for glucose.

5. Amino sugar and mucopolysaccharide metabolism. The synthesis of amino sugars and other sugars for the formation of mucopolysaccharides and glycoproteins.

6. Synthesis of non-essential amino acids. The intermediates of carbohydrate metabolism, mainly to keto-acids (e.g. pyruvic acid), serve as precursors for the synthesis of non-essential amino acids.

7. Synthesis of fat. Excess consumption of carbohydrates leads to the formation of fat which is stored in the adipose tissue.

DIGESTION OF CARBOHYDRATES

The principal dietary carbohydrates are **polysaccharides** (starch, glycogen), **disaccharides** (lactose, sucrose) and, to a minor extent, **monosaccharides** (glucose, fructose). The digestion of carbohydrates occurs briefly in mouth and largely in the intestine. The polysaccharides get hydrated during heating which is essential for their digestion. The hydrolysis of glycosidic bonds is carried out by a group of enzymes called **glycosidases**. These enzymes are specific to the bond, structure and configuration of monosaccharide units.

1. 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 and cleaves α-1,4 glycosidic bond. The products formed include **α-limit dextrins** (containing about 8 glucose units with one or more α-1,6 glycosidic bonds), **maltotriose** and **maltose**.

2. 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.

3. 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. The resultant products are disaccharides (maltose, isomaltose) and oligosaccharides.

4. The final digestion of di- and oligosaccharides to monosaccharides primarily occurs at the mucosal lining of the upper jejunum. This is carried out by **oligosaccharidases** and **disaccharidases**. The capacity of most of these enzymes is much greater than required for the digestion of the respective substrates. Particularly important is the enzyme **sucrase** which is capable of hydrolyzing a large quantity of table sugar (sucrose). In contrast, **lactase** is the rate-limiting, and, consequently, the utilization of milk sugar (lactose) is limited in human. Enzyme **isomaltase** hydrolyzes the isomaltose (α-1,6-glucose) to glucose, **maltase —** maltose, maltotriose (α 1,4-glucose) to glucose, **trehalase —** trehalose (α-1,1-glucose; trehalose is non-reducing disaccharide found in young mushrooms) to glucose.

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 sugar mostly takes place in the **duodenum** and **upper jejunum** of small intestine.

The relative rates of absorption of important monosaccharides in comparison with glucose are: glucose — 100%, galactose — 110%, fructose — 43%, mannose — 20%, xylose — 15%, arabinose — 9%. It is observed that hexoses are more rapidly absorbed than pentose. Further, among the monosaccharides, galactose is most efficiently absorbed followed by glucose and fructose. Insulin has no effect on the absorption of sugar.

Mechanism of absorption

Different sugars possess different mechanisms for their absorption.

1. Glucose is transported onto the intestinal mucosal cells by a carrier mediated and energy requiring process. **Glucose** and **Na^+** share the same transport system (symport). 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. The reabsorption of glucose that occurs in the proximal convoluted tubules of kidney is also mediated by Na^+ and the mechanism is similar to that describe. Once within the mucosal cell, glucose is transported into the capillaries by facilitated passive diffusion. The maximal rate of glucose absorption is about 120 g/h.

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

3. 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, lignine and gums. Some of fibers are digestible by the enzymes of intestinal bacteria. For a long time, fiber was regarded as nutritional waste. And now nutritionists attach a lot of importance to the role of fiber in human health. Dietary fiber is involved in several functions.

1. Prevents constipation. Fiber helps to maintain the normal motility of gastrointestinal tract and prevents constipation.

2. Eliminates bacterial toxins. Fiber adsorbs large compounds produced by intestinal bacteria that lead to increased fecal mass and its easier expulsion.

3. Decreases gastrointestinal cancer. The lower incidence of cancer of gastrointestinal tract (e.g. colon and rectum) in vegetarians compared to non-vegetarians is attributed to dietary fiber.

4. Improves glucose tolerance. Fiber improves glucose tolerance by the body. This is mainly done by a diminished rate of glucose absorption from the intestine.

5. Reduced plasma cholesterol level. Fiber decreases the absorption of dietary cholesterol from the intestine. Further, fiber binds with the bile salts and reduced their enterohepatic circulation. This causes increased degradation of cholesterol to bile salts and its disposal from the body.

6. Satiety value. Dietary fiber significantly adds to the weight of the food-stuff ingested and gives a sensation of stomach-fullness. Therefore, satiety is achieved without the consumption of excess calories.

Abnormalities of carbohydrates digestion

1. 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 (increased intestinal motility, cramps and irritation). This occurs after ingestion of certain carbohydrates and is explained as follows. 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 utilized by intestinal bacteria causing flatulence. **Raffinose** containing galactose, glucose and fructose is a predominant oligosaccharide found in leguminous seeds.

2. 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.

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

4. 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. I 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.

5. 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.

Entry of glucose into cells

Glucose concentration is very low in the cells compared to blood (for humans < 100 mg/dl). However, glucose does not enter the cells by simple diffusion. Two specific transport systems are recognized for the entry of glucose into the cells:

1. Insulin-independent transport system of glucose. This is a carrier mediated uptake of glucose which is not dependent on the hormone insulin. This is operative in hepatocytes, erythrocytes and brain.

2. Insulin-dependent transport system. This occurs in muscle and adipose tissue.

3. Glucose transporters. In recent years, at least five glucose transporters (GLUT-1 to GLUT-5) in the cell membranes have been identified. They exhibit tissue specificity. For instance, GLUT-1 is abundant in erythrocytes whereas GLUT-4 is abundant in skeletal muscle and adipose tissue. Insulin increases the number and promotes the activity of GLUT-4 in skeletal muscle and adipose tissue.

Glycolysis

Glycolysis is derived from the *Greek* words (glycose — sweet or sugar; lysis — dissolution). It is a universal pathway in the living cells. The complete pathway of glycolysis was elucidated in 1940. This pathway is often referred to as Embden-Meyerhof pathway (E.M. pathway) in honor of the two biochemists who made a major contribution to the knowledge of glycolysis.

Glycolysis is defined as the sequence of reactions converting glucose (or glycogen) to pyruvate or lactate, with the production of ATP.

Salient features

1. Glycolysis takes place in all cells of the body. The **enzymes** of this pathway are present in the **cytosomal fraction** of the cell.

2. Glycolysis occurs in the absence of oxygen (**anaerobic**) or in the presence of oxygen (**aerobic**). **Lactate** is the end product under **anaerobic condition**. **In the aerobic condition**, pyruvate is formed, which is then oxidized to **CO_2 and H_2O**.

3. Generally, Embden-Meyerhof pathway is an emergency energy-yielding pathway for cells in the absence of oxygen.

4. The occurrence of glycolysis is a prerequisite for the aerobic oxidation of carbohydrates, the latter takes place in the cells possessing mitochondria.

5. Glycolysis is a major pathway for ATP synthesis in tissues lacking mitochondria, e.g. erythrocytes, cornea, lens etc.

6. In some other tissues which have relatively few mitochondria (e.g. testes, leucocytes and kidney medulla), glycolysis is significant for ATP production.

7. Glycolysis is very **essential for brain** which is dependent on glucose for energy. The glucose in brain has to undergo glycolysis before it is oxidized to CO_2 and H_2O.

8. The intermediates formed in glycolysis are useful for the synthesis of non-essential amino acids and glycerol, the latter used for fat formation.

9. Reversal of glycolysis along with the alternate arrangements made at the irreversible steps will result in the synthesis of glucose (gluconeogenesis).

Reactions of glycolysis (Fig. 11—1).

The pathway can be divided into three distinct phases

1. Energy investment phase or priming stage

2. Splitting phase

3. Energy generation phase.

Energy investment phase or priming stage

1. Glucose is phosphorylated to **glucose 6-phosphate** by **hexokinase** or **glucokinase**. This is an irreversible reaction, dependent on ATP and Mg^{2+}. The enzyme **hexokinase** is present in almost all the tissues. It catalyses the phosphorylation of various hexoses (fructose, mannose etc.), has low K_m for substrates (about 0.1 mM) and is inhibited by glucose 6-phosphate.

Glucokinase present in liver, catalyses the phosphorylation of only glucose, has high K_m for glucose (10 mM) and is not inhibited by glucose 6-phosphate.

Due to high affinity (low K_m), glucose is utilized by hexokinase even at low concentration, whereas glucokinase acts only at higher levels of glucose i.e., after a meal when blood glucose concentration is above 100 mg/dl.

Glucose-6-phosphate is impermeable to the cell membrane. It is a **central molecule with a variety of metabolic fates —** glycolysis, glycogenesis, gluconeogenesis and pentose phosphate pathway.

2. Glucose-6-phosphate undergoes isomerization to give fructose 6 phosphate in the presence of the enzyme **phosphoglucose isomerase** and Mg^{2+}.

3. Fructose 6-phosphate is phosphorylated to **fructose-1,6-bisphosphate** by **phosphofructokinase** (PFK). This is an irreversible and regulatory step in glycolysis. PFK is an allosteric enzyme, the activity of which is controlled by several allosteric modulators.

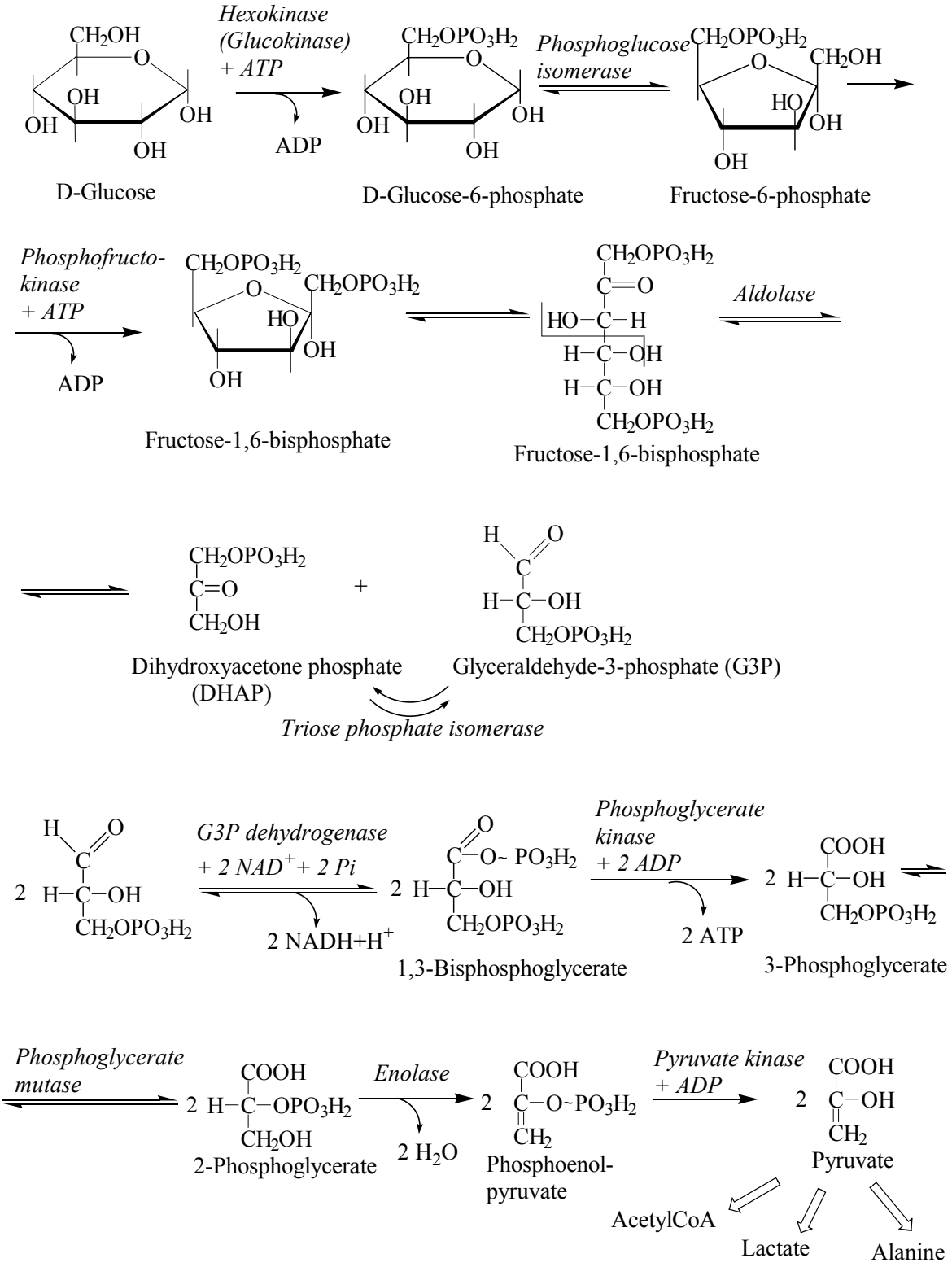


Figure 11—1. The pathway of glycolysis.

Splitting phase

4. The six carbon fructose-1,6-bisphosphate is split (hence the name glycolysis) to two three-carbon compounds, **glyceraldehyde 3-phosphate** (G3P) and **dihydroxyacetone phosphate** (DHAP) by the enzyme **aldolase** (fructose 1,6 bisphosphate aldolase).

5. The enzyme **phosphotriose isomerase** catalyses the reversible interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Although the reaction is reversible, because G3P is used in the subsequent reaction, its removal shift the equilibrium in the direction of conversion of essentially all of the DHAP to G3P. Thus, two molecules of glyceraldehyde 3-phosphate are obtained from one molecule of glucose. This isomerase enzyme is inhibited by **bromohydroxyacetone phosphate.**

Energy generation phase

6. Glyceraldehyde 3-phosphate dehydrogenase converts glyceraldehyde 3 phosphate to **1,3-bisphosphoglycerate**. This reversible reaction requires NAD^+ as an electron carrier. This enzyme brings about two reactions, namely oxidation, where the hydrogens are transferred to NAD^+ and phosphorylation of substrate adding one phosphate moiety. This step is important as it is involved in the formation of $NADH+H^+$ and a high energy compound 1,3-bisphosphoglycerate. **Iodoacetate and arsenite** inhibit the enzyme glyceraldehydes 3-phosphate dehydrogenase. In aerobic condition, NADH passes through the electron transport chain and 6 ATP (2 x 3 ATP) are synthesized by oxidative phosphorylation.

7. The enzyme **phosphoglycerate kinase** acts on 1,3-bisphosphoglycerate resulting in the synthesis of ATP and formation of 3-phosphoglycerate. This step is a good example of **substrate level phosphorylation,** since ATP is synthesized from the substrate without the involvement of electron transport chain. **Phosphoglycerate kinase reaction is reversible, a rare example among the kinase reactions.**

8. 3-Phosphoglycerate is converted to **2-phosphoglycerate** by **phosphoglycerate mutase.** This is an isomerization reaction, involving the transfer of phosphate group from position 3 to position 2 in the substrate. The enzyme requires Mg^{2+}.

9. The high energy compound **phosphoenol pyruvate** is generated from 2 phosphoglycerate by the enzyme **enolase.** This enzyme requires Mg^{2+} or Mn^{2+} and is inhibited by **fluoride.** For blood glucose estimation in the laboratory, fluoride is added to the blood to prevent glycolysis by the cells, so that blood glucose is correctly estimated.

10. The enzyme **pyruvate kinase** catalyses the transfer of high energy phosphate from phosphoenol pyruvate to ADP, leading to the formation of ATP and pyruvate. This step also is a **substrate level phosphorylation.** Pyruvate kinase requires K^+ and either Mg^{2+} or Mn^{2+}. This reaction is irreversible.

Irreversible steps in glycolysis

Most of the reactions of glycolysis are reversible. However, the three steps catalysed by the enzymes **hexokinase** (or glucokinase), **phosphofructokinase and pyruvate kinase,** are irreversible. These three stages mainly regulate glycolysis. The reversal of glycolysis, with alternate arrangements made at the three irreversible stages, leads to the synthesis of glucose from pyruvate (gluconeogenesis).

Regulation of glycolysis

The three enzymes namely hexokinase (and glucokinase), phosphofructokinase and pyruvate kinase, catalyzing the irreversible reactions regulate glycolysis.

1. Hexokinase is inhibited by glucose 6-phosphate. This enzyme prevents the accumulation of glucose 6-phosphate due to product inhibition. Glucokinase, which specifically phosphorylates glucose, is an inducible enzyme. The substrate glucose, probably through the involvement of insulin, induces glucokinase.

2. Phosphofructokinase (PFK) is the most important regulatory enzyme in glycolysis. This enzyme catalyses the **rate limiting committed step.** PFK is an allosteric enzyme regulated by allosteric effectors. **ATP, citrate** and **H^+ ions** (low pH) are the most important allosteric inhibitors, whereas, fructose-2,6-bisphosphate, AMP and Pi are the allosteric activators. **Fructose 2,6-bisphosphate** (F2,6BP) is considered to be the most regulatory factor (activator) for controlling PFK and, ultimately, glycolysis **in the liver only**. F2,6BP is synthesized from fructose-6-phosphate by the enzyme phosphofructokinase called PFK-2 (PFK-1 is the glycolytic enzyme). F2,6BP is hydrolysed by fructose-2,6-bisphosphatase. The activity of PFK-2 and fructose 2,6-bisphosphatase is controlled by covalent modification which, in turn, is regulated by cyclic AMP. Cyclic AMP brings about phosphorylation of the bifunctional enzyme, resulting in inactivation of active site responsible for the synthesis of F2, 6BP but activation of the active site responsible for the hydrolysis of F2, 6BP.

3. Pyruvate kinase also regulates glycolysis. This enzyme is inhibited by ATP and activated by F1,6BP. Pyruvate kinase is active (a) in dephosphorylated state and inactive (b) in phosphorylated state. Inactivation of pyruvate kinase by phosphorylation is brought about by cAMP-dependent protein kinase. The hormone — **glucagon inhibits** hepatic glycolysis by this mechanism.

Conversion of pyruvate to acetyl CoA

Pyruvate is converted to acetyl CoA by **oxidative decarboxylation.** This is an irreversible reaction, catalyzed by a multienzyme complex, known as **pyruvate dehydrogenase complex** (PDH), which is found only in the **mitochondria.** High concentrations of PDH are found in cardiac muscle and kidney. PDH links glycolysis and the citric acid cycle.

The enzyme PDH requires five cofactors (coenzymes), namely — thiamine pyrophosphate (TPP), lipoamide (lipoamide contains lipoic acid linked to ε-amino group of lysine), FAD, coenzyme A and NAD^+ (Fig. 11—2).

Reactions of PDH complex

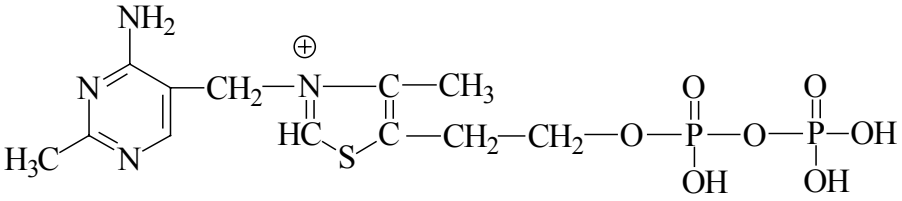
1. Pyruvate is decarboxylated to give hydroxyethyl TPP, catalyzed by **pyruvate dehydrogenase** (decarboxylate activity) (Fig.11—3).

2. Dihydrolipoyl transacetylase brings about the formation of acetyl lipoamide (from hydroxethyl-TPP) and then catalyses the transfer of acetyl group to coenzyme A to produce **acetyl CoA**.

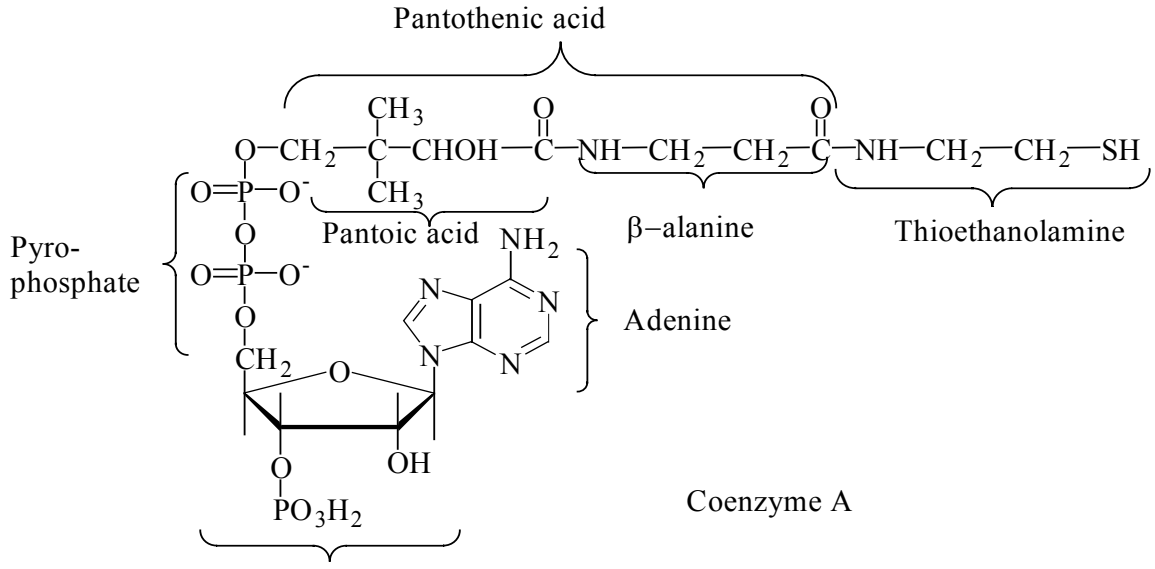
3. The cycle is complete when reduced lipoamide is converted to oxidized lipoamide by **dihydrodpoyl dehydrogenase**, transferring the reducing equivalents to FAD. $FADH_2$, in turn, transfers the reducing equivalents to NAD^+ to give NADH + H^+, which can pass through the respiratory chain to give 3 ATP (6 ATP from 2 moles of pyruvate formed from glucose) by oxidative phosphorylation.

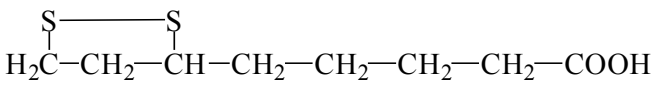
The intermediates of PDH catalyzed reaction are not free but bound with enzyme complex. **In mammals, the PDH complex has an approximate molecular weight of $9x10^6$. It contains 60 molecules of dihydrolipoyltransacetylase and about 20—30 molecules each of the other two enzymes (pyruvate dehydrogenase and dihydrolipoyl dehydrogenase).**

A comparable enzyme with PDH is **α-ketoglutarate dehydrogenase complex** of citric acid cycle which catalyses the oxidative decarboxylation of α-ketoglutarate to succinyl CoA.



Thiamine pyrophosphate (TPP)





Lipoic acid (L)

Figure 11-2. Coenzyme of PDH complex

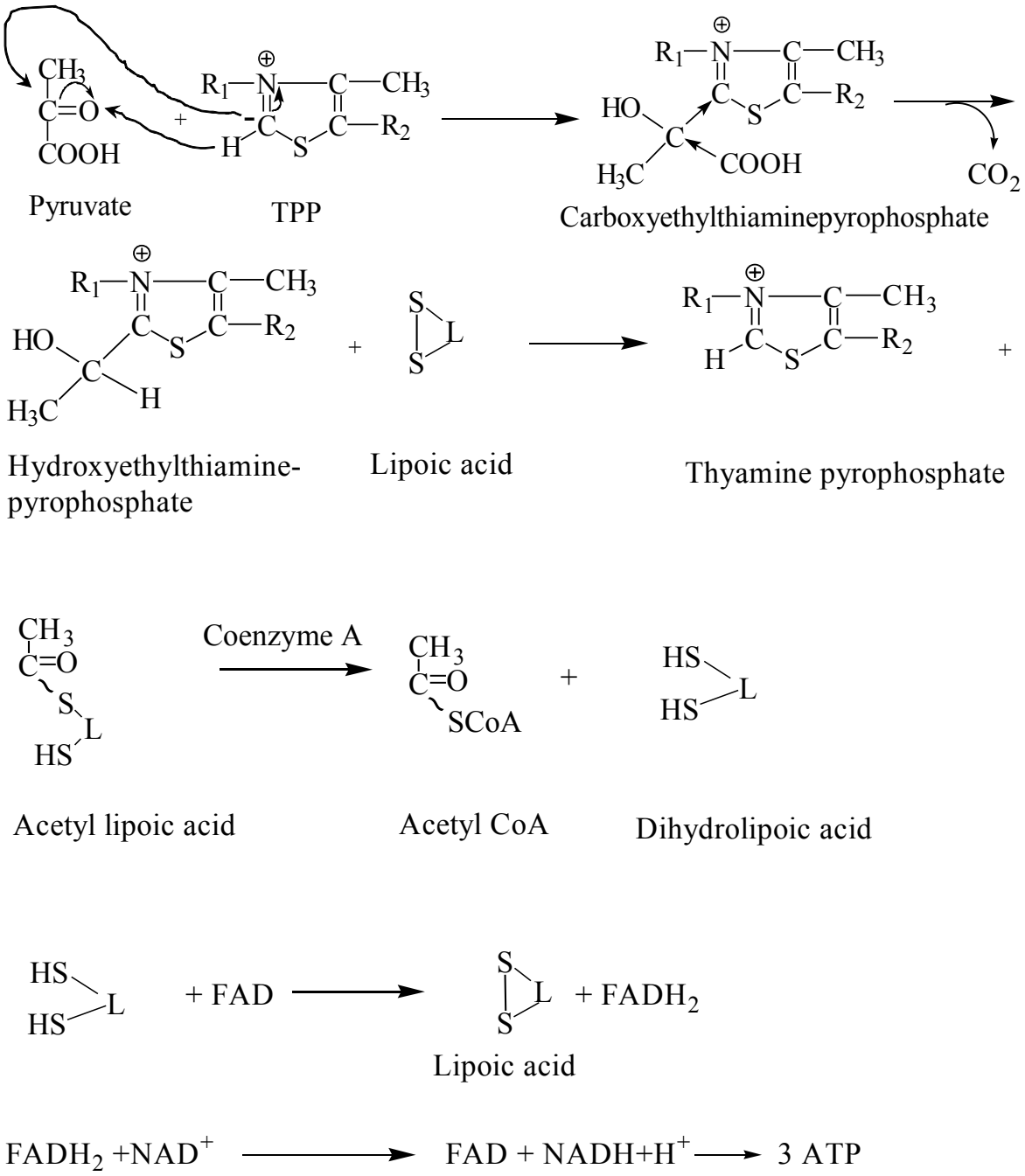


Figure 11—3. Mechanism of oxidative decarboxylation of pyruvate

Biochemical importance of PDH

1. Lack of TPP (due to deficiency of thiamine) inhibits PDH activity resulting in the accumulation of pyruvate.

2. In the thiamine deficient alcoholics, pyruvate is rapidly converted to lactate, resulting in lactic acidosis.

3. In patients with inherited deficiency of PDH, lactic acidosis (usually after glucose load) is observed.

4. PDH activity can be inhibited by **arsenic** and **mercuric** ions. These ions bind with -SH groups of lipoic acid.

PDH regulation

1. Product inhibition. Both acetyl CoA and NADH inhibit PDH.

2. Availability of substrates. Adequate concentrations of CoA and NAD^+ must be present.

3. Covalent modification. PDH exist in two forms: inactive, phosphorylated and active (dephosphorylated).

4. Hormonal regulation. Insulin can activate PDH in adipose tissue. **Catecholamines** can activate PDH in cardiac muscle.

Glycolysis and shuttle pathways

Glycolysis takes place in the cytosol. In the presence of **mitochondria** and **oxygen,** the NADH produced in glycolysis can participate in the synthesis of ATP. NADH cannot permeate the inner mitochondrial membrane. To overcome this problem, the NAD produced in the cytosol during glycolysis must be shuttled into the mutochondria to serve as a substrate for ATP production via oxidative phosphorylation.

1. Glycerol-3-phosphate shuttle. This shuttle functions primarily in **skeletal muscle** and **brain**. It results in the production of **2 moles of ATP** per mole of NADH. Cytosolic NADH is used to catalyze the reduction of DHAP to glycerol-3-phosphate. Glycerol-3-phosphate enters the mitochondrion and is oxidized back to DHAP by a mitochondrial enzyme that uses flavin adenine dinucleotide (FAD) as an electron acceptor. Reduced FAD ($FADH_2$) formed in the reaction supports the synthesis of 2 moles of ATP via the mitochondrial electron transport chain and oxidative phosphorylation. The DHAP formed is returned to the cytosol to continue the shuttle.

2. Malate shuttle. This shuttle functions primarily in the **heart, kidney, and liver.** It the results in the production of **3 moles of ATP** per mole of NADH. Cytosolic NADH is used to catalyze the reduction of oxaloacetate (OAA) to malate. Malate enters the mitochondrion and is oxidized back to OAA by a mitochondrial malate dehydrogenase that uses NAD^+ as an electron acceptor. Reduced NADH formed in the reaction supports synthesis of 3 moles of ATP via the mitochondrial electron transport chain and oxidative phosphorylation. OAA is transported across the membrane in the form of aspartate. Aspartate aminotransferase converts OAA to aspartate in the mitochondrion. After transport to the cytosol, another aspartate aminotransferase converts aspartate back to OAA to complete the cycle.

Generation of ATP in glucose metabolism under aerobic condition.

Glycolysis:

Glyceraldehyde-3-phosphate dehydrogenase (2 NADH, ETC, oxidative phosphorylation) — **6 ATP**

Phosphoglycerate kinase (substrate level phosphorylation) — **2 ATP**

Pyruvate kinase (substrate level phosphorylation) — **2 ATP**

Two ATP are consumed in the reaction catalyzed by hexokinase and phosphofructokinase — **2 ATP**

Pyruvate dehydrogenase (2 NADH, ETC, oxidative phosphorylation) — **6 ATP**

Citric acid cycle:

Isocitrate dehydrogenase (2 NADH, ETC, oxidative phosphorylation) — **6 ATP**

α-Ketoglutarate dehydrogenase — **6 ATP**

Succinate thiokinase (substrate level phosphorylation) — **2 ATP**

Succinate dehydrogenase (2 $FADH_2$, ETC, oxidative phosphorylation) — **4 ATP.**

Malate dehydrogenase (2 NADH, ETC, oxidative phosphorylation) — **6 ATP**.

Total ATP per mole of glucose under aerobic condition — **38 ATP.**

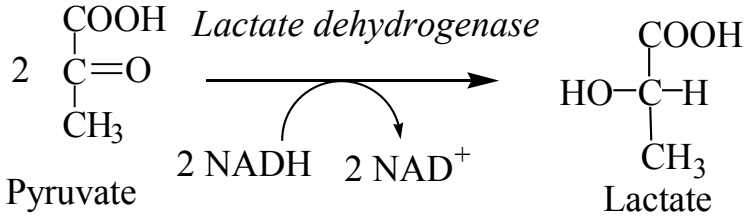
LECTURE 12

METABOLISM OF CARBOHYDRATES. METABOLISM GLUCOSE UNDER ANAEROBIC CONDITION. GLUCONEOGENESIS. PENTOSE PHOSPHATE PATHWAY

METABOLISM GLUCOSE UNDER ANAEROBIC CONDITION

Conversion of pyruvate to lactate

The NADH produced by the enzyme, glyceraldehyde 3-phosphate dehydrogenase, is utilized later by the enzyme lactate dehydrogenase and NAD^+ is regenerated.



So far as the synthesis and utilization of reducing equivalents are coupled, glycolysis proceeds uninterrupted and the end product is **lactate**. The perfect coupling is possible only in cells that **lack mitochondria** or under **anaerobic conditions.**

The fate of pyruvate produced in glycolysis depends on the presence or absence of oxygen in the cells. Under anaerobic conditions (lack of O_2), pyruvate is reduced by NADH to lactate in presence of the enzyme lactate dehydrogenase (competitive inhibitor — **oxamate**). The NADH utilized in this step is obtained from the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase. The formation of lactate allows the regeneration of NAD^+ which can be reused by glyceraldehyde 3-phosphate dehydrogenase so that glycolysis proceeds even in the absence of oxygen to supply ATP.

The occurrence of uninterrupted glycolysis is very essential in skeletal muscle during strenuous exercise where oxygen supply is very limited. **Glycolysis in the erythrocytes leads to lactate production,** since mitochondria — the centers for aerobic oxidation — are absent. **Brain, retina, skin, renal medulla and gastrointestinal tract** derive most of their energy from glycolysis.

Production of ATP in glycolysis

Under **anaerobic conditions, 2 ATP** are synthesized.

Pasteur effect

The **inhibition of glycolysis by oxygen** (aerobic condition) is known as Pasteur effect. This effect was discovered by Louis Pasteur, more than a century ago, while studying fermentation by yeast. He observed that when anaerobic yeast cultures (metabolizing yeast) were exposed to air, the utilization of glucose decreased by nearly sevenfold.

1. In the aerobic condition, the levels of glycolytic intermediates from fructose 1,6-bisphosphate onwards decrease while the earlier intermediates accumulate.

2. This clearly indicates that Pasteur effect is due to the inhibition of the enzyme phosphofructokinase. The inhibitory effect of citrate and ATP (produced in the presence of oxygen) on phosphofructokinase explains the Pasteur effect.

Lactic acidosis

Blood level. Normal blood lactate levels are less than 1.2 mM. With lactic acidosis, the blood lactate level may be 5 mM or more.

1. The high concentration of lactate results in **lowered blood pH and bicarbonate levels.**

2. The high blood lactate levels can result from **increased formation or decreased utilization of lactate.**

Hypoxia, or lack of oxygen, is a common cause of high blood lactate levels.

1. The shortage of oxygen reduces mitochondrial production of ATP with the consequent activation of PFK, which increases glycolysis and lactate production via pyruvate under anaerobic conditions.

2. Tissue hypoxia may occur in conditions that impair blood flow (e.g. shock), in respiratory disorders, and in severe anemia.

GLUCONEOGENESIS

The synthesis of glucose or glycogen from non-carbohydrate compounds is known as **gluconeogenesis.** The major substrates/precursors for gluconeogenesis are **lactate, pyruvate, glucogenic amino acids, propionate and glycerol.**

Location of gluconeogenesis

Gluconeogenesis occurs mainly in the **cytosol,** although some precursors are produced in the mitochondria. Gluconeogenesis mostly takes place **in liver** and, to some extent, in **kidney matrix** (about one-tenth of liver capacity).

Importance of gluconeogenesis

Glucose occupies a key position in the metabolism and its continuous supply is absolutely essential to the body for a variety of functions

1. Brain and central nervous system, erythrocytes, testes and kidney medulla are dependent on glucose for continuous supply of energy. Human brain alone requires about 120 g of glucose per day, out of about 160 g needed by the entire body.

2. Glucose is the only source that supplies energy to the **skeletal muscle**, under anaerobic conditions.

3. All other carbohydrates are synthesized from glucose, e.g. lactose, amino sugars etc.

4. Glucose is **converted to glycerol** and utilized for the synthesis of fat.

5. In fasting even more than a day, gluconeogenesis must occur to meet the basal requirements of the body for glucose and to maintain the intermediates of citric acid cycle. This is essential for the survival of humans and other animals.

6. Certain metabolites produced in the tissues accumulate in the blood, e.g. lactate, glycerol, propionate etc. Gluconeogenesis effectively clears them from the blood.

Reactions of gluconeogenesis

Gluconeogenesis closely resembles the **reversed pathway of glycolysis**, although it is not the complete reversal of glycolysis. **Essentially, 3** (out of 10) **reactions of glycolysis are irreversible.** The seven reactions are common for both glycolysis and gluconeogenesis. The **three irreversible steps** of glycolysis are catalysed by the enzymes, namely **hexokinase, phosphofructokinase and pyruvate kinase**. These three stages — by passed by alternate enzymes specific to gluconeogenesis — are discussed

1. Conversion of pyruvate to phosphoenolpyruvate. This takes place in two steps. **Pyruvate carboxylase** is a biotin – dependent mitochondrial enzyme that converts pyruvate to oxaloacetate in presence of ATP and CO_2. This enzyme regulates gluconeogenesis and requires acetyl CoA for its activity (Fig. 12—1).

2. Oxaloacetate is synthesized in the mitochondrial matrix. It has to be transported to the cytosol to be used in gluconeogenesis, where the rest of the pathway occurs.

3. Due to membrane impermeability, oxaloacetate cannot diffuse out of the mitochondria. It is converted to malate and then transported to the cytosol. Within the cytosol, oxaloacetate is regenerated. The reversible conversion of oxaloacetate and malate is catalyzed by **malate dehydrogenase,** an enzyme present in both mitochondria and cytosol.

4. In the cytosol, **phosphoenolpyruvate carboxykinase** converts oxaloacetate to **phosphoenolpyruvate**. GTP or ITP (not ATP) is used in this reaction and the CO_2 (fixed by carboxylase) is liberated. For the conversion of pyruvate to phosphoenol pyruvate, **2 ATP equivalents are** utilized. This is in con-

trast to only one ATP that is liberated in glycolysis for this reaction.

5. Conversion of fructose 1, 6-bisphosphate to fructose 6-phosphate. Phosphoenolpyruvate undergoes the reversal of glycolysis until fructose-1,6-bisphosphate is produced. The enzyme **fructose 1,6-bisphosphatase** converts fructose-1,6-bisphosphate to fructose-6-phosphate. This enzyme requires Mg^{2+} ions. Fructose-1,6-bisphosphatase **is absent in smooth muscle and heart muscle**. This enzyme is also regulatory in gluconeogenesis.

6. Conversion of glucose 6-phosphate to glucose. Glucose 6-phosphatase catalyses the conversion of glucose 6-phosphate to glucose. The presence or absence of this enzyme in a tissue determines whether the tissue is capable of contributing glucose to the blood or not. It is mostly **present in liver and kidney** but **absent in muscle, brain and adipose tissue.** The overall summary of gluconeogenesis for the conversion of pyruvate to glucose is shown :

$$2\ \text{Pyruvate} + 4\text{ATP} + 2\text{GTP} + 2\text{NADH} + 2\text{H}^+\ 6\text{CO}_2 \rightarrow$$
$$\text{Glucose} + 2\text{NAD}^+ + 4\text{ADP} + 2\text{GDP} + 6\text{Pi} + 6\text{H+}$$

After the formation of glucose, its conversion to glycogen occurs through the formation of uridine diphosphate glucose (UDP glucose) by the enzyme glycogen synthase.

Gluconeogenesis from amino acids

The carbon skeleton of glucogenic amino acids (all except leucine and lysine) results in the formation of pyruvate or the intermediates of citric acid cycle that, ultimately, result in the synthesis of glucose (Fig.12—1).

Gluconeogenesis from glycerol

Glycerol is liberated **mostly in the adipose tissue** by the hydrolysis of fats (triacylglycerols). The enzyme glycerokinase (found in liver and kidney, absent in adipose tissue) activates glycerol to glycerol 3-phosphate. The latter is converted to dihydroxyacetone phosphate by glycerol 3-phosphate dehydrogenase. Dihydroxyacetone phosphate is an intermediate in glycolysis that can be conveniently used for glucose production.

Gluconeogenesis from propionate

Oxidation of odd chain fatty acids and the breakdown of some amino acids (methionine, isoleucine) yield a three-carbon **propionyl CoA**. Propionyl **CoA carboxylase** acts on this in presence of ATP and **biotin** and converts to **methyl malonyl CoA** that is then converted **to succinyl CoA** in presence of **B_{12} coenzyme**. Succinyl CoA formed from propionyl CoA enters gluconeogenesis via citric acid cycle. Propionate is an important precursor for gluconeogenesis in ruminant animals such as cattle. Propionate is activated to propionyl CoA which is then converted to glucose as described above.

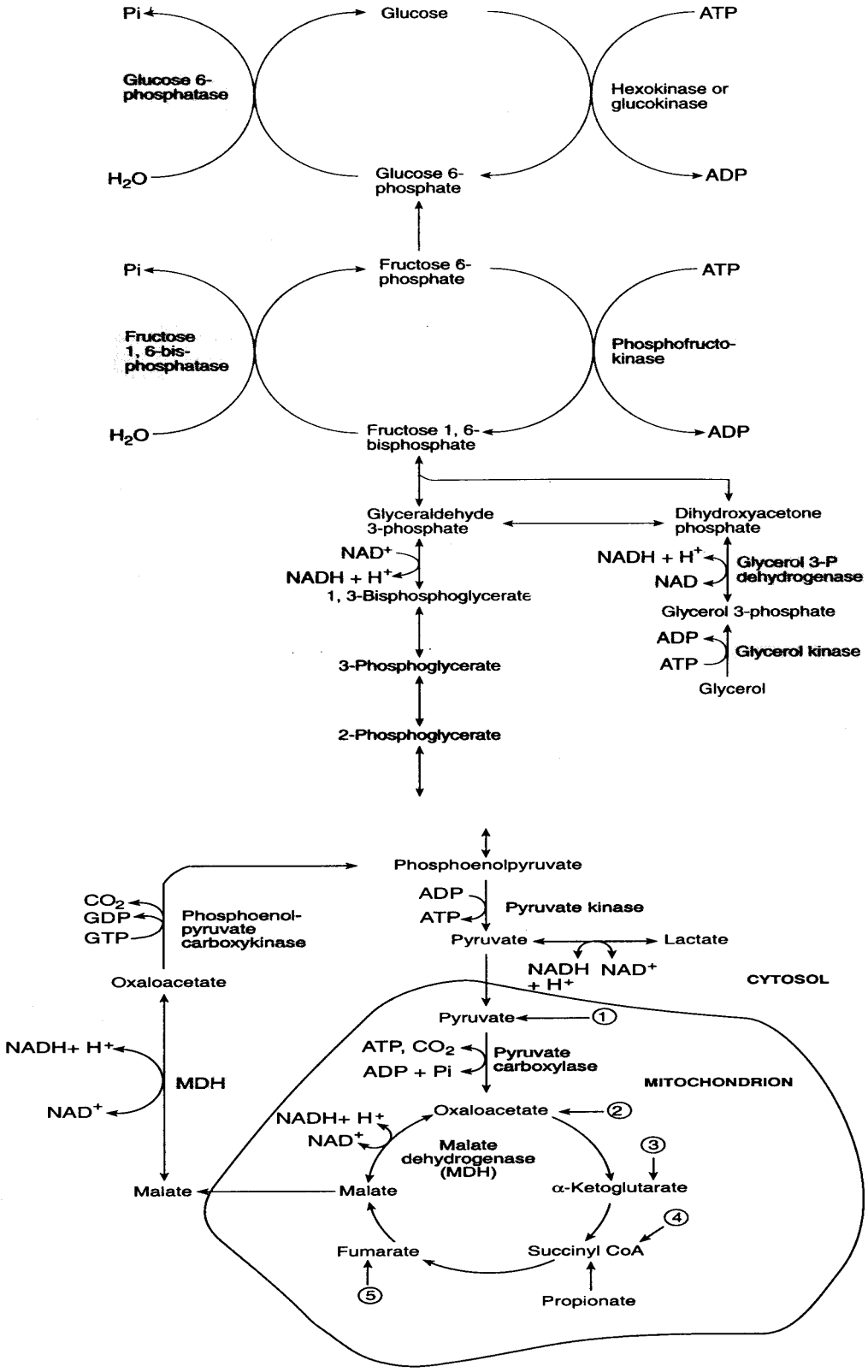


Figure 12—1. The pathways of gluconeogenesis. The number represent the entry of glucogenic amino acids: (1) Alanine, glycine, serine, cysteine, threonine and tryptophan; (2) Aspartate and asparagines; (3) Arginine, glutamate, glutamine, histidine, praline; (4) Isoleucine, methionine, valine; (5) Phenylalanine, tyrosine (by U. Satyanarayana, 2002).

Gluconeogenesis from lactate (Cori cycle)

1. Lactate produced **by active skeletal muscle** is a major precursor for gluconeogenesis. Under anaerobic conditions, **pyruvate is reduced to lactate** by **lactate dehydrogenase** (LDH).

2. Lactate is a dead glycolysis, since it must be reconverted to pyruvate for its further metabolism. The very purpose of lactate production is to regenerate NADH so that glycolysis proceeds uninterrupted in skeletal muscle. Lactate or pyruvate produced in the muscle cannot be utilized for the synthesis of glucose due to the absence of the key enzymes of gluconeogenesis (glucose-6-phosphatase and fructose-1,6-bisphosphatase).

3. The plasma membrane is freely permeable to lactate. Lactate is carried from the skeletal muscle through blood and handed over to liver, where it is oxidized to pyruvate. Pyruvate, so produced, is converted to glucose by gluconeogenesis, which is then transported to the skeletal muscle.

4. The cycle involving the synthesis of glucose in liver from the skeletal muscle lactate and the reuse of glucose thus synthesized by the muscle for energy purpose is known as Cori cycle.

Glucose-alanine cycle

There is a continuous transport of amino acids from muscle to liver, which predominantly occurs during starvation. Alanine dominates among the amino acids. It is postulated that pyruvate in skeletal muscle undergoes transamination to produce alanine. Alanine is transported to liver and used for gluconeogenesis. This cycle is referred to as glucose-alanine cycle.

PENTOSE PHOSPHATE PATHWAY

Pentose phosphate pathway (hexose monophosphate pathway) is also called **hexose monophosphate shunt or phosphogluconate pathway.** This is an alternative pathway to glycolysis and TCA cycle for the oxidation of glucose. However, pentose phosphate pathway is more anabolic in nature, since it is concerned with the biosynthesis of **NADPH and pentoses**.

The pathway starts with glucose 6-phosphate. As such, no ATP is directly utilized or produced in pentose phosphate pathway**. It is a unique multifunctional pathway**, since there are several interconvertible substances produced which may proceed in different directions in the metabolic reactions.

Location of the pathway

The enzymes of pentose phosphate pathway are located in the **cytosol.** The tissues such as **liver, adipose tissue, adrenal gland, erythrocytes, testes** and **lactating mammary gland,** are highly active in pentose phosphate pathway. Most of these tissues are involved in the biosynthesis of fatty acids and steroids which are dependent on the supply of NADPH.

The sequence of reactions of pentose phosphate pathway (Fig. 12—2) is divided into two phases—**oxidative** and **non-oxidative.**

1. Oxidative phase. Glucose-6-phosphate dehydrogenase (G6PD) is an NADP-dependent enzyme that converts glucose-6-

phosphate to 6-**phosphogluconolactone.** This latter is then hydrolyzed by **the gluconolactone hydrolase** to 6-phosphogluconate. The next reaction involving the synthesis of NADPH is catalyzed by **6-phosphogluconate dehydrogenase** to produce 3-keto-6-phosphogluconate which then undergoes decarboxylation to give ribulose-5-phosphate. **NADH competitively inhibits** G6PD. It is the ration of NADPH/NAD^+ that ultimately determines the flux of this cycle.

2. Non-oxidative phase. The non-oxidative reactions are concerned with the interconvertion of three, four, five and seven carbon monosaccharides. Ribose-5-phosphate is acted upon by an **epimerase** to produce **xylulose-5-phosphate** while **ribose-5-phosphate isomerase** converts ribulose-5-phosphate to **ribose-5-phosphate.**

3. The enzyme **transketolase** catalyses the transfer of two carbon moiety from xylulose-5-phosphate to ribose-5-phosphate to give a 3-carbon **glyceraldehydes-3-phosphate** and a 7-carbon **sedoheptolose-7-phosphate.** Transkelolase is dependent on the coenzyme thiamine pyrophosphate (TPP) and Mg^{2+}ions.

4. Transaldolase brings about the transfer of a 3-carbon fragment (active dihydroacetone) from sedoheptulose-7-phosphate to glyceraldehydes-3-phosphate to give **fructose-6-phosphate and four carbon erythrose-4-phosphate.**

5. Transketolase acts on xylulose-5-phosphate and transfers a 2-carbon fragment (glyceraldehyde) from it to erythrose-4-phosphate to generate **fructose-6-phosphate and glyceraldehyde-3-phosphate.**

6. Fructose 6-phosphate and glyceraldehyde 3-phosphate can be further catabolized through glycolysis and citric acid cycle. Glucose may also be synthesized from these two compounds.

Functions of pentose phosphate pathway

1. Provides a source of reduced **nicotinamide adenine dinucleotide phosphate (NADPH)**

a. NADPH is required for the **reductive biosynthesis** of fatty acids and steroids, hence pentose phosphate pathway is more active in the tissues concerned with lipogenesis, e.g. adipose tissue, liver etc.

b. Antioxidant reactions involve NADPH. NADPH is responsible for the regeneration of reduced **glutathione** from the oxidized one.

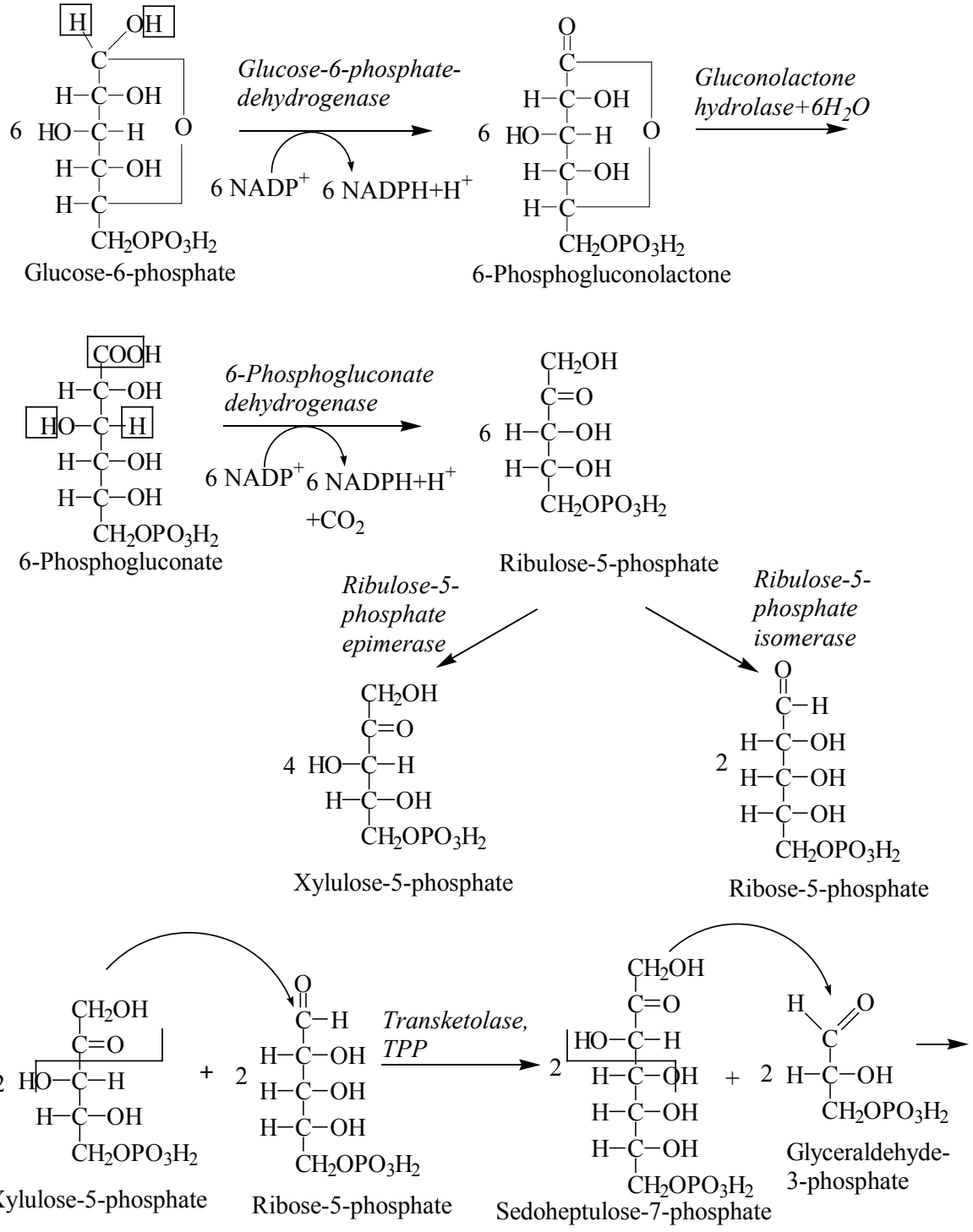
c. Microsomal cytochrone P_{450} system (in liver) brings about the **detoxification of drugs** and foreign compounds by hydroxylation reactions involving NADPH.

d. Phagocytosis is the engulfment of foreign particles, including microorganisms, carried out by white blood cells. This process requires the supply of NADPH.

e. NADPH produced in erythrocytes has special functions to perform. It maintains the concentration of reduced glutathione that is essentially required to preserve the **integrity of the red blood cell membrane.** NADPH is also necessary to keep the ferrous iron (Fe^{2+}) of hemoglobin in the reduced state so that accumulation of methemoglobin (Fe^{3+}) is prevented.

2. Provides a source of ribose-5-phosphate. Ribose-5-phosphate or its derivatives are useful for the **synthesis of nucleic acids** (RNA and DMA) and many **nucleotides** such as ATP, NAD^+, FAD and CoA.

3. Provides a route for the **conversion of pentoses** to fructose-6-phosphate and glyceraldehydes-3-phosphate.



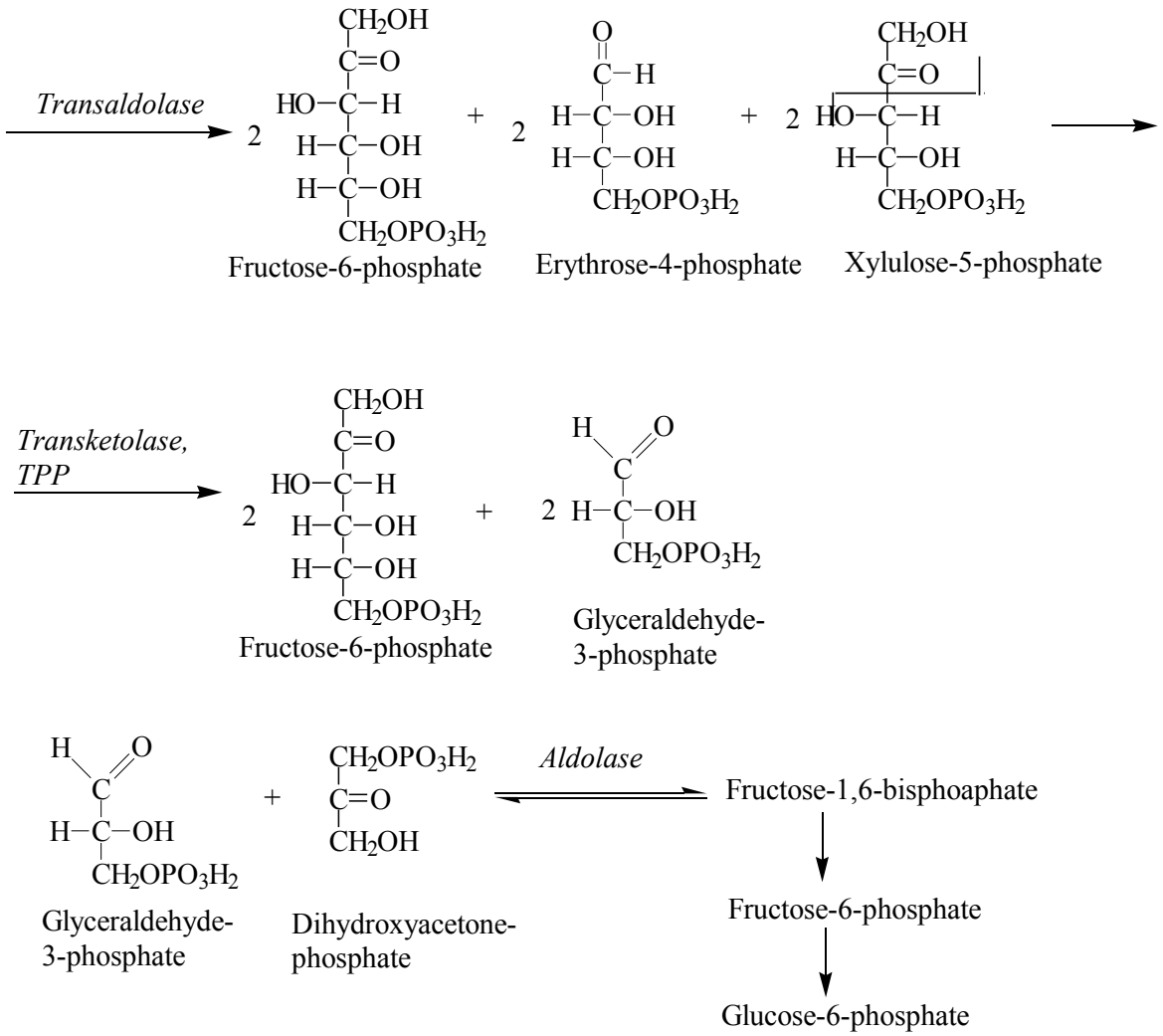


Figure 12—2. The pentose phosphate pathway

ETHANOL METABOLISM

Oxidation to acetate in the liver

1. Ethanol is oxidized in the liver by a cytosolic **alcohol dehydrogense** to acetaldehyde, leading to excess production of NADH. $C_2H_5OH + NAD^+ \rightarrow CH_3CHO + NADH + 2\ H^+$. The NADH generated competes with reducing equivalents from other substrates for the respiratory chain, inhibiting their oxidation.

2. The acetaldehyde is further oxidized to acetate by a mitochondrial **aldehyde dehydrogenase** $CH_3CHO + NAD^+ + H_2O \rightarrow CH_3COO^- + NADH + 2\ H^+$. Aldehyde dehydrogenase is the enzyme that is inhibited by drug **disulfiram,** which is given to alcoholics to discourage drinking. It causes acetaldehyde to accumulate, which leads to severe nausea. However, if alcohol consumption is not discourages, aldehyde levels may become lethal.

3. Much of the acetate produced from ethanol leaves the liver and is converted to acetyl coenzyme A, which can be used to provide energy via the citric acid cycle.

4. Acetyl CoA may also be formed in the liver and used as precursor for lipid biosynthesis.

5. Ethanol also may be oxidized by a **microsomal ethanol oxidizing system**, which is induced by ethanol. $C_2H_5OH + NADPH + H^+ + O_2 \rightarrow CH_3CHO + NADP^+ + 2\ H_2O$. Ethanol will also inhibit the metabolism of some drugs, e.g. barbiturates, by competing for cytochrome P_{450}-dependent enzymes.

Mechanism of methanol and ethylene glycol poising

Alcohol dehydrogenase is not a very specific enzyme and also converts methanol to formaldehyde and ethylene glycol to oxalate, both of which are very toxic.

LECTURE 13

METABOLISM OF CARBOHYDRATES. METABOLISM OF GLYCOGEN. METABOLISM OF FRUCTOSE AND GALACTOSE

METABOLSIM OF GLYCOGEN

Glycogen is the storage form of glucose in animals.

Structure

1. Glycogen is a large branched polymer of glucose molecules linked by α-1,4-glycosidic linkages; branches arise by α-l,6-glycosidic bonds at approximately every tenth residue.

2. Glycogen exists in the cytosol as granules, which also contain the enzymes that catalyze its formation and use.

Storage

The polymeric nature of glycogen allows energy to be sequestered without the problems from osmotic effects that glucose would cause.

1. Sites of glycogen storage are primarily the muscle (1—2%) and liver (6—8%). Due to more muscle mass, the quantity of glycogen in muscle (250 g) is about three times higher than that in the liver (75 g).

2. Liver can mobilize its glycogen for the release of glucose to the rest of the body, but muscle can only use its glycogen for its own energy needs.

3. In humans, liver glycogen stores are typically adequate for up to 12 hours without support from gluconeogenesis.

Glycogenesis

The synthesis of glycogen from glucose is glycogenesis. Glycogenesis takes place in the cytosol and requires ATP and UTP, besides glucose.

1. Synthesis of UDP-glucose (Fig.13—1).

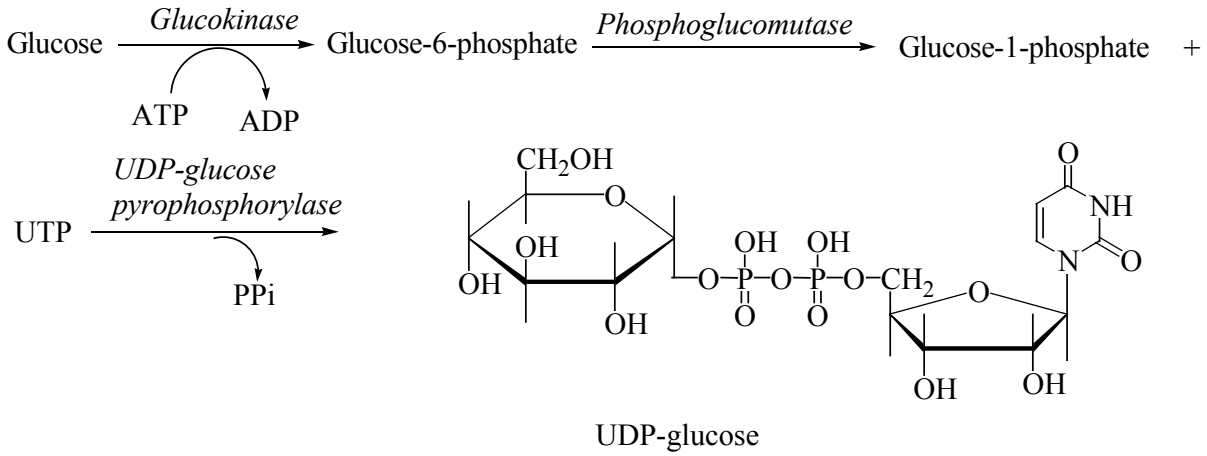


Figure 13—1. **Synthesis of UDP-glucose**

The enzymes **hexokinase** (in muscle) and **glucokinase** (in liver) convert glucose to glucose 6-phosphate. Phosphoglucomutase catalyses the conversion of glucose 6-phosphate to glucose 1-phosphate. **Uridine diphosphate glucose** (UDPG) is synthesized from glucose 1-phosphate and UTP by **UDP-glucose pyrophosphorylase**. Pyrophosphate (PPi) produced in this reaction is hydrolysed to inorganic phosphate (Pi) by pyrophosphatase. This will ensure the optimal synthesis of UDPG.

2. Requirement of primer to initiate glycogenesis. A small fragment of preexisting glycogen must act as a **'primer'** to initiate glycogen synthesis. It is recently found that in the absence of glycogen primer, a specific protein — namely **'glycogenin'** — can accept glucose from UDPG. The hydroxyl group of the amino acid tyrosine of glycogenin is the site at which the initial glucose unit is attached. The enzyme glycogen initiator synthase transfers the first molecule of glucose to glycogenin. Then glycogenin itself takes up a few glucose residues to form a fragment of primer which serves as an acceptor for the rest of the glucose molecules.

3. Glycogen synthesis by glycogen synthase. Glycogen synthase is responsible for the formation of 1,4-glycosidic linkages. This enzyme transfers the glucose from UDP-glucose to the non-reducing end of glycogen to form α-**1,4 linkages**. The UDP released can be converted back to UTP by nucleoside diphosphate kinase.

4. Formation of branches in glycogen. Glycolgen synthase can catalyse the synthesis of a linear unbranched molecule with α1,4-glycosidic linkages. Glycogen, however, is a branched treelike structure. The formation of branches is brought about by the action of a branching enzyme, namely **glycosyl α-4-6-transferase** (amylo α1,4→1,6 transglucosidase, **branching enzyme**). This enzyme transfers a small fragment of five to eight glucose residues from the non-reducing end of glycogen chain (by breaking α-1,4 linkages) to another glucose residue where it is linked by α-1,6 bond. This leads to the formation of a new non-reducing end, besides the existing one. Glycogen is fur-

ther elongated and branched, respectively, by the enzymes glycogen synthase and glycosyl 4-6 transferase.

Glycogenolysis

The **degradation of stored glycogen** in liver and muscle constitutes **glycogenolysis**. The pathway for the synthesis and degradation of glycogen are not reversible. An independent set of enzymes present in the cytosol carry out glycogenolysis. Glycogen is degraded by breaking α-1,4- and α-1,6-glycosidic bonds.

1**. Action of glycogen phosphorylase**. The α-1,4 glycosidic bonds (from the non-reducing ends) are cleaved sequentially by the enzyme **glycogen phosphorylase** to yield glucose 1-phosphate. This process — called phosphorolysis — continues until four glucose residues remain on either side of branching point (α-1,6-glycosidic link). The glycogen so formed is known as **limit dextrin** which cannot be further degraded by phosphorylase. Glycogen phosphorylase possesses a molecule of **pyridoxal phosphate**, covalently bound to the enzyme.

2. **Action of debranching enzyme**. The branches of glycogen are cleaved by two enzyme activities present on a single polypeptide called **debranching enzyme**, hence it is a **bifunctional enzyme**. Glycosyl 4:4 transferase (oligo α-1, 4→1, 4 glycan transferase) activity removes a fragment of three or four glucose residues attached at a branch and transfers them to another chain. Here, one α-1,4-bond is cleaved and the same α-1, 4 bond is made, but the places are different. **Amylo α-1,6-glucosidase** breaks the α-1,6 bond at the branch with a single glucose residue and **releases a free glucose**.

The remaining molecule of glycogen is again available for the action of phosphorylase and debranching enzyme to repeat the reactions stated in 1 and 2.

3. **Formation of glucose 6-phosphate and glucose**. Through the combined action of glycogen phosphorylase and debranching enzyme, glucose 1-phosphate and free glucose in ratio of 8:1 are produced. Glucose 1-phosphate is converted to glucose 6-phosphate by the enzyme **phosphoglucomutase**. The fate of glucose 6-phosphate depends on the tissue. The liver, kidney and intestine contain the enzyme **glucose 6-phosphatase that** cleaves glucose 6-phosphate to glucose. This enzyme is absent **in muscle and brain**, hence free glucose cannot be produced from glucose 6-phosphate in these tissues. Therefore, **liver** is the major glycogen storage organ to **provide glucose into the circulation** to be utilized by various tissues.

In the peripheral tissues, glucose 6-phosphate produced by glycogenolysis will be **used for glycolysis.** It may be noted that though glucose 6-phosphatase is absent in muscle, some amount of free glucose (8—10% of glycogen) is produced in glycogenolysis due to the action of debranching enzyme (α-1,6-glucosidase activity).

Phosphorylation cascades

The enzymes of glycogen metabolism undergo a sequential **covalent modification** by means of **phosphorylation**. This process provides a very large amplification of the initial stimulus (Fig. 13—2).

Cascade regulation of glycogen synthase activity

1. Glycogen synthase exists in two forms.

a. **The inactive form** is designated D because it is dependent. The D form is the phosphorylated form of the enzyme. The D form is an allosteric enzyme form that may be **activated by high concentrations of glucose-6-phosphate (G6P).**

b. **The active form** is designated I because it is independent. The I form of glycogen synthase is the **dephosphorylated** form of the enzyme. The I form does not require G6P for activity.

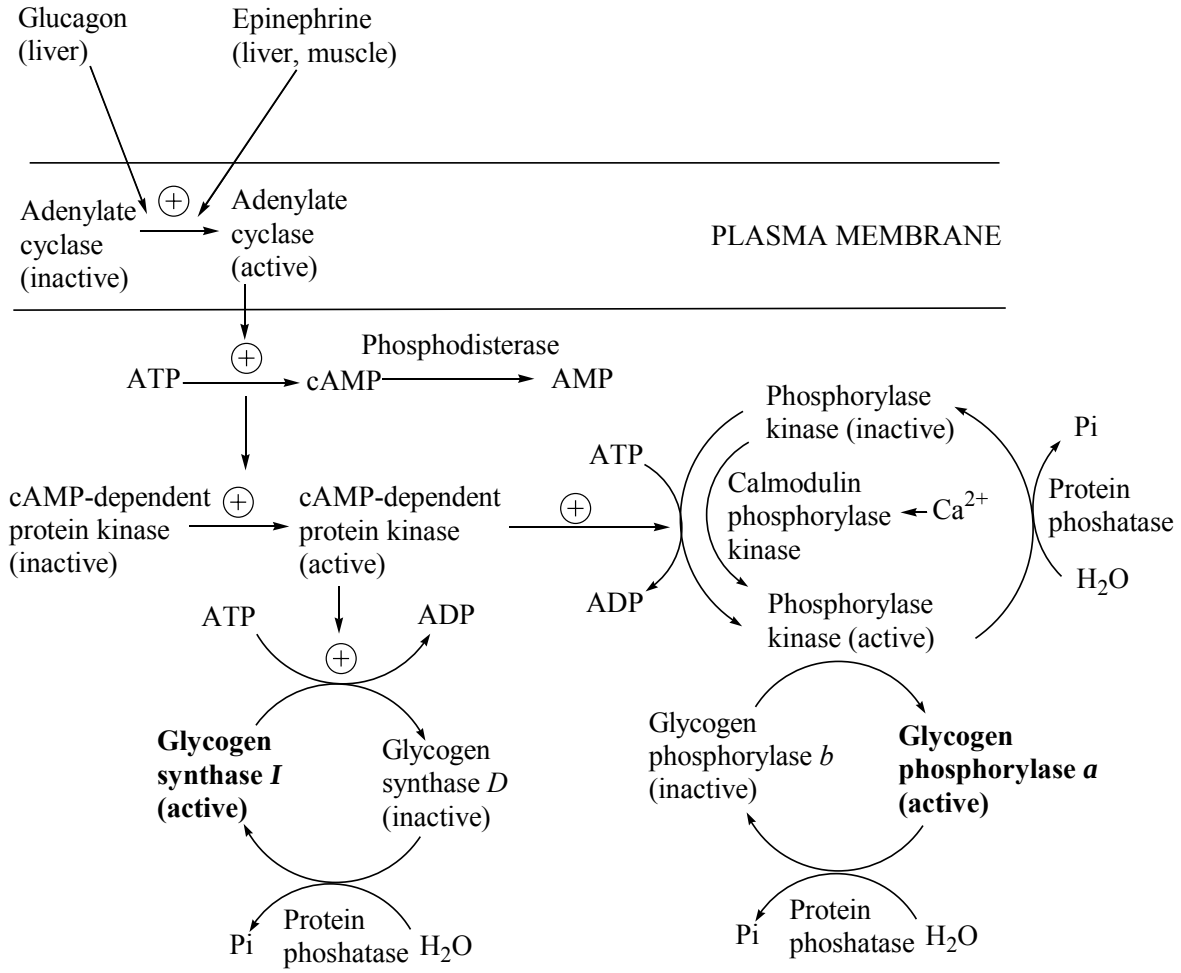


Figure 13—2. Hormonal regulation of glycogen synthesis and degradation

2. The interconversion of the D and I forms **of glycogen synthase is catalyzed by a** cyclic adenosine monophosphate (cAMP)-dependent protein kinase.

3. cAMP-dependent protein kinase**. The cAMP-dependent protein kinase is a tetramer having two types of subunits: two regulatory (R) subunits and two catalytic (C) subunits. The R_2C_2 tetramer is inactive.** Two molecules of cAMP **bind to each R subunit, whereupon the R_2C_2 complex disassociates into an R_2 subunit and two C units that are each catalytically active. The active protein kinase** transfers the γ-phosphate group **from ATP to a specific amino acid of a specific cell protein.**

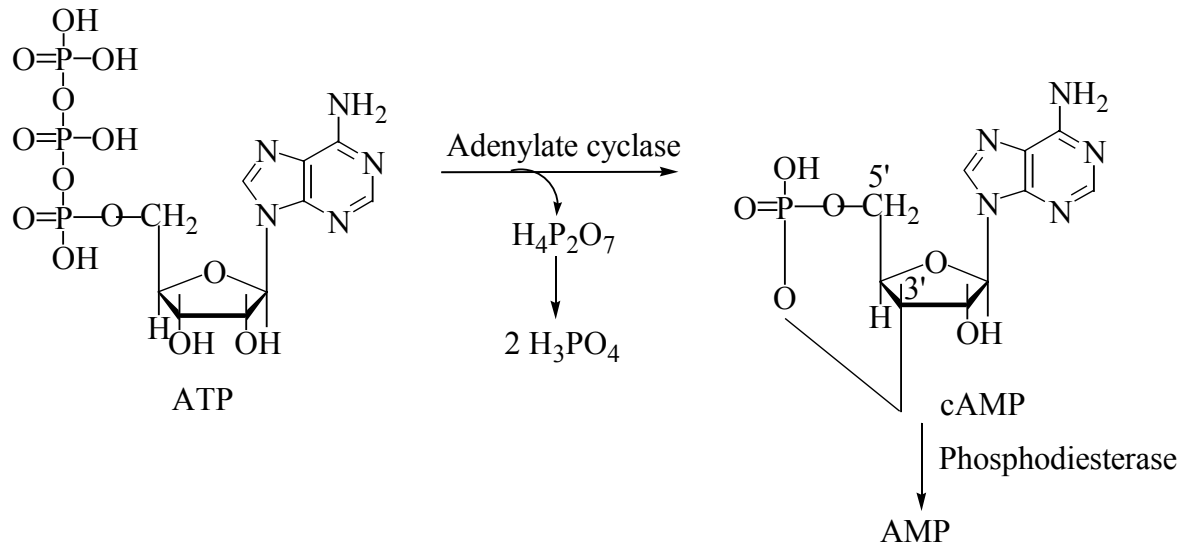


Figure 13—3. Synthesis and degradation of cAMP

4. Regulatory cascade

a. **Adenylate cyclase** regulates the activity of the protein kinase by regulating the level of cAMP. It produces cAMP from ATP (Fig. 13—3).

b. Adenylate cyclase is activated by the hormones **epinephrine and glucagon**, depending on the tissue.

c. Only a few molecules of hormone are needed to activate adenylate cyclase, which then produces a large number of cAMP molecules, each of which can activate a cAMP-dependent protein kinase enzyme molecule.

d. This active enzyme in turn phosphorylates a large number of glycogen synthase molecules, converting each of them to the **D** form.

Cascade regulation of glycogen phosphorylase activity

1. Glycogen phosphorylase exists in two forms.

a. Phosphorylase *a* is the **phosphorylated active form** of the enzyme.

b. Phosphorylase ***b*** is the **dephosphorylated inactive form** of the enzyme.

2. The interconversion of phosphorylases *a* and *b* is catalyzed by two enzymes.

a. Phosphorylase kinase phosphorylates a specific serine residue on each subunit of phosphorylase ***b*** to convert it to phosphorylase ***a.*** The activity of this protein kinase is regulated by adenylate cyclase, which regulates the level of cAMP and consequently is regulated by epinephrine and glucagon.

b. A phosphatase dephosphorylates that serine residue of phosphorylase ***a*** to regenerate the ***b*** form.

Regulation of glycogen metabolism

Hormonal regulation

1. In muscle

a**. Epinephrine** promotes glycogenolysis and inhibits glycogenesis.

(1) It stimulates the formation of cAMP by activating adenylate cyclase.

(2) When epinephrine is released and acts upon the muscle cell membrane, glycogenolysis is activated via the phosphorylation cascade, and simultaneously, glycogenesis is retarded.

b. Insulin increases glycogenesis and decreases glycogenolysis.

(1) It heightens the entry of glucose into the muscle cells.

(2) It reduces cAMP levels, probably by speeding up the destruction of cAMP by phosphodiesterase.

2. In the liver

a. Glucagon activates adenylate cyclase in the liver cell membranes and thus turns on glycogenolysis and reduces glycogenesis.

b. Insulin increases glycogenesis in the liver by increasing the activity of glycogen synthase by a mechanism that is not yet clear.

c. The glucagon:insulin ratio appears to be more important than the absolute level of either hormone.

(1) Insulin domination provides for the storage of glycogen after a meal.

(2) Glucagon domination favors mobilization of glycogen stores as the blood glucose level declines.

3. Effect of Ca^{2+} ions on glycogenolysis. When the muscle contracts, Ca^{2+}-ions are released from the sarcoplasmic reticulum. Ca^{2+} binds to **calmodulin** — **cal**cium **modul**ating prote**in** and directly activates phosphorylase kinase without the involvement of cAMP-dependent protein kinase.

Antithetic effects of covalent modification. With separate systems for the synthesis and degradation of glycogen, and with glucose 1-phosphate acting as a common intermediate, the possibility of a futile cycling of glycogen must be considered. The futile cycle is avoided because covalent modification, by phosphorylation, has opposite effects on the enzymes concerned with the synthesis and degradation of glycogen.

Glycogen storage diseases are caused by genetic defects that result in deficiencies in certain enzymes of glycogen metabolism. These deficiencies lead to excessive accumulation of glycogen or the inability to use that glycogen as a fuel source. **Type II glycogen storage disease (Pompe's disease)** is due to a defect in the lysosomal α-1,4-glucosidase. Glycogen accumulates and causes problems in the central nervous system (CNS), which leads to psychomotor retardation, an enlarged heart, and eventually failure of the heart and lungs.

Type III glycogen storage disease (Cori's disease, Forbes' disease) is due to a defect in the debranching enzyme (see III B 1). This disease also causes heart and lung problems, stunted growth, an enlarged liver, hypoglycemia, and acidosis.

Type V glycogen storage disease (McArdle's disease) is due to a defect in the isozyme of glycogen phosphorylase present in muscle tissue. People with this disease suffer from skeletal muscle cramps, and they demonstrate a low blood lactate level during exercise.

Type VI glycogen storage disease (Hers' disease) is due to a defect in the isozyme of glycogen phosphorylase present in liver. Patients with this disease suffer hepatomegaly, moderate hypoglycemia, mild acidosis, and growth retardation.

Table 13—1.

Genetic Diseases of Glycogen Metabolism

Type	Disease Name	Defective enzyme	Glycogen levels	Glycogen Structure	Principal Tissue Affected
I	**von Gierke's disease**	**Glucose 6-phosphatase - (G6Pase)**	**High**	**Normal**	**Liver, kidney**
II	**Pompe's disease**	**α-1 ,4-Glucosidase**	**Very high**	**Normal**	**All organs**
III	**Cori's, Forbes' disease**	**Debranching enzyme**	**High**	**Short outer branches**	**Liver, heart, muscle**
IV	**Andersen's disease**	**Branching enzyme**	**Normal**	**Long outer branches**	**Liver, spleen, muscle**
V	**McArdle's disease**	**Phosphorylase**	**High**	**Normal**	**Muscle**
VI	**Hers' disease**	**Phosphorylase**	**High**	**Normal**	**Liver**
VII	**Tarui disease**	**Phosphofructokinase**	**High**	**Normal**	**Muscle**
VIII	**Hepatic phosphorylase kinase deficiency**	**Phosphorylase kinase**	**High**	**Normal**	**Liver**

METABOLISM OF GALACTOSE

The disaccharide **lactose,** present in milk and milk products, is the principal dietary **source of galactose**. Lactase (β-galactosidase) of intestinal mucosal cells hydrolyses lactose to galactose and glucose. Galactose is also produced within the cells from the lysosomal degradation of **glycoproteins** and **glycolipids**. As is the case for fructose, galactose entry into the cells is not dependent on insulin.

The specific enzyme, namely **galactokinase**, phosphorylates galactose to galactose-1-phosphate (Fig. 13—4).

This reacts with UDP-glucose in an exchange reaction to form **UDP-galactose** in presence of the enzyme **galactose-1-phosphate uridyltransferase. UDP-galactose** is an active **donor** of galactose for many synthetic reactions involving the formation of compounds like **lactose, glycosaminoglycans, glycoproteins** and **glycolipids**. UDP-gaiactose can be converted to UDP-glucose by UDP hexose-4-epimerase. In this way, galactose can enter the metabolic pathways of glucose. It may be noted that **galactose is not an essential nutrient** since UDP-glucose can be converted to UDP-galactose by the enzyme UDP-hexose-4-epimerase.

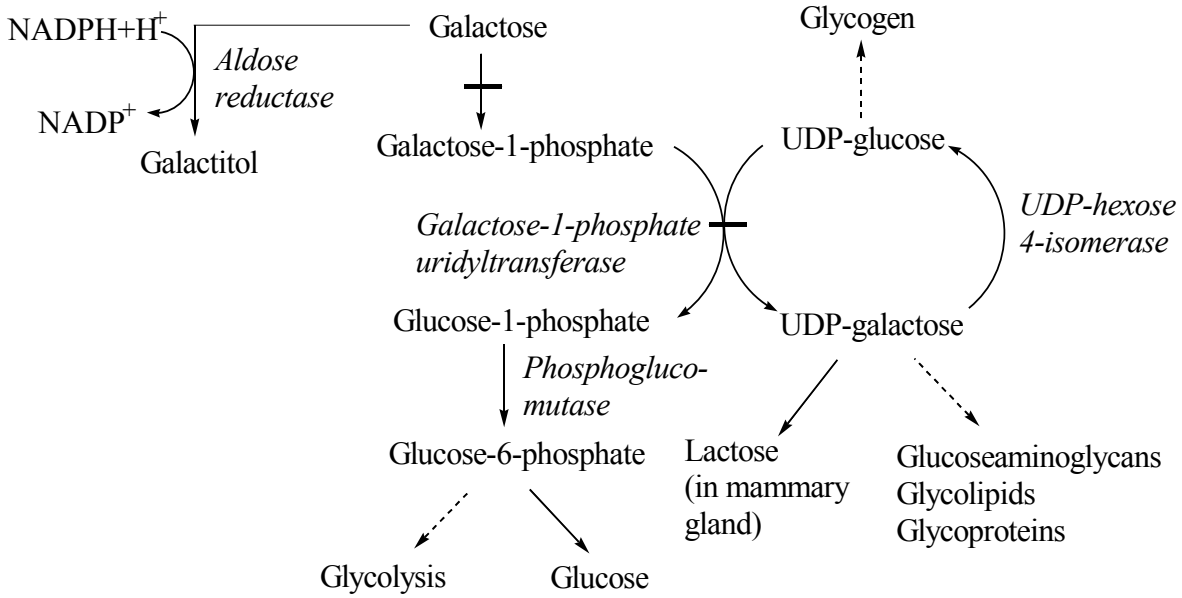


Figure 13—4. Metabolism of galactose

Disorders of galactose metabolism

Classical galactosemia. This is due to the deficiency of the enzyme galactose-1-phosphate uridyltransferase. It is a rare congenital disease in infants, inherited as an autosomal recessive disorder. The salient features of galactosemia are

1. Galactose metabolism is impaired leading to **increased galactose levels** in circulation (**galactosemia)** and urine (**galactosuria).**

2. The accumulated galactose is diverted for the production of **galactitol** by the enzyme **aldose reductase** (the same enzyme that converts glucose to sorbitol). Aldose reductase is present in lens, liver, nervous tissue, seminal vesicles etc. The conversion of galactose to galactitol is insignificant in routine galactose metabolism. However, with increased levels of galactose (galactosemia), this pathway assumes significance. Galactitol (like sorbitol) has been implicated in the development of **cataract.**

3. The accumulation of galactose-1-phosphate and galactitol in various tissues like liver, nervous tissue, lens and kidney leads to impairment in their function.

4. The clinical symptoms of galactosemia are — loss of weight (in infants) hepatospleenomegaly, jaundice, mental retardation etc. In severe cases, cataract, amino aciduria and albuminuria are also observed.

Diagnosis. Early detection is possible (biochemical diagnosis) by measuring the activity of galactose-1-phosphate uridyltransferase in erythrocytes.

Treatment. The therapy includes the supply of diet deprived of galactose and lactose.

Galactokinase deficiency. The defect in the enzyme **galactokinase,** responsible for phosphorylation of galactose, will also result in galactosemia and galactosuria. Here again galactose is shunted to the formation of galactitol. Generally, galactokinase-deficient individuals do not develop hepatic and renal complications. Development of cataract occurs at a very early age, sometimes

within year after birth. The treatment is the removal of galactose and lactose from the diet.

METABOLISM OF FRUCTOSE

Fructose is a ketohexose. The major dietary source of fructose is the disaccharide sucrose (cane sugar), containing equimolar quantities of fructose and glucose. It is also found in free form in **honey** and many fruits. In the body, entry of **fructose** into the cells is **not controlled** by the hormone **insulin**. This is in contrast to glucose that is regulated for its entry into majority of the tissues.

Fructose is mostly phosphorylated by fructokinase to fructose 1-phosphate (Fig.13—5).

Fructokinase has been identified in **liver, kidney** and **intestine**. Hexokinase, which phosphorylates various monosaccharides, can also act on fructose to produce fructose 6-phosphate. However, hexokinase has low affinity (high K_m) for fructose, hence this is a **minor pathway**.

Fructose 1-phosphate is cleaved to glyceraldehyde and dihydroxyacetone phosphate (DHAP) by **aldolase B**. Glyceraldehyde is phosphorylated by the enzyme **triokinase** to glyceraldehydes-3-phosphate which, along with DHAP, enters glycolysis or gluconeogenesis.

The fructose is more rapidly metabolized (via glycolysis) by the liver than glucose. This is due to the fact that the rate limiting reaction in glycolysis catalyzed by phosphofructokinase is bypassed. Increased dietary intake of fructose significantly elevates the production of acetyl CoA and lipogenesis (fatty acid, triacylglycerol and very low density lipoprotein synthesis).

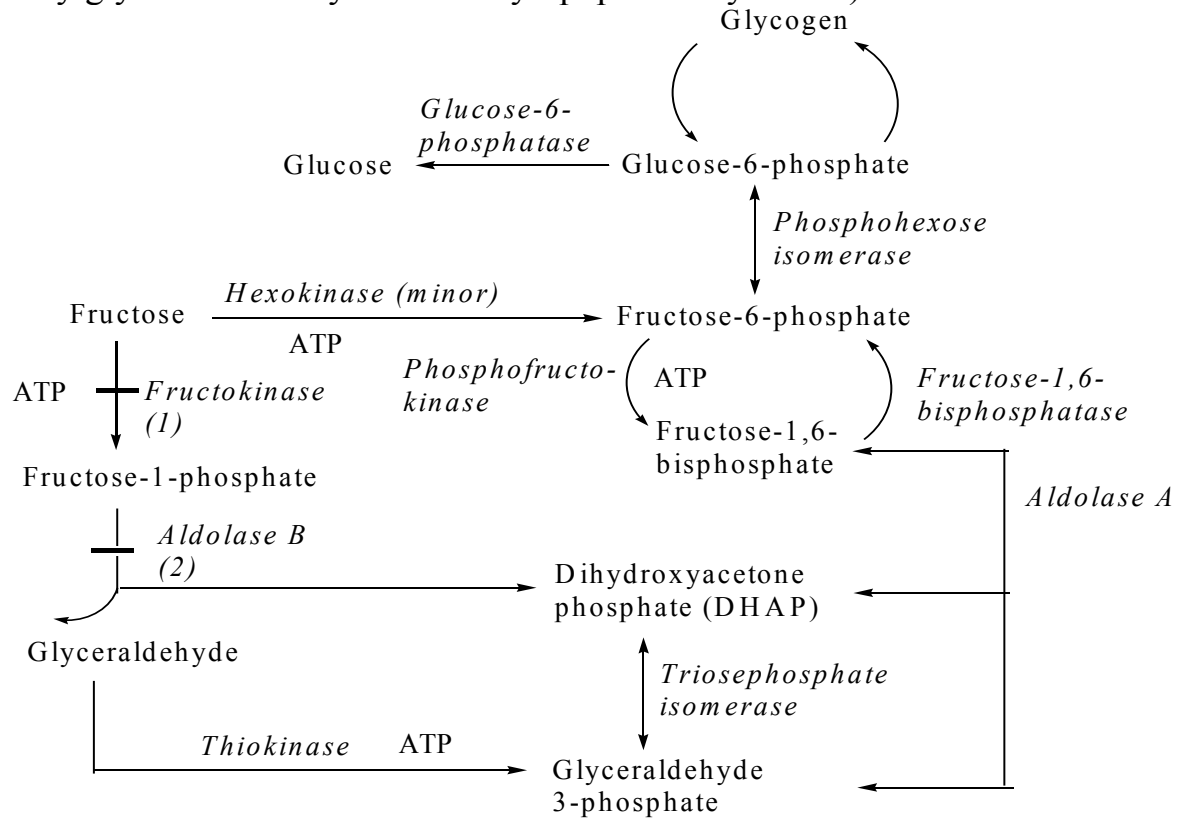


Figure 13—5. Metabolism of fructose.

Defects in fructose metabolism

1. Essential fructosuria. Due to the deficiency of the enzyme hepatic **fructokinase,** fructose is not converted to fructose-1-phosphate. This is an asymptomatic condition with excretion of fructose in urine. Treatment involves the restriction of dietary fructose.

2. Hereditary fructose intolerance. This is due to the absence of the enzyme **aldolase B.** Hereditary fructose intolerance causes intracellular accumulation of fructose-1-phosphate, **severe hypoglycemia, vomiting, hepatic failure and jaundice.** Fructose-1-phosphate allosterically inhibits liver phosphorylase and blocks glycogenolysis leading to **hypoglycemia.** Early detection and intake of diet free from fructose and sucrose are advised to overcome fructose intolerance.

3. Consumption of high fructose. Fructose is rapidly converted to fructose-1-phosphate by fructokinase. The activity of the enzyme aldolase B is relatively less, and, due to this, fructose 1-phosphate accumulates in the cell. This leads to the depletion of intracellular **inorganic phosphate (Pi) levels**. The phenomenon of binding of Pi to the organic molecules (like fructose here) — that leads to the less availability of Pi for the essential metabolic functions—is known as **sequestering of phosphate**. Due to the decreased availability of Pi, the liver metabolism is adversely affected. This includes the lowered synthesis of ATP from ADP and Pi. High consumption of fructose over a long period is associated with increased uric acid in blood leading to gout. This is due to the excessive breakdown of ADP and AMP (accumulated due to lack of Pi) to uric acid.

LECTURE 14

METABOLISM OF LIPIDS. EICOSANOIDS. DIGESTION OF LIPIDS. LIPID TRANSPORT. FATTY ACID OXIDATION

Lipids may be regarded as organic **substances relatively insoluble in water, soluble in organic solvents** (alcohol, ether etc.), actually or potentially related to fatty acids and utilized by the living cells.

Classification of lipids

Lipids are broadly classified into **simple, complex, derived** and **miscellaneous** lipids, which are further subdivided as follow

1. Simple lipids are esters of fatty acids with alcohol. These are mainly of two types

a. Fats and oils (triacylglycerols). These are eaters of fatty acids with glycerol. The difference between fat and oil is only physical. Thus, oil is a liquid while fat is a solid at room.

b. Waxes: esters of fatty acids (usually long chain) with alcohol other than glycerol. These alcohols may be aliphatic or alicyclic.

2. Complex (or compounds) lipids are esters of fatty acids with alcohols containing additional groups such as phosphate, nitrogenous base, carbohydrate, protein etc. They are further divided:

a. Phospholipids

(1) Glycerophospholipids. These phospholipids contain glycerol as the alcohol e.g. lecithin, cephalin.

(2) Sphingophospholipids. Sphingosine is the alcohol in this group of phospholipids e.g., sphingomyelin.

b. Glycolipids. These lipids contain a fatty acid, carbohydrate and nitrogenous base. The alcohol is sphingosine, hence they are also called as glycosphingolipids. Glycerol and phosphate are absent e.g., **cerebrosides**, **gangliosides**.

c. Lipoproteins. Macromolecular complexes of lipids with proteins.

d. Other complex lipids. Sulfolipids, aminolipids and lipopolysaccharides are among the other complex lipids.

3. Derived lipids. These are the derivatives obtained on the hydrolysis of group I and group 2 lipids which possess the characteristics of lipids. These include glycerol and other alcohols, fatty acids, mono- and diacylglycerols, lipid soluble vitamins, steroid hormones, hydrocarbons and ketone bodies.

4. Miscellaneous lipids. These include a large number of compounds possessing the characteristics of lipids e.g., carotenoids, squalene, hydrocarbons such as pentacosane (in bees wax), terpenes etc.

Functions of lipids

Lipids perform several important functions

1. They are the concentrated fuel reserve of the body (triacylglycerols).

2. Lipids are the constituents of membrane structure and regulate the membrane permeability (phospholipids and cholesterol).

3. They serve as a source of fat-oluble vitamins (A, D, E and K).

4. Lipids are important as cellular metabolic regulators (steroid hormones and prostaglandins).

5. Lipids protect the internal organs, serve as insulating materials and give shape and smooth appearance to the body.

6. As compounds of the inner mitochondrial membranes, lipids (phospholipids) participate in electron transport chain.

Fatty acids

Fatty acids are carboxylic acids with hydrocarbon side chain. They are the simplest form of lipids. Fatty acids mainly occur in the esterified form as major constituents of various lipids. They are also present as free (unesterified) fatty acids. Fatty acids of animal origin are much simpler in structure in contrast to those of plant origin that often contain groups such as epoxy, keto, hydroxy and cyclopentane rings.

Even and odd carbon fatty acid

Most of the fatty acids that occur in natural lipids are of **even carbons** (usually 14C — 20C). This is due to the fact that biosynthesis of fatty acids mainly occurs with the sequential addition of 2 carbon units. **Palmitic acid** (16C) and **stearic acid** (18C) are the most common. Among the odd chain fatty acids, propionic acid (3C) and valeric acid (5C) are well known.

Saturated and unsaturated fatty acids

Saturated fatty acids do not contain double bonds, while unsaturated fatty acids contain one or more double bonds. Both saturated and unsaturated fatty acids almost equally occur in the natural lipids. Fatty acids with one double bond are known as monounsaturated and those with 2 or more double bonds are collectively known as **polyunsaturated fatty acids** (PUFA).

Naturally occurring unsaturated fatty acids belong to ω 9, ω 6 and ω3 series (the terminal carbon containing methyl group is known as ω-carbon)

ω 3 series Linolenic acid (18:3; $\Delta^{9,12,15}$)

ω 6 series Linoleic acid (18:2; $\Delta^{9,12}$) and arachidonic aicd (20:4; $\Delta^{5,8,11,14}$)

ω 9 series Oleic acid (18:1; Δ^{9})

The fatty acids that cannot be synthesized by the body and, therefore, should be supplied in the diet are known as **essential fatty acids**. Chemically, they are polyunsaturated fatty acids, namely linoleic and linolenic acid. Arachidonic acid becomes essential, if its precursor linoleic acid is not provided in the diet in sufficient amount. **Function of essential fatty acids**: essential fatty acid are required for the **membrane structure and function**, **transport of cholesterol**, **formation of lipoproteins**, **prevention of fatty liver**. They are also needed for the synthesis of another important group of compounds, namely **eicosanoids**.

EICOSANOIDS

Eicosanoids are products of arachidonic acid (5,8,11,14-eocosatetraenoic acid) (Fig. 14—1).

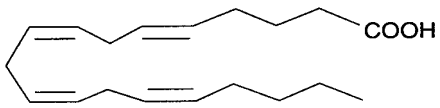


Figure 14—1. Arachidonic acid

Arachidonic acid is the precursor of **prostaglandins, thromboxanes,** and **leukotrienes.**

1. Storage. There is little free arachidonic acid in cells. It is stored almost completely as esters of the 2-position of the glycerol backbone of cell membrane phospholipids.

2. Regulation. The synthesis of prostaglandins requires its immediate release from membrane phospholipids.

a. Stimulation of cells by an appropriate agonist causes the release of arachidonic acid. Agonists have specific target cells. **Thrombin** causes release of arachidonic acid in platelets and endothelial cells. **Bradykinin** acts similarly in renal tubular cells.

b. Inhibition. Release of arachidonate from membrane phospholipids is inhibited by a protein whose synthesis is induced by **glucocorticoid hormones.** This inhibition of arachidonate release accounts for the **anti-inflammatory action of steroids** because prostaglandins play important roles in the inflammatory reaction.

Synthesis of prostaglandins, thromboxanes, and leukotrienes

Once arachidonate is released in the cell, it activates both **cyclooxygenase** and **lipoxygenase** enzymes.

1. The cyclooxygenase system converts arachidonate (and other C—20 unsaturated fatty acids) to endoperoxides, which are key precursors for thromboxanes and prostaglandins.

a. The cyclooxygenase enzyme (prostaglandin synthase) is inhibited by the **nonsteroidal anti-inflammatory agents,** such as aspirin, indomethacin, and ibuprofen.

b. Inhibition of platelet cyclooxygenase by aspirin is important because it is irreversible and, because platelets cannot synthesize protein, the inactivated enzyme cannot be replaced.

2. The lipoxygenase system converts arachidonic acid to 5-hydroperoxyeicosatetraenoic acid, which is the precursor for the synthesis of **leukotrienes** LTA_4, LTB_4, LTC_4, LTD_4, and LTE_4 (Fig. 14—2).

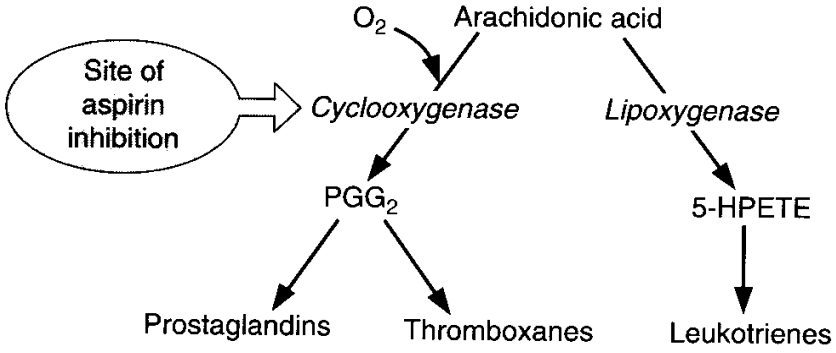


Figure 14—2. Biosynthesis of prostaglandins, thromboxanes and leucotrienes from arachidonic acid.

3. Degradation of prostaglandins. Almost all the eicosanoids are metabolized rapidly. The lung and liver are the major sites of PG degradation. Two enzymes, namely **15-α-hydroxyPG dehydrogenase** and **13-PG reductase**, convert hydroxyl group at 15 to keto group and then to C_{13} and C_{14} dihydroderivate.

Prostaglandins

Prostaglandins (PGs) were first discovered in human semen by Ulf von Euler (of Sweden) in 1930. These compounds were found to stimulate uterine contraction and reduce blood pressure. It was later realized that PGs and other eicosanoids are synthesized in almost all the tissues (exception erythrocytes).

1. Structure. Prostaglandins are analogs of **prostanoic acid** (Fig. 14—3). This has a cyclopentane ring (formed by carbon atoms 8 and 12) and two side chains, with a carboxyl group on one side.

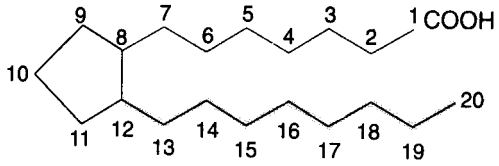


Figure 14—3. Prostanoic acid.

2. Nomenclature. Prostaglandins are abbreviated PG, with an additional capital letter that denotes the ring type and a numeral (or, in one case, a Greek letter) as a subscript. **The subscript number** (e.g., PGE_1, PGE_2, and PGE_3) denotes the **number of unsaturated bonds** that a prostaglandin contains in the **hydrocarbon** chains. In the 1 series, the double bond is $\Delta^{13,14}$; in the 2 series they are $\Delta^{5,6}$ and $\Delta^{13,14}$, and in the 3 series, they are $\Delta^{5,6}$, $\Delta^{13,14}$, and $\Delta^{17,18}$. A subscript α-denotes that the hydroxyl group at C_9 of the ring and the carboxyl group are on the same side of the ring.

3. Function. The prostaglandins E and F were first isolated from the biological fluids. They were so named due to their solubility in ether (PGE) and phosphate buffer (PGF, F for fosfat, in Swedish) (Fig. 14—4).

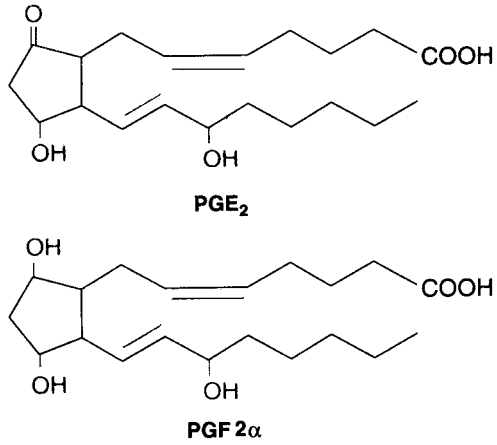


Figure 14—4. Structure of PGE_2 and PGF2α

a.Prostaglandins act as **local hormones** in their function. PGs are produced in almost all the tissues in contrast to hormonal synthesis which occur in specialized glands. PGs are not stored and they are degraded to inactive products at the site of their production. Further, PGs are produced in very small amounts and have low half-lives.

b. Overproduction of PGs results in many symptoms which includes pain, fever, nausea, vomiting, inflammation etc.

c. Regulation of blood pressure. The prostaglandins (PGE, PGA and PGI_2) are **vasodilators** in function. This result in increased blood flow and decreased peripheral resistance to lower the blood pressure.

d.Inflammation. The PGE induce the symptoms of inflammation (redness, swelling, edema etc) due to arteriolar vasodilatation. Corticosteroids are frequently used to treat these inflammatory reactions, since they inhibit PG synthesis.

e. Reproduction. PGE_2 and PGF_2 are used for the medical termination of pregnancy and induction of labor.

f. Pain and fever. It is believed that pyrogens (fever producing agent) promote prostaglandin biosynthesis leading to the formation of PGE_2 in the hypothalamus, the site of regulation of body temperature. PGE_2 along with histamine and bradykinin cause pain. Migraine is also due to PGE_2. Aspirin and other non-steroidal drugs inhibit PG synthesis and thus control fever and relieve pain.

g.Regulation of gastric secretion. PGE inhibit gastric secretion. PGs are used for the treatment of gastric ulcers.

h. Effects on respiratory function. PGE is a bronchodilator whereas PGF acts as a constrictor of bronchial smooth muscle. PGE_1 and PGE_2 are used in the treatment of asthma.

Thromboxanes

1. Structure and nomenclature. Thromboxanes are analogs of prostanoic acid that possess a six-membered, oxygen-containing ring (Fig. 14—5).

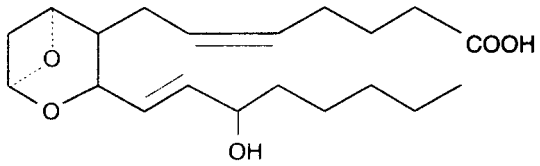


Figure 14—5. Thromboxane A_2

2. Thromboxanes are abbreviated TX, with different capital letters used to designate different ring substituents. A subscript, if present, denotes the number of unsaturated bonds.

3. Function

a. Thromboxane A_2 (TXA_2) is produced by platelets. It causes contraction of arteries and triggers platelet aggregation.

b. These effects are exactly the opposite of those caused by **prostacyclin** (PGI_2), which is produced by the endothelial cells of the vascular system.

c. TXA_2 and PGI_2 are antagonistic and have a balanced working relationship.

Leukotrienes

1. Structure and nomenclature

a. Leukotrienes are formed from hydroperoxyeicosatetraenoic acids (HPETEs) by lipoxygenase and have a common feature of **three conjugated double bonds.**

b. All leukotrienes are abbreviated **LT**. Those derived from **arachidonic acid** have a subscript numeral four to denote that they contain a total of **four double bonds.** An additional letter is included to indicate modifications to the carbon chain of the parent compound (Fig. 14—6).

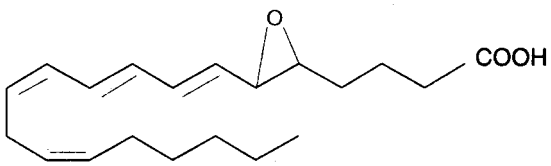


Figure 14—6. Structure of leukotrien A (LTA_4)

c. Some leukotrienes (e.g., LTC_4, LTD_4, and LTE_4) have one or more amino acids covalently attached.

2. Functions

a. Leukotriens are synthesized by leucocytes, mast cells, lung, heart, spleen etc. Leukotrienes are involved in **chemotaxis**, **inflammation, and allergic reactions**.

b. Leukotriene D_4 (LTD_4) has been identified as the **slow-reacting substance of anaphylaxis** (SRS-A), which causes smooth muscle contraction and is approximately 1000 times more potent than histamine in constricting the pulmonary airways. SRS-A also increases fluid leakage from small blood vessels and constricts coronary arteries.

c. Leukotriene B_4 (LTB_4) attracts neutrophils and eosinophils, which are found in large number at sites of inflammation.

DIGESTION OF 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**. In addition to the dietary intake, about 1—2 g cholesterol and 3—5 g. phospholipids (mostly lecithin) are daily secreted into the intestine through bile.

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, catalyzed 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** that 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 unfavorable for the action of gastric lipase. In case of **infants**, the milk fat (with short chain fatty acids) can be hydrolyzed 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.

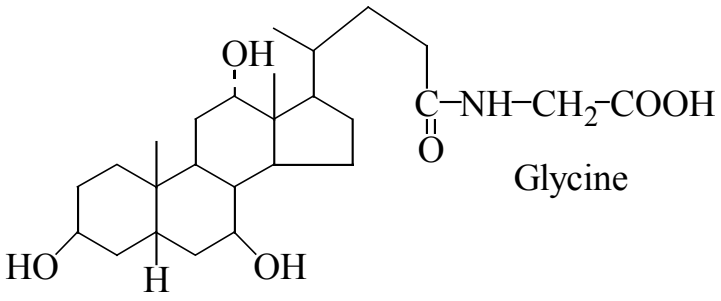
1. Bile salts. The bile acids possess **24 carbon atoms**, **2 or 3 hydroxyl groups** in the steroid nucleus and a side chain ending in carboxyl group. The bile acids are **amphipathic** in nature since they possess both polar and non-polar groups. At physiological pH, the bile acids are mostly present as anions. Bile salts are the biological detergents synthesized from cholesterol in the liver. **Cholic acid** and **chenodeoxycholic acid** are the **primary bile acids** and the former is found in the largest amount in bile. On conjugation with **glycine** or **taurine**, conjugated bile acids **(glycocholic acid, taurocholic acid etc.)** (Fig.14—8) are formed which are more efficient in their function as surfactants.

In the bile, the conjugated bile acids exist as sodium and potassium salts which are known as **bile salts.** In the intestine, a portion of primary bile acids undergo deconjugation and dehydroxylation to form **secondary bile acids** (**deoxycholic acid** and **lithocholic acid**). Bacterial enzymes in the intestine catalyze these reactions.

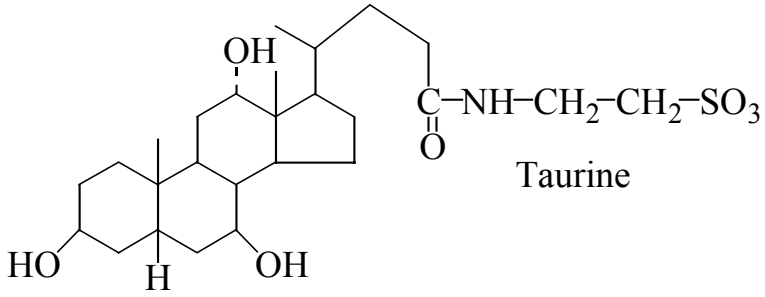
Bile salts are the most effective biological emulsifying agents. They interact with lipid particles and the aqueous duodenal contents and **convert them into smaller particles** (emulsified droplets). Further, bile salts **stabilize the smaller particles** by preventing them from coalescing.

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 **sur-**

factants. 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.



Glycocholic acid



Taurocholic acid

Figure 14—8. Structure of glycocholic and taurocholic acid

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**. 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). Colipase binds at the lipid-aqeuous 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

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. **Snake venom and bee venom** are rich sources of phospholipase A_2. This enzyme is found in many tissues and pancreatic juice.

The lysophospholipid is further hydrolyzed by lysophospholipase to fatty acid and glycerylphosphoryl choline. The latter may be further degraded and absorbed or secreted in the feces.

ABSORPTION OF LIPIDS

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. 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.

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. 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 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 in the intestinal cells and used for **triacylglycerol synthesis.** Within the intestinal cells, cholesterol is converted to cholestrylester and phospholipids are regenerated from the absorbed lysophos-

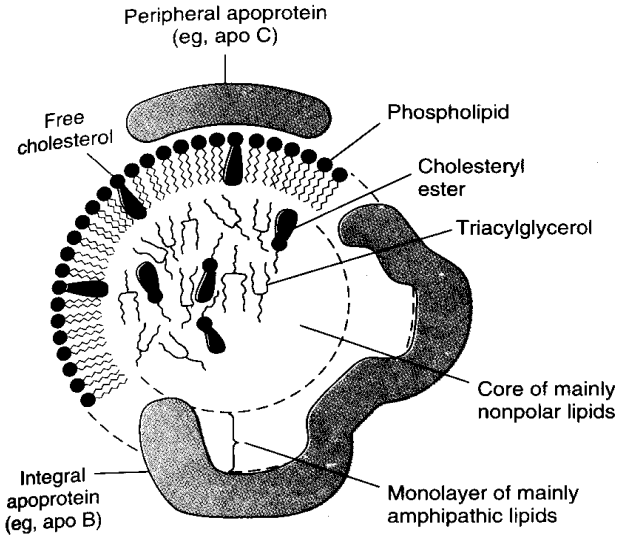
pholipids. The newly synthesized lipids are usually different from those consumed in the diet.

LIPOPROTEINS

Lipoproteins are molecular complexes that consist of **lipids** and **proteins** (conjugated proteins). They function as **transport vehicles for lipids in blood** plasma. Lipoproteins deliver the lipid components (cholesterol, triacylglycerol etc.) to various tissues for utilization.

Structure of lipoproteins

A lipoprotein basically consists of a neutral lipid core (with triacylglycerol and/or cholesteryl ester) surrounded by a coat shell of phospholipids, apoproteins and cholesterol. The polar portions (amphiphilic) of phospholipids and cholesterol are exposed on the surface of lipoproteins so that lipoprotein is soluble in aqueous solution.



Classification of lipoproteins

Four major classes of lipoproteins are identified in human plasma, based on their separation by electrophoresis.

1. Chylomicrons. They are synthesized in the **intestine** and transport **exogenous (dietary) triacylglycerol** to various tissues. They consist of highest (99%) quantity of lipid and lowest (1%) concentration of protein. The chylomicrons are the least in density and the largest in size, among the lipoproteins.

2. Very low density lipoproteins (VLDL). They are produced in **liver** and **intestine** and are responsible for the transport of **endogenously synthesized triacylglycerols**.

3. Low density lipoproteins (LDL). They are formed from VLDL in the **blood circulation**. They transport **cholesterol from liver to other tissues**.

4. High density lipoproteins (HDL). They are mostly synthesized in liver. Three different fractions of HDL (1, 2 and 3) can be identified by ultracentrifugation. HDL particles transport cholesterol from **peripheral tissues to liver** (reverse cholesterol transport). HDL serves as **reservoir** of apoproteins. The accept apopro-

teins (CII and E) and donate the same to other lipoproteins-chylomicrons and VLDL. The apoprotein CII of HDL served as an activator of lipoprotein lipase.

Apolipoproteins (apoproteins)

The protein components of lipoproteins are known as **apolipoproteins** or, simply, apoproteins. They perform the following functions

1. Act as structural components of lipoproteins.

2. Recognize the cell membrane surface receptors.

3. Activate enzymes involved in lipoprotein metabolism.

Metabolism of lipoproteins

Chylomicrons (nascent) are synthesized in the **small intestine** during the course of **fat absorption**. They contain apoprotein **B_{48}** and mostly **triacylglycerols**. Apo B_{48} name given since this apoprotein contains 48% of protein coded by apo B gene (apo B_{100} is found in LDL and VLDL). Chylomicrons pass into the lymphatic system draining the intestine and then into the blood. Chylomicrons are produced when nascent particles combine with apo C II and apo E, derived from HDL (Fig. 14—7).

The liver synthesizes nascent VLDL containing apo B_{100} which are rich in **triacylglycerols** and **cholesterol**. Circulating HDL donates apo C II and apo E to convert nascent VLDL to VLDL (Fig. 14—8).

The enzyme **lipoprotein lipase** is present in the capillary walls of **adipose tissue, cardiac and skeletal muscle**, besides other tissues. It hydrolyses a portion of triacylglycerols present in chylomicrons and VLDL to liberate free fatty acids and glycerol. Lipoprotein lipase is activated by apo C II.

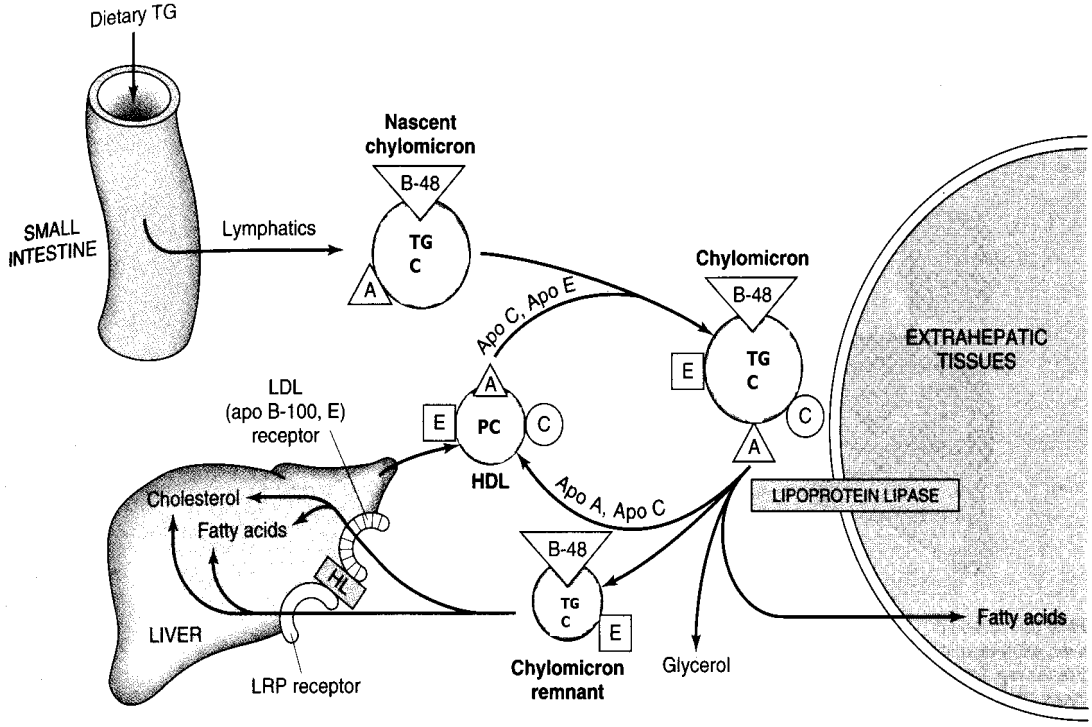


Figure 14—7. Metabolism of chylomicrons (by Murray R.K.et al, 2003)

As the triacylglycerols of chylomicrons and VLDL are degraded, they lose the apo C II which is returned to HDL. The chylomicron remnants are taken up by receptors present on the hepatocytes of liver.

During the course of VLDL metabolism, intermediate density lipoprotein (IDL) is formed which lose apo-E and get converted to LDL. The apo E is returned to HDL. LDL contains high cholesterol (free and esterified) and less triacylglycerol.

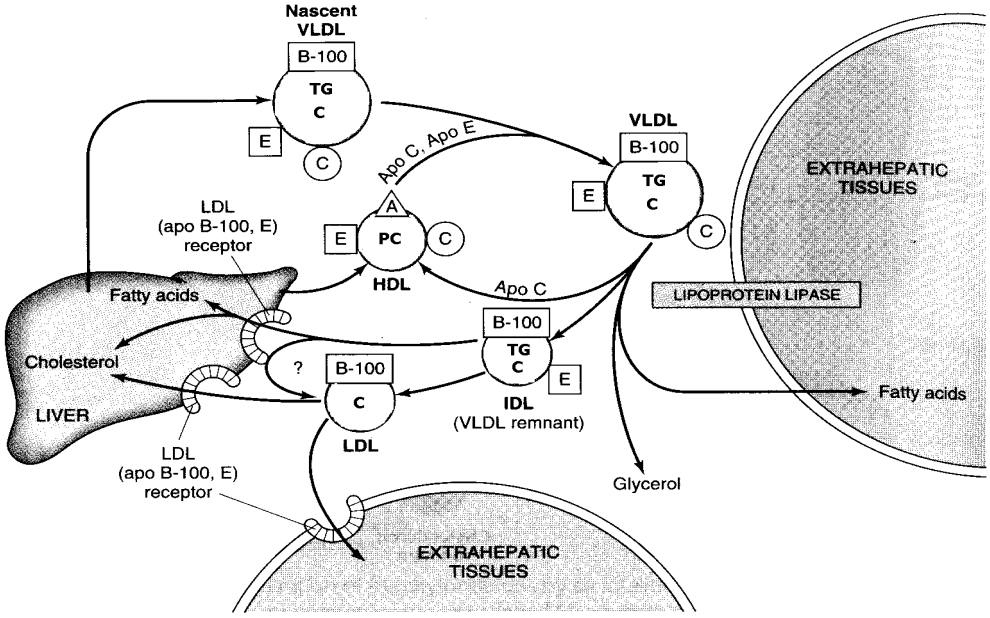


Figure 14—8. Metabolism of VLDL (by Murray R.K.et al, 2003)

LDL receptors and supply of cholesterol to tissues

The most important function of LDL is to supply cholesterol to the extrahepatic tissues. The LDL particles bind to the specific receptor pits (identified as glycoprotein) on the cell membrane. The shape of the pit is stabilized by a protein called **clatrin.** Apo B_{100} is responsible for the recognition of LDL receptor sites. A defect in LDL receptors results in the elevation of plasma LDL, hence plasma cholesterol. However, plasma triacylglycerol concentration remains normal. This disorder is associated with a very high **risk of atherosclerosis** (particularly of coronary artery).

Free fatty acids produced in metabolism of lipoproteins in the circulation are in a bound form to albumin. Each molecule of albumin can hold about 20—30 molecules of free fatty acids. Fatty acids are transported in tissues and oxidized for ATP production.

FATTY ACID OXIDATION

The fatty acids in the body are mostly oxidized by **β-oxidation.** β-Oxidation may be defined as the **oxidation of fatty acids on the β-carbon atom.** This results in the sequential removal of a two carbon fragment, acetyl CoA.

The β-oxidation of fatty acids involves three stages

I. Activation of fatty acids occurring in the cytosol.

II. Transport of fatty acids into mitochondria.

III. β-Oxidation proper in the mitochondrial matrix.

Fatty acids are oxidized by most of the tissues in the body. However, **brain, erythrocytes and adrenal medulla cannot utilize fatty acids for energy requirement.**

I. Fatty acid activation

Fatty acids are activated to acyl CoA by **thiokinases or acyl CoA synthetases.** The reaction occurs in two steps and requires ATP, coenzyme A and Mg^{2+}. Fatty acid reacts with ATP to form acyladenylate which then combines with coenzyme A to produce acyl CoA (Fig. 14—10). In the activation, **two high energy phosphates are utilized,** since ATP is converted to pyrophosphate (PPi).

The enzyme **inorganic pyrophosphatase** hydrolyses PPi to phosphate (Pi). The immediate elimination of PPi makes this reaction totally irreversible.

Three different thiokinases, to activate long chain (10—20 carbon), medium chain (4—12 carbon) and short chain (<4 carbon) fatty acids have been identified.

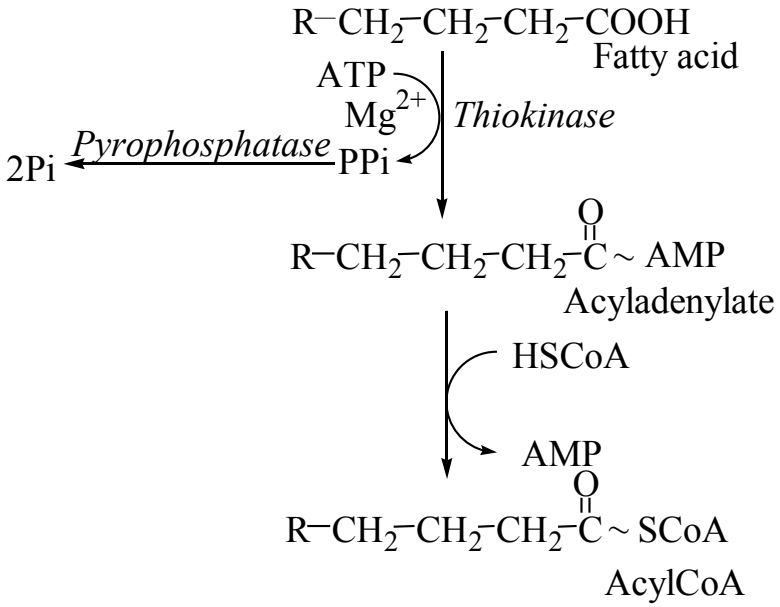


Figure 14—10. Activation of fatty acid to acylCoA

II. Transport of acyl CoA into mitochondria

The inner mitochondrial membrane is impermeable to fatty acids. A specialized **carnitine carrier system** operates to transport activated fatty acids from cytosol to the mitochondria. This occurs in four steps (Fig. 14—11).

1. Acyl group of acyl CoA is transferred to **carnitine** (β-hydroxy-γ-trimethyl aminobutyrate), catalysed by the carnitine acyltransferase I (present on the outer surface of inner mitochondrial membrane).

2. The acyl-carnitine is transported across the membrane to mitochondrial matrix by a specific carrier protein.

3. Carnitine acyl transferase II (found on the inner surface of inner mitochondrial membrane) converts acyl-carnitine to acyl CoA.

4. The carnitine released returns to cytosol for reuse.

It should be noted that the coenzyme A used for activation is different from the one that finally combines with fatty acid in the mitochondria to form acyl CoA. Thus, the **cell has two separate pools (cytosolic and mitochondrial) of coenzyme A.**

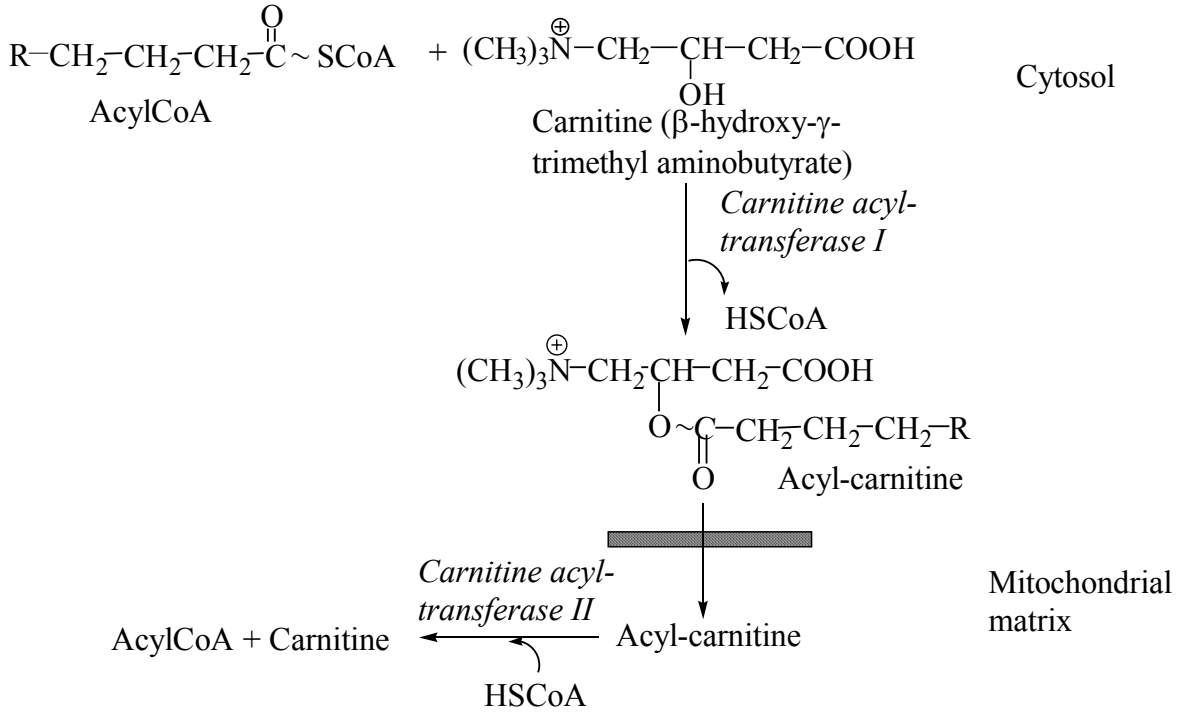


Figure 14—11. Carnitine shuttle for transport of activated fatty acid into mitochondria.

III. β-Oxidation proper

Each cycle of β-oxidation, liberating a two-carbon unit-acetyl CoA, occurs in a sequence of four reactions (Fig. 14—12).

1. Oxidation. Acyl CoA undergoes dehydrogenation by an FAD-dependent flavoenzyme, acyl CoA dehydrogenase. A double bond is formed between α and β carbons (i.e., 2 and 3 carbons).

2. Hydration. Enoyl CoA hydratase brings about the hydration of the double bond to form β-hydroxyacyl CoA.

3. Oxidation. β-Hydroxyacyl CoA dehydrogenase catalyses the second oxidation and generates NADH. The product formed is β-ketoacyl CoA.

4. Cleavage. The final reaction in β-oxidation is the liberation of a 2 carbon fragment, acetyl CoA from acyl CoA. This occurs by a thiolytic cleavage catalyzed by **β-ketoacyl CoA thiolase** (or simply **thiolase**).

The new acyl CoA, containing two carbons less than the original, reenters the β-oxidation cycle. The process continues till the fatty acid is completely oxidized.

The overall reaction for each cycle of β-oxidation

$$C_n \text{ Acyl CoA} + FAD + NAD^+ + H_2O + CoASH \rightarrow C_{(n-2)} \text{ Acyl CoA} + \text{Acetyl CoA} + FADH_2 + NADH + H^+$$

The scheme of fatty acid oxidation discussed above corresponds to saturated (no double bond) and even carbon fatty acids. This occurs most predominantly in biological system.

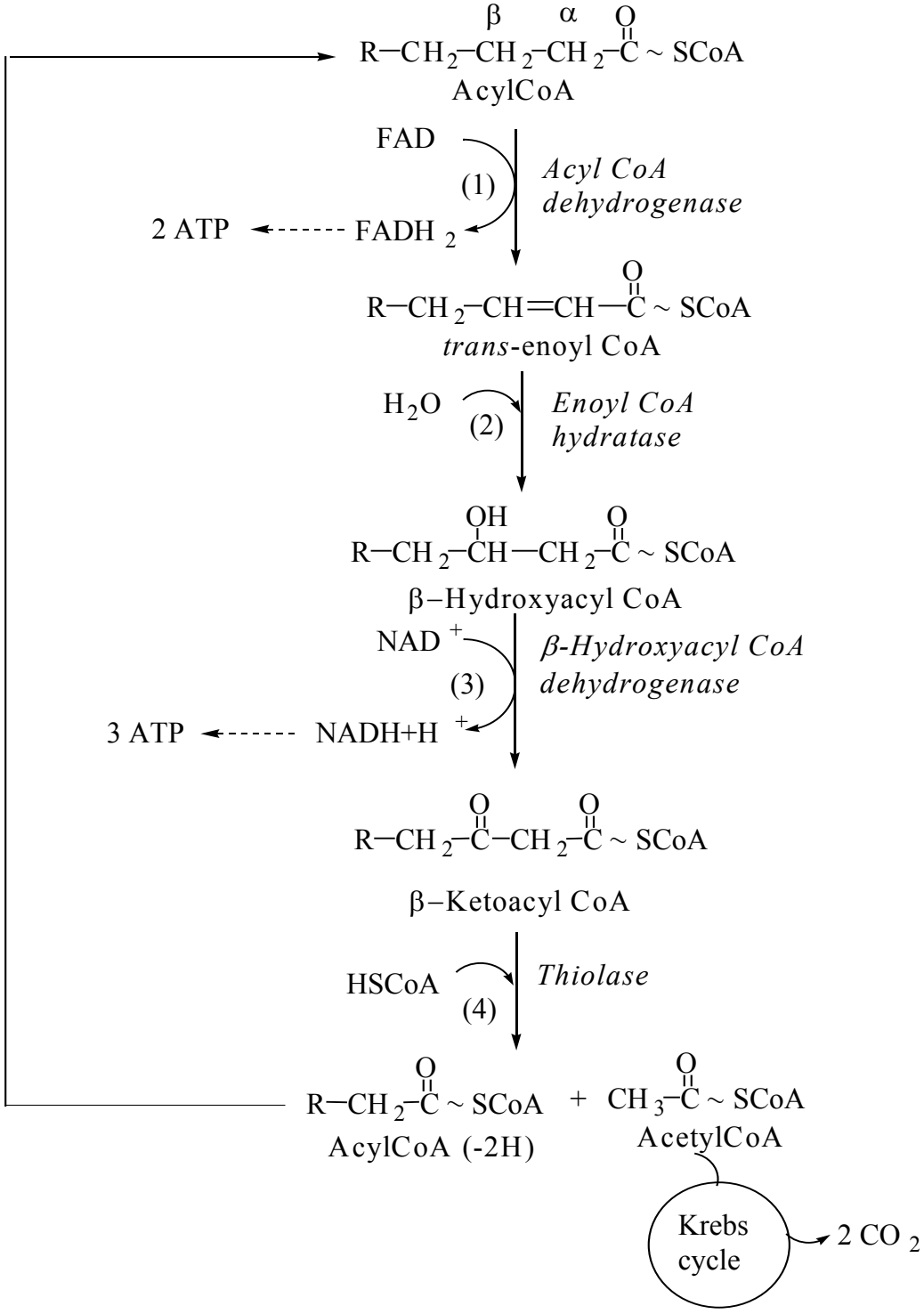


Figure 14—12. β-Oxidation of fatty acids.

Energetic of β-oxidation

The ultimate aim of fatty acid oxidation is to generate energy. The follow equation may be proposed for estimation of energy production of a fatty acid

$[5 \cdot (n/2 - 1) + n/2 \cdot 12] - 2$

5 — ATP produced in each cycles of β-oxidation

n — number of carbon atoms of fatty acid

n/2 — 1 — number of cycles of β-oxidation

n/2 — number of acetyl CoA produced from fatty acid; $n/2 \cdot 12$ — number of ATP produced in citric acid cycle and oxidative phosphorylation.

– 2 — energy utilized for activation

E.g. palmitoyl CoA undergoes 7 cycles of β-oxidation to yield 8 acetyl CoA. Acetyl CoA can enter citric acid cycle and get completely oxidized to Co_2 and H_2O. Hence, net yield of oxidation of one molecule of palmitate is $[5 \cdot 7 + 8 \cdot 12] - 2 = 129$ ATP

Oxidation of odd carbon chain fatty acids

The β-oxidation of saturated fatty acids containing odd number of carbon atoms proceeds in the same manner, as described above for even carbon fatty acids. The only difference is that in the last and final β-oxidation cycle, a three-carbon fragment is left behind (in place of 2 carbon unit for saturated fatty acids). This compound is **propionyl CoA** which is converted to succinyl CoA as follows

1. Propionyl CoA is carboxylated in the presence of ATP, CO_2 and vitamin **biotin** to D-methylmalonyl CoA (Fig. 14—13).

2. Methylmalonyl CoA **epimerase** converts the methylmalonyl CoA to L-form. This reaction (D→L) is essential for the entry of this compound into the metabolic reactions of the body.

3. The next enzyme, **methylmalonyl CoA mutase**, is dependent on **vitamin B_{12}** (deoxyadenosyl cobalamin). It catalyses the conversion of methylmalonyl CoA (a branched compound) to succinyl CoA (a straight chain compound), which can enter citric acid cycle.

Oxidation of unsaturated fatty acids

Due to the presence of double bonds, the unsaturated fatty acids are not reduced to the same extent as saturated fatty acids. Therefore, oxidation of unsaturated fatty acids, in general, **provides less energy** than that of saturated fatty acids.

Most of the reactions involved in the oxidation of unsaturated fatty acids are the same as found in the β-oxidation of saturated fatty acids. However, the presence of double bonds poses problem for β-oxidation to proceed. This is overcome by two additional enzymes — an **isomerase** and an **epimerase.**

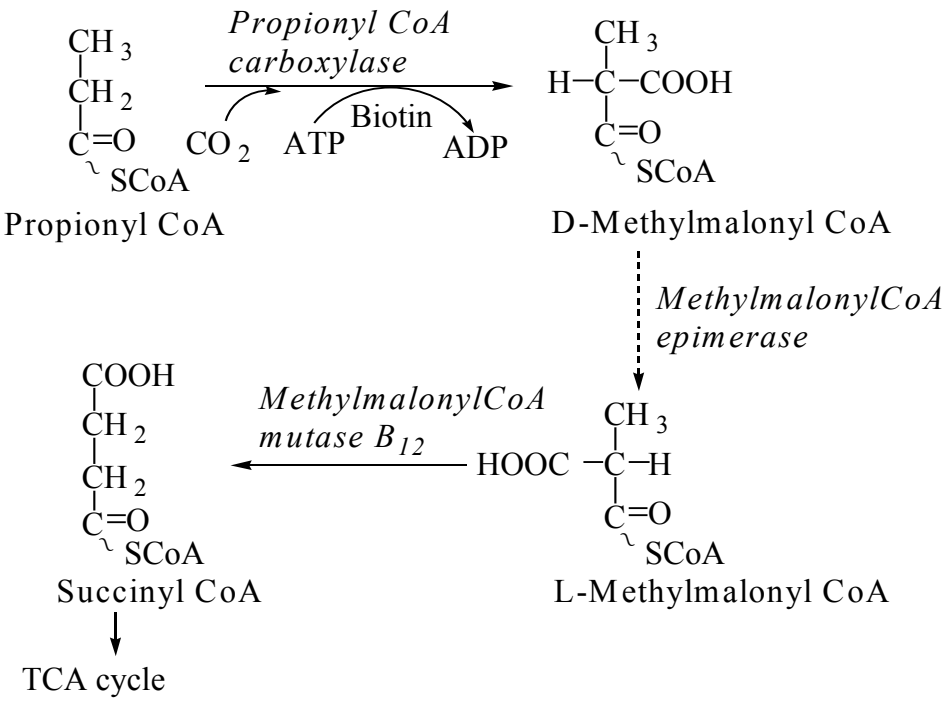


Figure 14—10. Conversion of propionyl CoA to succinyl CoA

LECTURE 15

METABOLISM OF LIPIDS. BIOSYNTHESIS OF FATTY ACID. BIOSYNTHESIS OF CHOLESTEROL. KETONE BODIES METABOLISM

BIOSYNTHESIS OF FATTY ACIDS

General features

1. Fatty acids may be synthesized from dietary **carbohydrates** via pyruvate and **amino acids**, when consumed in excess and can be stored as **triacylglycerol**.

2. The major site of fatty acid synthesis is the **liver.** This system is present in many tissues, including **kidney**, **brain**, **mammary gland** and **adipose tissue**.

3. The enzymes that synthesize fatty acids are localized in the **cytosol,** and they are completely different from the mitochondrial enzymes that catalyze fatty acid degradation.

4. The major synthetic pathway involves polymerization of two-carbon units derived from **acetyl coenzyme A (acetyl CoA)** to form a sixteen-carbon saturated fatty acid, palmitic acid.

5. Reduced nicotinamide adenine dinucleotide phosphate **(NADPH), ATP, Mn^{2+}, biotin, and HCO_3^-** (as a soures of CO_2) are required for fatty acid synthesis.

Production of acetyl CoA and NADPH

1. Cytosol

a. Acetyl CoA is derived from the oxidation of glucose via glycolysis and pyruvate dehydrogenase.

b. NADPH is generated by the pentose phosphate pathway (50%).

2. Mitochondria. The acetyl CoA shuttle system. Acetyl CoA cannot cross the inner mitochondrial membrane and is transported to the cytosol via this shuttle, which also produces NADPH in the cytosol (Fig.15—1).

a. Citrate synthase catalyzes the reaction of acetyl CoA with oxaloacetate to form citrate in the mitochondria.

b. Citrate is transported into the cytosol by a tricarboxylic acid transport system.

c. Citrate reacts with CoA in the cytosol to form acetyl CoA and oxaloacetate.

d. Oxaloacetate is converted to malate by an oxidized nicotinamide adenine dinucleotide (NAD^+)-dependent cytosolic malate dehydrogenase.

e. Malate is decarboxylated to form pyruvate by the **malic enzyme**, which also forms NADPH (50%) from oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$).

f. Pyruvate is transported into the mitochondria by an active transport system.

g. Oxaloacetate is regenerated from pyruvate by pyruvate carboxylase.

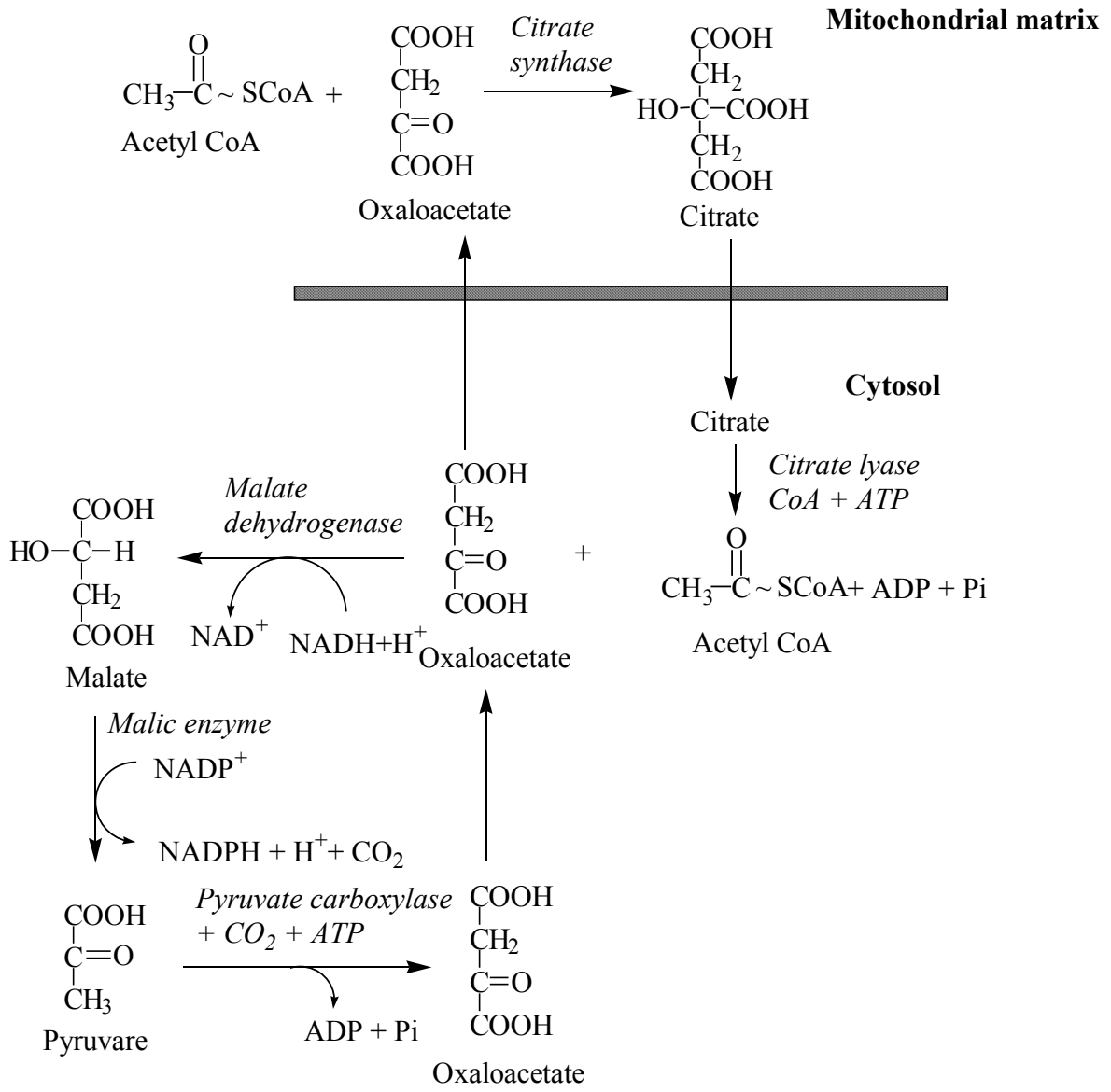


Figure 15—1. The acetyl coenzyme A shuttle system.

Formation of malonyl CoA

1. Acetyl CoA is carboxylated to malonyl CoA by the enzyme **acetyl CoA carboxylase**. This is an ATP-dependent reaction and requires biotin for CO_2 fixation (Fig.15—2).

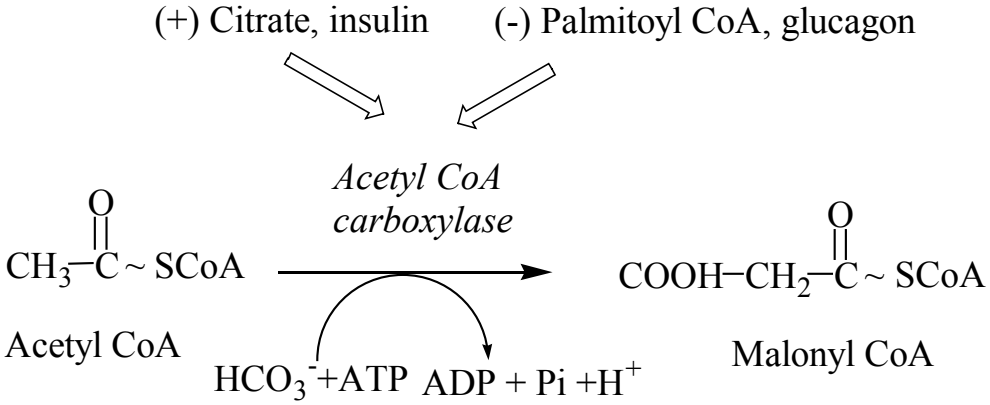


Figure 15—2. Conversion of acetyl CoA to malonyl CoA

2. This is the **key regulatory site** for fatty acid synthesis. **Activators** are citrate and insulin. **Inhibitors** are palmitoyl CoA and glucagon.

Fatty acid synthase complex

The remaining reactions of fatty acid synthesis are catalyzed by a multifunctional enzyme known as **fatty acid synthase** (FAS) **complex** (Fig.15—3)**.**

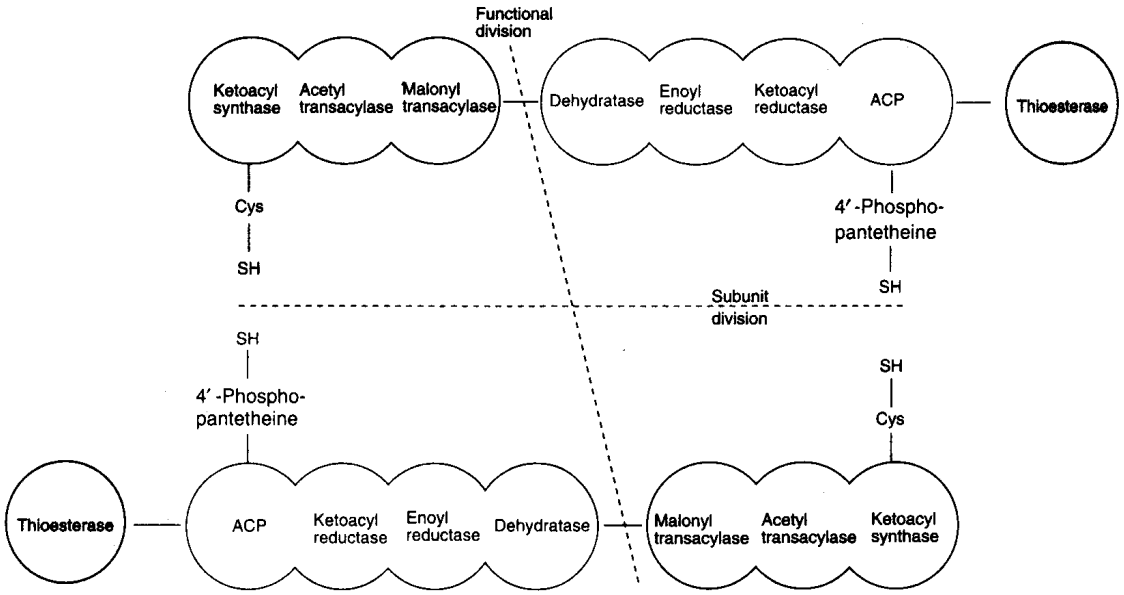


Figure 15—3. Fatty acid synthase multienzyme complex (by U. Satyanarayana, 2002).

In eukaryotic cells, including man, the fatty acid synthase exists as a **dimer** with two identical subunits (monomers). Each subunit contains the activities of **seven** different enzymes and an **acyl carrier protein** (ACP) with 4'-phosphopantetheine –SH-group. The two subunits lie in **antiparallel** (head-to-tail) orientation. The –SH group of phosphopantetheine of one subunit is in

close proximity to the –SH of cysteine residue (of the enzyme ketoacyl synthase) of the other subunit. Each monomer of FAS contains all the enzyme activities of fatty acid synthesis. But only the dimer is **functionally active**. This is because the functional unit consists of half of each subunit interacting with the complementary half of the other. Thus, the FAS structure has both **functional** and **subunit** division. The two functional subunits of FAS independently operate and synthesize two fatty acids simultaneously.

Reactions of fatty acids synthesis (Fig. 15—4)

1. A priming molecule of acetyl CoA combines with the cystein –SH group catalyzed by **acetyl transacylase.** Malonyl CoA combines with adjacent –SH on the 4'-phosphopanteteine of ACP of the other monomer, catalyzed by **malonyl transacylase.**

2. The acetyl group attached to cysteine is transferred to malonyl residue, catalyzed by **ketoacyl synthase,** and liberates CO_2, **forming β-ketoacyl-ACP.**

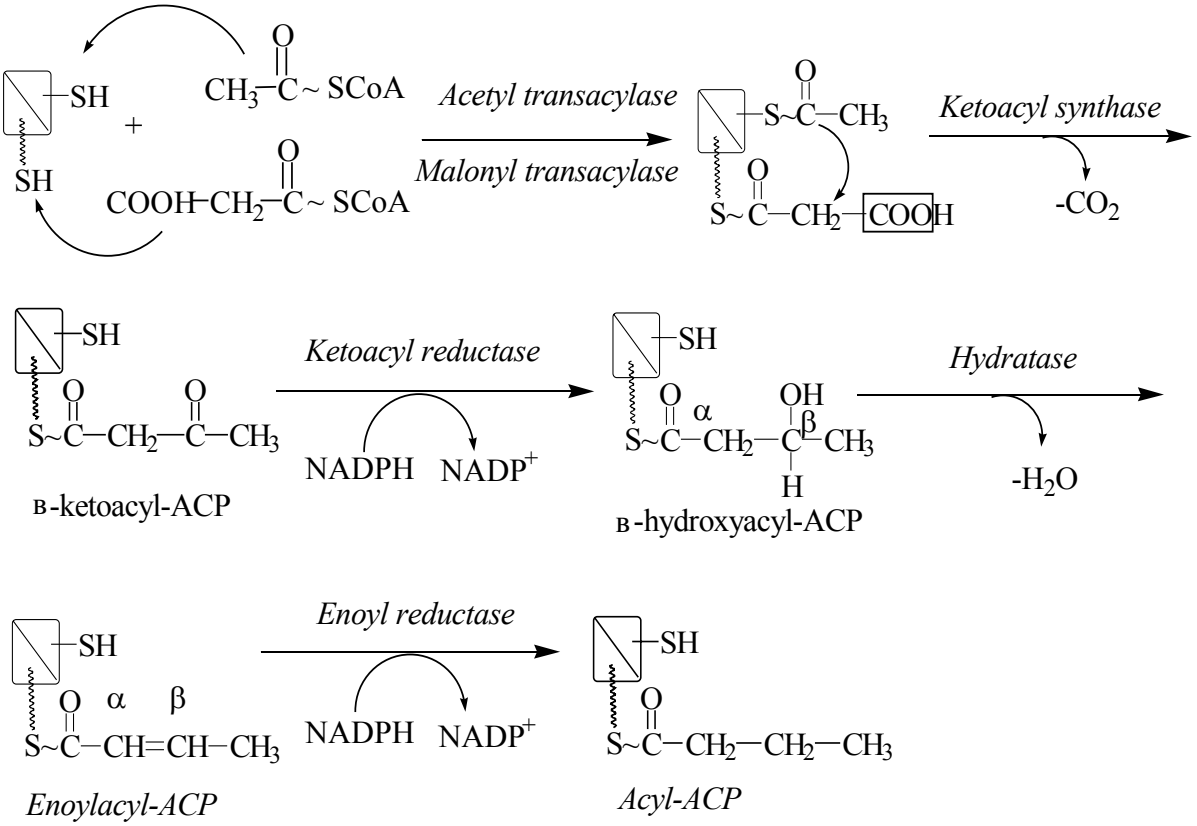


Figure 15—4. Reaction of fatty acid synthesis. ACP – acyl carrier protein

3. The **ketoacyl reductase** catalyzes the NADPH-dependent reduction of β-ketoacyl-ACP forming **β-hydroxyacyl-ACP**.

4. The **hydratase** catalyzes the dehydration of β-hydroxyacyl ACP forming **enoylacyl-ACP.**

5. The **enoyl reductase** catalyzes the NADPH-dependent reduction of enoylacyl-ACP forming saturated **acyl-ACP.**

6. A new malonyl CoA molecule combines with the –SH of 4' - phosphopantetheine, displacing the saturated acyl residue onto the free cysteine –SH group.

7. The sequence of reactions is repeated 6 more times, a new malonyl residue being incorporation during each sequence, until a saturated 16-carbon acyl radicals has been assembled.

8. It is liberated from the enzyme complex by the activity of a seventh enzyme in the complex **thioesterase (deacylase).**

Elongation of fatty acids

Palmitate is the end product of the reactions of fatty acid synthase system that occurs in cytosol. Fatty acids longer than 16 carbons can be formed via one of two **elongation systems** adding 2-carbon units.

1. The endoplasmic reticulum system (the "**microsomal system**") is the most active. It adds **malonyl CoA** onto palmitate in a manner similar to the action of fatty acid synthase, except that CoASH is used rather than the ACP. Stearic acid (an 18-carbon unit) is the product.

2. A mitochondrial elongation system is almost a reversal of β-oxidation of fatty acid. **Acetyl CoA** are successively added to fatty acid to lengthen the chain. The reducing equivalents are derived from NADPH.

Desaturation of fatty acids

1. The two most common monounsaturated fatty acids in mammals are **palmitoleic acid** ($16{:}1,\Delta^9$) and **oleic acid** ($18{:}1,\Delta^9$).

2. In the endoplasmic reticulum, double bonds are introduced between carbons 9 and 10 by **fatty acid oxygenase (fatty acyl CoA desaturase),** which requires molecular oxygen (O_2) and NADPH.

SYNTHESIS OF KETONE BODIES

The compounds namely **acetone, acetoacetate** and **β-hydroxybutyrate** (or 3-hydroxybutyrate) are known as **ketone bodies**. Only the first two are true ketones while β-hydroxybutyrate does not possess a keto (C=O) group. **Ketone bodies are water-soluble and energy yielding.** Acetone, however, is an exception since it cannot be metabolized.

Ketogenesis

The synthesis of ketone bodies occurs in the **liver**. The enzymes for ketone body synthesis are located in the **mitochondria matrix.** Acetyl CoA, formed by oxidation of fatty acids, pyruvate or some amino acids, is the precursor for ketone bodies. Ketogenesis occurs through the following reactions (Fig. 15-4)

1. Two moles of acetyl CoA condense to form **acetoacetyl CoA**. This reaction is catalyzed by **thiolase**, an enzyme involved in the final step of β-oxidation. Hence, acetoacetate synthesis is appropriately regarded as the reversal of thiolase reaction of fatty acid oxidation.

2. Acetoacetyl CoA combines with another molecule of acetyl CoA to produce **β-hydroxy-β-methyl glutaryl CoA** (HMG CoA). **HMG CoA synthase,** catalyzing this reaction, **regulates the synthesis of ketone bodies.**

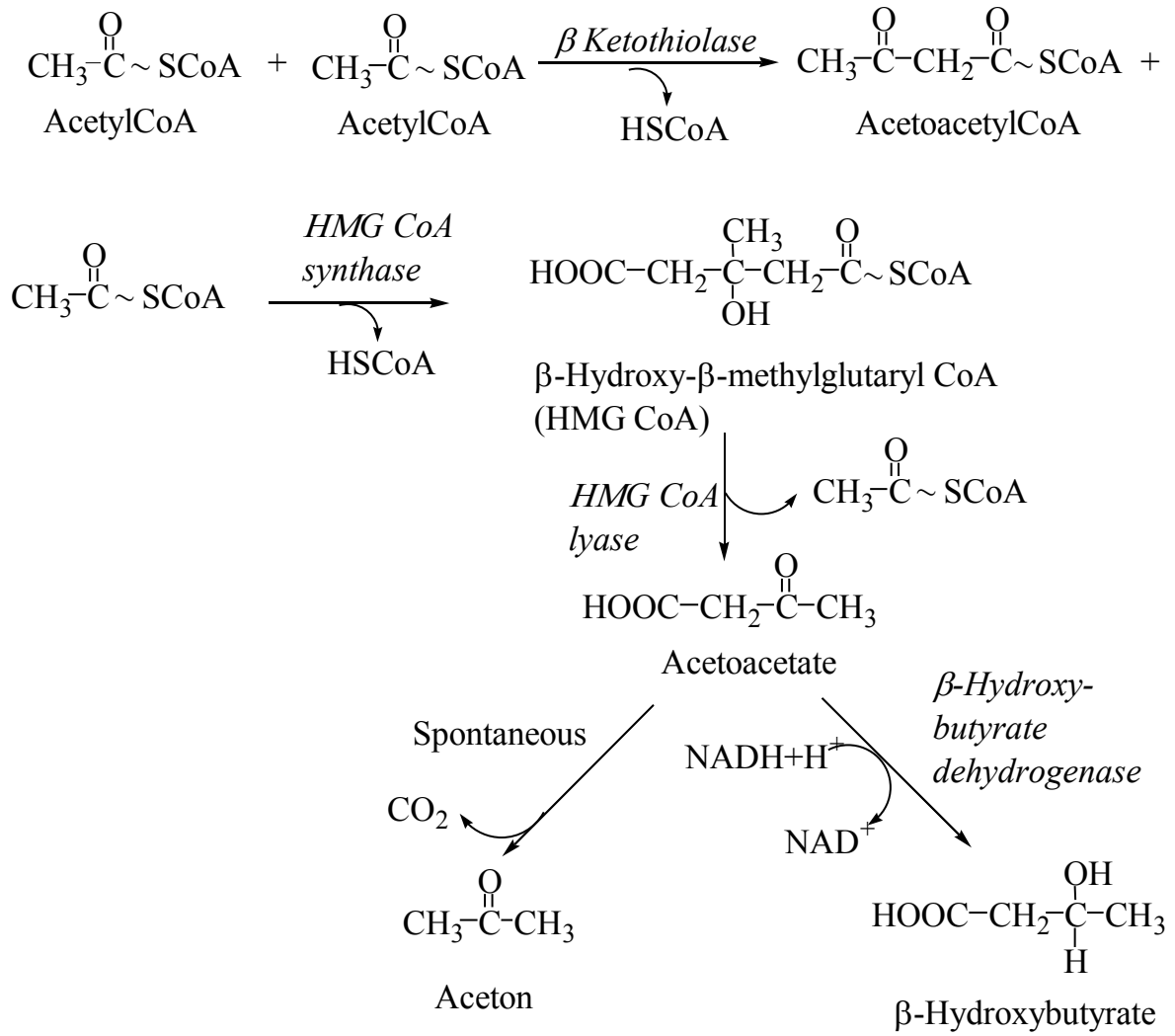


Figure 15—4. Synthesis of ketone bodies (ketogenesis).

3. HMG CoA lyase cleaves HMG CoA to produce **acetoacetate** and **acetyl CoA**.

4. Acetoacetate can undergo spontaneous decarboxylation to form acetone.

5. Acetoacetate can be reduced by a dehydrogenease to β-hydroxybutyrate.

The carbon skeleton of some amino acids (ketogenic) is degraded to acetoacetate or acyl CoA and, therefore, to ketone bodies, e.g. leucine, lysine, phenylalanine etc.

Utilization of ketone bodies

The ketone bodies, being water-soluble, are easily transported from the liver to various tissues. The two ketone bodies — acetoacetate and β-hydroxybutyrate serve as an important source of energy for the **peripheral tissues** such as skeletal muscle, cardiac muscle, renal cortex etc. The tissues which lack mitochondria (e.g. erythrocytes) however, cannot utilize ketone bodies. The production of ketone bodies and their utilization become more significant when glucose is in short supply to the tissues, as observed in **starvation and diabetes mellitus.**

During prolonged **starvation,** ketone bodies are the major **fuel source for the brain** and other parts of central nervous system. It should be noted that the ability of the brain to utilize fatty acids for energy is very limited. The ketone bodies can meet 50—70% of the brain's energy needs. This is an **adaptation for the survival** of the organism **during the periods of food deprivation.**

Reactions of ketone bodies

β-Hydroxybutyrate is first converted to acetoacetate (reversal of synthesis) and metabolized. Acetoacetate is activated to acetoacetyl CoA by a mitochondrial enzyme **thiophorase** (succinyl CoA acetoacetate CoA transferase) (Fig.15—5).

The coenzyme A is donated by succinyl CoA, an intermediate in citric acid cycle. **Thiophorase is absent in liver,** hence ketone bodies are not utilized by the liver. Thiolase cleaves acetoacetyl CoA to two moles of acetyl CoA.

Overproduction of ketone bodies

In normal individuals, there is a constant production of ketone bodies by liver and their utilization by extrahepatic tissues. Their excretion in urine is very low and undetectable by routine tests (Rothera's test).

When the rate of synthesis of ketone bodies exceeds the rate of utilization, their concentration in blood increases, this is known as **ketonemia.** This is followed by **ketonuria** — excretion of ketone bodies in urine. The overall picture of ketonemia and ketonuria is commonly referred to as **ketosis.** Smell of acetone in breath is a common feature in ketosis. Ketosis is most commonly associated with starvation and severe uncontrolled diabetes mellitus.

Starvation. Starvation is accompanied by increased degradation of fatty acids (from the fuel reserve triacylglycerol) to meet the energy needs of the body. This causes an overproduction of acetyl CoA which cannot be fully handled by citric acid cycle. Furthermore, TCA cycle is impaired due to deficiency of oxaloacetate, since most of it is diverted for glucose synthesis to meet the essential requirements (often unsuccessful) for tissues like brain. The result is an accumulation of acetyl CoA and its diversion for overproduction of ketone bodies.

Diabetes mellitus. Diabetes mellitus is associated with insulin deficiency. This results in impaired carbohydrate metabolism and increased lipolysis, both of them ultimately leading to the accumulation of acetyl CoA and its conversion to ketone bodies. In severe diabetes, the ketone body concentration in blood plasma may reach 100 mg/dl and the urinary excretion may be as high as 500 mg/day.

Regulation of ketogenesis

The ketone body formation (particularly overproduction) occurs primarily due to nonavailability of carbohydrates to the tissues. This is an outcome of excessive utilization of fatty acids to meet the energy requirements of the cells. The hormone **glucagon stimulates ketogenesis** whereas **insulin inhibits.**

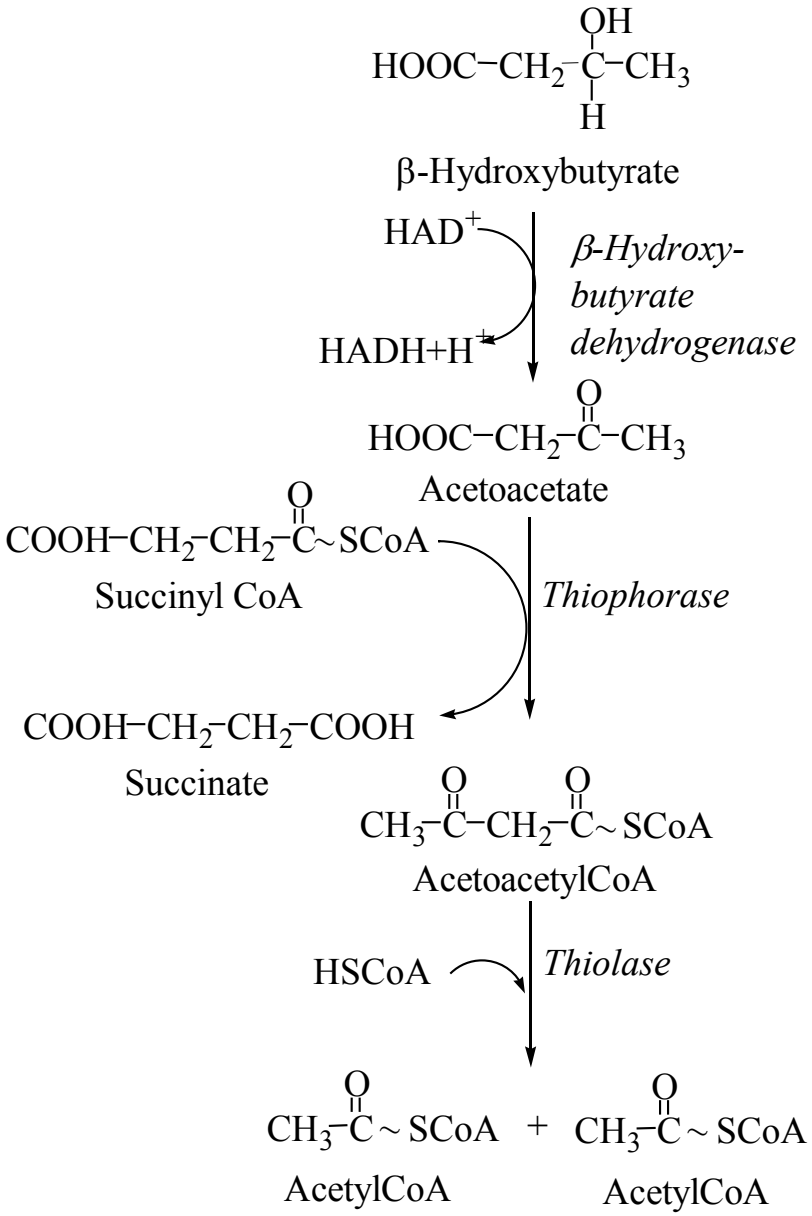


Figure 15—5. Utilization of ketone bodies to acetyl CoA

METABOLISM OF CHOLESTEROL

Cholesterol is found exclusively in animals, hence it is often called as animal sterol. The total body content of cholesterol in an adult man weighing 70 kg is about 140 g i.e., around 2 g/kg body weight. Cholesterol is **amphipathic** in nature, since it possesses both hydrophilic and hydrophobic regions in the structure.

Functions of cholesterol

Cholesterol is essential to life, as it performs a number of important functions

1. It is a structural component of cell membrane.

2. Cholesterol is the precursor for the synthesis of all other steroids in the body. These include steroid hormones, vitamin D and bile acids.

3. It is an essential ingredient in the structure of lipoproteins in which form the lipids in the body are transported.

4. Fatty acids are transported to liver as cholesteryl esters for oxidation.

Cholesterol biosynthesis

About 1 g of cholesterol is synthesized per day in adults. Almost all the tissues of the body participate in cholesterol biosynthesis. The largest contribution is made by **liver** (50%), **intestine** (15%), skin, adrenal cortex, reproductive tissue etc.

The enzymes involved in cholesterol synthesis are found in the **cytosol and microsomal fractions** of the cell. Acetate of **acetyl CoA** provides all the carbon atoms in cholesterol. The reducing equivalents are supplied by **NADPH** while **ATP** provides energy.

The synthesis of cholesterol may be learnt in 5 stages (Fig. 15—6)

1. Synthesis of HMC CoA.

2. Formation of mevalonate (6C)

3. Production of isoprenoid units (5C)

4. Synthesis of squalene (30C)

5. Conversion of squalene to cholesterol (27C).

1. Synthesis of (β-hydroxy-β-methylglutaryl CoA (HMG CoA). Two moles of acetyl CoA condense to form acetoacetyl CoA. Another molecule of acetyl CoA is then added to produce HMG CoA. These reactions are similar to that of ketone body synthesis. However, the two pathways are distinct, since ketone bodies are produced in mitochondria while cholesterol synthesis occurs in cytosol. Thus, there exist **two pools of HMG CoA** in the cell.

Further, two isoenzymes of HMG CoA synthase are known. The cytosomal enzyme is involved in cholesterol synthesis whereas the mitochondrial HMG CoA synthase participates in ketone body formation.

2. Formation of mevalonate. HMG CoA reductase is the **rate limiting enzyme** in cholesterol biosynthesis. This enzyme is present in endoplasmic reticulum and catalyses the reduction of HMG CoA to mevalonate. The reducing equivalents are supplied by NADPH.

3. Production of isoprenoid units. In a three-step reaction catalyzed by kinases, mevalonate is converted to **3-phospho-5-pyrophosphomevalonate** which on decarboxylation forms **isopentenyl pyrophosphate** (IPP). The latter isomerizes to dimethylallyl pyrophosphate (DPP). Both IPP and DPP are 5-carbon isoprenoid units.

4. Synthesis of squalene. IPP and DPP condense to produce a 15-carbon farnesyl pyrophosphate (FPP). Two units of farnesyl pyrophosphate unite and get reduced to produce a 30-carbon squalene.

5. Conversion of squalene to cholesterol. Squalene undergoes hydroxylation and cyclization utilizing O_2 and NADPH and gets converted to lanosterol. The formation of cholesterol from lanosterol is a multistep process with a series of about 19 enzymatic reactions. The following are the most important reactions

a. Reducing the carbon atoms from 30 to 27.

b. Removal of two methyl groups from C_4 and one methyl group from C_{14}.

c. Shift of double bond from C_8 to C_5.

d. Reduction in the double bond present between C_{24} and C_{25}.

Cholesterol biosynthesis is now believed to be a part of a major metabolic pathway concerned with the synthesis of several other isoprenoid compounds. These include ubiquinone (coenzyme Q of electron transport chain) and dolichol (found in glycoprotein). Both of them are derived from farnesyl pyrophosphate.

Regulation of cholesterol synthesis

Cholesterol biosynthesis is controlled by the rate limiting enzyme **HMG CoA reductase,** at the beginning of the pathway. HMG CoA reductase is found in association with endoplasmic reticulum and is subjected to different metabolic controls.

1. Feedback control. The end product cholesterol controls its own synthesis by a feedback mechanism. Increase in the cellular concentration of cholesterol reduces the synthesis of the enzyme HMG CoA reductase. This is achieved by **decreasing the transcription** of the gene responsible for the production of HMG CoA reductase. Feedback regulation has been investigated with regard to LDL-cholesterol taken up by the cells and the same mechanism is believed to operate whenever cellular cholesterol level is elevated.

2. Hormonal regulation. The enzyme HMG CoA reductase exists in two interconvertible forms. The dephosphorylated form of HMG CoA is more active while the phosphorylated form is less active. The hormones exert their influence through cAMP by a series of reactions which are comparable with the control of the enzyme glycogen synthase. The net effect is that **glucagon** and **glucocorticoids** favour the formation of inactive HMG CoA reductase (phosphorylated form) and, thus, decrease cholesterol synthesis. On the other hand, **insulin and thyroxine** increase cholesterol production by enhancing the formation of active HMG CoA reductase (dephosphorylated form).

3. Inhibition by drugs. The drugs **compactin** and **lovastatin** (mevinolin) are fungal products. They are used to decrease the serum cholesterol level in patients with hypercholesterolemia. Compactin and lovastatin are competitive inhibitors of the enzyme HMG CoA reductase and, therefore, reduce cholesterol synthesis. About 50 to 60% decrease in serum cholesterol level has been reported by a combined use of these two drugs.

4. HMG CoA reductase activity is inhibited by **bile acids.** Fasting also reduces the activity of this enzyme.

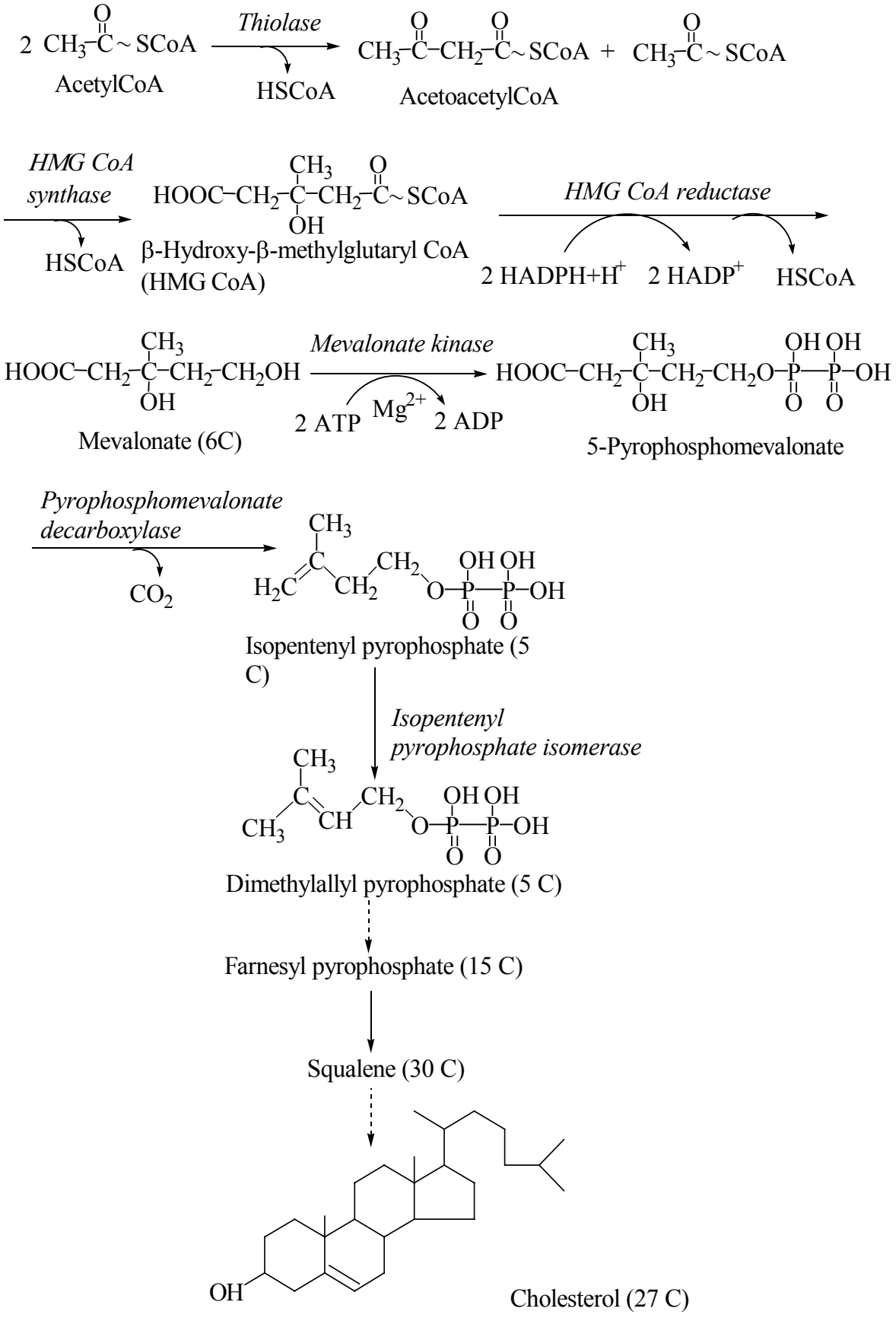


Figure 15—6. Biosynthesis of cholesterol

Degradation of cholesterol

The steroid nucleus (ring structure) of the cholesterol cannot be degraded to CO_2 and H_2O. Cholesterol (50%) is converted to bile acids, excreted in feces, serves as a precursor for the synthesis of steroid hormones, vitamin D, coprostanol and cholestanol. The latter two are the fecal sterols, besides cholesterol.

Synthesis of bile acids

They serve as emulsifying agents in the intestine and actively participate in the digestion and absorption of lipids. The synthesis of primary bile acids takes place in the liver and involves a series of reactions. The step catalyzed by **7α-hydroxylase** is inhibited by bile acids and this is the **rate limiting** reaction.

Enterohepatic circulation. The conjugated bile salts synthesized in the liver accumulate in gall bladder. From there they are secreted into the small intestine where they serve as emulsifying agents for the digestion and absorption of fats and fat soluble vitamins. A large portion of the bile salts (primary and secondary) are reabsorbed and returned to the liver through portal vein. Thus the bile salts are recycled and reused several times in a day. This is known as enterohepatic circulation. About 15—30 g of bile salts are secreted into the intestine each day and reabsorbed. However, a small portion of about 0.5 g/day is lost in the feces. An equal amount (0.5 g/day) is synthesized in liver to replace the lost bile salts. The fecal excretion of bile salts is the only route for the removal of cholesterol from the body.

Cholelithiasis. Bile salts and phospholipids are responsible for keeping the cholesterol in bile in a soluble state. Due to their deficiency (particularly bile salts), cholesterol crystals precipitate in the gall bladder often resulting in cholelithiasis — **cholesterol gall stone disease.** Cholelithiasis may be due to defective absorption of bile salts from the intestine, impairment in liver function, obstruction of biliary tract etc.

LECTURE 16

METABOLISM OF LIPIDS. METABOLISM OF TRIACYLGLYCEROLS AND GLYCEROPHOSPHOLIPIDS. CHOLESTEROL TRANSPORT. DISORDERS OF PLASMA LIPOPROTEINS. FATTY LIVER. OBESITY. ATHEROSCLEROSIS

TRIACYLGLYCEROLS

Lipids constitute about 15—20% of the body weight in humans. Triacylglycerols **(formerly triglycerides)** are the most abundant lipids comprising **85—90%** of body lipids. Most of the triacylglycerols (TG; also called neutral fat or depot fat) are stored in the adipose tissue and serve as energy reserve of the body. This is in contrast to carbohydrates and proteins which cannot be stored to a significant extent for energy purposes. Fat also acts as an insulating material for maintaining the body temperature of animals.

There are two main reasons for fat being the fuel reserve of the body

1. Triacylglycerols are highly **concentrated forms of energy**, yielding **9 Cal/g**, in contrast to carbohydrates and proteins that produce only **4 Cal/g**. This is because fatty acids found in TG are in the reduced form.

2. The triacylglycerols are non-polar and hydrophobic in nature, hence **stored in** pure form without any association with water **(anhydrous form).** On the other hand, glycogen and proteins are polar. One gram of glycogen combines with 2 g of water for storage.

The fuel reserve in the form of fat stores will meet the energy requirements for several weeks of food deprivation in man. Hibernating animals provide good example for utilizing fat reserve as fuel. For instance, bears go on hibernation for about 7 months and, during this entire period, the energy is derived from the degradation of fat stores. The ruby-throated humming birds fly non-stop between New England and West Indies (2,400 km!) at a speed of 40 km/hr for 60 hours! This is possible only due to the stored fat.

SYNTHESIS OF TRIACYLGLYCEROLS

Triacylglycerol (TG) synthesis mostly occurs in **liver and adipose tissue**, and to a lesser extent in **other tissues**. Fatty acids and glycerol must be activated prior to the synthesis of triacylglycerols. Conversion of fatty acids to acyl CoA by thiokinase is already described (lecture № 14).

Synthesis of glycerol 3-phosphate

Two mechanisms are involved for the synthesis of glycerol 3-phosphate (Fig.16—1).

1. In the liver, glycerol is activated by glycerol kinase. This enzyme is absent in adipose tissue.

2. In both liver and adipose tissue, glucose serves as a precursor for glycerol 3-phosphate. Dihydroxyacetone phosphate (DHAP) produced in glycolysis is reduced by glycerol 3-phosphate dehydrogenase to glycerol 3-phosphate.

Addition of acyl groups to form TG

Glycerol 3-phosphate **acyltransferase** catalyses the transfer of an acyl group to produce **lysophosphatidic acid**. Another acyl group is added to lysophosphatidic acid to form phosphatidic acid (1, 2-diacylglycerol phosphate). The enzyme **phosphatase** cleaves off phosphate of phosphatidic acid to produce **diacylglycerol**. Incorporation of another acyl group finally results in synthesis of **triacylglycerol.**

The three fatty acids found in triacylglycerol are not of the same type. A saturated fatty acid is usually present on carbon 1, an unsaturated fatty acid is found on carbon 2 and carbon 3 may have either.

The intermediates of TG synthesis phosphatidic acid and diacylglycerol are also utilized for phospholipid synthesis (described later).

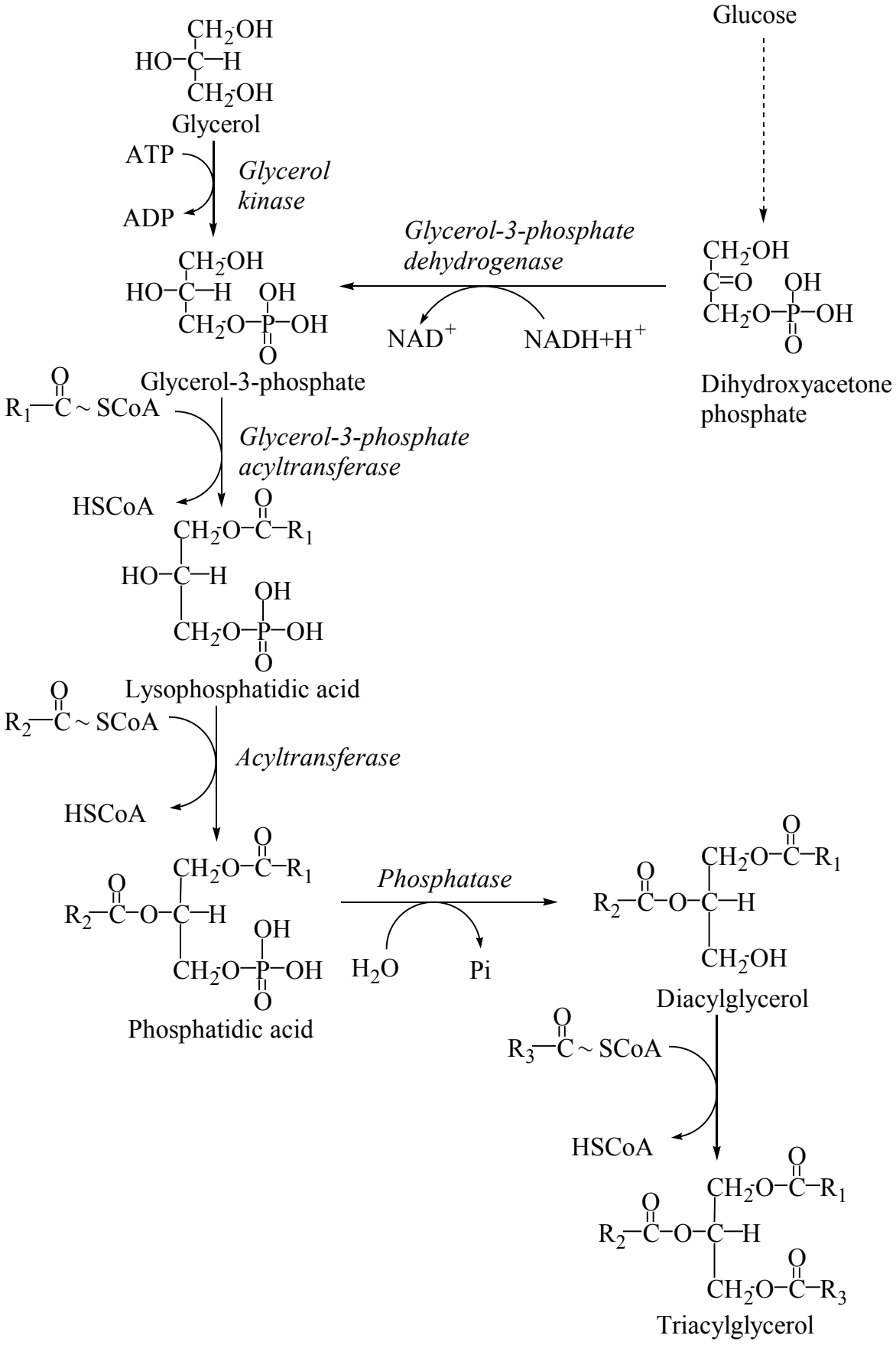


Figure 16—1. Synthesis of triacylglycerol

SYNTHESIS OF GLYCEROPHOSPHOLIPIDS

Phospholipids are a specialized group of lipids performing a variety of functions. These include the membrane **structure and functions**, involvement in **blood clotting** and supply of **arachidonic acid** for the synthesis of prostaglandins.

Phospholipids are synthesized from phosphatidic acid and 1, 2-diacylglycerol, intermediates in the production of triacylglycerols (Fig. 16—2). Phospholipid synthesis occurs in the **smooth endoplasmic reticulum**.

1. Formation of lecithin and cephalin. Choline and ethanolamine first get phosphorylated and then combine with CTP to form, respectively, CDP-choline and CDP-ethanolamine**. Phosphatidylcholine (lecithin)** is synthesized when CDP-choline combines with 1, 2-diacylglycerol. **Phosphatidyl ethanolamine (cephalin)** is produced when CDP-ethanofamine reacts with 1, 2-diacylglycerol. Phosphatidyl ethanolamine can be converted to phosphatidyl choline on methylation.

2. Synthesis of phosphatidylserine. Phosphatidyl ethanolamine can exchange its ethanolamine group with free serine to produce phosphatidyl serine. The latter, on decarboxylation, gives the former.

3. Formation of phosphatidylinositol. CDP-diacylglycerol produced from phosphatidic acid combines with inositol to form phosphatidyl inositol (PI). This phospholipid contains arachidonic acid on carbon 2 of glycerol which serves as a substrate for prostaglandin synthesis.

Mobilization of fat from adipose tissue

Triacylglycerol is the stored fat in the adipose tissue. The enzyme, namely **hormone-sensitive triacylglycerol lipase**, removes the fatty acid either from carbon 1 or 3 of the triacylglycerol to form diacylglycerol. The other two fatty acids of TG are cleaved by additional lipases specific for diacylglycerol and monoacylglycerol. The complete degradation of triacylglycerol to glycerol and free acids is known as **lipolysis.**

Regulation of hormone-sensitive TG-lipase

Hormone-sensitive TG-lipase is so named because its activity is mostly controlled by hormones. Lipase is present in an inactive form b and is activated (phosphorylated) by a cAMP dependent protein kinase to lipase a. Several hormones — such as **epinephrine** (most effective), norepinephrine, glucagon, thyroxine, ACTH etc. — enhance the activity of adenylate cyclase and, thus, increase lipolysis. On the other hand, **insulin** decreases cAMP levels and thereby inactivates lipase. Caffeine promotes lipolysis by increasing cAMP levels through its inhibition on phosphodie6sterase activity.

As is evident from the foregoing discussion, increased levels of cAMP **promote lipolysis**. In contrast, cAMP decreases fatty acid synthesis by inhibiting acetyl CoA carboxylase activity. It should be therefore kept in mind that lipolysis and lipogenesis are not simultaneously operative.

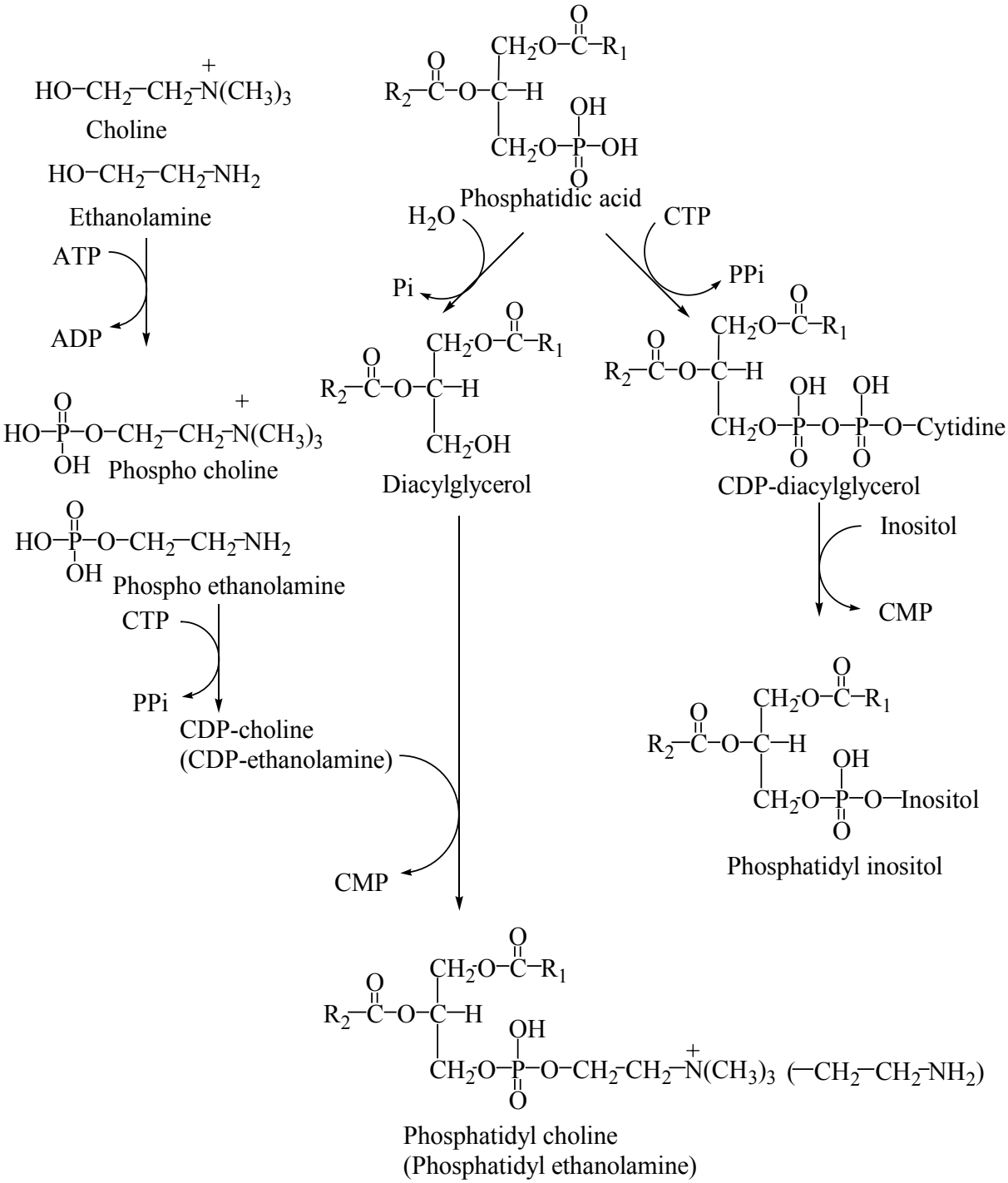


Figure 16—2. Biosynthesis of phospholipids

Fate of glycerol. The adipose tissue lacks the enzyme glycerol kinase, hence glycerol produced in lipolysis cannot be phosporylated here. It is transported to liver where it is activated to glycerol 3-phosphate. The latter may be used for the synthesis of triacylglycerols and phospholipids. Glycerol 3-phosphate may also enter glycolysis by getting converted to dihydroxyacetone phosphate.

Fate of free fatty acids. The fatty acids released by lipolysis in the adipocytes enter the circulation and are transported in a bound form to albumin. The

free fatty acids enter various tissues and are utilized for the energy. About 95% of the energy obtained from fat comes from the oxidation of fatty acids.

TRANSPORT OF CHOLESTEROL

Cholesterol is present in the plasma lipoproteins in two forms

1. About 70—75% of it is in an esterified form with long chain fatty acids.

2. About 25—30% as free cholesterol. This form of cholesterol readily exchanges between different lipoproteins and also with the cell membranes.

High density lipoproteins (HDL) and the enzyme **lecithin-cholesterol acyltransferase (LCAT)** are responsible for the transport and elimination of cholesterol from the body. High density lipoproteins are synthesized in the liver as discoidal particles — nascent HDL. They contain free cholesterol and phospholipids (mostly lecithin) and apoproteins (A, CM, E etc.).

LCAT is a plasma enzyme, synthesized by the liver. LCAT catalyses the transfer of fatty acid from the second position of phosphatidyl choline (lecithin) to the hydroxyl group of cholesterol. HDL-cholesterol is the real substrate for LCAT and this reaction is freely reversible. LCAT activity is associated with apo-A_1 of HDL.

The cholesterol (cholesteryl) ester forms an integral part of HDL. Due to the addition of cholesterol, HDL particles become spherical. In this manner, the cholesterol from the peripheral tissues is trapped in HDL, by a reaction catalyzed by LCAT and then transported to liver. The HDL enter the hepatocytes by a receptor-mediated endocytosis. In the liver, the cholesteryl esters are degraded to cholesterol. The cholesteryl is utilized for the synthesis of bile acids and lipoproteins or excreted into bile (as cholesterol). This mechanism is commonly known as reverse cholesterol transport.

Biomedical importance of plasma cholesterol

In healthy individuals, the total plasma cholesterol is in the range of 150—250 mg/dl. The women have relatively lower plasma cholesterol which is attributed to the hormones-estrogens.

Cholesterol level increases with increasing age (in women particularly after menopause) and also in pregnancy.

Plasma cholesterol is associated with different lipoprotein fractions (LDL, VLDL and HDL).

Total cholesterol can be estimated by many methods such as Libermann-Burchard reaction, Carr and Dructor method and, more recently, cholesterol oxidase method. HDL– cholesterol can be determined after precipitating LDL and VLDL by polyethylene glycol (PEC). VLDL cholesterol is equivalent to 1/5th of plasma triacylglycerol (TG) in a fasting state. LDL– cholesterol can be calculated from the following formula

LDL-cholesterol = Total cholesterol – (HDL-cholesterol – TG/5).

Hypercholesterolemia

Increase in plasma cholesterol (>250 mg/dl) concentration is known as hypercholesterolemia and is observed in many disorders

1. Diabetes mellitus. Due to increased cholesterol synthesis since the availability of acetyl CoA is increased.

2. Hypothyroidism (myxoedema). This is believed to be due to decrease in the HDL receptors on hepatocytes.

3. Obstructive jaundice. Due to an obstruction in the excretion of cholesterol through bile.

4. Nephrotic syndrome. Increase in plasma globulin concentration is the characteristic feature of nephrotic syndrome. Cholesterol elevation is due to increase in plasma lipoprotein fractions in this disorder.

Hypercholesterolemia is associated with **atherosclerosis** and **coronary heart disease.**

Control of hypercholesterolemia

Several measures are advocated to lower the plasma cholesterol level

1. Consumption of PUFA. Dietary intake of polyunsaturated fatty acids (PUFA) reduces the plasma cholesterol level. PUFA will help in transport of cholesterol by LCAT mechanism (described earlier) and its excretion from the body. The oils with rich PUFA content include cottonseed oil, soya bean oil, sunflower oil, corn oil, fish oils etc. Ghee and coconut oil are poor sources of PUFA.

2. Dietary fiber. Fiber present in vegetables decreases the cholesterol absorption from the intestine.

3. Avoiding high carbohydrate diet. Diets rich in carbohydrates (particularly sucrose) should be avoided to control hypercholesterolemia.

4. Use of drugs. Drugs such as **lovastatin** which inhibit HMG CoA reductase and decrease cholesterol synthesis are used. Certain drugs — **cholestyramine** and **colestipol** — bind with bile acids and decrease their intestinal reabsorption. This helps in the conversion of more cholesterol to bile acids and its excretion through feces**. Clofibrate** increases the activity of lipoprotein lipase and reduces the plasma cholesterol and triacylglycerols.

Hypocholesterolemia

A decrease in the plasma cholesterol, although less common, is also observed. Hyperthyroidism, pernicious anemia, malabsorption syndrome, hemolytic jaundice etc., are some of the disorders associated with hypocholesterolemia.

DISORDERS OF PLASMA LIPOPROTEINS

Inherited disorders of lipoproteins are encountered in some individuals resulting in **primary** hyper- or hypolipoproteinemias. These are due to **genetic defects** in lipoprotein metabolism and transport. The **secondary** acquired lipoprotein disorders are due to some other diseases (e.g. diabetes mellitus, nephrotic

syndrome, atherosclerosis, hypothyrodism etc.), resulting in abnormal lipoprotein pattern which often resembles the primary inherited condition.

Hyperlipoproteinemias

Elevation in one or more of the lipoprotein fractions constitutes hyperlipoproteinemias. These disorders may be either primary or secondary. Some authors use **hyperlipidemias** instead of hyperlipoproteinemias. **Frederickson's classification of hyperliporoteinemias** — based on the electrophoretic patterns of plasma lipoproteins — is widely accepted to understand these disorders.

1. Type I. This is due to familial **lipoprotein lipase deficiency**. The enzyme defect causes increase in plasma chylomicron and triacylglycerol levels.

2. Type IIa. This is also known as hyperbetalipoproteinemia and is caused by a **defect in LDL receptors**. Secondary type IIa hyperlipoproteinemia is observed in association with diabetes mellitus, hypothyroidism, nephrotic syndrome etc. This disorder is characterized by hypercholesterolemia.

3. Type IIb. Both LDL and VLDL increase along with elevation in plasma cholesterol and triacylglycerol. This is believed to be due to **overproduction of apo B.**

4. Type III. This is commonly known as broad beta disease and characterized by the appearance of a broad β-band corresponding to **intermediate density lipoprotein** (IDL) on electrophoresis.

5. Type IV. This is due to overproduction of endogenous triacylglycerols with a concomitant **rise in VLDL**. Type IV disorder is usually associated with obesity, alcoholism, diabetes mellitus etc.

6. Type V. Both chylomicrons and VLDL are elevated. This is mostly an acquired condition, due to disorders such as obesity, diabetes and excessive alcohol consumption etc.

FATTY LIVER

The **normal concentration of lipid** (mostly phospholipid) in liver is around **5%.** Liver is **not a storage organ** for fat, unlike adipose tissue. However, in certain conditions, lipids — especially the **triacylglycerols — accumulate** excessively in liver, resulting in fatty liver. In the normal liver, Kupffer cells contain lipids in the form of droplets. In fatty liver, droplets of triacylglycerols are found in the entire cytoplasm of hepatic cells. This causes impairment in metabolic functions of liver. Fatty liver is associated with fibrotic changes and cirrhosis. Fatty liver may occur due to two main causes.

1. Increased synthesis of triacylglycerols

2. Impairment in lipoprotein synthesis.

1. Increased triacylglycerol synthesis. Mobilization of free fatty acids from adipose tissue and their influx into liver is much higher than their utilization. This leads to the overproduction of triacylglycerols and their accumulation in liver. **Diabetes mellitus, starvation, alcoholism and high fat diet** are associated with increased mobilization of fatty acids that often cause fatty liver. Alcohol also inhibits fatty acid oxidation and, thus, promotes fat synthesis and its deposition.

2. Impaired synthesis of lipoproteins. The synthesis of very low density lipoproteins (VLDL) actively takes place in liver. VLDL formation requires phospholipids and apoprotein B. Fatty liver caused by impaired lipoprotein synthesis may be due to:

a)a defect in phospholipid synthesis;

b)a block in apoprotein formation;

c)a failure in the formation/secretion of lipoprotein.

Among the three causes, fatty liver due to impairment in phospholipid synthesis has been studied in some detail. This is usually associated with the dietary **deficiency of lipotropic factors** such as choline, betaine, inositol etc. Deficiency of **essential fatty acids** leads to a decreased formation of phospholipids. Further, excessive consumption of cholesterol competes with essential fatty acids and impairs phospholipid synthesis.

Certain **chemicals** (e.g. puromycin, ethionine, carbon tetrachloride, chloroform, lead, phosphorus etc.) that **inhibit protein synthesis** cause fatty liver. This is due to a blockade in the synthesis of apoprotein B required for VLDL production.

Lipoprotein synthesis and their secretion require ATP. Decrease in the availability of ATP sometimes found in **pyridoxine** and **pantothenic acid** deficiency impairs lipoprotein formation. The action of **ethionine** in the development of fatty liver is believed to be due to a reduction in the availability of ATP. Ethionine competes with methionine and traps the available adenosine (as adenosylethionine) — thereby reducing ATP levels.

Deficiency of vitamin E is associated with fatty liver. Selenium acts as a protective agent in such a condition.

Endocrine factors. Certain hormones like ACTH, insulin, thyroid hormones, adrenocorticoids promote deposition of fat in liver.

LIPOTROPIC FACTORS

These are the substances the **deficiency of which causes fat** (triacylglycerol) to **accumulate in liver**. This may happen despite the fatty acid synthesis and uptake by liver being normal.

Important lipotropic factors

These include **choline, betaine, methionine and inositol. Folic acid, vitamin B_{12}, glycine and serine** also serve as lipotropic factors to some extent.

Action of lipotropic factors

Choline and inositol are components of phospholipids and, hence, required for their synthesis. The other lipotropic factors are directly or indirectly concerned with transmethylation reactions and, ultimately, the synthesis of choline. Severe protein deficiency (e.g. kwashiorkor) causes fatty liver. This is due to a defect in the synthesis of choline as a result of insufficient amino acid (particularly methionine) supply.

Choline deficiency and fatty liver

Several explanations are offered to understand choline deficiency causing fatty liver:

1. Decreased phospholipid synthesis.

2. Impaired formation of lipoprotein membrane.

3. Reduced synthesis of carnitine due to insufficient supply of methyl groups

4. Impairment in fatty acid oxidation.

OBESITY

Obesity is an abnormal increase in the body weight due to **excessive fat deposition.**

Nutritional basis

Men and women are considered as obese if their weight due to fat (in adipose tissue), respectively, exceeds more than 20% and 25% of body weight. Obesity is basically a disorder of excess calorie intake, in simple language — **overeating.** It has to be remembered that every 7 calories of excess consumption leads to 1 g fat deposit and increase in body weight. Overeating — coupled with **lack of physical exercise** — contribute to obesity.

Types of obesity

Many types of obesity are known e.g. juvenile-onset, adult-onset, genetic, hormone-related, drug-induced, psychic etc.

In the **juvenile-onset** obesity, the **number of adipose cells** is highly **increased**. In contrast, in **adult-onset** obesity, the number of adipose cells is constant but enlarged due to **excessive fat deposition**. Hence this is often referred to as **hypertrophic** obesity.

Hormonal-related disorders like Cushing's syndrome, hypothyroidism, insulinoma cause obesity. **Heredity** and **genetic** predisposition play a significant role in the risk of obesity.

Adipose tissue

There are two types of adipose tissues

1. White adipose tissue. The fat is mostly stored and this tissue is metabolically less active.

2. Brown adipose tissue. The stored fat is relatively less but the tissue is metabolically very active.

Brown adipose tissue possesses high proportion of mitochondria and cytochromes but low activity of ATP synthase. This is an active centre for the oxidation of fatty acids and glucose and is responsible for the **diet-induced thermogenesis**. Oxidation and phosphorylation are not coupled. Mitochondrial oxidation produces **more heat and less ATP**. A specific protein — namely **thermogenin** — has been isolated in the inner membrane of these mitochondria. Thermogenin functions like an **uncoupler** and dissipates the energy in the form of heat and thus blocks the formation of ATP.

Brown adipose tissue is mostly found in **hibernating animals**, and the animals exposed to cold, besides the **newborn.** In adult humans, though not a prominent tissue, it is located in the thoracic region. It is significant to note that **brown adipose tissue is almost absent in obese persons**.

Some individuals are fortunate to have active brown adipose tissue. They eat and liberate it as heat with the result that they do not become obese. Thus, the brown adipose tissue partly explains why only some of the overeating individuals become fat.

ATHEROSCLEROSIS

Atherosclerosis *(Creek:* athere — mush) is a complex disease characterized by thickening or **hardening of arteries due to the accumulation of lipids** (particularly cholesterol, free, and esterified) collagen, fibrous tissue, proteoglycans, calcium deposits etc. in the inner arterial wall. Atherosclerosis is a progressive disorder that narrows and ultimately blocks the arteries. Infarction is the term used to indicate the stoppage of blood flow resulting in the death of affected tissue. **Coronary arteries** — the arteries supplying blood to heart — are the most commonly affected leading to myocardial infarction or heart attacks.

The incidence of atherosclerosis and coronary heart diseases are higher in developed countries (e.g. USA, U.K.) than in the developing countries (India, Africa fete.).

Causes of atherosclerosis and CHD. The development of atherosclerosis and the risk for the coronary heart disease (CHD) is directly correlated with plasma cholesterol and LDL. On the other hand, plasma HDL is inversely correlated with CHD.

Disorders that may cause atherosclerosis

Certain diseases are associated with atherosclerosis. These include **diabetes mellitus, hyperlipoproteinemias, nephrotic syndrome, hypothyroidism etc**. Many other factors like **obesity, high consumption of saturated fat, excessive smoking, lack of physical exercise, hypertension, stress etc**. are the probable causes of atherosclerosis.

Relation between HDL and CHD

The increased levels of plasma HDL are correlated with a low incidence of cardiovascular disorders. Women have higher HDL and are less prone to heart diseases compared to men. This is attributed to estrogens in women. Strenuous physical exercise, moderate alcohol intake, consumption of unsaturated fatty acids (vegetable and fish oils), reduction in body weight — all tend to increase HDL levels and reduce the risk of cardiovascular diseases.

Lipoprotein a and CHD

Lipoprotein a (Lp-a) is almost identical in structure to LDL. Lp-a contains an additional apoprotein, apo-a. Lp-a inhibits fibrinolysis. Recent studies have shown that elevation of lipoprotein-a in the plasma (>30 mg/dl) suggests increased risk of CHD. More studies are needed to confirm the relation between Lp-a and CHD.

ALCOHOL METABOLISM

Walker has rightly said “alcohol can be a food, a drug or a poison depending on the dose.” In small quantities, alcohol relieves tension and anxiety. Unfortunately, consumption of alcohol seldom ends with small doses, hence the beneficial effects are over-shadowed by the harmful effects.

Alcohol (ethanol) gets oxidized in the liver by alcohol dehydrogenase to acetaldehyde. It is believed that the acetaldehyde reacts with neurotransmitters and depletes their levels. Increased production of NADH in alcoholism competes with other reducing equivalents of electron transport chain. Further, elevation in the ratio of NADH/NAD^+ impairs many other metabolic reactions.

Alcohol (over-) consumption for a long period leads to:

1. Decreased fatty acid oxidation resulting in their accumulation.

2. Impairment in citric acid cycle.

3. Increased synthesis of triacylglycerols and their accumulation in liver, causing fatty liver.

4. Enhanced synthesis of cholesterol.

5. Hyperlipidemia leading to atherosclerosis.

6. Increased concentration of uric acid in plasma. This is due to elevation in NADH/NAD^+ ratio that leads to increased lactate/pyruvate ratio that, in turn, causes decreased capacity of kidneys to excrete uric acid.

LECTURE 17

METABOLISM OF AMINO ACIDS. TRANSAMINATION AND DEAMINATION OF AMINO ACIDS

Proteins are the **most abundant organic compounds** and constitute a major part of the body dry weight (10—12 kg in adults). They perform a wide variety of static (structural) and dynamic (enzymes, hormones, clotting factors, receptors etc.) functions. About half of the body protein (predominantly collagen) is present in the supportive tissue (skeleton and connective) while the other half is intracellular. **Protein metabolism is more appropriately learnt as metabolism of amino acids.**

DIGESTION OF 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 from 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.

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.

1. Hydrochloric acid. The pH of the stomach is < 2 due to the presence of HCI, 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.

2. Pepsin.

a. Pepsin *(Creek:* 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 HCI** (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.

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

c. 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.

3. 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.

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.

1. 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). Trypsin cleaves the peptide bonds,

the carbonyl (-CO-) group of which is contributed by **arginine** or **lysine**. Chymotrypsin is specific for peptide bonds containing uncharged amino acid residues, such as **aromatic amino acids**.

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

3. Mechanism of action of pancreatic proteases. 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.** The enzyme inhibitor diisopropyl fluorophosphate binds with serine residue of the proteases and inactivates.

4. 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.

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

Digestion of proteins by small intestinal enzymes

The luminal surface of intestinal epithelial cells contains **aminopeptidases** and **dipeptidases. Aminopeptidases** 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.

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 hydrolyzed 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.**

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 that takes place by a simple diffusion.

Transport systems of amino acid absorption

At least **six transport** systems responsible for the absorption of amino acids have been identified:

1. For short chain neutral amino acids (Ala, Ser, Thr).

2. For long chain, neutral and aromatic amino acids (Val, Leu, lie, Met, Phe, Tyr).

3. For acidic amino acids (Asp, Glu).

4. For basic amino acids and cystine (Lys, Arg, Cys-Cys).

5. For imino acids (Pro, Hyp).

6. For β-amino acids (β-Ala, taurine).

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. 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).

The free amino acid is transferred to glutathione to form γ-glutamyl- amino acid and cysteinyl-glycine. This reaction is catalysed by a membrane bound enzyme **γ-glutamyl transpeptidase**. In the cytosol, the bound amino acid (being transported) is freed and the γ-glutamyl residue is converted to 5-oxoproline that then forms glutamate by an ATP dependent reaction. Glutamate is converted back to glutathione by first combining with cysteine and then with glycine. Both these reactions require ATP. Thus 3 ATP are utilized for the transport of a single molecule of an amino acid that appears to be a needless extravagance. However, this may be necessary for the rapid transport of certain amino acids, namely 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.

AMINO ACID POOL

An adult has about **100 g of free amino acids** which represent the amino acid pool in the body. The amino acid pool may be an oversimplification of the facts, since there is no single compartment — rather, several compartments exist.

Glutamate and glutamine together constitute about 50%, and essential amino acids about 10% of the body pool. The concentration of **intracellular amino acids is always higher than the extracellular amino acids**. Amino acids enter the cells against a concentration gradient by active transport.

The amino acid pool of the body is maintained by the sources that contribute (input) and the metabolic pathways that utilize (output) the amino acids.

Sources of amino acid pool

Turnover of body protein, intake of dietary protein and the synthesis of non-essential amino acids contribute to the body amino acid pool

1. Protein turnover. The protein present in the body is in a **dynamic state**. It is estimate that about **300—400 g of protein per day is constantly degraded** and **synthesized** which represents body protein turnover. There is a wide variation in the turnover of individual proteins. For instance, the plasma proteins and digestive enzymes are rapidly degraded, their half-lives being in hours or days. The structural proteins (e.g. collagen) have long half-lives often in month and years.

Factors affecting the rate of protein degradation are:

a. Denaturation (i.e. loss of its native configuration) accelerates proteolysis.

b. Activation of lysosomes increases the rate of intracellular proteolysis.

c. Glucocorticoids increase protein degradation in muscle tissue.

d. Excessive thyroid hormones increase protein turnover.

e. Insulin reduces proteolysis and increases protein synthesis.

Abnormal, defective, and damaged proteins must be removed because they are of no use to the body, and they may inhibit processes that require the functional proteins. **Inducible enzymes** must be removed when their activities are no longer beneficial.

2. Dietary protein. There is a regular **loss of nitrogen** from the body due to degradation of amino acids. In healthy adults, it is estimated that about 30—50 g of protein is lost everyday from the body. This amount of protein (30—

50 g/day) must, therefore, be supplied daily in the diet to maintain nitrogen balance. The purpose of dietary protein is to supply amino acids (particularly the essential ones) for the synthesis of proteins and other nitrogen compounds.

Nitrogen balance is determined by comparing the intake of nitrogen (chiefly by proteins) and the excretion of nitrogen (mostly undigested protein in feces, urea and ammonia in urine).

a. A normal healthy adult is in **nitrogen equilibrium** since the daily dietary intake is equal to the loss through urine, feces and sweat

b. Positive nitrogen balance. This is a state in which **the nitrogen intake is higher than the output**. Some amount of nitrogen is retained in the body causing a net increase in the body protein. Positive nitrogen balance is observed in **growing children, pregnant women or during recovery after serious illness.**

c. Negative nitrogen balance. This is a situation in which the **nitrogen output is higher than the input**. The result is that some amount of nitrogen is lost from the body depleting the body protein. Prolonged negative nitrogen balance may even lead to death. Negative nitrogen balance may occur due to **inadequate dietary intake of protein** (deficiency of even a single essential amino acid) or **destruction of tissues or serious illness**. In all these cases, the body adapts itself and increases the breakdown of tissue proteins causing loss of nitrogen from the body.

3. Synthesis of non-essential amino acids. The body that contributes to the amino acid pool can synthesize ten out the 20 naturally occurring amino acids.

There is **no storage form of amino acids** as is the case for carbohydrates (glycogen) and lipids (triacylglycerols). The excess intake of amino acids is metabolized — oxidized to provide energy, converted to glucose or fat. The amino groups are lost as urea and excreted. The protein consumption in developed countries is much higher than the recommended dietary allowance.

Utilization of amino acids from body pool

Most of the body proteins (300—400 g/day) are synthesized from the amino acid pool. These include enzymes, hormones, immunoproteins, contractile proteins etc.

1. Many important nitrogenous compounds (porphyrins, purines, pyrimidines, etc) are produced from the amino acids. About 30 g of protein is daily utilized for this purpose.

2. Generally, about 10—15% of body energy requirements are met from the amino acids.

3. The amino acids are converted to carbohydrates and fats. This becomes predominant when the protein consumption is in excess of the body requirements.

Metabolism of amino acids

The amino acids obtained from dietary source or body protein turnover are utilized for protein biosynthesis and the production of a wide range of nitrogen-containing compounds (creatine, amines, porphyrin etc) (Fig. 17—1).

The amino acids undergo certain common reactions like **transamination** followed by **deamination** for the liberation of **ammonia**. The amino group of amino acids is utilized for the formation of **urea** which is an excretory end product of protein metabolism. The carbon skeleton of the amino acids is first converted to keto acids (by transamination) which meet one or more of the following fates.

1. Utilized to generate energy.
2. Used for the synthesis of glucose.
3. Diverted for the formation of fat or ketone bodies.
4. Involved in the production of non-essential amino acids.

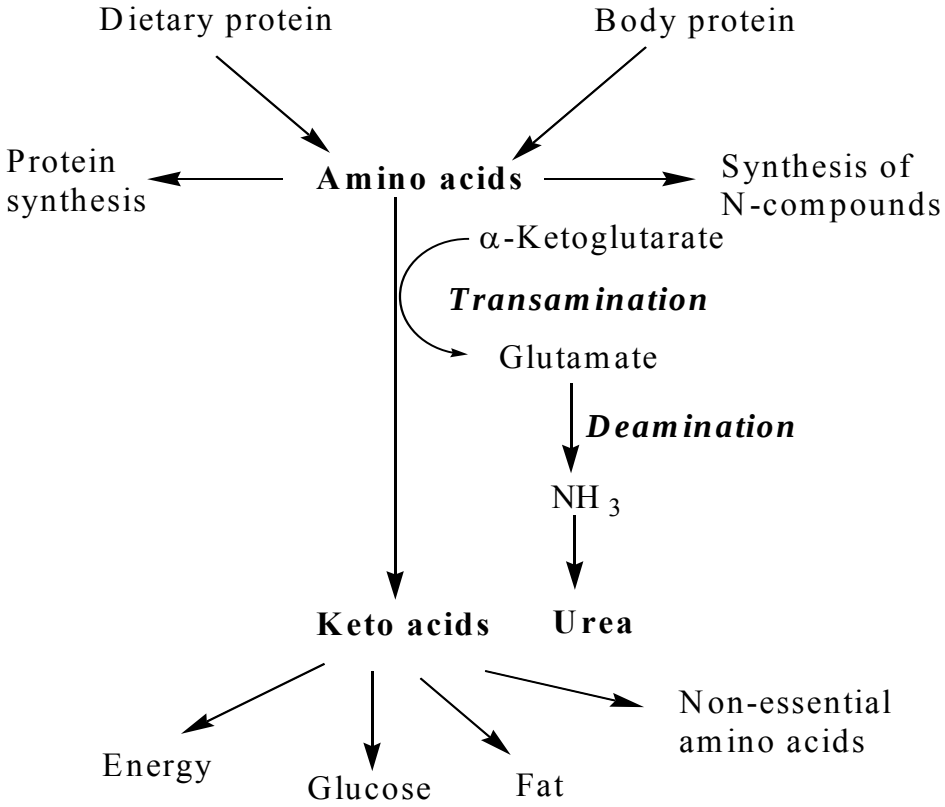


Figure 17—1. An overview of amino acid metabolism.

TRANSAMINATION OF AMINO ACIDS

The transfer of an amino ($-NH_2$) group from amino acid to a keto acid is known as **transamination**. This process involved the interconvention of a pair of amino acids and a pair of keto acids, catalysed by a group of enzymes called **transaminases** (recently, **aminotransferases**) (Fig. 17—2).

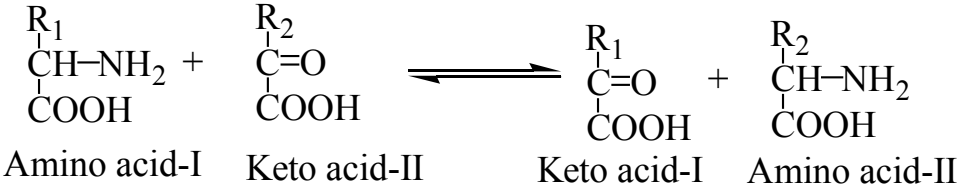


Figure 17—2. Transamination reaction.

Transamination is the most important reaction in the amino acid metabolism. The salient features of transamination are:

1. All transaminases require **pyridoxal phosphate**, a coenzyme derived from vitamin B_6

2. Specific transaminases exist for each pair of amino and keto acids. However, only two — **aspartate transaminase** and **alanine transaminase** — make a significant contribution for transamination.

3. There is **no free NH_3 liberated**; only the transfer of amino group occurs.

4. Transamination is **reversible**.

5. It involves both catabolism (degradation) and anabolism (synthesis) of amino acids. Transamination is very important for the redistribution of amino groups and **production of non-essential amino acids**, as per the requirement of the cell.

6. Transamination diverts the excess amino acids towards **energy generation**.

7. The amino acids undergo transamination to finally concentrate nitrogen in glutamate. **Glutamate** is the only amino acid that undergoes oxidative deamination to a significant extent to liberate free NH_3 for urea synthesis.

8. All amino acids except lysine, threonine, proline and hydroxyproline participate in transamination.

9. Serum transaminases are important for diagnostic and prognostic purposes. Serum glutamate pyruvate transaminase (SGPT) or **alanine transaminase** (**ALT**) is elevated in all **liver diseases**. Serum glutamate oxaloacetate transaminase (SGOT) or **aspartate transaminase** (**AST**) is increased in **myocardial infarction**.

Mechanism of transamination

Transamination occurs in two stages

1. Transfer of the amino group to the coenzyme pyridoxal phosphate (bound to the coenzyme) to form pyridoxamine phosphate.

2. The amino group of pyridoxamine phosphate is then transferred to a keto acid to produce a new amino acid and the enzyme with PLP is regenerated.

DEAMINATION OF AMINO ACIDS

The **removal of amino group from the amino acids as NH_3 is deamination**. Deamination results in the liberation of ammonia for urea synthesis. Simultaneously, the carbon skeleton of amino acids is converted to keto acids. Deamination may be either **oxidative** or **non-oxidative**. Transamination and deamination occur simultaneously, often involving glutamate as the central molecule. For this reason, some authors use the term **transdeamination** while describing the reactions of transamination and deamination, particularly involving glutamate.

Oxidative deamination

Oxidative deamination is the liberation of free ammonia from the amino group of amino acids coupled with oxidation. This takes place mostly in **liver** and

kidney. The purpose of oxidative deamination is to provide NH_3 for urea synthesis and α-keto acids for a variety of reactions, including energy generation.

Role of glutamate dehydrogenase

In the process of transamination, the amino groups of most amino acids are transferred to α-ketoglutarate to produce glutamate. Thus, glutamate serves as a **"collection center" for amino groups** in the biological system. Glutamate rapidly undergoes oxidative deamination, catalysed by glutamate dehydrogenase (GDH) to liberate ammonia. This enzyme is unique in that it can utilize either NAD^+ or $NADP^+$ as coenzyme. Conversion of glutamate to α-ketoglutarate occurs through the formation of an intermediate, α-iminoglutarate (Fig 17—3).

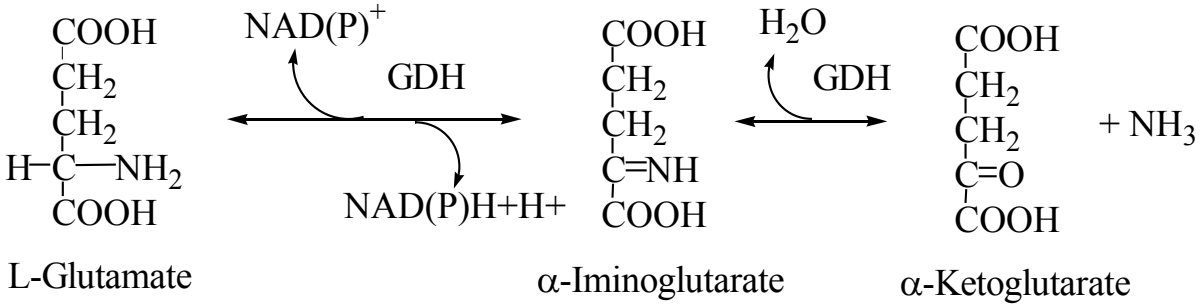


Figure 17—3. Oxidation of glutamate by glutamate dehydrogenase (GDH).

Glutamate dehydrogenase catalyzed reaction is important as it reversibly links up glutamate metabolism with citric acid cycle through α-ketoglutarate. GDH is involved in both catabolic and anabolic reactions.

Regulation of GDH activity

Glutamate dehydrogenase is zinc containing mitochondrial enzyme. It is a complex enzyme consisting of six identical units with a molecular weight of 56000 each. GDH is controlled by allosteric regulation. **GTP** and **ATP inhibit** – whereas **GDP** and **ADP activate** — glutamate dehydrogenase. **Steroid** and **thyroid hormones** inhibits GDH.

After ingestion of a protein-rich meal, liver glutamate level is elevated. It is converted to α-ketoglutarate with liberation of NH_3. Further, when the cellular energy levels are low, the degradation of glutamate is increased to provide α-ketoglutarate which enters citric acid cycle to liberate energy.

Oxidative deamination by amino acid oxidases

L-**Amino acid oxidase** and **D-amino acid oxidase** are **flavoproteins**, possessing FMN and FAD, respectively. They act on the corresponding amino acids (L or D) to produce α-keto acids and NH_3. In this reaction, oxygen is reduced to H_2O_2, which is later decomposed by catalase (Fig.17—4).

The activity of **L-amino acid oxidase** is much low while that of **D-amino oxidase** is high in tissues (mostly liver and kidney). L-Amino acid oxidase, due to its very low activity, does not appear to play any significant role in the amino acid metabolism.

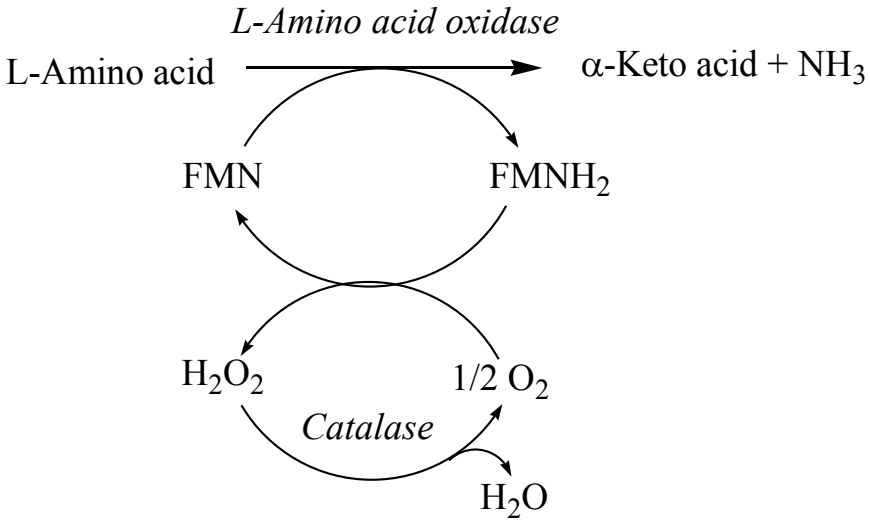


Figure 17—4. Oxidative deamination of amino acids

D-amino acids are found in plants and microorganisms. They are, however, not present in the mammalian proteins. But D-amino acids are regularly taken in the diet and metabolized by the body. D-amino acid oxidase converts them to the respective α-keto acids so produced undergo transamination to be converted to L-amino acids which participate in various metabolism. Keto acids may be oxidized to generate energy or serve as precursor for glucose and fat synthesis. Thus, D-amino acid oxidase is important as it initiates the first step for the conversion of unnatural D-amino acids to L-amino acids in the body.

LECTURE 18

METABOLISM OF AMINO ACIDS. UREA CYCLE. DECARBOXYLATION OF AMINO ACIDS

METABOLISM OF AMMONIA

Disposal of ammonia

The organisms, during the course of evolution, have developed different mechanisms for the disposal of ammonia from the body. The animals in this regard are of tree different types:

1. Ammoniotelic. The aquatic animals dispose off NH_3 into the surrounding water.

2. Uricotelic. Ammonia is converted mostly to uric acid, e.g. reptiles and birds.

3. Ureotelic. The mammals including man convert NH_3 to urea. Urea is a non-toxic and soluble compound, hence easily excreted.

Ammonia is constantly being liberated in the metabolism of amino acids (mostly) and other nitrogenous compounds. At the physiological pH, ammonia **exists as ammonium (NH_4^+) ion**.

Formation of ammonia

The production of NH_3 occurs from the **amino acids** (transamination and deamination), **biogenic amines**, **amino group of purines and pyrimidines** and by the **action of intestinal bacteria** (urease) on urea.

Transport and storage of NH_3

Despite a regular and constant production of NH_3 from various tissues, its concentration in the circulation is surprisingly low (normal plasma 10—20 μg/dl). This is mostly because the body has an efficient mechanism for NH_3 transport and its immediate utilization for urea synthesis. The transport of ammonia mostly occurs in the form of **glutamine** or **alanine** and not as free ammonia. Alanine is important for NH_3 transport from muscle to liver by **glucose-alanine cycle**. Pyruvate in skeletal muscle undergoes transamination to produce alanine. Alanine is transported to liver and used for gluconeogenesis.

Role of glutamine. Glutamine is **a storehouse of NH_3**. It is present at the highest concentration in blood among the amino acids. Glutamine serves as a storage and transport form of NH_3. Its is synthesis mostly occurs in **liver**, **brain** and **muscle**. Ammonia is removed from the brain predominantly as glutamine. Glutamine is freely diffusible in tissues, hence easily transported.

Glutamine synthetase (a mitochondrial enzyme) is responsible for the synthesis of glutamine from glutamate and ammonia. This reaction is unidirectional and requires ATP and Mg^{2+} ions. Glutamine can be deaminated by hydrolysis to release ammonia by glutaminase (Fig. 18—1) an enzyme mostly found in kidney and intestinal cells.

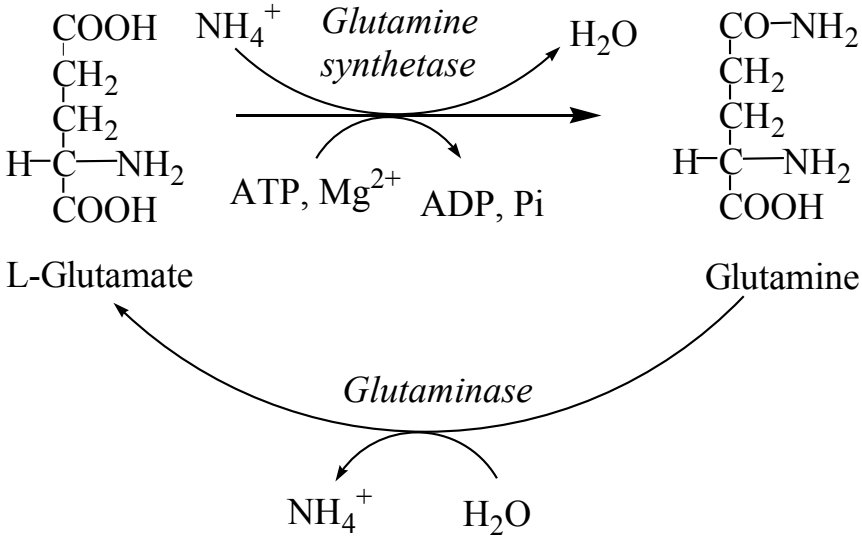


Figure 18—1. Synthesis of glutamine and its conversion to glutamate.

Functions of ammonia

Ammonia is not just a waste product of nitrogen metabolism. It is involved (directly or via glutamine) for the **synthesis** of many compounds in the body. These include **non-essential amino acids, purines, pyrimidines, amino sugar** etc. Ammonium ions (NH_4^+) are very important to maintain **acid-base balance** of the body.

Toxicity of ammonia

Even a marginal elevation in the blood ammonia concentration is harmful to the brain. Ammonia, when it accumulates in the body, results in slurring of

speech and blurring of the vision and causes tremors. It may lead to coma and, finally, death, if not correct.

Hyperammonemia. Elevation in blood NH_3 level may be genetic or acquired. All disorders lead to hyperammoniemia and cause mental retardation. The acquired hyperammoniemia may be due to hepatitis, alcoholism etc. Where the urea synthesis becomes defective, hence NH_3 accumulates.

Explanation of NH_3 toxicity. The reaction catalyzed by glutamate dehydrogenase probably explains the toxic affects of NH_3 in brain

$$\alpha\text{-Ketoglutarate} + NH_3 + NADPH + H^+ \leftrightarrow \text{Glutamate} + NAD^+$$

Accumulation of NH_3 shifts the equilibrium to the right with more glutamate formation, hence more utilization of α-ketoglutarate. α-Ketoglutarate is a key intermediate in citric acid cycle and its depleted levels impair the citric acid cycle. The net result is that production of energy (ATP) for brain is reduced. The toxic effects of NH_3 on brain are, therefore, due to impairment in ATP formation.

UREA CYCLE

Urea is the end product of protein metabolism (amino acid metabolism). The nitrogen of amino acids converted to ammonia is toxic to the body. As such, urea accounts for 80—90% of nitrogen containing substance excreted in urine.

Urea is **synthesized in liver** and transported to kidney for excretion in urine (Fig.18—2). Urea cycle is the **first metabolic cycle** that was elucidated by Hans Krebs and Kurt Henseleit (1932), hence it is known as **Krebs-Henseleit** cycle. Urea has two amino (-NH_2) groups, one derived from **NH_3** and the other from **aspartate.** Carbon atom is supplied by **CO_2.** Urea synthesis is a five-step cyclic process, with five distinct enzyme. The first two enzymes are present in mitochondria while the rest are localized in cytosol.

1. Synthesis of carbamoyl phosphate. **Carbamoyl phosphate synthase I** (CPS I) of mitochondria catalyses the condensation of NH_4^+ions with CO_2 to form **carbamoyl phosphate**. This step consumes **two ATP** and is **irreversible**, and rate-limiting. CPS I requires **N-acetylglutamate** for its activity. Another enzyme — **carbamoyl phosphate synthase II** (CPS II) — involved in pyrimidine synthsis — is present in cytosol. It accept amino group from glutamine and does not require N-acetylglutamate for its activity.

2. Formation of citrulline. **Citrulline** is synthesized from **carbamoyl phosphate** and **ornithine** by **ornithine transcarbamoylase**. Ornitine is regenerated and used in urea cycle. Therefore, its role is comparable to that of oxaloacetate in citric acid cycle. Ornithine and citrulline are basic amino acids. Citrulline produced in this reaction is transported to cytosol by transporter system.

3. Synthesis of arginosuccinate. **Arginosuccinate synthase** condenses **citrullin** with **aspartate** to produce **arginosuccinate**. The second amino group of urea is incorporated in this reaction. This step requires **ATP** which is cleaved to AMP and pyrophosphate (PPi). The latter is immediately broken down to inorganic phosphate (Pi).

4. Cleavage of arginosuccinate. **Arginosuccinase** cleaves **arginosuccinate** to give **arginine and fumarate**. Arginine is the immediate precursor for urea. Fumarate liberated here provides a connecting link with citric acid cycle, gluconeogenesis etc.

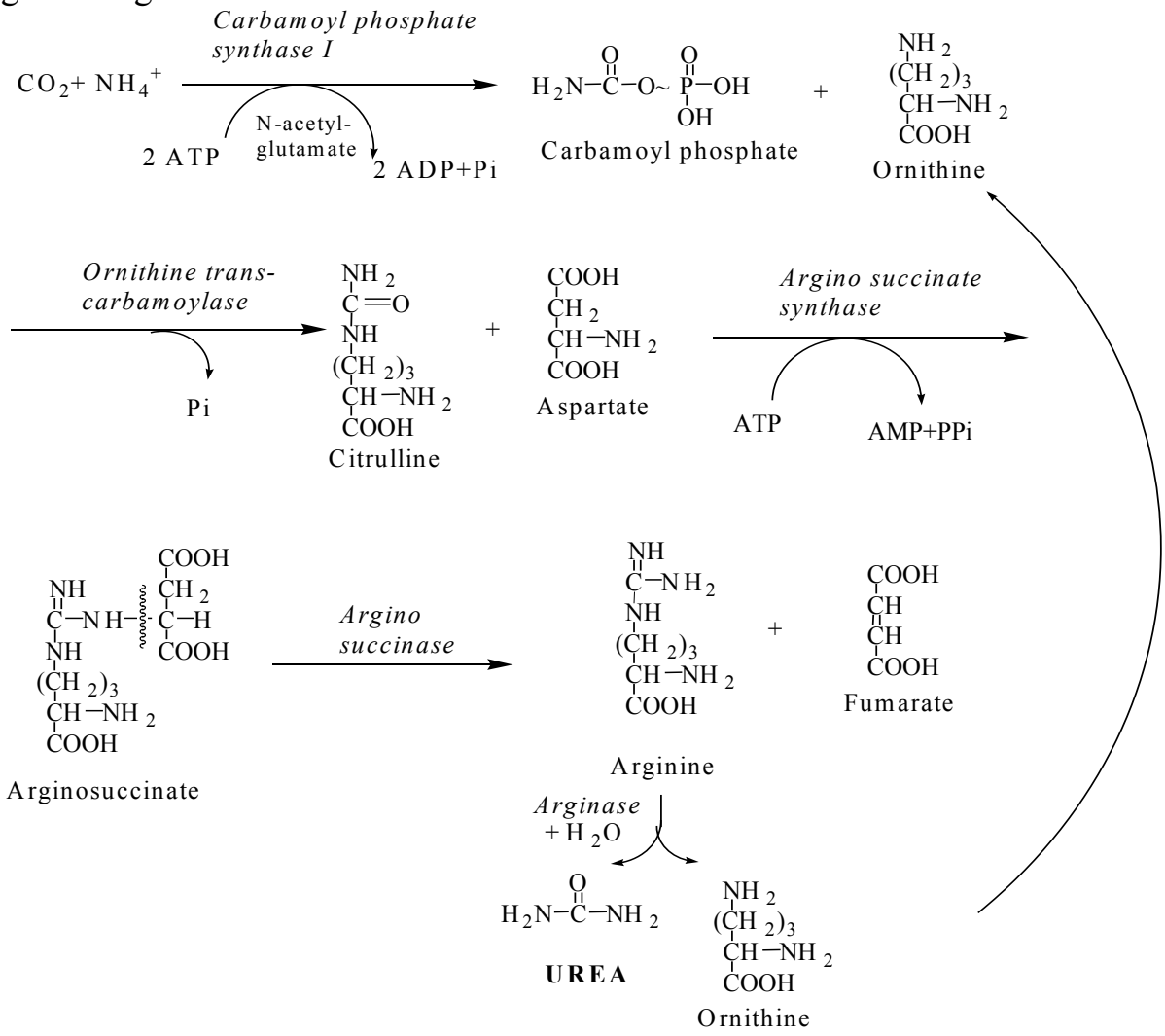


Figure 18—2. Reactions of urea cycle

5. Formation of urea. Arginase is the fifth and final enzyme that cleaves arginine to yield urea and ornithine. Ornithine, so regenerated, enters mitochondria for its reuse in the urea cycle. Arginase is activated by CO^{2+} and Mn^{2+}. Ornithine and lysine compete with arginine (competitive inhibition). Arginase is mostly found in the liver, while the rest of the enzymes (four) of urea cycle are also present in other tissues. For this reason, arginine synthesis may occur to varying degree in many tissues. But only the liver can ultimately produce urea.

Overall reaction and energetics

The urea cycle is irreversible and consumes 4 ATP. Two ATP are utilized for the synthesis of carbamoyl phosphate. One ATP is converted to AMP and PPi to produce arginosuccinate which equals 2 ATP. Hence 4 ATP are actually consumed.

$NH_4^+ + CO_2 +$ Aspartate + 3 ATP→Urea + Fumarate + 2 ADP + 2 Pi + AMP+PPi

Regulation of urea cycle

The first reaction catalyzed by **carbamoyl phosphate synthase I** is **rate-limiting** reaction or committed step in urea synthsis. CPS I is a**llosterically** activated by **N-acetylglutamate**. It is synthesized from glutamate and acetyl CoA by synthase and degraded by a hydrolase.

The rate of urea synthesis in liver is correlated with the concentration of N-acetylglutamate. The consumption of a protein-rich meal increases the level of N-acetylglutamete in liver, leading to enhanced urea synthesis.

Carbamoyl phosphate synthase I and **glutamate dehydrogenase** are localized in the mitochondria. They coordinate with each other in the formation of NH_3 and its utilization for the synthesis of carbamoyl phosphate. The remaining four enzymes of urea cycle are mostly controlled by the concentration of their respective substrates.

Disposal of urea

Urea produced in the liver freely diffuses and is transported in blood to **kidney** and excreted. A small amount of urea enters the intestine where it is broken down to CO_2 and NH_3 by bacterial enzyme **urease**. This ammonia is either lost in the feces or absorbed into the blood. In renal failure, the blood urea level is elevated (uremia), resulting in diffusion of more urea into intestine and its breakdown to NH3. Hyperammoniemia (increased blood NH3) is commonly seen in patients of kidney failure. For these patients, oral administration of antibiotics (neomycin) to kill intestinal bacteria is advised.

Integration between urea cycle and citric acid cycle

Urea cycle is linked with TCA cycle in three different ways

1. The production of **fumarate** in urea cycle is the most important integrating point with TCA cycle. Fumarate is converted to malate and then to oxaloacetate in TCA cycle. Oxaloacetate undergoes transamination to produce aspartate which enters urea cycle. Here, it combines with citrulline to produce arginosuccinate. Oxaloacetate is an important metabolite which can combine with acetyl CoA to form citrate and get finally oxidized. Oxaloacetate can also serve as a precursor for the synthesis of glucose (gluconeogenesis).

2. ATP (12) are generated in the citric acid cycle while ATP (4) are utilized for urea synthesis.

3. Citric acid cycle is an important metabolic pathway for the compete oxidation of various metabolites to CO_2 and H_2O. The CO_2 liberated in TCA cycle (in the mitochondria) can be utilized in urea cycle.

Metabolic disorders of urea cycle

Metabolic defects associated with each of the five enzyme of urea have been reported (Tabl.18—1).

All the disorders invariably lead to a build-up in blood ammonia (**hyperammonemia**), leading to toxicity. Other metabolites of urea cycle also accumulate which, however, depends on the specific enzyme defect. The clinical symptoms associated with defect in urea cycle enzymes include vomiting, lethargy, irritability, ataxia and mental retardation.

Table 18—1.

Metabolic defects in urea cycle

Defect	Enzyme involved
Hyperammonemia type I	Carbamoyl phosphate synthase I
Hyperammonemia type II	Ornithine transcarbamoylase
Citrullinemia	Arginosuccinate synthase
Arginosuccinic aciduria	Arginosuccinase
Hyperargininemia	Arginase

Clinical importance of blood urea

In healthy people, the normal blood urea concentration is **10—40 mg/dl (2.5—8.3 mmol/l).** Higher protein intake marginally increases blood urea level; however this is well within normal range. About 15—30 g of urea is excreted in urine per day.

Blood urea estimation is widely used as a screening test for the evaluation of **kidney** (renal) **function**. It is estimated in the laboratory either by **urease method** or **diacetyl monooxime procedure**. Elevation in blood urea may be broadly classified into three groups.

1. Pre-renal. This is associated with **increased protein breakdown**, leading to a negatively nitrogen balance, as observed after major surgery, prolonged fevers, diabetic coma, thyrotoxicosis etc. In leukemia and bleeding disorders also, blood urea is elevated.

2. Renal. In renal disorders like **acute glomerulonephritis**, chronic nephritis, nephrosclerosis, polycyctic kidney, blood urea is increased.

3. Post-renal. Whenever there is an **obstruction** in the **urinary tract** (e.g. tumors, stones, enlargement of prostate gland etc.) blood urea is elevated. This is due to increased reabsorption of urea from the renal tubules.

The term **"uremia"** is used to indicate increased blood urea levels due to renal failure. **Azotemia** reflects a condition with elevation in blood urea/or other nitrogen metabolites which may or may not be associated with renal disease.

DECARBOXYLATION OF AMINO ACIDS

Some of the α-amino acids undergo decarboxylation to form the respective **biogenic amines.** This is carried out by a group of enzymes called decarboxylases which are dependent on **pyridoxal phosphate** (vitamin B_6). Many biogenic amines with important functions are synthesized.

1. Serotonin (5-hydroxytryptamine), produced from tryptophan (Fig. 18—3) is important in nerve impulse transmission (neurotransmitter).

a. Serotonin is a powerful vasoconstrictor and results in smooth muscle contraction in bronchioles.

b. It is closely involved in the regulation of cerebral activity (excitation).

c. Serotonin controls the behavioural patterns, sleep, blood pressure and body temperature.

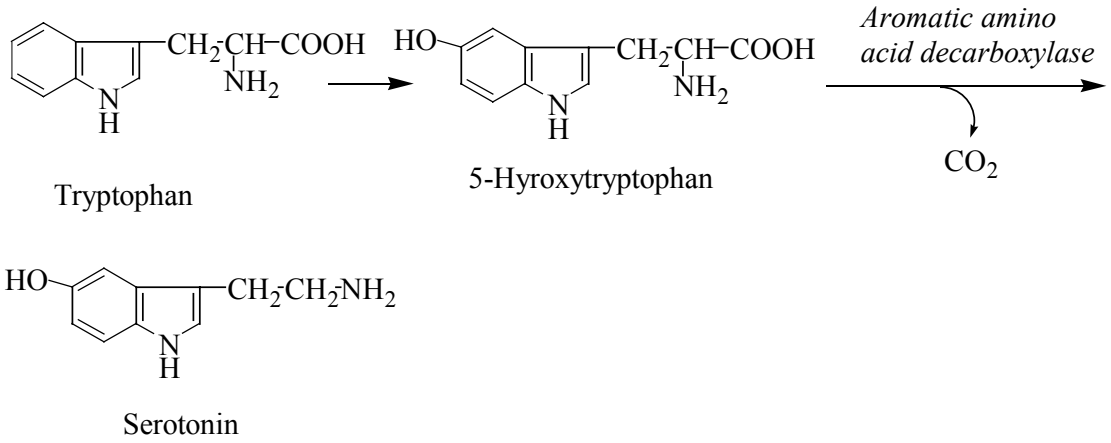


Figure 18—3. Biosynthesis of serotonin

d. Serotonin evokes the release of peptide hormones from gastrointestinal tract.

e. It is also necessary for the motility of gastrointestinal tract.

2. Histamine on decarboxylation gives the corresponding amine — **histamine** (Fig. 18—4). Histamine is a **vasodilator** and **lowers blood pressure**. It stimulates **gastric HCl secretion** and is involved in **inflammation and allergic** reactions.

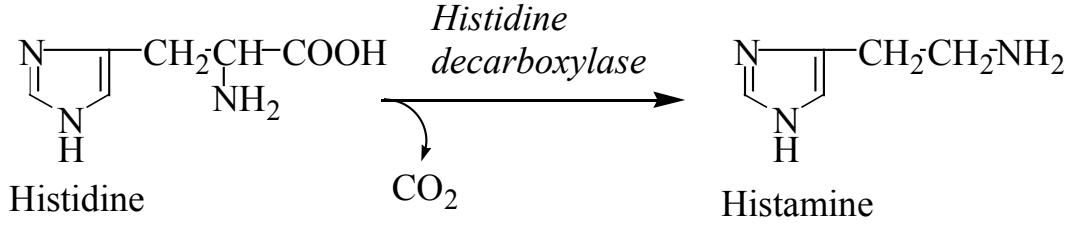


Figure 18—4. Biosynthesis of histamine

3. Glutamate on decarboxylation gives **γ-amino butyric acid** (GABA) (Fig.18—5). **GABA** inhibits the transmission of nerve impulses, hence it is an inhibitory neurotransmitter in the brain.

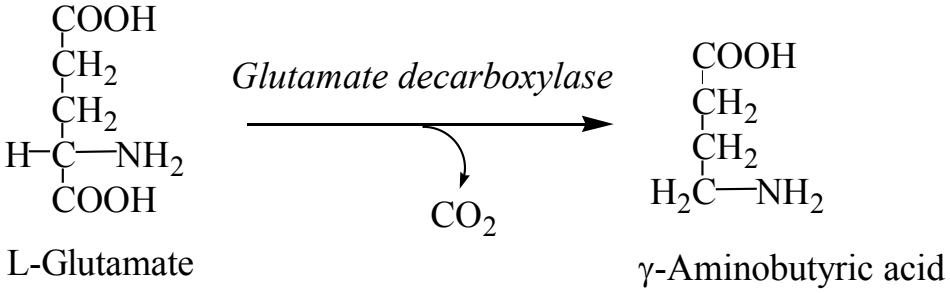


Figure 18—5. Biosynthesis of γ-amino butyric acid

4. The synthesis of **catecholamines** (dopamine, norepinephrine and epinephrine) from tyrosine requires **aromatic amino acid decarboxylase** (Figure 18—6). Catecholamines are involved in metabolic and nervous regulation.

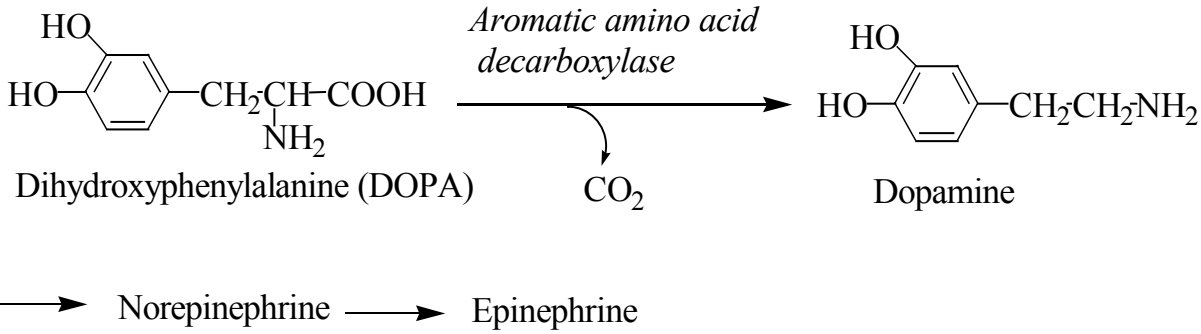


Figure 18—6. Biosynthesis of dopamine and catecholamines

5. Ornithine decarboxylase acts on ornithine so split off CO_2 and produce **putrescine** (Fig. 18—7).

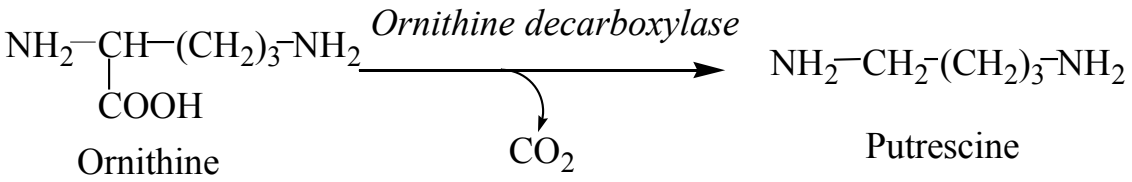


Figure 18—7. Biosynthesis of putrescine

Putrescine is converted to spermidine and then spermine. Putrescine, spermine and spermidine are the biologically important polyamines.

Functions of polyamines:

1. Polyamines **are basic** in nature and possess multiple positive charges. Hence they are **readily associated with nucleic acid** (DNA and RNA).

2. They are involved in the **synthesis of DNA, RNA and protein**.

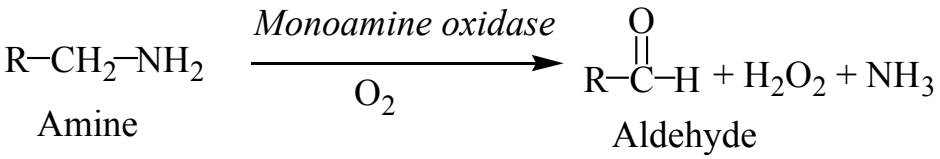
3. They are essential for **cell growth and proliferation**.

4. They are believed to be involved in the **stabilization of the membrane structure** (cell and cellular organelles).

The excretion of polyamines is found to be elevated in almost all types of cancer, e.g. leukemia, carcinoma of lungs, bladder, kidney etc. Diagnostically, putrescine is an ideal marker for cell proliferation whereas spermidine is suitable for the assessment of cell destruction.

Deamination of amines

Monoamine oxidase (MAO) is an oxidoreductase that deaminates monoamines.



It is located in many tissues, but it occurs in highest concentrations in the liver, stomach, kidney, and intestine. At least two isozymws of MAO have been described. **MAO-A** is found in neural tissue and deaminated serotonin, epinephrine, and norepinephrine, while MAO-B if found in extraneural tissues. MAO inhibitors have been used to treat **hypertension and depression**.

The drug, **iproniazid** inhibits monoamine oxidase (MAO) and elevates serotonin level, therefore, this drug is a **psychic stimulant**. On the other hand, **reserpine** increases the degradation of serotonin, hence acts as a depressant drug. Lysergic acid diethylamide competes with serotonin, therefore, acts as a depressant.

LECTURE 19

METABOLISM OF AMINO ACIDS. TRANSMETHYLATION. METABOLISM OF PHENYLALANINE AND TYROSINE

TRANSMETHYLATION

The transfer of **methyl group** (-CH_3) from **active methionine** to an **acceptor** is known as **transmethylation**. **Methionine** has to be activated to **S-adenosylmethionine** (SAM) or active methionine to donate the methyl group.

Synthesis of S-adenosylmethionine

The synthesis of S-adenosylmethionine occurs by the transfer of an adenosyl group from ATP to sulfur atom of methionine. This reaction is catalyzed by **methionine S-adenosyltransferase** (Fig. 19—1).

The activation of methionine is unique as the sulfur becomes a sulfonium atom (SAM is a sulfonium compound) by the addition by a third carbon. This reaction is also unusual since all the three phosphates of ATP are eliminated as pyrophosphates (PPi) and inorganic phosphates (Pi). Three high energy phosphates (3 ATP) are consumed in the formation of SAM.

Function of S-adenosylmethionine

S-Adenosylmethionine is highly reactive due to the presence of a positive charge. The enzymes involved in the transfer of methyl group are collectively known as **methyltransferase** (Fig. 19—1). S-Adenosylmethionine transfers the methyl group to an acceptor and gets itself converted to **S-adenosylhomocysteine**. The loss of free energy in this reaction makes the methyl transfer essentially irreversible. S-Adenosylhomocysteine is hydrolyzed to homocysteine and adenine. **Homocysteine** can be remethylated to methionine by **N^5-methyl tetrahydrofolate** and this reaction is dependent on methylcobalamin (vitamin B_{12}). In this manner, **methionine can be regenerated** for reuse. It should be noted that there is no net synthesis of methionine in the S-adenosylmethionine cycle. Hence, **methionine** is an **essential amino acid**.

The sources of methyl groups for transmethylation are **choline** and **betaine**.

Significance of transmethylation

1. Transmethylation is of great biological significance since many compounds become functionally active only after methylation.

2. Protein (amino acid residues) methylation helps to control protein turnover. In general, methylation protects the proteins from immediate degradation.

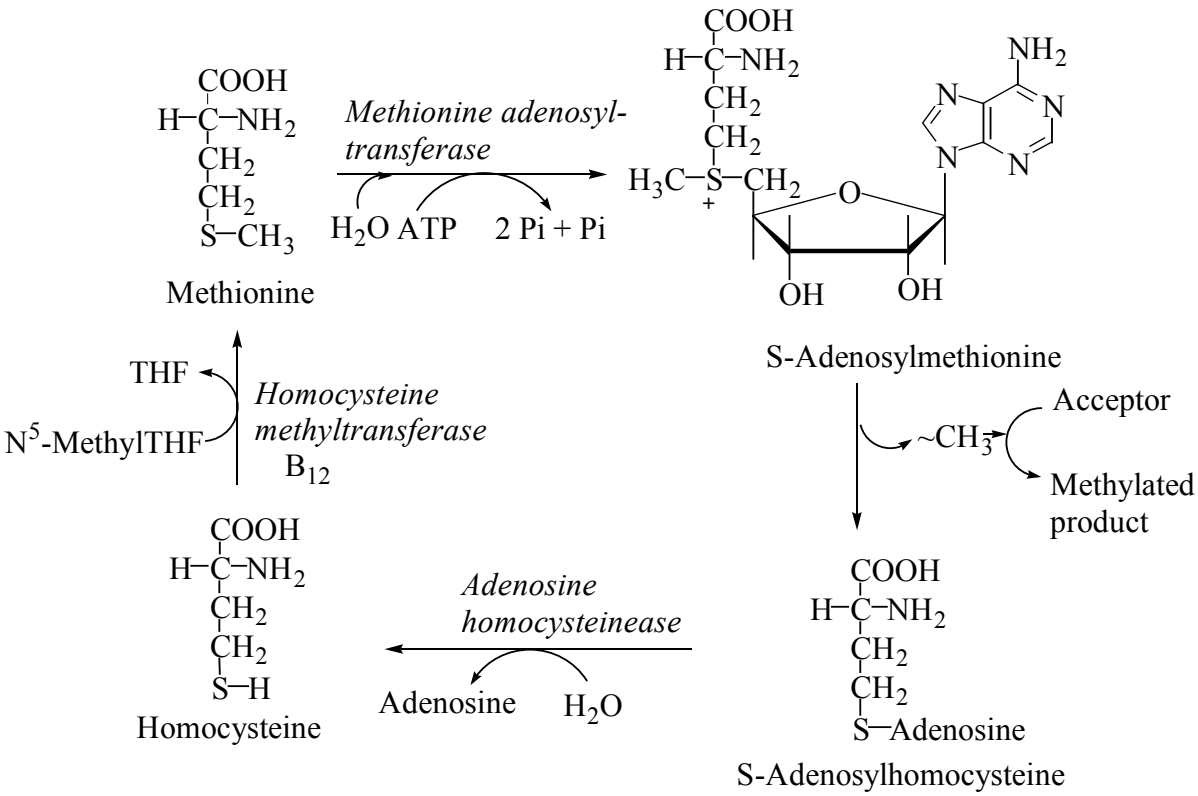


Figure 19—1. S-Adenosylmethionine cycle — synthesis, utilization and regeneration

The most important transmethylation reactions

Biosynthesis of creatine

Creatine is present in the tissues (muscle, brain, blood etc.) as the **high energy** compound, **phosphocreatine** and as free **creatine**.

Three amino acids — **glycine, arginine and methionine** — are required for creatine formation (Fig. 1—2).

The first reaction occurs in the **kidney**. It involves the transfer of guanidine group of arginine to glycine, catalyzed by **arginine-glycine transamidinase** to produce **guanidoacetate**. S-Adenosylmethionine donates methyl group to guanidoacetate to produce **creatine**. This reaction occurs in **liver**. Creatine is reversibly phosphorylated to phosphocreatine **(creatine phosphate)** by creatine kinase.

Creatinine is an anhydride of creatine. It is formed by spontaneous cyclization of creatine or creatine phosphate. Creatinine is excreted in **urine**.

Estimation of serum creatinine (along with urea) is used as a diagnostic test to assess **kidney function**. The normal concentration of creatine and creatinine in human serum and urine are as follows: serum creatine 0.2—0.6 mg/dl, creatinine — 0.6—1 mg/dl; urine creatine — 0-50 mg/day, creatinine — 1—2 mg/day. Increased output of creatine in urine is referred to as **creatinuria**. Creatinuria is observed in muscular dystrophy, diabetes mellitus, hyperthyroidism, starvation etc.

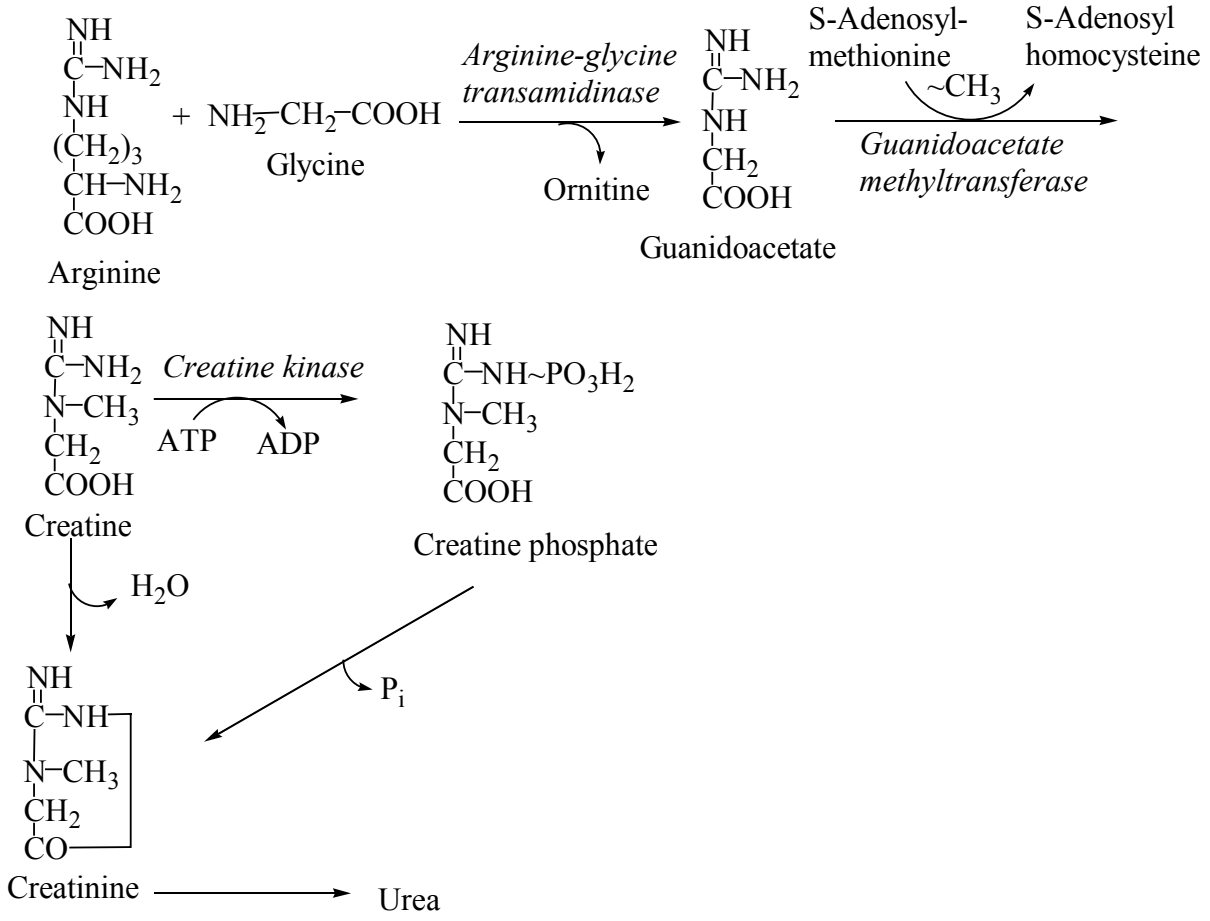


Figure 19—2. Metabolism of creatine

Biosynthesis of epinephrine

Methylation of norepinephrine by S-adenosylmethyonine gives epinephrine (Fig.19—3). The difference between epinephrine and norepinephrine is only a methyl group (remember that norepinephrine has no methyl group).

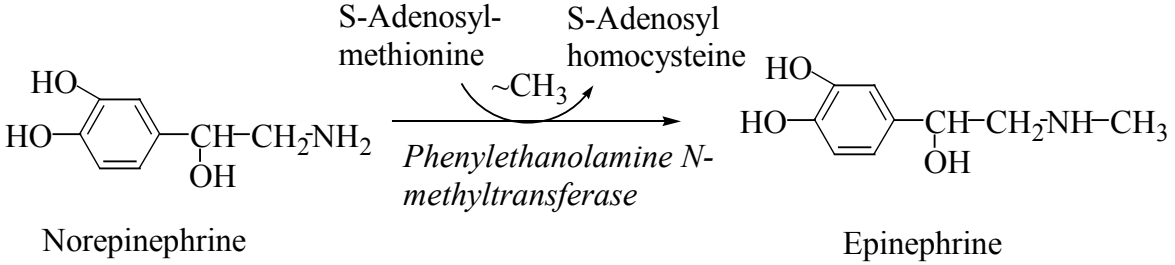


Figure 19—3. Biosynthesis of epinephrine

Biosynthesis of phosphatidylcholine (Fig. 19—4).

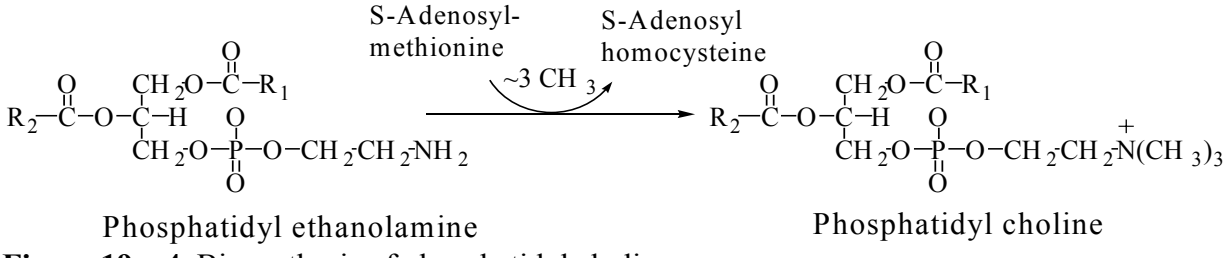


Figure 19—4. Biosynthesis of phosphatidyl choline

METABOLISM OF PHENYLALANINE AND TYROSINE

Phenylalanine (Phe) and **tyrosine** (Tyr) are structurally related aromatic amino acids. Phenylalanine is an **essential amino acid** while tyrosine is non-essential. Besides its incorporation into proteins, the only **function** of phenylalanine is its **conversion to tyrosine**. For this reason, ingestion of tyrosine can reduce the dietary requirement of phenylalanine. The phenomenon is referred to as **"sparing action" of tyrosine on phenylalanine**.

The predominant **metabolism of phenylalanine** occurs **through tyrosine**. **Tyrosine** is incorporated into proteins and is involved in the synthesis of a variety of biologically important compounds — **epinephrine, norepinephrine**, **dopamine** (catecholamines), **thyroid hormones** and the pigment **melanin**. During the course of degradation, Phe and Tyr are converted to metabolites which can serve **as precursors for the synthesis** of glucose and serve as **precursors for the synthesis of glucose and fat**. Hence, these amino acids are both **glucogenic** and **ketogenic**. Biochemists attach special significance to Phe and Tyr metabolism for two reasons — **synthesis of biologically important compounds** and the **metabolic disorders due to enzyme defects**.

Conversion of phenylalanine to tyrosine (Fig. 19—5)

Under normal circumstances, the degradation of phenylalanine mostly occurs **through tyrosine**. Phenylalanine is hydroxylated at para-position by **phenylalanine hydroxylase** to produce **tyrosine** (p-hydroxy phenylalanine). This is an irreversible reaction and requires the participation of a specific coenzyme biopterin (containing pteridine ring) which is structurally related to folate. The active form of biopterin is tetrahydropterin (H_4-biopterin). The enzyme phenylalanine hydroxylase is present in the **liver.** In the conversion of phenylalanine to tyrosine, the reaction involves the incorporation of one atom of molecular oxygen into the para position of phenylalanine while the other atom of O_2 is reduced to form water.

Degradation of tyrosine (phenylalanine)

The metabolism of phenylalanine and tyrisone is considered together.

1. As phenylalanine is converted to tyrosine, a single pathway is responsible for the degradation of both these amino acids, which occurs mostly in liver. In a sequence of reactions, tyrosine is converted to **fumarate** and **acetoacetate** (Fig. 19—5).

2. Tyrosine first undergoes transamination to give **p-hydroxyphenylpyruvate**. This reaction is catalyzed by **tyrosine transaminase** (pyridoxal phosphate dependent).

3. p-**Hydroxyphenylpyruvate hydroxylase** (or **dioxygenase**) is a **copper**-containing enzyme. It catalyse oxidative decarboxylation as well as hydroxylation of the phenyl ring of p-hydroxyphenylpyruvate to produce **homogentisate**. This step in tyrosine metabolism requires **ascorbic acid**.

4. Homogentisate oxidase (**iron** metalloprotein) cleaves the benzene of **homogentisate** to form 4-maleylacetoacetate. **Molecular oxygen** is required for this reaction to break the aromatic ring.

5. 4-Maleylacetoacetate is converted to liberate **fumarate** and **acetoacetate**. Fumarate is an intermediate of **citric acid cycle** and can also serve as **precursor for gluconeogenesis**. **Acetoacetate is a ketone body** from which fat can be synthesized.

Synthesis of melanine

The synthesis of melanin occurs in **melanosomes present in melanocytes**, the pigment-producing cells. Tyrosine is the precursor for melanin and only one enzyme, namely **tyrosinase** (a copper-containing oxyganase) is involved in its formation. Tyrosinase hydroxylates tyrosine to form 3,4-dihydroxyphenylalanine (DOPA). DOPA is converted in some stages to black melanin. The skin color of the individual is determined by the relative concentration of **black** and **red** melanins. This, in turn, is dependent on many factors, both genetic and environmental. These include the activity of **tyrosinase**, the density of melanocytes, availability of tyrosine etc.

Biosynthesis of thyroid hormones

Thyroid hormones — thyroxine (tetraiodothyronin) and triiodothyronin – are synthesized from the tyrosine residue of the protein thyroglobulin and activated iodine.

Biosynthesis of catecholamines

Tyrosine is hydroxylated to 3,4-dihydroxyphenylalanine (DOPA) by **tyrosine hydroxylase**. This enzyme catalyses the rate limiting reaction and is required **tetrahydrobiopterin** as coenzyme. In contrast to this enzyme, tyrosinase present in melanocytes converts tyrosine to DOPA. Hence, two different enzyme systems exist to convert tyrosine to DOPA. DOPA undergoes PLP-dependent decarboxylation to produce norepinephrine. Methylation of norepinephrine by S-adenosylmethyonine gives epinephrine.

DISORDERS OF TYROSINE (PHENYLALANINE) METABOLISM

Several enzyme defects in phenylalanine/tyrosine degradation leading to metabolic disorders are known. In Fig. 19—5, the deficient enzymes and the respective inborn errors are depicted and they are discussed here.

Phenylketonuria

1. Phenylketonuria (PKU) is the most common metabolic disorder in amino acid metabolism. The incidence of PKU is 1 in 10,000 births. It is due to the deficiency of the hepatic enzymes, **phenylalanine hydroxylase (1),** caused by an autosomal recessive gene. The net outcome in PKU is that **phenylalanine is not converted to** tyrosine.

2. Phenylketonuria primarily causes the accumulation of **phenylalanine** in tissues and blood, and results in its increased excretion in urine. Due to disturbances in the routine metabolism, phenylalanine is diverted to alternate pathways (Fig. 19—6), resulting in the excessive production of **phenylpyruvate**, **phenylacetate**, **phenyllactate**.

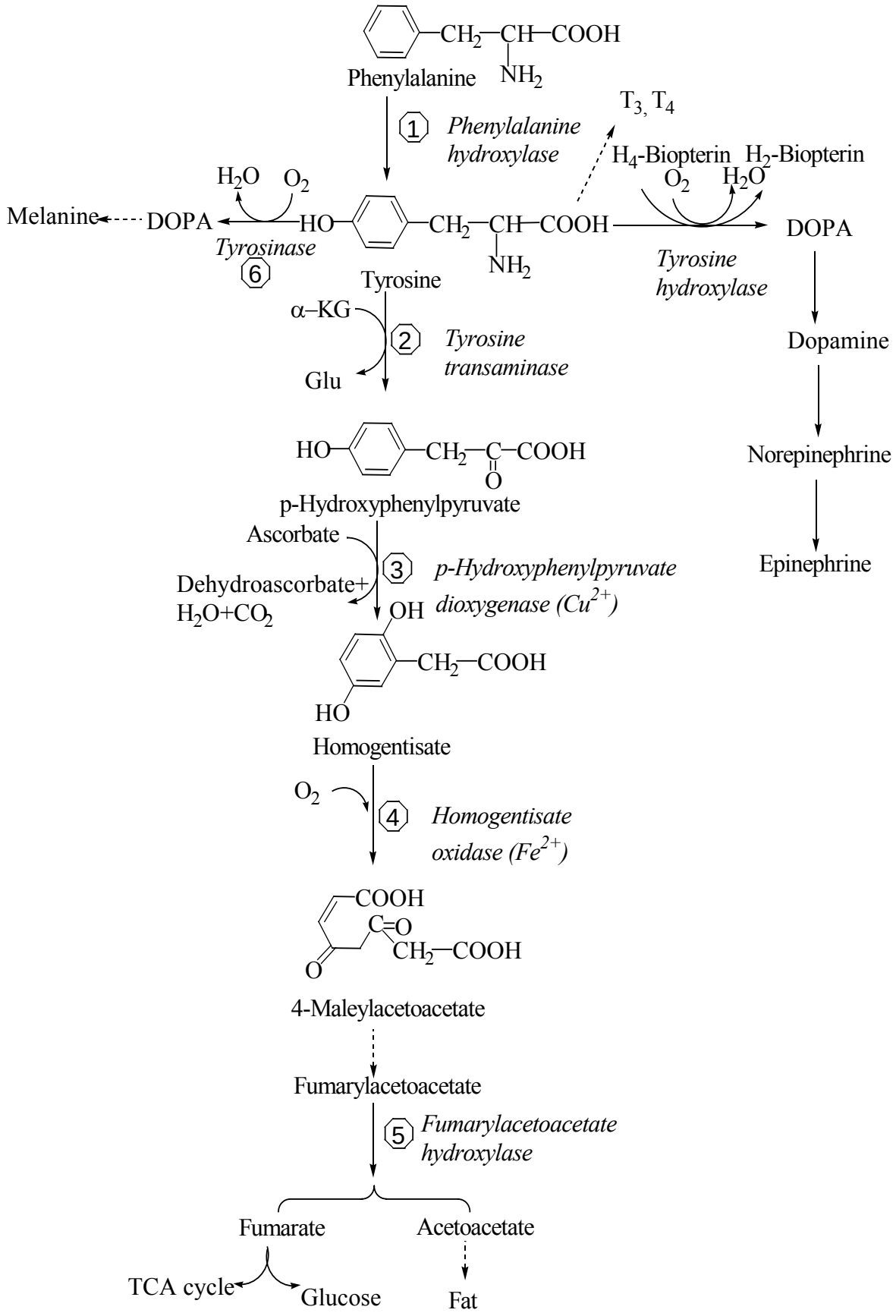


Figure 19—5. Metabolism of phenylalanine and tyrosine

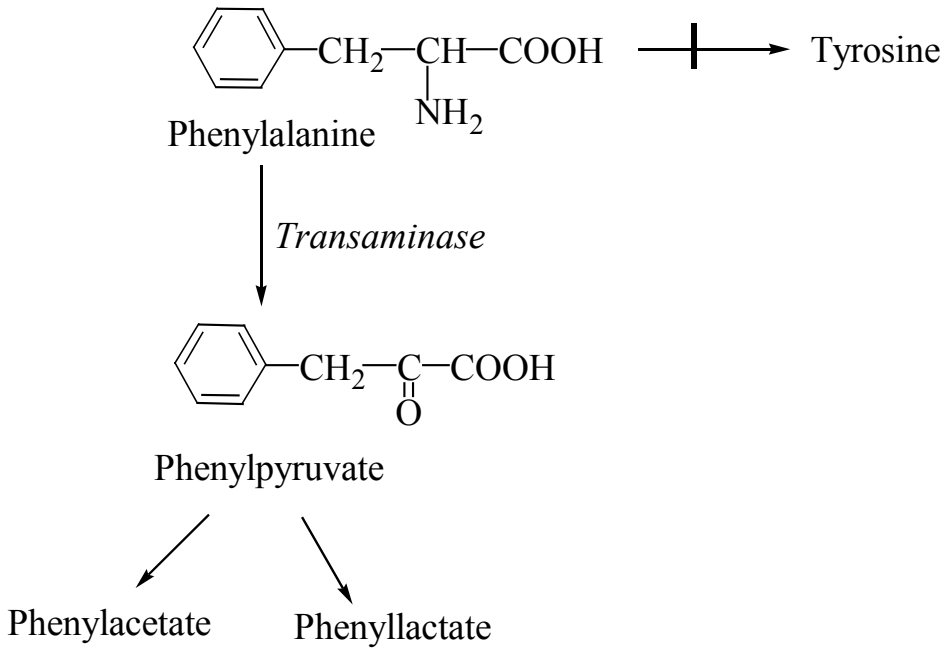


Figure19—6. Metabolites that accumulate in phenylketonuria

All these metabolites are excreted in urine in high concentration in PKU. The name phenylketonuria is coined due to the fact that the metabolite phenylpyruvate is a **keto** acid excreted in urine in high amounts.

3. Biochemical manifestations of PKU. The disturbed metabolism of phenylalanine — resulting in the increased concentration of phenylalanine and its metabolites in the body — causes many clinical and biochemical manifestations.

a. Effects on central nervous system. Mental retardation, failure to walk or talk, failure of growth, seizures and tremor are the characteristic findings in PKU. The biochemical basis of mental retardation in PKU is not well understood.

b. Accumulation of phenylalanine in brain impairs the transport and metabolism of other **aromatic amino acids** (tryptophan and tyrosine).

c. The synthesis of **serotonin** (an excitatory neurotransmitter) from tryptophan is insufficient. This is due to the competition of phenylalanine and its metabolites with tryptophan that impairs the synthesis of serotonin.

d. Defect in **myelin** formation is observed PKU patients.

e. Effect on pigmentation. Melanin is the pigment synthesized from tyrosine by tyrosinase. Accumulation of phenylalanine competitive inhibits tyrosinase and impairs melanin formation. The result is hypopigmentation that causes light skin colour, fair hair, blue eyes etc.

f. Elevated levels of phenylalanine, phenyl pyruvate, phenyllactate, and phynylacetate are found in plasma and urine. Phenylacetate gives the urine a mouse odor.

4. Diagnosis of PKU. PKU is mostly detected by screening the newborn babies for the increased plasma levels of phenylalanine (PKU, 20—65 mg/dl; normal

1—2 mg/dl). This is usually carried out by **Guthrie test,** which is a bacterial *(Bacillus subtilis)* bioassay for phenylalanine. The test is usually performed after the baby is fed with breast milk for a couple of days. All the babies born in USA are screened for PKU by testing elevated levels of phenylalanine. Phenylpyruvate in urine can be detected by **ferric chloride test** (a **green colour** is obtained). This test is **not specific**, since many other compounds give a false positive test.

5. Treatment of PKU. The maintenance of plasma phenylalanine concentration within the normal range is a challenging task in the treatment of PKU. This is done by selecting foods with **low phenylalanine content** and/or feeding synthetic amino acid preparations, low in phenylalanine. Dietary intake of phenylalanine should be adjusted by measuring plasma levels. Early diagnosis (in the first couple of months of baby's life) and treatment for 4—5 years can prevent the damage to brain. However, the restriction to protein diet **should be continued** for many more years in life. Since the amino acid tyrosine cannot be synthesized in PKU patients, it becomes essential and should be provided in the diet in sufficient quantity. In some seriously affected PKU patients, treatment includes administration of 5-hydroxytryptophan and dopa to restore the synthesis of serotonin and catecholamines.

Tyrosinemia

This disorder — also known as **Richner-Hanhart** syndrome, is due to a defect in the enzyme **tyrosine transaminase (2)**. The result is a blockade in the routine degradative pathways of tyrosine. Accumulation and excretion of tyrosine and its metabolites and tyramine are observed. Tyrosinemia is characterized by **skin** (dermatitis) and **eye lesions** and, rarely, **mental retardation**. A disturbed self-coordination is seen in these patients.

Alkaptonuria

Alkaptonuria has great historical importance. It was first described by Lusitanus in 1649 and characterized in 1859. Garrod conceived the idea of inborn errors of metabolism from his observation on alkaptonuria. The prevalance of this autosomal recessive disorder is 1 in 25,000.

1. Enzyme defect. The defective enzyme in alkaptonuria is **homogentisate oxidase (4)** n tyrosine metabolism (Fig. 19—5). Homogentisate accumulates in **tissues and blood**, and is excreted into urine. Homogentisate, on standing, gets oxidized to the corresponding quinones, which polymerize to give black or brown colour. For this reason, the urine of alkaptonuric patients resembles **coke in colour.**

2. Biochemical manifestations. Homogentisate gets oxidized by polyphenol oxidase to benzoquinone acetate which undergoes polymerization to produce a pigment called **alkapton.** Alkapton deposition occurs in connective tissue, bones and various organs (nose, ear etc.) resulting in a condition known as **ochronosis.** Many alkaptonuric patients suffer from **arthritis** and this is believed to be due to the deposition of pigment alkapton (in the joints), produced from homogentisate.

3. Diagnosis. Change in colour of the urine on standing to brown or dark has been the simple traditional method to identify alkaptonuria. The urine gives a positive test with ferric chloride and silver nitrate. This is due to the strong reducing activity of homogentisate. Benedict's test — employed for the detection of glucose and other reducing sugars — is also positive with homogentisate.

4. Treatment. Alkaptonuria is not a dangerous disorder and, therefore, does not require any specific treatment. However, consumption of protein diet with relatively low phenylalanine content is recommended.

Tyrosinosis or tyrosinemia

This is due to the deficiency of the enzyme **maleylacetoacetate isomerase (6).** Tyrosinosis is a **rare** but serious disorder. It causes liver **failure, rickets**, **renal tubular dysfunction and polyneuropathy**. Tyrosine, its metabolites and many other amino acids are excreted in urine. In acute tyrosinosis, the infant exhibits diarrhea, vomiting and 'cabbage-like' odor. Death may even occur due to liver failure within one year. For the treatment, diets low in tyrosine, phenylalanine and methionine are recommended.

Albinism *(Greek:* albino — white) is an inborn error, due to the lack of synthesis of the pigment melanin. It is an autosomal recessive disorder with a frequency of 1 in 20,000.

1. Biochemical basis. The color of skin and hair is controlled by a large number of genes. About 150 genes have been identified in mice. The melanin synthesis can be influenced by a variety of factors. The most common cause of albinism is a defect in **tyrosinase,** the enzyme most responsible for the synthesis of melanin (Fig. 19.5).

2. Clinical manifestations. The most important function of melanin is the protection of the body from sun radiation. Lack of melanin in albinos makes them sensitive to sunlight. Increased susceptibility to **skin cancer** (carcinoma) is observed. **Photophobia** (intolerance to light) is associated with lack of pigment in the eyes. However, there is no impairment in the eyesight of albinos.

Neonatal tyrosinemia

The absence of the enzyme **p-hydroxyphenylpyruvate dioxygenase** (Fig.19—5) causes neonatal **tyrosinemia**. This is mostly a temporary condition and usually responds to ascorbic acid. It is explained that the substrate inhibition of the enzyme is overcome by the presence of ascorbic acid.

DISORDERS OF SULFUR-CONTAINING AMINO ACIDS

Cystinuria. Cystinuria is one of the most common inherited diseases with a frequency of 1 in 7000. It is primarily characterized by increased excretion of cystine (25—40 times normal). Elevation in the urinary output of lysine, arginine and ornithine is also observed. In cystinuria, the carrier system for the reabsorption of cysteine becomes **defective** leading to the excretion of all these four amino acids in urine.

Cysteine is relatively insoluble and its increased concentration leads to its precipitation to its precipitation and formation of cystine **stones** in **kidney** and **urinary tract**. Cysteinuria is usually identified in the laboratory by cyanide nitroprusside test. The treatment includes restricted ingestion of dietary cysteine and high intake of fluid.

Cystinosis. Cysteine crystals are deposited in many tissues and organs of reticuloendothelial system throughout the body. These include spleen, lymph nodes, liver, kidney, bone marrow etc. Impairment in renal function is commonly seen in cystinosis. It is characterized by generalized amino aciduria. The affected patients die usually within 10 years, mostly due to renal failure.

Homocystinurias. Homocystinurias are a group of metabolic disorders characterized by the accumulation and increased urinary excretion of homocysteine and S-adenosylmethionine. Accumulation of homocysteine results in various complications — thrombosis, osteoporosis and, very often, mental retardation.

LECTURE 20

STRUCTURE OF NUCLEIC ACIDS

Nucleic acids are polymers of nucleotides. Nucleic acids are responsible for the storage and passage of the information needed for the production of proteins.

Types of nucleic acids

Nucleic acids are found in two basic structural forms: **deoxyribonucleic acid** (DNA) and **ribonucleic acid** (RNA). Each plays a different role in the storage and passage of cellular information.

1. Role of DNA. In most organisms DNA serves as the genetic material.

2. Role of RNA. RNA plays multiple roles.

a. RNA serves as the **genetic material** for some viruses (e.g., tobacco mosaic virus, poliovirus, and influenza virus).

b. RNA serves as the **carrier of genetic information** to the site of protein synthesis.

c. RNA forms the crucial **link between messenger RNA and amino acids** being coupled in protein synthesis.

d. RNA is an essential component of **ribosomes** and some enzymes. In fact, RNA can have catalytic activity without interacting with proteins.

Nucleotide structure (Fig. 20-1). Nucleic acids, both DNA and RNA, are polymers of **nucleotides (nucleoside monophosphates).** Each nucleotide consists of a pentose sugar, a nitrogenous base, and a phosphate group.

1. A pentose sugar is a five-carbon sugar in a pentose ring form. DNA and RNA have different sugar moieties.

a. Ribose sugar. RNA nucleotides, or **ribonucleotides,** contain ribose sugars, which have a hydroxyl group in both the 2' and 3' position of the sugar ring.

b. Deoxyribose sugar. DNA nucleotides, or **deoxyribonucleotides,** have 2'-deoxyribose sugars. These sugars have only a single hydroxyl group, in the 3' position of the sugar ring.

2. Base. A nitrogenous base is attached by a glycosidic bond to the 1' carbon atom of the nucleotide's sugar. The bases of nucleic acids are of two structural types (see Fig. 20—1).

a. Purines consist of linked five-membered and six-membered rings. There are two purines commonly found in nucleic acids: **adenine** (A) and **guanine** (G). Each can be found in DNA or RNA.

b. Pyrimidines consist of six-membered rings. There are three pyrimidines commonly found in nucleic acids: **cytosine (C), thymine (T), and uracil** (U). Cytosines can be found in DNA or RNA. Thymines are found in DNA. Uracils are found in RNA.

3. Phosphate. A nucleotide contains a single phosphate group, which is a strong acid. The phosphate can be attached through the oxygen of a hydroxyl at either the 5' or 3' position of the sugar. It is more commonly attached to the 5' position.

4. The nomenclature of nucleotides and nucleosides is presented in Table 20—1. A nucleoside is the term for a sugar and a base (see Figure 6-1). From one to three phosphates can be attached to nucleosides to form nucleoside mono-, di-, or triphosphates (see Fig. 20—1). A nucleoside monophosphate can also be called a nucleotide.

Table 20—1.

Nomenclature of nucleotides and nucleosides

Base	Purines		Pyrimidines	
	Adenine (A)	**Guanine (G)**	**Cytosine(C)**	**Uracil (U) (Thymine [T])**
Nucleosides	Adenosine Deoxyadenosine	Guanosine Deoxyguanosine	Cytidine Deoxycytidine	Uridine Deoxythymidine
Nucleotides	Adenylate (AMP) Deoxyadenylate (dAMP)	Guanylate (GMP) Deoxyguanylate (dGMP)	Cytidylate (CMP) Deoxycytidylate (dCMP)	Uridylate (UMP) Thymidylate (dTMP)

Primary polymeric structure (Fig. 20—2)

1. Linkage of nucleotides. Nucleotides are linked (in DNA and RNA) together by **phosphodiester bonds** between the 3' hydroxyl on the sugar of one nucleotide through a phosphate molecule to the 5' hydroxyl on the sugar of another nucleotide. The sugar-phosphate linkages form a symmetrical "backbone," with the 5' end of one sugar always linked through a phosphate molecule to the 3' end of the adjacent sugar. The bases are variable and stick out from the backbone. The order of bases determines the coding or structural capacity of the nucleic acid.

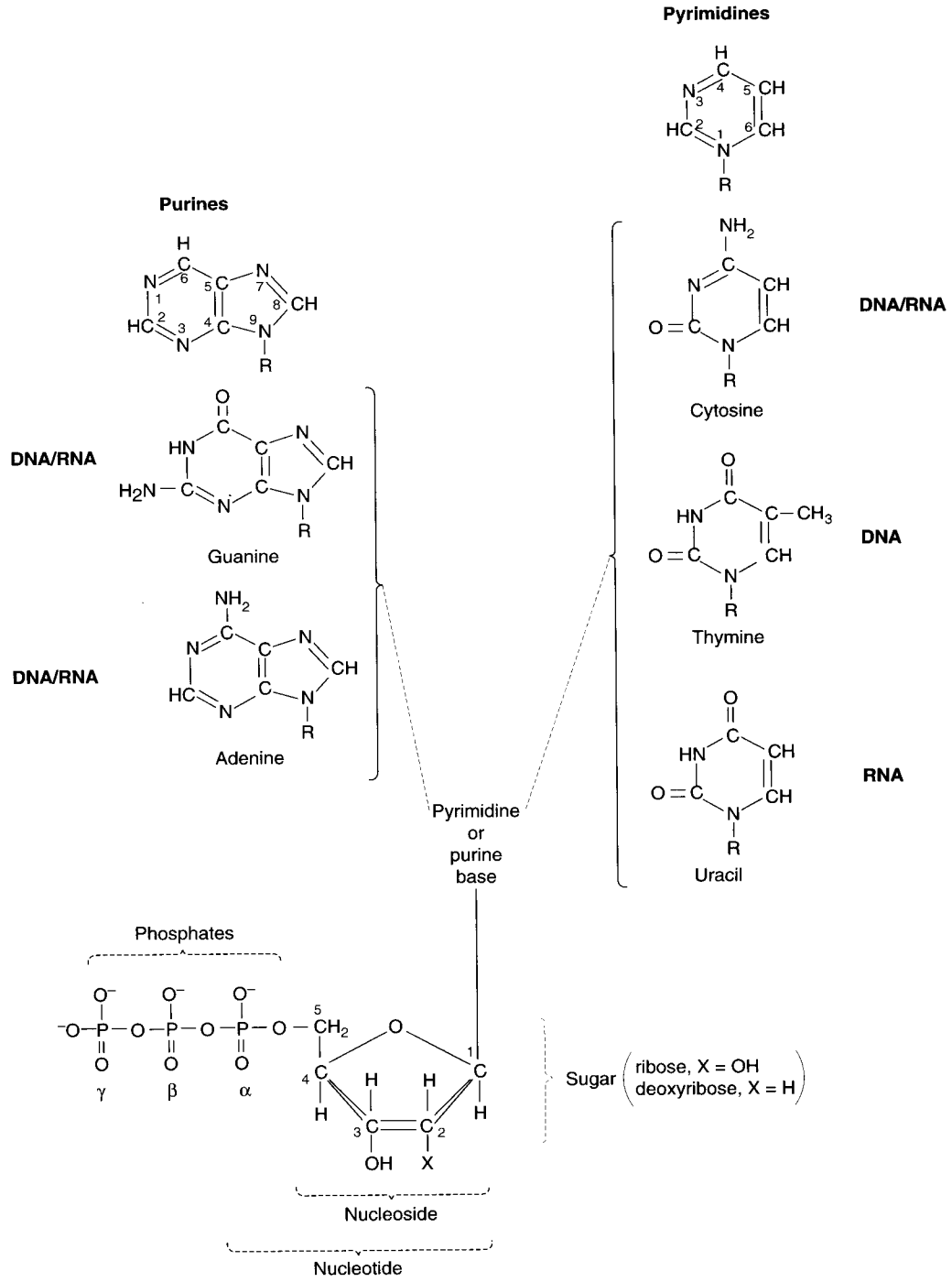


Figure 20—1. Nucleotide structure (by Davidson V.L. et al., 1999).

2. Polarity. The symmetry of the sugar-phosphate backbone imparts a polarity to nucleic acid polymers. The terminal nucleotide of one end usually has a free 3'-hydroxyl group on its sugar moiety and the other end usually has a free phosphate group attached to the 5' position of the sugar.

3. Notation. It is standard to use the one-letter abbreviation for the bases (see Table 6—1) when writing the order of a nucleic acid polymer. By convention, DNA sequences are written in the 5' to 3' direction. The sequence of the nucleic acid presented in Figure -2 is written as CCA.

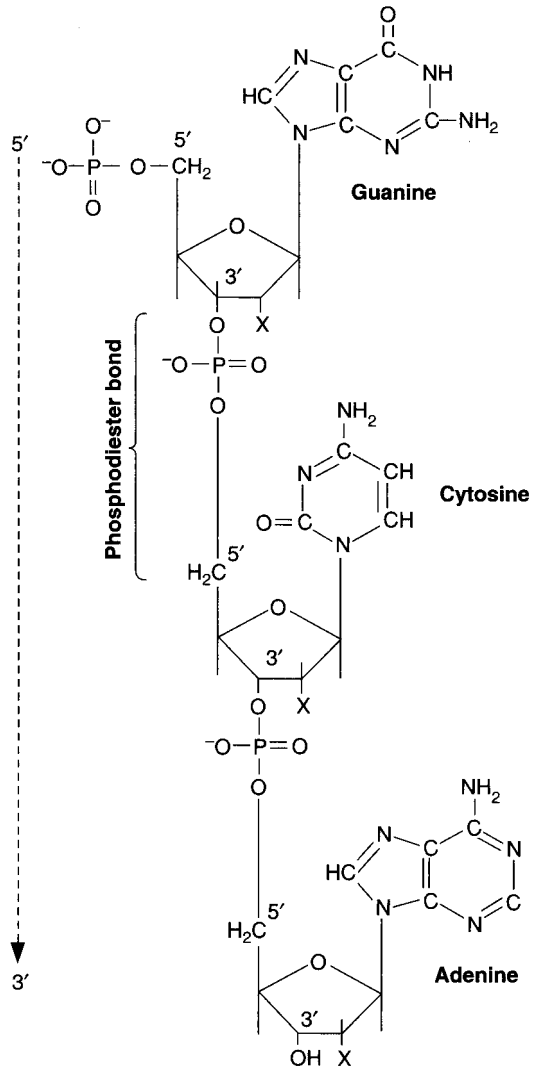


Figure 20—2. Structure of a trinucleotide. The bases of the three linked nucleotides are guanine, cytosine, and adenine. If *X* is OH, then the sugar is ribose, and the structure is RNA. If *X* is H, then the sugar is deoxyribose, and the structure is DNA (by Davidson V.L. et al., 1999).

DNA secondary structure

Double-helical (B-form) DNA (Fig. 20—3). In 1953, Watson and Crick, using x-ray diffraction data of Franklin and Wilkins, proposed a structure for DNA that became known as the **double helix.** It is now known that DNA can adopt different conformations; however, Watson and Crick's double helix is the predominant conformation and is now referred to as **B-form DNA.**

1. Two antiparallel strands form a right-handed helix. B-form DNA consists of two polymers, or strands, of DNA paired to each other and coiled around a common axis in a right-handed manner. Each strand has an opposite polarity to the other. That is, where one sugar-phosphate backbone has a 5' to 3' symmetry, the adjacent, paired strand is oriented oppositely in a 3'-to-5' direction. The two strands are said to be **antiparallel.**

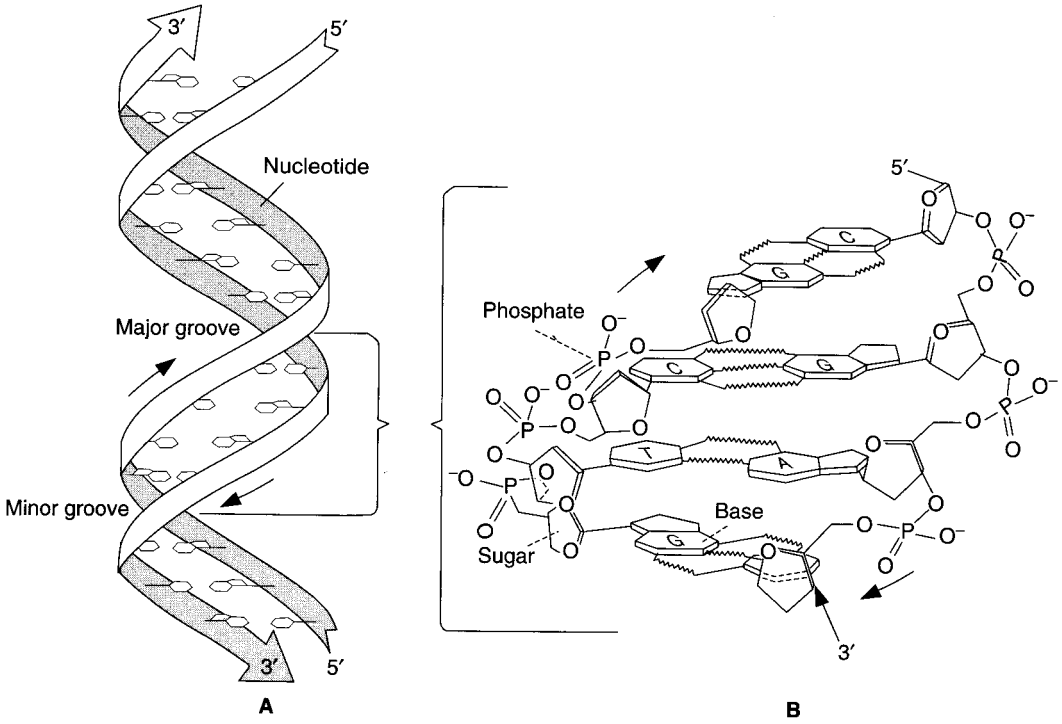


Figure 20—3. Structure of B-form DNA. *(A)* Two complementary strands of DMA base pair to form a double helix. The antiparallel sugar-phosphate backbones are presented as ribbons. *(B)* An expanded view of a subregion of the helix showing the paired bases and their position in relation to the sugar groups of the sugar-phosphate backbone (by Davidson V.L. et al., 1999).

2. Complementary base pairing. The two DNA strands of the double helix are held together by complementary base pairing. Specific hydrogen bonds can only form between complementary bases in a double helix (see Fig. 20—3). The purine adenine pairs with the pyrimidine thymine through two hydrogen bonds (AT), and the purine guanine pairs with the pyrimidine cytosine through three hydrogen bonds (GC). Therefore, in double-helical DNA, there are always the same number of adenine bases as thymine bases, and always the same number of guanosine bases as there are cytosine bases.

3. Base stacking. The complementary base pairs lie inside the helix, perpendicular to the sugar-phosphate backbone, which lies outside the helix. The base pairs inside the helix are stacked one above the other. The **hydrogen** bonding of the base pairs and the van der Waals interactions of the stacked base pairs provide the thermodynamic stability of the double helix.

4. Spiral staircase. Each base pair is offset approximately 36° from its neighboring base pairs. The helix therefore appears much like a spiral staircase in which **there are 10 steps or base pairs for each complete turn of the helix.**

5. Dimensions. The B-form double helix is 2 nm wide. There are 0.34 nm between each stacked base pair. A turn of the helix (10 base pairs) is therefore 3.4 nm long. From outside the helix two grooves are apparent: a **major groove** and a **minor groove**. It is through these grooves that many drugs and proteins can make contact with the bases without requiring the helix to open.

Alternate structural forms of DNA

In the aqueous environment of the cell, DNA is a dynamic structure that can bend and adopt many alternate structures. B-form DNA remains the predominant form of duplex DNA in the cell, although in the cell it is believed to be a slightly more tightly compacted molecule. Other structures of DNA have been described and are believed to be present in the cell, at least for short distances. Two well-described structures are A-form DNA and Z-form DNA.

1. A-form DNA is more compact than B-form DNA, with 11 base pairs per turn. This structure is probably the structure that double-stranded RNA adopts.

2. Z-form DNA is dramatically different from B-form or A-form DNA. It is a left-handed helix and is more elongated than B-form DNA. There are 12 base pairs perturn. Z-form DNA only occurs in sequences of alternating purines and pyrimidines. In particular, if the cytosine residues in a stretch of alternating guanines and cytosines are methylated, then Z-form DNA can exist under physiologic conditions.

Dissociation (denaturation) and reassociation (renaturation) of DNA

Double-helical strands of DNA have a remarkable ability to dissociate from one another and to reassociate again. This behavior is essential to the process of **replication** and **transcription**.

1. Denaturation. The hydrogen bonds that hold the two strands of a double helix together can be broken with an **increase in temperature (melting)** or by **treatment with alkali.** When all the hydrogen bonds holding the two strands together are broken, the strands separate, or denature.

2. Hyperchromic effect. DNA absorbs ultraviolet light maximally at the wavelength of 260 nm. The absorption of light at 260 nm by DNA increases upon denaturation. This is called the hyperchromic effect.

3. Renaturation. Denaturation is reversible. The process whereby denatured, complementary strands of DNA can reform a duplex DNA structure is called renaturation or **annealing.**

4. Hypochromic effect. As single-stranded, complementary DNA reforms a duplex structure, its absorbance at 260 nm decreases. This is called the hypochromic effect.

Higher order (tertiary) structure of DNA

The DNA in a single human cell, if stretched to its full length, is 1.74 meters. Clearly, to get DNA into a cell's nucleus, it must be packaged into a more tightly compacted form. The structural flexibility of DNA allows it to adopt more compacted structures than simple linear B-form DNA.

Supercoils

1. Description. Studies of circular DNA have shown that it can be twisted into a compact supercoiled or superhelical form. DNA supercoils can be either **right-handed (positive) or left-handed (negative).**

a. Positive supercoils are twisted in the same direction as the right-handed helix of B-form DNA about its axis, whereas **negative supercoils** are twisted in the opposite direction.

b. Supercoils have a **higher free energy** than nonsupercoiled, "relaxed" DNA.

2. Supercoils exist naturally. Bacterial genomic DNA and plasmid DNA are supercoiled. Eukaryotic DNA also becomes supercoiled as it becomes packaged into higher order chromatin structures. Most of these naturally occurring supercoils are negative.

Chromatin

In humans, DNA is divided and packaged into 46 separate structures known as chromosomes, which during mitosis are visible with a light microscope. The packing of DNA in a mitotic chromosome represents a 10,000-fold shortening of its length from primary B-form DNA. During interphase, when DNA needs to be accessible to the transcription and replication enzymes, it is packaged less densely than in mitotic chromosomes, in a structure known as **chromatin.** Chromatin was originally detected by its ability to be stained with a variety of microscopy stains. The DNA of chromatin is packaged with approximately double its mass of protein.

1. Histones. The major class of proteins associated with chromatin is the histones, which exist in a mass approximately equal to DNA in the chromatin.

a. Histones are **small (11,000—21,000 MW), basic proteins** that bind to the acidic DNA by noncovalent interactions to form nucleosomes.

b. There are **five types of histones:**

(1) The **four core histones: H2a, H2b, H3,** and **H4**

(2) The **one linker histone: H1**

c. In many eukaryotes there are **amino acid sequence variants of all of the histones except H4.** These variants likely have slightly different functional roles in chromatin.

d. All of the **histones are post-translationally modified** (e.g., phosphorylated, methylated, acetylated) at various stages of the cell cycle. These modifications are expected to play a functional role in changing the structure of chromatin. However, the specific role of each type of modification is not yet known.

2. Nucleosomes. If chromatin is placed in a low salt buffer and viewed with an electron microscope, it resembles a "string of beads." The repeating beads-on-a-string structure is also called the **10-nm fiber,** based on measurements of its width, and represents a sevenfold shortening of linear, B-form DNA. The repeating, bead-like structures are the **basic packaging unit of chromatin** called nucleosomes, and they occur an average of once for every 200 base pairs of DNA.

a. Core particles (Fig. 20—5). The fundamental **structural feature of nucleosomes** is the core particle, the structure of which has been determined by x-ray crystallography. It consists of two each of the core histones, which form a disc that is wrapped, in two turns, by 146 base pairs of DNA.

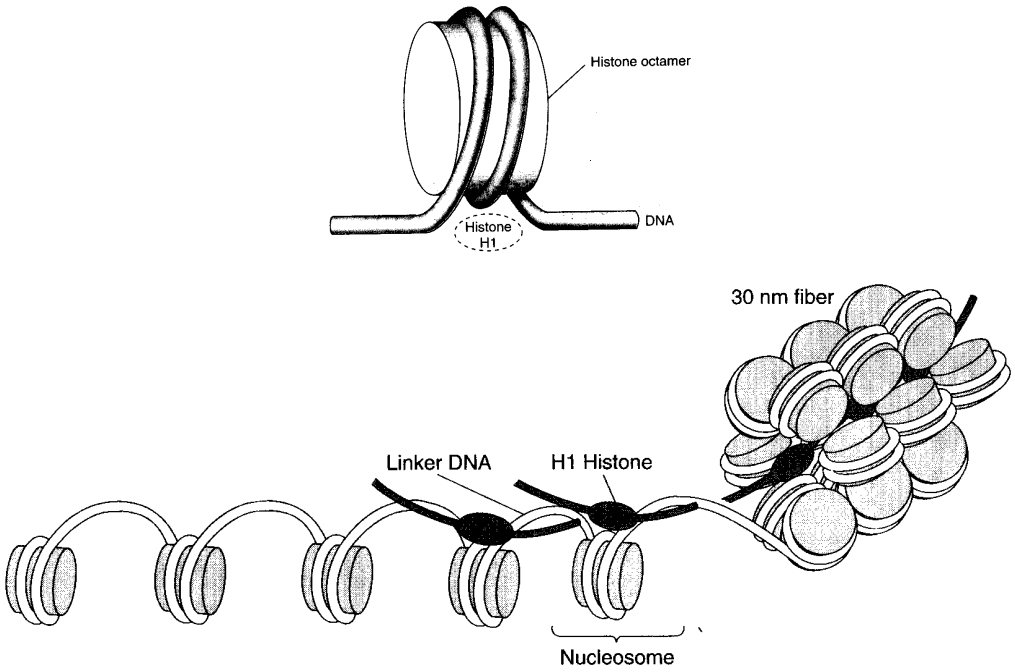


Figure 20—5. Illustration of nucleosomes (by Davidson V.L. et al., 1999).

b. Linker DNA. The amount of DNA wrapped around core particles is constant. However, the **DNA that connects core particles,** called linker DNA, is variable in length. Linker DNA averages 60 base pairs in length; however, depending on the organism and tissue, its length ranges from 8—120 base pairs.

c. The histone H1 binds to core particles at the point where DNA enters and exits the core particle (see Fig. 20—5). Histone H1 may sometimes be present in nucleosomes. Its presence in nucleosomes is **required for DNA to be packaged in a higher order form than nucleosomes.**

3. The 30-nm fiber. The next level of packaging leads to a 40-fold shortening of B-form DNA. The formation of 30-nm fibers requires the presence of the histone H1. The exact structure of the 30-nm fiber is not known but it is believed to look much like a **solenoid** (see Fig.20—5).

4. Higher-order chromatin structure. Few details are known of how DNA is condensed and packaged beyond the 30-nm fiber to its ultimate packaged form in mitotic chromosomes. It is, however, known that all DNA is not packaged equally. There are at least three forms of packaged DNA in most cells.

a. Heterochromatin. Portions of chromatin stain darkly and represent **densely packaged DNA.** These DNA regions, called heterochromatin, are thought to be **transcriptionally inactive** and do not appear to be unpackaged from their mitotic chromosome form.

b. Euchromatin. Portions of the chromatin stain poorly and are not tightly packaged like heterochromatin. These portions of the chromatin are thought to be **transcriptionally active** and are called euchromatin.

c. Loop domains. There is some evidence that DNA is attached to a proteinaceous matrix, called a **scaffold** in mitotic chromosomes, or a **nuclear matrix** in interphase nuclei. The DNA projects out as **loops** from these matrices, which are believed to contain active genes.

RNA

Three functionally distinct classes of RNA are produced in prokaryotes, and four are produced in eukaryotes.

1. Messenger RNA (mRNA) carries information from genes to ribosomes, where it is translated into proteins.

a. Most **prokaryotic** mRNAs are **polycistronic.** That is, they carry the information for the production of multiple polypeptides. Not all portions of prokaryotic mRNA code for polypeptides, Messenger RNA accounts for only **5%** of the total cellular RNA in prokaryotes. The **lifetime** of prokaryotic mRNA is **short**. Most mRNAs are stable for just a **few minutes**.

b. Eukaryotic mRNA is monocistronic; that is, it carries only the information for the production of a **single** polypeptide. Most, but not all, eukaryotic mRNAs arise by extensive post-transcriptional processing of large precursors. This processing occurs during the passage of the sequences that become mRNA from the nucleus to the cytoplasm. These large precursors of mRNA are called **heterogeneous nuclear RNA (hnRNA).** Eukaryotic mRNA accounts for **3%** of the total cellular RNA. Its precursor, **hn**RNA, accounts for **7%** of the total cellular RNA. Compared with prokaryotic mRNA, most eukaryotic mRNAs are relatively stable and exhibit half-lives on the order of hours to days.

2. Transfer RNAs (tRNAs) serve to transfer amino acids to the ribosomes and to facilitate the incorporation of the amino acids into newly synthesized proteins in a template-dependent manner. For each amino acid, there is one or more specific tRNA.

a. Prokaryotic tRNA. Transfer RNAs are small RNAs with an average size of 80 nucleotides. All tRNAs have common structural features that allow them to function in the ribosome. They also have unique structural features that are necessary for recognition by the enzymes that catalyze the attachment of amino acids to tRNAs. The sequences that pair with the appropriate codons in the ribosome are also unique for each tRNA. All tRNAs arise from the processing of large precursor tRNA. The tRNAs account for **15%** of the total cellular RNA in prokaryotes.

b. Eukariotic tRNA. Eukariotic tRNA are very similar to prokariotic tRNAs in size and in structural features. The total amount of small RNAs in the eukaryotic cell, of which the tRNAs are the most abundant, is 15%.

c. A secondary structure tRNA appears like a **cloverleaf**. All tRNA molecules contain four main arms (Fig. 20—6).

The **acceptor arm** consists of a base-pair stem that terminates in the sequence CCA (5’ to 3’).It is through an ester bond to the 3’-hydroxyl group of the

adenosyl moiety that the carboxyl groups of amino acids are attached. The **anti-codon arm** at the end of a base-paired stem recognized the triplet nucleotide or codon of the template m RNA. It has a nucleotide sequence complementary to the codon and is responsible for specificity of the tRNA. The **D** arm is named for the presence of the base dihydrouridine, and the **TψC** arm for the sequence T, pseudouridine, and C. The **extra arm** is most variable feature of tRNA, and it provides a basis for classification

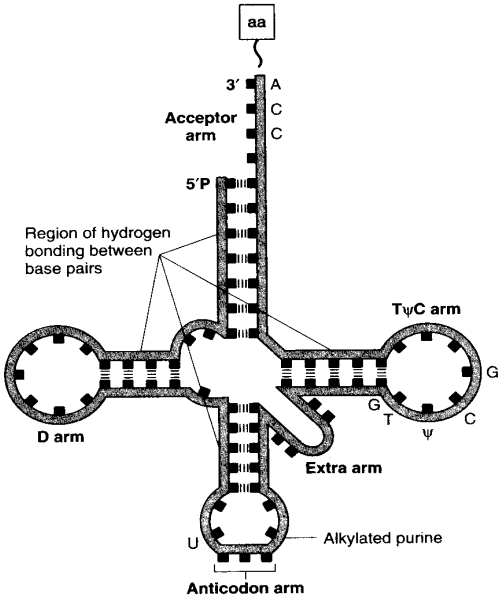


Figure 20—6. Secondary structure of tRNA (by Murray R.K. et al., 1996).

3. Ribosomal RNA (rRNA) comprises approximately **50%** of the mass of ribosomes. The function of rRNA is both structural as well as catalytic.

a. Prokaryotic rRNA. There are **three kinds of prokaryotic rRNA**: **23S** rRNA is a component of the large, 50S ribosomal subunit; **16S** rRNA is a component of the small, 30S ribosomal subunit; **5S rRNA** is a component of the large, 50S ribosomal subunit. Ribosomal RNAs are the most abundant RNA class. They account for 80% of the total cellular RNA in prokaryotes. Prokaryotic rRNA arise from the processing of a large 30S precursor rRNA.

b. Eukaryotic rRNA. The rRNAs of eukaryotes are typically bigger than those of prokaryotes. Also, **eukaryotes have four kinds of rRNA: 28S** rRNA is a component of the large, 60S ribosomal subunit; **18S rRNA** is a component of the small, 40S ribosomal subunit; **5.8**S rRNA is a component of the large, **60S** ribosomal subunit; **5S** rRNA is a component of the large, 60S ribosomal subunit. Similar to those of prokaryotes, three of the eukaryotic rRNAs arise from the processing of a large (45S) precursor rRNA. The **5S rRNA** is the transcription product of a separate gene. Approximately 4% of the total eukaryotic cellular RNA is 45S precursor rRNA, and 71% is fully processed rRNAs.

4. Small RNAs. In addition to tRNA, eukaryotes have numerous other small nuclear RNAs that participate in the splicing reactions needed to process hnRNA to mRNA.

LECTURE 21

METABOLISM OF PURINE AND PYRIMIDINE NUCLEOTIDES

METABOLISM OF PURINE NUCLEOTIDES

Synthesis of purine nucleotides

It should be remembered that are not synthesized as such, but they are formed as **ribonucleotides**. The purines ate build upon a pre-existing ribose-5-phosphate.

1. Many compounds contribute to the purine ring of the nucleotides. $\mathbf{N_1}$ of purine is derived from amino group of **aspartate**; $\mathbf{C_2}$ and $\mathbf{C_8}$ arise from **formate** of N^{10}-formyl tetrahydrofolate; $\mathbf{N_3}$ and $\mathbf{N_9}$ are obtained from amide group of **glutamine**; $\mathbf{C_4}$, $\mathbf{C_5}$ and $\mathbf{N_7}$ are contributed by **glycine**; $\mathbf{C_6}$ directly comes from $\mathbf{CO_2}$.

2. Liver is the major site for purine nucleotide synthesis; erythrocytes, polymorphonuclear leucocytes and brain cannot produce purines.

3. Ribose-5-phosphate, produced in the pentose phosphate pathway of carbohydrate metabolism is the starting material for purine nucleotide synthesis. It reacts with ATP to form phosphorybosyl pyrophosphate (PRPP) (Fig.21—1).

4. Glutamine transfers its amide nitrogen to PRPP to replace pyrophosphate and produce **5-phosphoribosylamine**. This reaction is the "**committed step**" in purine nucleotide biosynthesis.

5. Inosine monophosphate is synthesized.

6. Inosine monophosphate (IMP) is the immediate precursor for the formation of AMP and GMP. **Aspartate** condenses with IMP in the presence of **GTP** to produce **AMP**. For the synthesis of GMP, IMP undergoes NAD^+ dependent dehydrogenation and in present of **ATP** and **glutamine** the GMP is formed.

7. 6-Mercaptopurine is an inhibitor of the synthesis of AMP and GMP. It acts on AMP synthesis or GMP synthesis.

8. The nucleoside monophosphate (AMP and GMP) have to be converted to the corresponding di-and triphosphates to participate in most of the metabolic reactions. This is achieved by the transfer of phosphate group from ATP, catalyzed by nucleoside monophosphate kinases and nucleoside diphosphate kinases.

Inhibitors of purine synthesis

Folic acid (THF) is essential for the synthesis of purine nucleotides. **Sulfonamides** are the structural analogs of para-aminobenzoic acid (PABA). These sulfa drugs can be used to inhibit the synthesis of folic acid by microorganisms. This is indirectly reduces the synthesis of purines and, therefore, the nucleic acids (DNA and RNA). Sulfonamides have no influence on human, since folic acid is not synthesized and is supplied through diet.

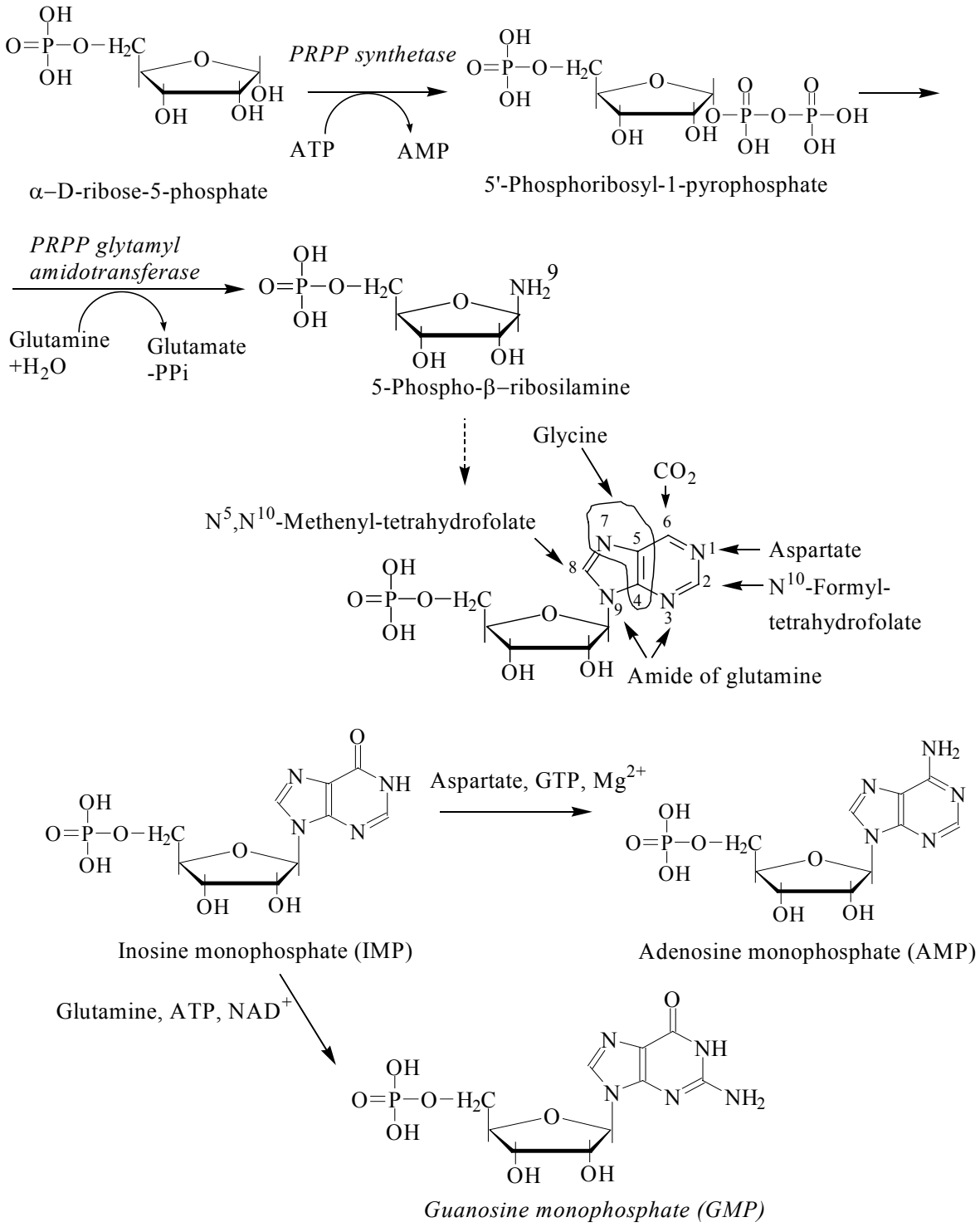


Figure 21—1. The main reactions of the pathways of de novo purine biosynthesis

The structural analogs of folic acid (e.g. **methotrexate**) are widely used to control cancer. They inhibit the synthesis of purine nucleotides and, thus, nucleic acids. These inhibitors also affect the proliferation of normally growing cells. This causes many side-effects including anemia, baldness, scaly skin etc.

Regulation of purine nucleotides synthesis

Synthesis of purine nucleotides is regulated by **feedback inhibition** (Fig. 21—2).

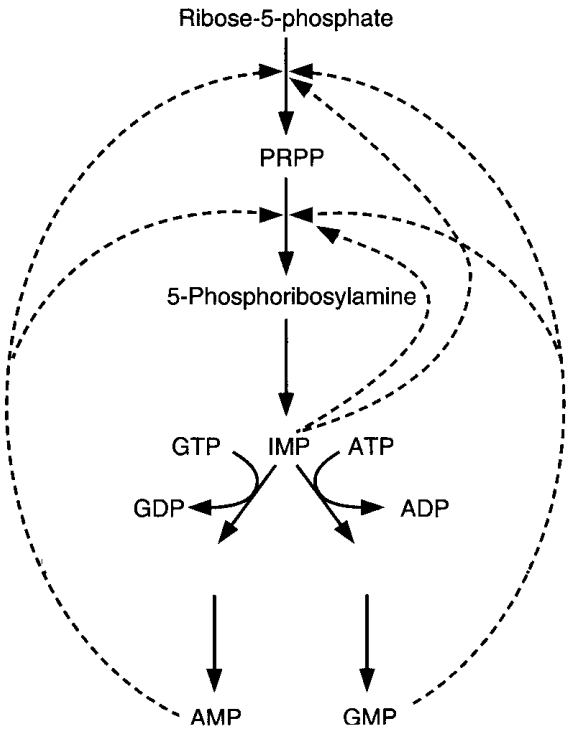


Figure 21—2. Regulation of synthesis of purine nucleotides.

1. Both of the **enzymes** that catalyze the first two steps of IMP synthesis — **PRPP synthetase and amidotransferase** — are inhibited by IMP, GMP, and AMP.

2. PRPP amidotransferase has two allosteric sites: one for IMP or GMP, and one for AMP. If both sites are occupied, then inhibition is synergistic.

3. Inhibition. The synthesis of AMP from IMP is inhibited by AMP, and the synthesis of GMP is inhibited by GMP.

Salvage pathways for purine nucleotides

There are two specific enzymes that catalyze the transfer of the ribose phosphate from PRPP to free purine bases, which are formed by the degradation of nucleotides.

1. Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) catalyzes the formation of nucleotides from either hypoxanthine or guanine. The enzyme is inhibited by high concentrations of its products, IMP and GMP.

$$\text{Hypoxanthine} + \text{PRPP} \rightarrow \text{IMP} + \text{PP}_i$$

$$\text{Guanine} + \text{PRPP} \rightarrow \text{GMP} + \text{PPi}$$

2. Adenine phosphoribosyltransferase (APRT) catalyzes the formation of AMP from adenine. The enzyme is inhibited by high concentrations of its product, AMP.

Degradation of purine nucleotides

The end product of purine metabolism in human is **uric acid**. The sequence of reactions in purine nucleotide degradation is given in Fig. 21—4.

1. The nucleotide monophosphate is converted to their respective nucleoside form (adenosine and guanosine) by action of **nucleotidase**.

2. The amino group from AMP can be removed to produce inosine.

3. Inosine and guanosine are, respectively, converted to hypoxanthine and guanine (purine bases) by **phosphorylase**. Adenosine is not degraded by this enzyme, hence it has to be converted to inosine.

4. Guanine undergoes deamination by guanase to form **xanthine**.

5. Xanthine oxidase is an important enzyme that converts hypoxanthine to xanthine, and xanthine to uric acid. This enzyme contains FAD, molybdenum and iron, and is exclusively found in liver and small intestine. Xanthine oxidase liberates H_2O_2 which is harmful to the tissues. Catalase cleaves H_2O_2 to H_2O and O_2.

6. Uric acid is the final excretory product of purine metabolism in humans. Uric acid can serve as an **important antioxidant**.

Disorders of purine metabolism

Uric acid is the end product of purine metabolism in human. The normal concentration of uric acid in the serum of adult is in the range of 3—7 mg/dl. The daily excretion of uric acid is about 500—700 mg.

Hyperuricemia refers to an **elevation in the serum uric acid** concentration.

Gout is a metabolic disease associated with **overproduction of uric acid**. At the physiological pH, uric acid is found in a more soluble form as sodium irate. In severe hyperuricemia, crystals of sodium urate get deposited in the soft tissues, particularly in the joints. Such deposits are commonly known as **tophi**. This causes inflammation in the joints resulting in a painful gouty **arthritis**. Sodium urate and/or uric acid may also precipitate in kidneys and ureters that result in renal damage and stone formation.

The **prevalence** of gout is about **3 per 1000 persons**, mostly affecting males. Gout is of two types — primary and secondary.

1. Primary gout. It is an inborn error of metabolism due to overproduction of uric acid. This is mostly related to increased synthesis of purine nucleotides. The hypoxanthine-guanidine phosphorybosyl transferase (HGPRT) is the important enzyme associated with primary gout. This is an enzyme of purine salvage pathway, and its defect causes **Lesch-Nychan syndrome**. It affects only the males and is characterized by **excessive uric acid production** (often gouty arthritis), and neurological abnormalities such as **mental retardation, aggressive behavior, learning disability** etc. The patients of this disorder have an **irresistible urge to bite their finger and lips, often causing self-mutilation.**

2. Secondary gout. Secondary hyperuricemia is due to various diseases causing increased synthesis or decreased excretion of uric acid. Increased degradation of nucleic acids (hence more uric acid formation) is observed in various cancers (leukemias, polycythemia, lymphomas, etc), psoriasis and increased tissue breakdown (trauma, starvation etc).

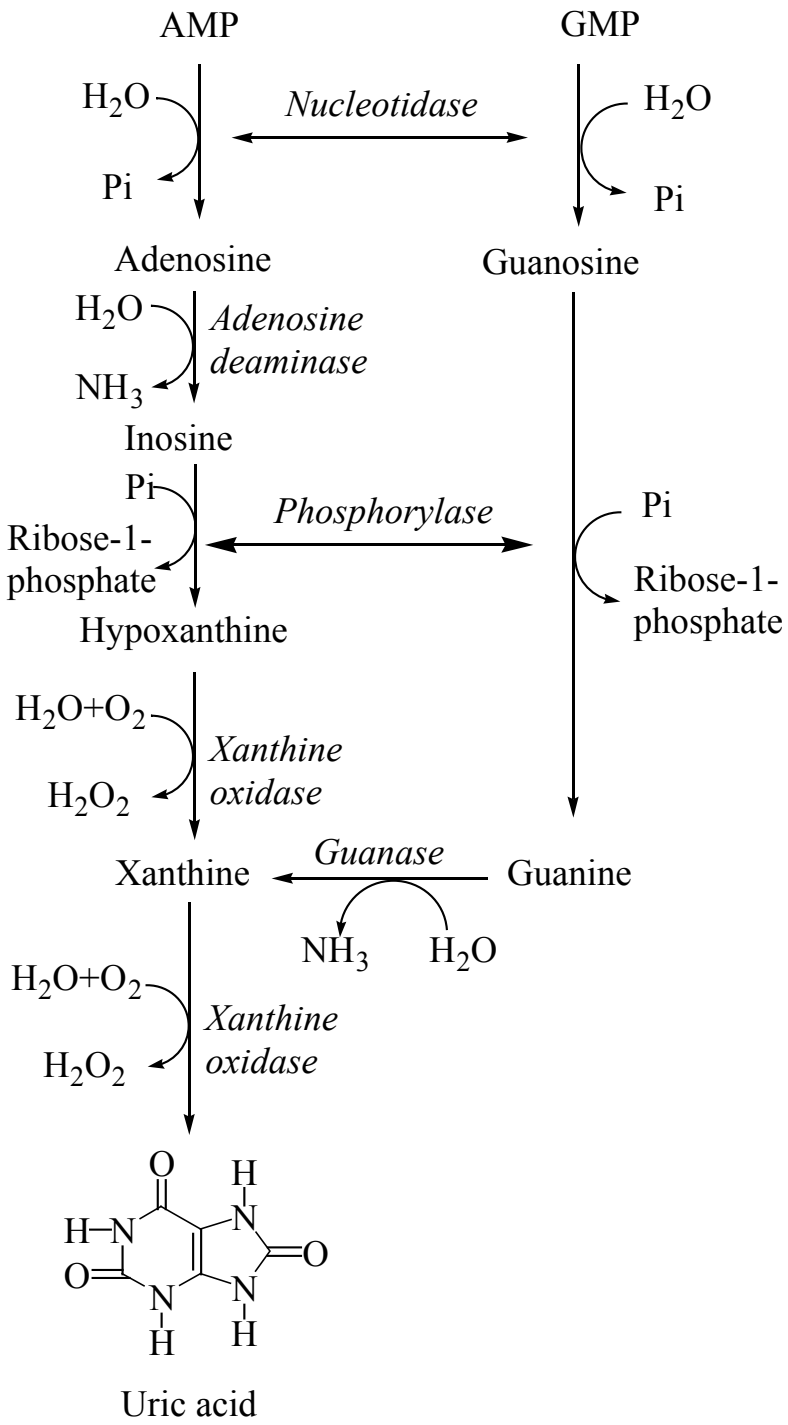


Figure 21—4. Degaradation of purine nucleotides to uric acid

3. Treatment of gout. The drug of choice for the treatment of primary gout is **allopurinol**. This is a structural analog of hypoxanthine that competitively inhibits the enzyme **xanthine oxidase**. Further, allopurinol is oxidized to alloxanthine by xanthine oxidase. Alloxanthine, in turn, is a more effective inhibitor of xanthine oxidase.

Inhibition of xanthine oxidase by allopurinol leads to the accumulation of hypoxanthine and xanthine. These two compounds are more soluble than uric acid, hence easily excreted.

Besides the drug therapy, restriction in dietary **intake of purines** and **alcohol** is **advised**. Consumption of plenty of water will also be useful.

The anti-inflammatory drug colchicine is used for the treatment of gouty arthritis. Other anti-inflammatory drugs such as phenylbutazone, indimethacine, oxyphenbutazone, corticosteroids — are also useful.

METABOLISM OF PYRIMIDINE NUCLEOTIDES

Synthesis of pyrimidine nucleotides

The synthesis of pyrimidines is a much simpler process compared to that of purines (Fig.21—3). **The precursors** of the ring are **glutamine, aspartate, and CO_2. Pyrimidine ring is first synthesized and then attached to ribose-5-phosphate.**

1. Carbamoyl phosphate is synthesized in the **cytosol** from glutamine and CO_2 by the enzyme **carbamoyl phosphate synthetase II.** This reaction is ATP-dependent. Carbamoyl phosphate also is synthesized in the **liver** as an intermediate in urea synthesis, but this synthesis takes place in the mitochondria and is catalyzed by a different enzyme, carbamoyl phosphate synthetase I.

2. Carbamoyl phosphate condenses with aspartate to form **carbamoyl aspartate**. This reaction is catalyzed by **aspartate transcarbamoylase**.

3. Dihydroorotase catalyses the pyrimidine ring closure with a loss of H_2O and dehydrogenase catalyses NAD^+-dependent dehydrogenation, leading to the formation of **orotate**.

4. Ribose-5-phosphate is now added to orotate to produce orotidine monophosphate (OMP). This reaction is catalyzed by orotate phosphoribosyltransferase.

5. OMP undergoes decarboxylation to **uridine monophosphate** (UMP).

By a ATP-dependent kinase reaction, UMP is converted to UDP which serves as a precursor for the synthesis of dUMP, dTMP, UTP and CTP. UDP undergoes an ATP-dependent kinase reaction to produce UTP.

6. An amino group from glutamine is donated to UTP to form cytidine triphosphate (CTP). This reaction is catalyzed by **CTP synthetase.**

7. Ribonucleotide reductase converts UDP to dUDP. **Thymidilate synthase** catalyses the transfer of a methyl group from N^5,N^{10}-methylen tetrahydrofolate to produce deoxythymidine monophosphate (dTMP).

Tetrahydrofolate is regenerated by **dihydrofolate reductase** in a reaction that requires NADPH.

Regulation of pyrimidine synthesis

1. The enzyme **aspartate transcarbamoylase**, which catalyses the committed step in pyrimidine synthesis **is inhibited by UTP and CTP**.

2. Carbamoyl phosphate synthetase is inhibited by UDP and UTP and it is activated by PRPP and ATP.

3. CTP feed back inhibits CTP synthetase.

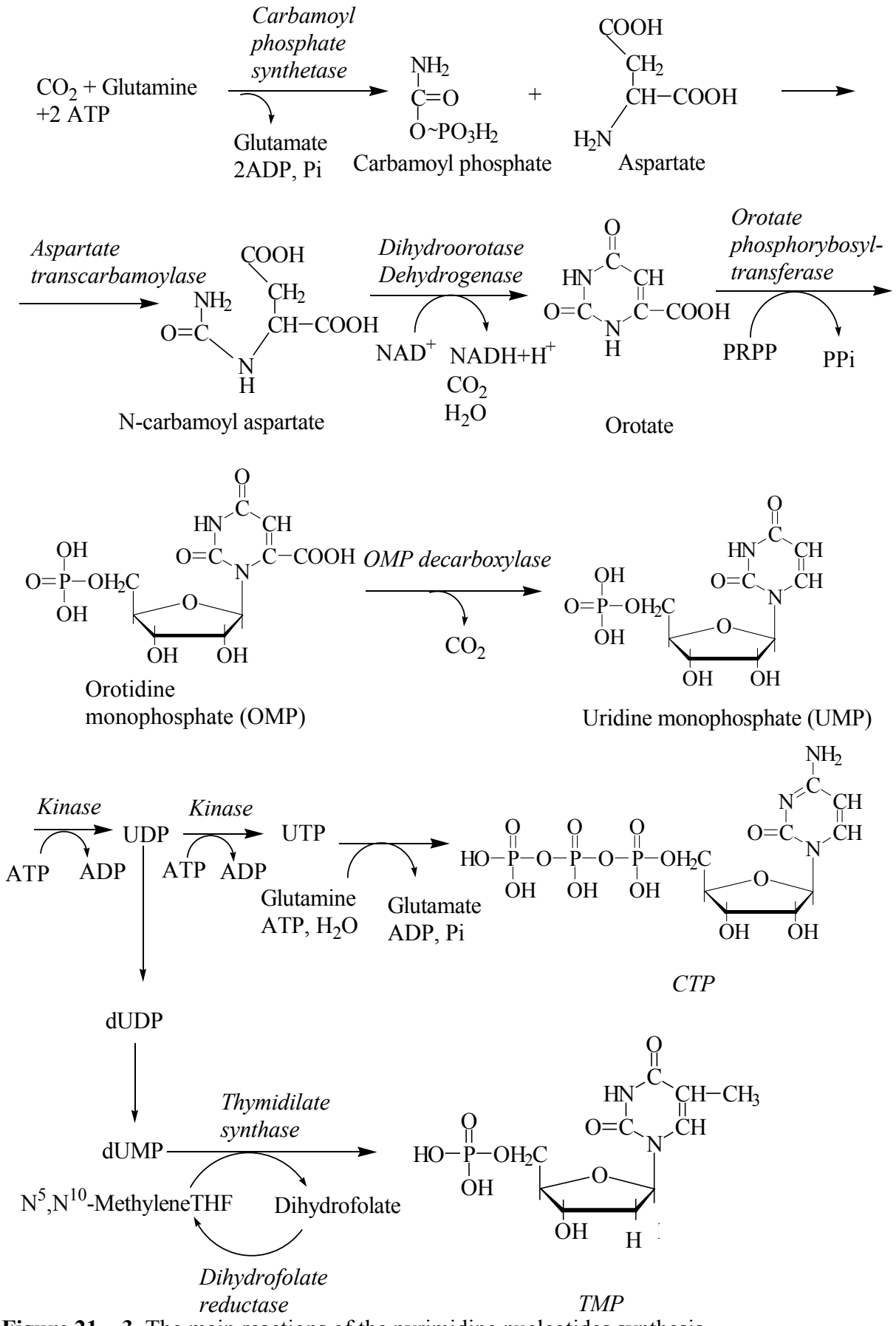


Figure 21—3. The main reactions of the pyrimidine nucleotides synthesis.

Degradation of pyrimidine nucleotides

The pyrimidine nucleotides undergo similar rections (dephosphorylation, deamination and cleavage of glycosidic bond) like that purine nucleotides to liberate the nitrogenous bases — cytosine, uracil and thymine. The bases are then degraded to highly soluble products — β-**alanine** and β-**aminoisobutyrate**. These are the amino acids which undergo transamination and other reactions to produce acetyl CoA and succinyl CoA.

Disorders of purimidine metabolism

1. Orotic aciduria. This is a rare metabolic disorder characterized by the excretion of orotic acid in urine, severe anemia and retarded growth. It is due to the deficiency of the enzymes **orotate phosphoribosyl transferase** and **OMP decarboxylase** of pyrimidine synthesis. Feeding **diet rich in uridine** and/or **cytidine** is an effective treatment for orotic aciduria.

2. Reye's syndrome. This is considered as a secondary aciduria. It is believed that a defect in ornithine transcarbamoylase (of urea cycle) causes the accumulation of carbamoyl phosphate.

LECTURE 22

NUCLEIC ACID METABOLISM. REPLICATION. DNA REPAIR. TRANSCRIPTION

Deoxyribonucleic acid (DNA) is a macromolecule that carries genetic information from generation to generation. It is responsible to preserve the identity of the species over millions of years. **DNA** may be regarded as a **reserve bank of information** or memory bank. A single mammalian fetal cell contains only a few picograms (10^{-12} g) of DNA. The **biological information flows from DNA to RNA and from there to proteins**. This is the **central dogma of life**. It is ultimately the DNA that controls every function of the cell through proteins synthesis. The word **genome** refers to the total **genetic information contained in the DNA**, in each cell of an organism.

REPLICATION OF DNA

Genetic material must be able to be **accurately replicated** and passed on from one generation to the next. Although elegant experiments indicated that DNA could carry genetic information from one generation to the next, not until Watson and Crick discovered the structure of DNA was it understood how DNA might be replicated. The **double-helical model** of DNA suggested that the **strands can separate and act as templates for the formation of a new, complementary strand.** However, the structure of DNA did not reveal whether the DNA was replicated conservatively or semiconservatively.

1. Conservative replication would occur if, after replication and cell division, the parental DNA strands remained **together** in one of the daughter cells, and the newly synthesized DNA strands went to the other daughter cell.

2. Semiconservative replication was shown in experiments by Meselson and Stahl to be the mechanism by which replication takes place. After replication and cell division, each daughter cell receives one parental DNA strand and one newly synthesized complementary strand for which the parental strand was the template.

The mechanism of replication in **prokaryotes** is much better understood than in eukaryotes. The **basic requirements** and **components** of replication are the **same** for prokaryotes as for eukaryotes. Therefore, an understanding of how prokaryotes replicate provides much insight into the understanding of how eukaryotes replicate.

Basic requirements for DNA synthesis

1. Substrates. The four deoxynucleoside triphosphates (dNTPs) — deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP), and deoxythymidine triphosphate (dTTP) are needed as substrates for DNA synthesis. Cleavage of the high-energy phosphate bond between the α and β phosphates provides the energy for the addition of the nucleotide.

2. Template. DNA replication cannot occur without a **template**. A template is required to direct the addition of the appropriate complementary deoxynucleotide to the newly synthesized DNA strand. In semiconservative replication, **each strand of parental DNA** serves as a template. Then, each template strand and its newly synthesized complementary strand serve as the DNA in daughter cells.

3. Primer. DNA synthesis cannot start without a primer, which prepares the template strand for the addition of nucleotides. Because new nucleotides are added to the 3' end of a primer that is properly base paired to the template strand of DNA, **new synthesis is said to occur in a 5' to 3' direction. Primosome** is a complex of proteins primes DNA synthesis at the origin. Driven by ATP hydrolysis, the primosome moves with the replication fork, making primer (RNA fragment).

4. Enzyme. The DNA synthesis that occurs during the process of replication is catalyzed by enzymes called **DNA-dependent DNA polymerases.** These enzymes depend on DNA to the extent that they require a DNA template. They are more commonly called DNA polymerases. The bacteria *Escherichia coli* contains three separate DNA polymerases.

a. DNA polymerase I (pol I) functions in the replication of DNA and in the repair of damaged DNA. Pol I has a **3' to 5'-exonuclease activity** and **5' to 3' exonuclease activity.**

b. DNA polymerase II (pol II) is a minor DNA polymerase in *E.coli.* Pol II may be involved in some DNA repair processes.

c. DNA polymerase III (pol III) is the primary DNA polymerase involved in cellular replication. Pol III catalyzes leading and lagging strand synthesis. Pol III holoenzyme has **3' to 5' exonuclease** activity but no excision-repair (5' to 3' exonuclease) activity.

It is believed that **all the replication enzymes and factors** are part of a large macromolecular complex called a **replisome**. It has been suggested that the replisome may be attached to the membrane and that instead of the replisome moving along the DNA during replication, DNA is passed through the stationary replisome.

Origin of replication

In prokaryotes, replication starts at particular DNA sequences called **origins** (an origin is often called by its genetic abbreviation, **ori).**

1. OriC is the single origin in *E. coli.* OriC is a sequence of approximately 240 base pairs, which is required to direct the initiation of replication that takes place within the oriC region.

2. dnaA protein is specific protein in *E.coli* that is required for proper initiation of replication at the origin. It **binds to specific sequences within oriC**, and in the presence of ATP and the other components of replication, dnaA protein **facilitates initiation** of replication.

Basic molecular events at replication forks (Fig. 22—1)

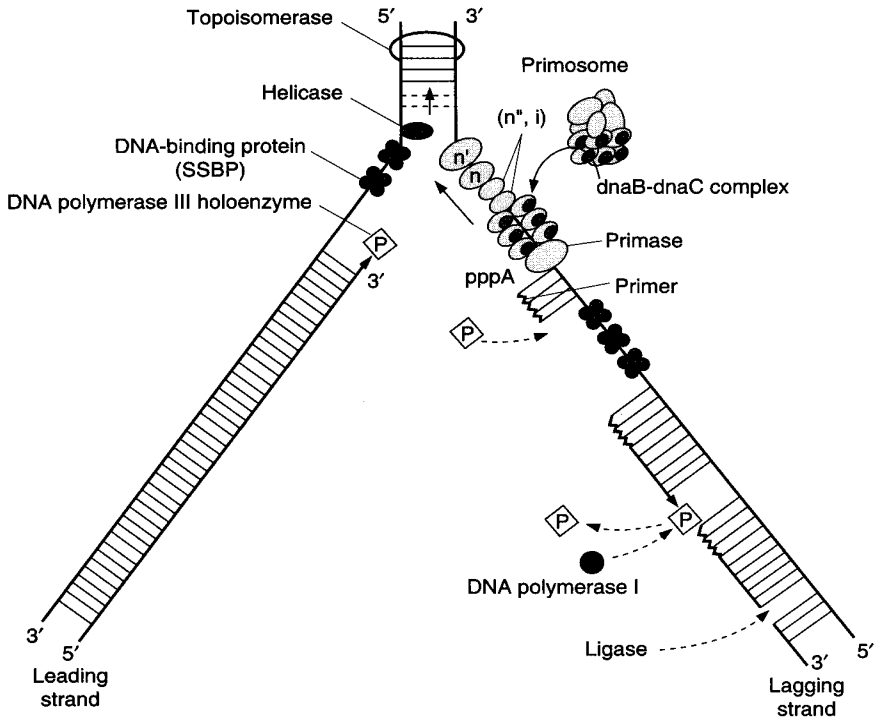


Figure 22—1. Schematic diagram of the basic molecular events at the replication fork of Escherichia coli (by Davidson V.L. et al., 1999).

1. Helicases are enzymes that catalyze the unwinding of the DNA helix. A helicase derives energy from cleavage of high-energy phosphate bonds of nucleoside triphosphates, usually **ATP**, to unwind the DNA helix. **Helicase activity provides single-strand templates for replication. The dnaB protein** is the principal helicase of E.*coli* replication and is a component of the primosome.

2. Positive supercoils would build up in advance of a moving replication fork without the action of **gyrase**, which is a topoisomerase.

3. Single-strand binding protein (SSBP) is an important component of replication. As its name implies, it binds to single-stranded DNA. SSBP **enhances the activity of helicase and binds to single-strand template** DNA until it can serve as a template. SSBP may serve to protect single-strand DNA from **degradation by nucleases**, and it may block formation of intrastrand duplexes of hairpins that can slow replication. SSBP is displaced from single-strand DNA when the DNA undergoes replication.

4. After initiation, replication has been observed proceeding away from the origin. In most organisms, replication proceeds **bidirectionally** from the origins as **replication forks**. Replication forks represent unwound parental template DNA strands to which newly synthesized complementary DNA is paired. As the replication fork moves forward, leading strand synthesis follows. A gap forms on the **opposite strand** because it is in the wrong orientation to direct continuous synthesis of a new strand. After a lag period, the gap that forms is filled in by 5' to 3' synthesis. This means that new DNA synthesis on the lagging strand is actually moving away from the replication fork.

5. Leading strand synthesis is the continuous synthesis of one of the daughter strands in a 5' to 3' direction. **Pol III catalyzes leading strand synthesis.**

6. Lagging strand synthesis. One of the newly synthesized daughter strands is made **discontinuously.** The resulting short fragments are called **Okazaki fragments**. An enzyme called **primase** is the catalytic portion of a **primosome** that makes the RNA primer needed to initiate synthesis of Okazaki fragments. It also makes the primer that initiates leading strand synthesis at the origin. **Primers** provide a 3'-hydroxyl group that is **needed to initiate DNA synthesis**. The primers made by primase are small pieces of RNA (4—12 nucleotides) complementary to the template strand. Discontinuous synthesis of the lagging strand occurs because DNA synthesis always occurs in a 5' to 3' direction. **Pol III catalyzes strand synthesis. The RNA primer is then removed by pol I and replaced with DNA.** Synthesis of each new Okazaki fragment takes place **until it reaches the RNA primer** of the preceding Okazaki fragment. This effectively leaves a nick between the newly synthesized Okazaki fragment and the RNA primer.

7. Joining of Okazaki fragments. After Pol I has removed the RNA primer and replaced it with DNA, an enzyme called **DNA ligase** catalyzes the formation of phosphodiester bonds between the adjoining fragments by the following reaction.

Termination of replication. Termination sequences (e.g., ter) direct termination of replication. A specific protein — the termination utilization substance (Tus) **protein** — binds to these sequences and prevents the helicase dnaB protein from further unwinding DNA. This facilitates the termination of replication.

EUKARYOTIC REPLICATION

Eukaryotes represent a diversity of organisms that may utilize slightly different mechanisms of replication. However, most of these mechanisms are very similar to those in prokaryotic replication. This section reviews replication only in mammalian cells. As with prokaryotic replication, mammalian replication is semiconservative and proceeds **bidirectionally from many origins.**

Replicons are basic units of replication. A replicon encompasses the **entire DNA** replicated from the growing replication forks that share a single origin. Replicons may vary in size from 50—120 μm. There are estimated to be anywhere from 10,000—100,000 replicons per cell in mammals. The large number of replicons is needed to replicate the large mammalian genomes in a reasonable period of time. It takes approximately 8 hours to replicate the human genome.

Replication rate

1. Prokaryotes. An E.*coli* replication fork progresses at approximately 1000 base pairs per second.

2. Eukaryotes. The eukaryotic replication rate is about **10 times slower** than the prokaryotic replication rate. Eukaryotic replication forks progress at approximately **100 base pairs per second**. Each replicon completes synthesis in approximately 1 hour. Therefore, during the total period of eukaryotic replication, not every replicon is active. The slow rate of eukaryotic replication is likely **due to interference of nucleosomes and chromosomal proteins**. Heterochromatin is known to replicate slower than euchromatin.

Multiple eukaryotic DNA polymerases

Eukaryotes contain at least four different nuclear DNA polymerases (i.e., α, β, δ, ε) and one mitochondrial DNA polymerase (γ).

1. DNA polymerase α is essential for replication. DNA polymerase α is responsible for initiation at origins and of Okazaki fragments. It is therefore required for both leading and lagging strand synthesis.

2. DNA polymerase δ is required for both leading and lagging strand synthesis.

3. DNA polymerase β plays **no role in replication and acts only in DNA repair synthesis.**

4. DNA polymerase ε also is essential for replication, although its exact role is not clearly defined.

4. DNA polymerase γ resides in and replicates **mitochondrial DNA.**

Other factors involved in eukaryotic replication

1. An SSBP called **replication protein A (RP-A)** has been isolated from mammalian cells.

2. Topoisomerases. Eukaryotes have several well-characterized topoisomerases that **relieve positive supercoils** that build up in advance of replication forks. There are two basic types of eukaryotic topoisomerases.

a. Topoisomerase I is the major topoisomerase used to relieve supercoils. It resolves or "unknots" DNA by breaking one strand of DNA, passing the other strand though the break, and then re-ligating the broken strand.

b. Topoisomerase II also is required during replication. It is needed to resolve the final knots that form when adjacent replication forks meet.

Telomeres

Unlike prokaryotes, eukaryotic DNA is linear and not circular. Because a DNA synthesis requires a primer, DNA would be lost at the lagging strand ends unless replication of ends proceeds by a different mechanism than all other DNA. Eukaryotic chromosomes have unique sequences at their ends called telomeres. A specialized DNA polymerase called **DNA telomerase** replicates telomeric ends.

1. Structure of telomeres. Mammalian telomeres are short, tandem repeats of the sequence TTAGGG. The 3' end of these repeats is single stranded.

2. The mechanism of telomere replication. The essence of this mechanism is that the telomerase provides an RNA template complementary to the telomeric repeat, and the free 3' end of the telomere is the primer for new DNA synthesis. After elongation of the telomere by telomerase, normal lagging strand synthesis presumably makes a complementary copy of all but the 3' most terminal sequences.

3. Clinical relevance of telomeres. The number of telomere repeats varies in the cells of different tissues. This difference in telomere length represents a shortening of telomeres that occurs during the replication in some cells but not in others. This shortening occurs because of the absence of telomerase. There is a correlation in tissue-culture cells of the presence of telomerase and the immortality of the cells. Of potential medical relevance is the fact that cancer cells, which are immortal in culture, have high levels of telomerase.

The cell cycle

Eukaryotic cell division occurs in four distinct phases, which are collectively called the cell cycle.

1. S phase is the phase in which DNA is synthesized.

a. Unlike the prokaryotes, in which a second round of DNA synthesis can begin before cell division takes place, **eukaryotes replicate their DNA only once per cell division cycle.**

b. The length of the S phase of DNA synthesis varies among organisms, but takes an invariable amount of time in any one species (e.g., human S phase always lasts about 8 hours).

2. G_2 and M phase. Before mitosis and cell division, or M phase, can take place, the cell must pass through a **gap phase** called G_2. **The time it takes for a cell to pass through G_2 and M phase is invariable,** like S phase.

3. G_1 phase. After M phase, the cell must pass through a gap phase called G, before it can initiate another round of replication in S phase. The normal cel-

lular functions take place in this phase. **The time it takes for a cell to progress through G_1 can vary.** Events that occur in G, determine if a cell is going to replicate and divide.

4. G_0 phase. Many cells go through prolonged periods without dividing. These cells leave G_1 and go into a resting phase called G_0. A feature of **tumor cells** is that they can no longer enter G_0, which is the nondividing state.

Drugs that affect replication

Some antibacterial and antiviral drugs and many chemotherapeutic drugs inhibit replication. These drugs can be classified according to the mechanism by which they inhibit replication.

Antimetabolites reduce or inhibit the production of the substrate for replication (i.e., dNTPs). Without substrate, DNA polymerases cannot make DNA.

Substrate analogs

Numerous analogs of dNTPs can be incorporated into DNA by DNA polymerases. Many analogs then inhibit further replication. A few examples are:

1. Dideoxynucleoside analogs: (zidovudine (azidothymidine, AZT), didanosine. On incorporation into cells, they become phosphorylated and incorporated into DNA. The lack of a 3'-hydroxyl group makes them unacceptable primers for further DNA synthesis. They are effective antiviral drugs because they are **accepted as substrate by viral DNA polymerases better than by the human DNA polymerases.** Dideoxynucleoside analogs are used to treat retroviral infections such as human immunodeficiency virus (HIV).

2. Cytarabine. The exact mechanism of action of this drug is unknown. The cell phosphorylates it, and, when incorporated into DNA, it slows the rate of replication. On incorporation into DNA, it is believed to alter the structure of DNA and make it more prone to breakage (and cell death if breakage is extensive). Ara-C is a potent acute myelocytic antileukemia drug. An analog, adenine arabinoside (ara-A), exhibits antiviral activity.

Inhibitors that interact directly with DNA

1. Intercalates are drugs, usually with aromatic rings, that insert between adjacent, stacked base pairs. **Intercalation causes a physical block** as **well as a disruption or change in the DNA conformation** that inhibits the action of replication enzymes. Many **intercalators are mutagenic** and induce mistakes in replication, which can cause disease. A few examples are:

a. Anthracycline glycosides (daunorubicin and doxorubicin). Daunorubicin is used to treat leukemia. Doxorubicin is used to treat a wide range of cancers.

b. Actinomycin D is an antibiotic. It is useful in treating a number of cancers, especially when used with other therapies.

2. Drugs that damage DNA

Alkylating agents. There are many alkylating agents in this class that share the feature of acting as strong electrophiles. These drugs evolved from the sulfur and nitrogen mustard gases of World War I. As strong electrophiles, alkylating

agents become linked to many cellular nucleophiles, in particular the seventh nitrogen in the purine ring of guanine in DNA. The alkyl linkage that is formed causes mispairing with guanines during replication, which results in mutations. The alkyl linkage also causes breakage of DNA and cross-linking of the double helix.

Inhibitors of replicative enzymes. Very few drugs have been found to inhibit DNA polymerases directly, and those that do have shown very limited clinical use.

DAMAGE AND DNA REPAIR

The survival of a species ultimately depends on the integrity and maintenance of the genetic material — DNA. The DNA, however, is constantly subjected to environmental insults and replication errors. This leads to an alteration or removal of nucleotide bases causing damage to DNA. It is estimated that at least six nucleotides of human DNA get damaged per year.

Types of DNA damages

There are four different types of damages to DNA

1. **Single base alteration** e.g. deamination of adenine; hypoxanthine.
2. **Two base alteration** e.g. UV light induced formation of thymine dimmer.
3. **Chain breaks** e.g. due to ionization.
4. **Cross-links** e.g. formed between bases of the same or opposite strand.

Serious consequences of DNA damage

If the damage caused to DNA is not repaired, it may result in mutation or even cell death. A mutation in DNA causes loss of control of cell proliferation and leads to cancer. Fortunately, cells possess an efficient repairing machinery to undo the damage done to DNA.

Repair of DNA damage. Exposure to ultraviolet light may result in the formation of thymine-thymine dimers. These dimmers block the DNA replication.

1. An enzyme — UV-specific **endonuclease** — can recognize the dimmer and cleave the phosphodiester bond to produce a nick in the DNA strand.

2. DNA polymerase I in microorganisms (polymerase β in humans) is responsible for the synthesis of the small fragments of DNA strand to replace the lost thymine and a few adjoining bases.

3. The unwanted fragment of DNA is excised and removed. This is catalyzed by DNA polymerase I.

4. The nick of the DNA strand is sealed by **DNA ligase**.

Xeroderma pigmentosum

Eukaryotes also have an excision-repair system that is called **nucleotide excision repair.** Xeroderma pigmentosum, an autosomal recessive disease that may involve up to nine genes, results in hypersensitivity of the skin to UV light damage. Xeroderma pigmentosum **is caused** by a deficiency in the repair of thymine-thymine dimers. Patients with this disease have many skin and eye problems and a high incidence of skin cancer due to exposure to UV light.

Transcription

The process of RNA synthesis directed by a DNA template is termed **transcription**, and it occurs in three phases: **initiation, elongation**, and **termination**.

1. Template. A single strand of DNA acts as a temple to direct the formation of complementary RNA during transcription.

2. Substrate. The substrates for RNA synthesis are the four ribonucleoside triphosphates: adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), and uridine triphosphate (UTP). Cleavage of the high-energy phosphate bond between the α and β phosphates of these nucleoside triphosphates provides the energy for the addition of nucleotides to the growing RNA chain.

3. Direction of synthesis. After the first nucleoside triphosphate subsequent nucleotides are added to the 3'-hydroxyl of the preceding nucleotide. **Therefore, RNA chain growth proceeds in the 5' to 3' direction.**

4. Enzyme

a. Prokaryotes have a single RNA polymerase responsible for all cellular RNA synthesis. The structure of this enzyme is complex.

(1) A core enzyme with the subunit structure α_2, β, and β' is required for the **elongation** steps of RNA synthesis.

(2) A holoenzyme, which is a core enzyme with an additional subunit σ, is required for proper **initiation** of transcription.

b. Eukaryotes have one mitochondrial and three nuclear RNA polymerases. The **nuclear RNA polymerases are distinct enzymes that function to synthesize different RNAs.**

Initiation of transcription

1. Promoter sequences. Unlike the initiation of replication, transcriptional initiation does not require a primer. **Promoter sequences are responsible for directing RNA polymerase to initiate transcription at a particular point.** Promoter sequences differ between prokaryotes and eukaryotes.

a. Nomenclature and numbering conventions are used to avoid confusion in the description of sequences, such as promoter sequences, and their location in genes.

(1) Because DNA is double-stranded, the **sequence of only one strand is presented.**

(2) Because RNA synthesis occurs in a 5' to 3' direction, and sequences are written in a 5' to 3' direction, the sequence of the DNA strand that is **identical to the RNA transcript** is presented.

(3) For any one gene, the location of particular sequences related to the expression of that gene is given relative to its transcriptional startpoint. Therefore, **position 1 of a gene is the base that is equivalent to the first base of the 5' end of the RNA transcript of that gene.**

(4) Sequences preceding the first base are numbered negatively and said to be **upstream** of the initiation point. Sequences following the first base are numbered positively and are said to be **downstream** of the initiation point.

b. Prokaryotic promoters. For most prokaryotic genes, there are **conserved sequences** that are necessary to promote accurate initiation of transcription. The **promoters for most prokaryotic genes have three sequence elements**.

(1)Initiation site (i.e., startpoint). Transcription for most genes always starts at the same base (position 1). **The startpoint is usually a purine.**

(2) Pribnow box. For all prokaryotic genes there is a sequence called the Pribnow box that lies 9—18 base pairs upstream of the startpoint. The sequence of a typical Pribnow box is either identical to or very similar to the sequence **TATAAT.** The Pribnow box also has been called the **-10 sequence** because it is usually found 10 base pairs upstream of the startpoint.

(3) The **-35 sequence** is a component of typical prokaryotic promoters. It is a sequence that is either identical to or very similar to the sequence **TTGACA.** It is named the -35 sequence because it is typically found 35 base pairs upstream of the startpoint.

c. Eukaryotic promoters. Each type of eukaryotic RNA polymerase uses a different promoter. The promoters used by RNA polymerase I and II are similar to the prokaryotic promoter in that they are upstream of the startpoint. However, the promoters used by RNA polymerase III are unique because they are usually downstream of the startpoint.

2. Initiation factors are needed to initiate transcription. In prokaryotes, only a single factor, **sigma (σ)**, is needed to initiate transcription. In eukaryotes, multiple factors are required, in part because of the diversity of promoters.

a. The prokaryotic σ-factor is required for accurate initiation of transcription. The σ factor enables the RNA polymerase holoenzyme to recognize and bind tightly to the promoter sequences.

3. Eukaryotic initiation factors. The initiation of transcription in eukaryotes is considerably more complex than in prokaryotes, partly because of the increased complexity of eukaryotic RNA polymerases and partly because of the diversity of their promoters.

Elongation

The basic requirements and fundamental mechanism of the elongation phase of RNA synthesis is the **same in prokaryotes and eukaryotes.** Upon binding, the **σ factor facilitates the opening or melting of the DNA double** helix. The enzyme then **catalyzes the formation of a phosphodiester bond** between the first two bases. The first base is usually a purine nucleoside triphosphate (pppA or pppG). Elongation proceeds after the formation of the first phosphodiester bond. By the time 10 nucleotides have been added, the **σ-factor dissociates.** The core enzyme then continues the elongation of the transcript. The released σ-factor can combine with free core enzyme to form an other holoen-

zyme that can initiate transcription. **Rifampin** is an effective antibacterial drug and one of the few therapeutic drugs that affects only transcription. Actions of rifampin include the following. **Rifampin binds to the β subunit of RNA polymerase when the polymerase is in the holoenzyme form.** Through binding of the β subunit of the holoenzyme, rifampin **specifically inhibits initiation of transcription** and not elongation. Rifampin has **no effect on eukaryotic nuclear RNA polymerases.**

Termination

Prokaryotes and eukaryotes use an identical mechanism of synthesizing RNA and share many similarities in the way that they initiate transcription. They appear, however, to have very little in common in the way they terminate transcription.

1. Prokaryotes. There are two basic classes of termination events in prokaryotes.

a. Factor-independent termination. Particular sequences can cause the core enzyme to terminate transcription. These sequences share several common **features**.

(1) All of these sequences have a sequence that codes for a self-complementary sequence that **can form a stable stem-loop structure**.

(2) The DNA codes for a **stretch of uracils** (Us) to be formed just after the stem-loop region.

(3)Termination becomes favorable when the transcript forms a stable stem-loop structure. This structure causes the RNA polymerase to slow its synthesis, and the transcript is displaced when the slowed RNA polymerase synthesizes the U-rich segment. Displacement occurs easily because only weak adenine-uracil bonds hold the transcript to the template.

(4) Because of the nature of this displacement, there is **no specific base where transcription stops** (i.e., different transcripts have a different number of uracil residues on their 3' end).

b. Factor-dependent termination. Particular sequences act as termination sequences in the presence of factor rho (p). **Rho-dependent termination sequences** do not appear to share common structural features as do the factor-independent termination sequences. **Rho binds as a hexamer** to the forming transcript at these unique sequences. **Rho is an ATPase.** The exact mechanism that rho uses to terminate transcription is unknown, but it requires the cleavage of ATP by rho.

2. Eukaryotes. Compared with prokaryotes, very little is known about how eukaryotes terminate transcription.

a. RNA polymerase I terminates transcription in a factor-dependent manner at a particular sequence.

b. RNA polymerase III terminates transcription by an unknown mechanism after.

Post-transcriptional RNA

Once a gene transcript has been synthesized, numerous post-transcriptional modification or processing events may be needed before the transcript is functional.

Prokaryotes. Post-transcriptional processing of RNA is not as extensive in prokaryotes as in eukaryotes; however, some processing does occur.

1. In prokaryotes, **mRNA is not post-transcriptionally processed.** Prokaryotic mRNA is functional immediately upon synthesis. In fact, its translation often begins before transcription is complete.

2. Seven genes produce **rRNA.** Each gene produces a **30S precursor rRNA** that is processed to discrete, functional rRNAs. All seven genes contain the sequences that become **23S**, **16S,** and **5S** rRNA. The final rRNAs are **methylated.** Methylation is needed for the rRNAs to be functional.

3. The **tRNAs** not formed from processing of the precursor rRNA arises from **large precursor transcripts.** The tRNA genes are clustered, and each transcript contains sequences for two to seven tRNAs. The portions of the transcript that form functional tRNAs are removed by the enzymes **ribonucleases.** After excision from the precursor, some of the tRNAs are left without the sequence CCA, which is common to all tRNAs, on the 3' end. This sequence is added to these tRNAs by the enzyme **tRNA nucleotidyl transferase.** Many of the **bases** of the tRNAs **are modified**. These modifications are various methylations and other more extensive modifications of some of the bases. These modifications are necessary for the tRNAs to adopt their unique, functional conformations.

Eukaryotes. Post-transcriptional processing is more extensive in eukaryotes than in prokaryotes. This partly is due to the presence of a nucleus from which most RNAs must be transported.

1. Eukaryotic rRNA processing is very similar to that of prokaryotes. Three of the eukaryotic rRNAs (28S, 18S, and 5.8S) are derived from a **45S precursor rRNA.** Unlike prokaryotes, there are no tRNA sequences in this precursor. The processing of the 45S rRNA and formation of the ribosomal subunits begin in the nucleolus. The 45S rRNA is highly **methylated** before it is cleaved to the functional rRNAs. As in prokaryotes, the **spacer sequences are removed by endonucleolytic cleavage** of the 45S rRNA by specific endonucleases.

2. Eukaryotic mRNA is formed from extensive processing of a large precursor called **hnRNA**.

a. 5' caps. Unlike prokaryotes, which have an unmodified pppA or pppG on the 5' end of their mRNAs, eukaryotes have a **cap structure** on the 5' ends of their mRNAs. A **7-methylguanylate** is linked to the 5' end of mRNAs by a 5' to 5' triphosphate linkage. All mRNAs are capped. **Cap** formation is a multistep process that begins during transcription or immediately after. Caps serve two functions:

(1) The mRNAs with caps are **translated more efficiently.**

(2) Caps help **stabilize mRNAs** by protecting them from digestion by ribonucleases that degrade RNAs from their 5' end (e.g., 5'-exonucleases).

b. Polyadenylation. The 3' ends of most mRNAs are **polyadenylated** (poly A). **Poly A tails** are polymers of 200-300 adenylate residues linked with phosphodiester bonds. Polyadenylation is template independent. **The signal that identifies the site of polyadenylation lies within the RNA.** Termination of transcription occurs downstream of the polyadenylation site. The enzyme **poly A polymerase** joins the complex and, after the RNA is cut, it catalyzes the polymerization of adenylate residues onto the free 3' end of the mRNA. **Polyadenylation occurs after capping and before splicing**. Polyadenylation is a **necessary event for all hnRNAs** to be successfully converted to mRNA. Polyadenylation helps **stabilize mRNA.** In the cytoplasm, poly A tails are slowly shortened. When the poly A tail is completely removed, the mRNA is rapidly degraded.

c. Splicing. All the sequences necessary to form an mRNA that codes or a protein product are contained in hnRNA. The coding sequences, however, are often split and separated by noncoding sequences. **The process by which noncoding sequences are removed to form a functional mRNA is called splicing**.

(1) Exons are the transcribed portions of genes that are retained in the processing of hnRNA to mRNA. The term exon stands for the **expressed** portion of genes.

(2) Introns are the transcribed portions of genes that are removed in the processing of hnRNA to mRNA. The term intron refers to the **intervening** sequences between exons.

(3). Different genes have different numbers of introns of different sizes.

(4). Intron/exon junctions. A few conserved sequences shared by introns and exons are sufficient to allow recognition by the splicing apparatus of the precise junction between introns and exons.

(5)Mechanism of splicing. Splicing occurs through a multistep process that is catalyzed by a large (50S—60S) ribonucleoprotein complex called a **spliceosome.** Spliceosomes are made of **five small nuclear RNAs** (snRNA). The snRNAs are responsible for recognition of the conserved sequences in introns and the bringing together of RNA sequences into perfect alignment for splicing.

(6) Transport of mRNA from the nucleus to the cytoplasm **is coupled to splicing** and does not occur until all the splicing is complete.

(7) Regulation of gene expression is often at the level of splicing.

3. The tRNAs are formed in eukaryotes similar to the way they are formed in prokaryotes, except that some have introns that are removed.

a. Eukaryotic tRNAs are processed from large precursor RNAs. In eukaryotes, these precursors may contain one or more tRNA sequences. The tRNA sequences are excised from the precursor by specific endonucleolytic cleavage.

The **sequence CCA** is added to the **3' end** after the tRNA is cleaved from the precursor. As in prokaryotes, the bases of eukaryotic tRNAs are also extensively modified before the tRNA can adopt its final functional structure.

b. Some, but not all, eukaryotic tRNAs have small (14—50 nucleotide) **introns.** The introns of different tRNAs have no sequence homology. The mechanism of tRNA splicing differs from mRNA splicing. The splicing enzymes recognize characteristic structural features of the tRNA to identify the intron/exon junctions.

REVERSE TRANSCRIPTION

Some of the viruses — known as **retroviruses** — possess RNA as the genetic material. These viruses cause cancers in animals, hence known as **oncogenic**. They are actually found in the transformed cells of the tumors. The enzyme RNA dependent DNA polymerase — or simple reverse **transcriptase** — is responsible for the formation of **DNA from RNA**. This DNA is complementary (cDNA) to viral RNA and can be transmitted into host DNA.

LECTURE 23

TRANSLATION. REGULATION OF GENE EXPRESSION. MUTATIONS

The genetic information stored in DNA is passed on to RNA through transcription. **Biosynthesis of a protein or a polypeptide** in a living cell is known as translation. The sequence of amino acids in the protein synthesized is determined by the nucleotide base sequence of mRNA.

GENETIC CODE

The **three nucleotide** (triplet) **base sequences in mRNA that act as code words for amino acids** in protein constitute the genetic code or simple **codons**. The genetic code may be regarded as a dictionary of nucleotide bases (A, G, C and U) that determines the sequence of amino aids in proteins.

The codons are composed of the four nucleotide bases, namely the purines — adenine (A) and guanine (G), and the pyrimidine — cytosine (C) and uracil (U). These four bases produce 64 different combinations (4^3) of three base codons. The nucleotide sequence of the codon on mRNA is written from the 5'end to 3' end. Sixty one codons code for the 20 amino acids found in protein.

The three codons **UAA**, **UAG** and **UGA** do not code for amino acids. They act as **stop signals** in proteins synthesis. These three codons are collectively known as **termination codons** or nonsense codons.

The codons **AUG** — and, sometimes, **GUG** — are the chain **initiating codons**.

Characteristics of genetic code

The genetic code is universal, specific, non-overlapping and degenerate.

1. Universality. The same codons are used to code for the same amino acids in all the living organisms. Thus, the genetic code has been conserved during the course of evolution. Hence genetic code is appropriately regarded as universal.

2. Specificity. A particular **codon** always codes for the **same amino acids**, hence the genetic code is highly specific or unambiguous e.g. UGG is the codon for tryptophan.

3. Non-overlapping. The genetic code is read from a fixed point as a continuous base sequence. It is non-overlapping, commaless and without any punctuations. Addition or deletion of one or two bases will radically change the message sequence in mRNA. And the protein synthesized from such mRNA will be totally different. This is encountered in **frame-shift mutations** which cause an alteration in the reading frame of mRNA.

4. Degenerate. Most of the amino acids have more than one codon. The codon is degenerate or redundant, since there are 61 codons available to code for only 20 amino acids. For instance, glycine has four codons. The codons that designate the same amino acid are called **synonyms**. Most of the synonyms differ only in the third (3' end) base of the codon.

Codon anticodon recognition

The codon of the mRNA is recognized by the anticodon of tRNA (Fig.23—1).

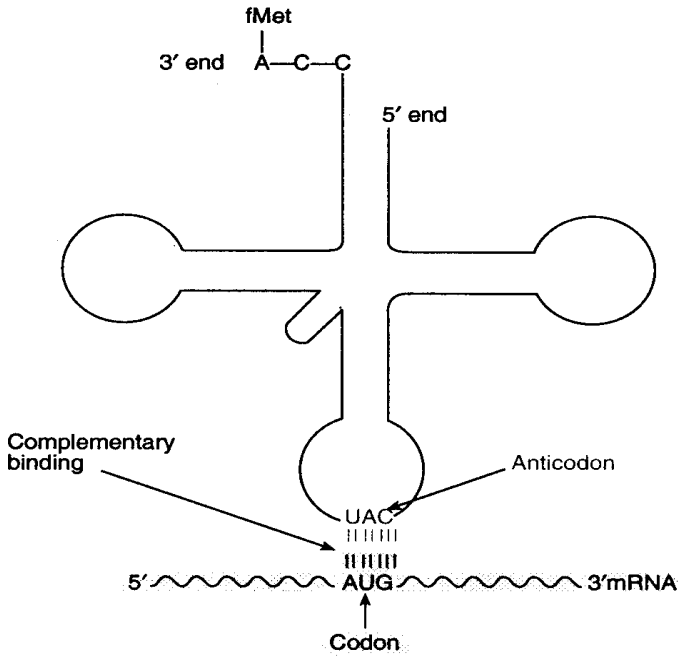


Figure 23—1. Complementary binding of codon (of mRNA) and anticodon (of tRNA) (by U. Satyanarayana, 2002).

They pair with each other in antiparallel direction (5'→3' of mRNA with 3'→5' of tRNA). The usual conventional complementary base pairing (A=U, C≡G) occurs between the first two bases of codon and the last two bases of anticodon. The third base of the codon is rather lenient or flexible with regard to the complementary base.

"Wobble hypothesis". This was put forth by Crick. Wobble hypothesis is a phenomenon in which a **single tRNA can recognize more than one codon**. This is due to the fact that the third base (3'-base) in the codon often fails to recognize the specific complementary base in the anticodon (5'-base). Wobbling is attributed to the difference in the spatial arrangement of the 5'-end of the anticodon.

Wobble hypothesis explains the degeneracy of the genetic code, i.e. existence of multiple codons for a single amino acid. Although there are 61 codons for amino acids, the number of tRNA is far less (around 40) which is due to wobbling.

The **protein synthesis** which involves the translation of nucleotide base sequence of mRNA into the language of amino acid sequence may be divided into the **following stages** for the convenience of understanding.

I. Requirement of the components.

II. Activation of amino acids.

III. Protein synthesis proper.

IV. Post-translational modifications.

I. Requirement of the components.

The protein synthesis may be considered as a biochemical factory operating on the ribosomes. As a factory is dependent on the supply of raw materials to give a final product, the protein synthesis also requires many components.

1. Amino acids. Proteins are polymers of amino acids. Of the **20 amino acids** found in protein structure, half of them (10) can be synthesized by man. About **10 essential amino acids** have to be provided through the diet. Protein synthesis can occur only when all the amino acids needed for a particular protein are available. If there is a deficiency in the dietary supply of any one of the essential amino acids, the translation stops. It is, therefore, necessary that a regular dietary supply of essential amino acids, in sufficient quantities, is maintained, as it is a prerequisite for protein synthesis.

2. Ribosomes. The functionally active ribosomes are the **centers** or **factories for protein synthesis**. Ribosomes are huge complex structures (70S for prokaryotes and 80S for eukaryotes) of proteins and ribosomal RNAs. Each ribosome consists of two subunits — one big and one small. The functional ribosome has two sites — A site and P site. Each site covers both the subunits. ***A*** site is for binding of aminoacyl tRNA and ***P*** site is for binding peptidyl tRNA, during the course of translation.

The ribosomes are located in the cytosomal fraction of the cell. They are found in association with rough endoplasmic reticulum (RER) to form clusters RER — ribosomes, where the protein synthesis occurs. The term **polyribosome** (polysome) is used when several ribosomes simultaneously translate on a single mRNA.

3. Messenger RNA (mRNA). The specific information required for the synthesis of a given protein is present on the mRNA. The DNA has passed on the genetic information in the form of **codons** to mRNA to translate into a protein sequence.

4. Transfer RNAs (tRNAs). They carry the amino acids and hand them over to the growing peptide chain. The amino acid is covalently bound to tRNA at the 3'-end. Each tRNA has a three nucleotide base sequence — the **anti-codon**, which is responsible to recognize the codon (complementary bases) of mRNA for protein synthesis.

5. Energy sources. Both **ATP** and **GTP** are required for the supply of energy in protein synthesis. Some of the reactions involve the breakdown of ATP or GTP, respectively, to AMP and GMP with the liberation of pyrophosphate. Each one of these reactions consumes two high energy phosphates (equivalent to 2 ATP).

6. Protein factors. The process of translation involves a number of protein factors. There are needed for initiation, elongation and termination of protein synthesis.

II. Activation of amino acids

Amino acids are activated and attached to tRNA in a two step reaction (Fig.23—2). A group of enzymes — namely **aminoacyl tRNA synthetases** — are required for this process. These enzymes are **highly specific** for the **amino acid** and the **corresponding tRNA**. The attachment of an amino acid to its appropriate tRNA, which is catalyzed y tRNA synthetases, requires ATP and proceeds by the formation of an **activated aminoacyl intermediate**. This is a three-step process.

1. Formation of an aminoacyl-adenylate complex is the first step of the reaction and is expressed as

$$\text{Amino acid} + \text{ATP} \leftrightarrow \text{Aminoacyl-AMP} + \text{PPi}$$

2. Transfer of the aminoacyl group to the 2'- or 3'-hydroxyl group of the 3' adenosine of the appropriate tRNA is the second step. It is expressed as

$$\text{Aminoacyl-AMP} + \text{tRNA} \leftrightarrow \text{Aminoacyl-tRNA} + \text{AMP}$$

The sum of the activation and transfer steps is expressed as:

$$\text{Amino acid} + \text{ATP} + \text{tRNA} \leftrightarrow \text{Aminoacyl-tRNA} + \text{PPi} + \text{AMP}$$

3. PPi is then hydrolyzed to form two free phosphates. This final reaction makes the overall reaction irreversible. A total of two high-energy phosphate bonds of ATP are expended in the formation of a single aminoacyl-tRNA.

III. Protein synthesis proper

The protein or polypeptide synthesis occurs on the ribosomes (rather polyribosomes). The **mRNA is read in the 5'→ 3' direction and the polypeptide synthesis proceeds from N-terminal end to C-terminal end**.

The prokaryotic mRNAs are **polycistronic**, since a single mRNA has many coding regions that code for different polypeptides. In contrast, eukaryotic mRNA is **monocistronic**, since it codes for a single polypeptide.

Translation proper is divided into 3 stages — **initiation, elongation** and **termination**. Protein synthesis in prokaryotes (E.coli) is described here, which almost resembles that of eukaryotes. The distinguishing features of eukaryotic translation are also given (Fig. 23—3).

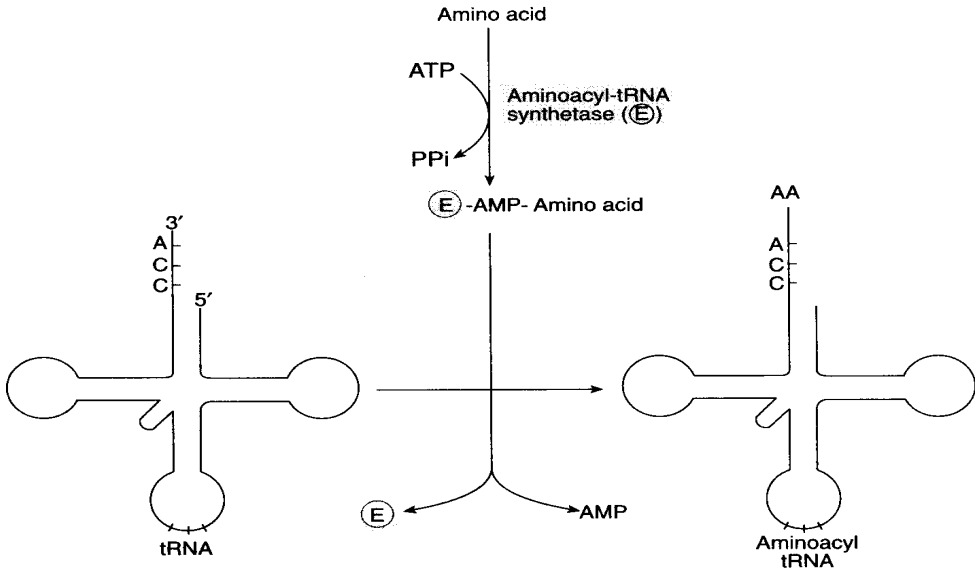


Figure 23—2. Formation of aminoacyl tRNA (AA — Amino acid; E — enzyme) (by U. Satyanarayana, 2002).

1. Initiation. The initiation factors — namely $\mathbf{IF_1}$ and $\mathbf{IF_3}$ — first bind with free 30S subunit. This is followed by the attachment of mRNA with 30S subunint. In the next step, IF_2 and N-formyl-methionine tRNA, in the presence of GTP, bind with 30S ribosome. There is still some controversy on the exact order of binding of initiation factors, mRNA and tRNA to 30S ribosomal subunits. The initiation factors of eukaryotes are designated as **eIF** and at least nine such factors are known. The 50S ribosome associates with 30S to form 70S initiation complex and the initiation factors are released.

Binding of mRNA with rRNA. The prokaryote mRNA possesses a specific sequence of nucleotide bases near 5'-end. That is, 5'-UAAGGAGG-3', which is known as **Shine-Dalgarno sequence**. The 16S ribosomal RNA component of 30S ribosome has a base sequence near its 3' end which is complementary to Shine-Dalgarno sequence (in part or full). The binding of mRNA to the 16S rRNA facilitates the correct positioning of mRNA with 30S ribosome to select the proper initiation codon.

Initiation codon. The codon **AUG** is responsible for the initiation of protein synthesis. It is found at the 5' end close to Shine-Dalgarno sequence. A special initiator tRNA carrying methionine is converted to N-formylmethionine (N^{10}-formyl THF donates formyl group). In eukaryotes, methionine is not formylated. The initiator tRNA with anticodon UAC recognizes the initiation codon AUG and starts protein synthesis.

2. Elongation. Ribosomes elongate the polypeptide chain by a sequential addition of amino acids to the growing carboxyl end. It is estimated that about 40 amino acids per second are added to the growing polypeptide chain. During elongation, the ribosome moves from 5' end to 3' end of the mRNA. This process

of elongation involves some non-ribosomal proteins. In prokaryotes, the elongation factors — **EF-Tu** and **EF-Ts** — in association with GTP, facilitate elongation. The corresponding elongation factors in eukaryotes are designated as **eEF**.

At the beginning, the initiation tRNA occupies the ***P*** site of the ribosome. The incoming next amino acid (as aminoacyltRNA) corresponding to the codon to mRNA is deposited at ***A*** site of the ribosome. This process depends on the elongation factors and the energy is derived from the hydrolysis of GTP.

Formation of peptide bond. The enzyme **peptidyltransferase** catalyses the formation of peptide bond. The activity of this enzyme lies on the 23S rRNA of 50S ribosomal subunit. It is, therefore, the **rRNA** (not protein) referred to as **ribosyme** that catalyses the peptide bond formation.

As the peptide bond formation occurs, the ribosome moves to the next codon (three bases) in the mRNA (3' end). This process, called **translocation**, basically involves the movement of growing peptide chain from A site to P site of the ribosome. The translocation requires EF-G and GTP.

The elongation process is repeated again and again, with addition of one amino acid each time, till the signal for termination is reached.

3. Termination. One of the three codons UAA, UAG and UGA act as stop signals and terminate the growing polypeptide. The signal codons do not have specific tRNA to bind with them. As the termination codon occupies the ribosomal A site, the release factors (RF—1, RF-2 and RF—3) bind with codon. These factors cause the hydrolytic breakdown of the peptidyl-tRNA and release the newly synthesized protein. The releasing factors are also responsible for the dissociation of ribosomes from mRNA.

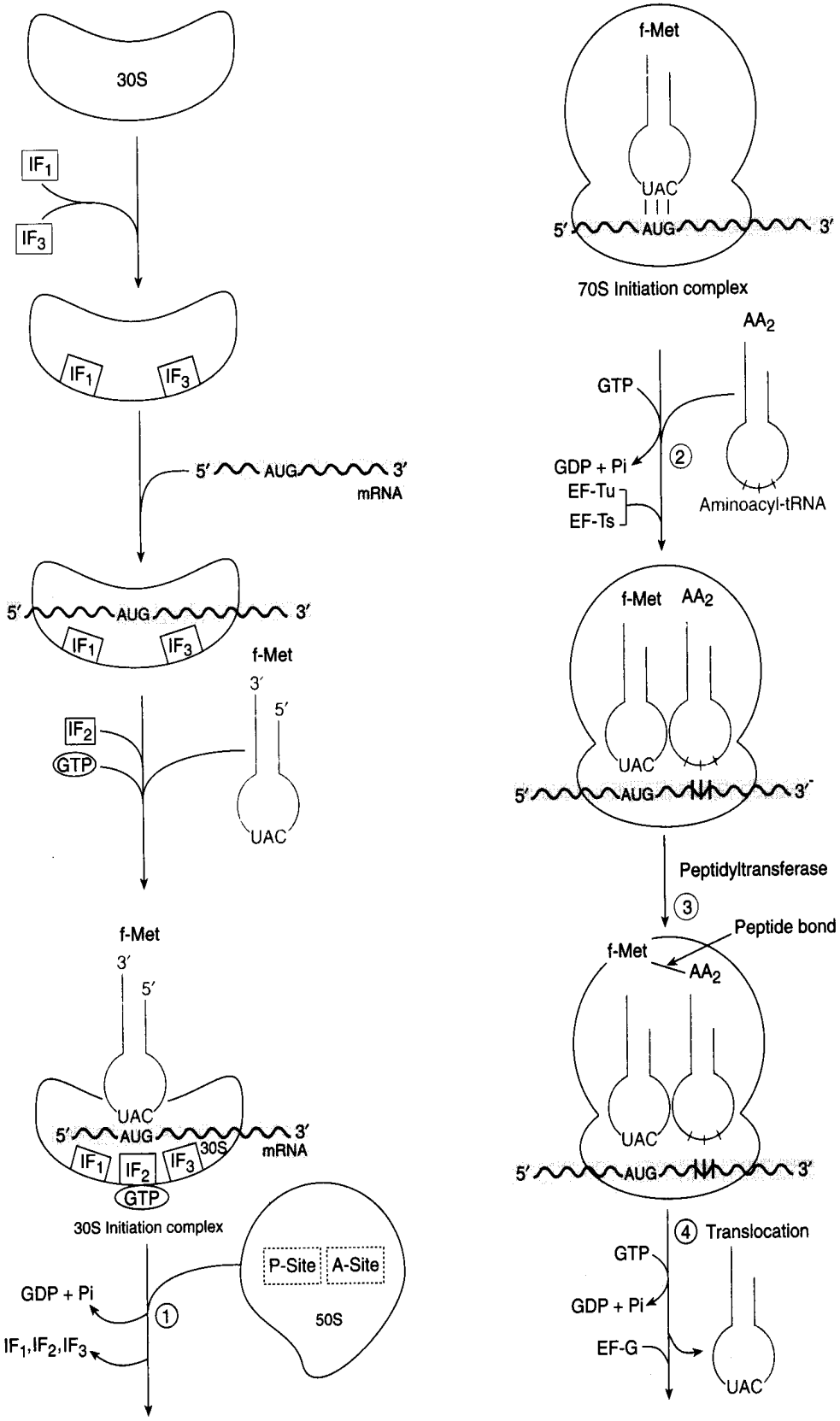
Chaperones and protein folding

The folding of a polypeptide chain into tertiary structure occurs during the course of translation to form a conformation specific to the biological activity of the protein. **Chaperones** are **heat shock proteins** (originally discovered in response to heat shock) which facilitate and favour the interactions on the polypeptide surfaces to finally give the specific conformation of a protein.

Inhibitors of protein synthesis

Translation is a complex process and it has become a favorite target for inhibition by **antibiotics**. Antibiotics are the substances produced by bacteria or fungi which inhibit the growth of other organisms. Majority of the antibiotics interfere with the bacterial protein synthesis and are harmless to higher organisms. This is due to the fact that the process of translation sufficiently differs between prokaryotes and eukaryotes.

1. Streptomycin. Initiation of protein synthesis is inhibited by streptomycin. It causes misreading of mRNA and interferes with the normal pairing between codons and anticodons.


30S
IF1
IF3
IF1
IF3
5′ AUG 3′
mRNA
5′ AUG 3′
IF1
IF3
f-Met
3′
5′
IF2
GTP
UAC
f-Met
3′
5′
UAC
5′ AUG 3′
IF1
IF2
IF3
30S
mRNA
GTP
30S Initiation complex
GDP + Pi
IF1,IF2,IF3
1
P-Site
A-Site
50S
f-Met
UAC
5′ AUG 3′
70S Initiation complex
AA2
GTP
GDP + Pi
EF-Tu
EF-Ts
2
Aminoacyl-tRNA
f-Met
AA2
UAC
5′ AUG 3′
Peptidyltransferase
3
Peptide bond
f-Met
AA2
UAC
5′ AUG 3′
4 Translocation
GTP
GDP + Pi
EF-G
UAC

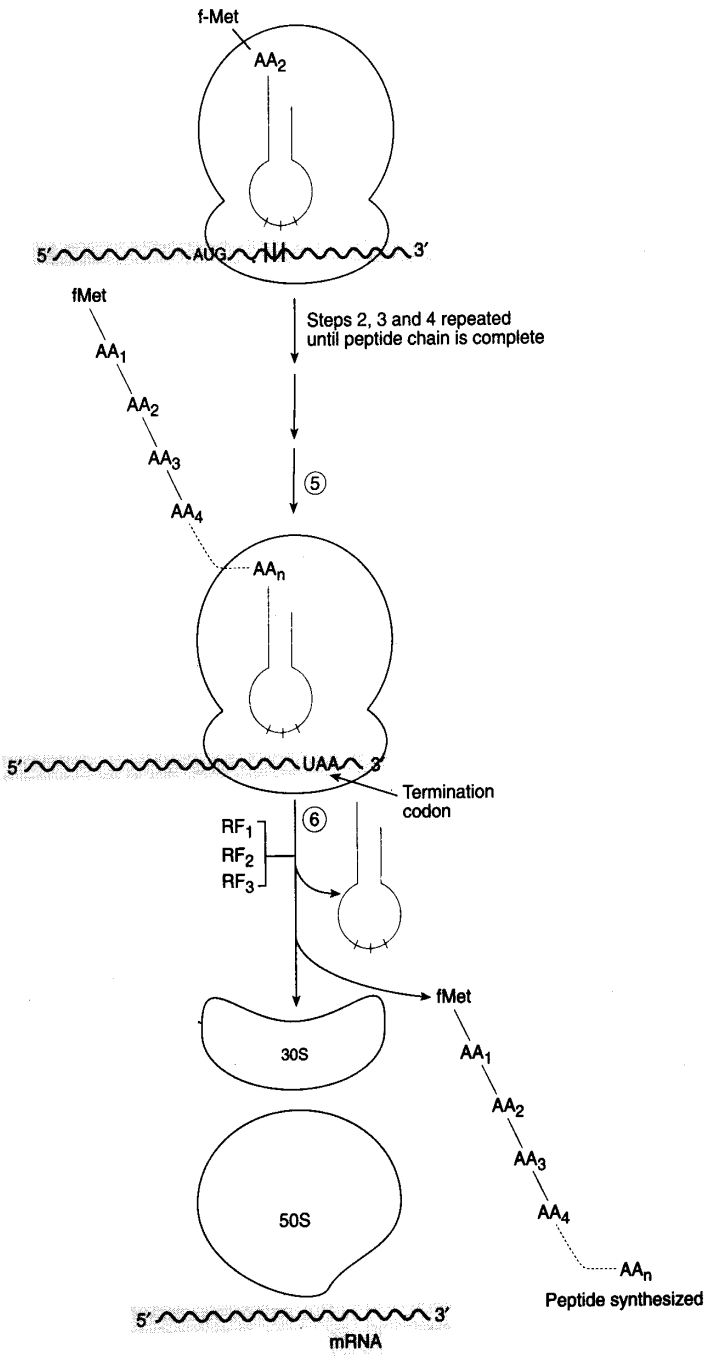


Figure 23—3. Protein biosynthesis (by U. Satyanarayana, 2002).

2. Tetracycline. It inhibits the binding of aminoacyl tRNA to the ribosomal complex. In fact, tetracycline can also block eukaryotic protein synthesis. This, however, does not happen since eukaryotic cell membrane is not permeable to this drug.

3. Puromycin. This has a structural resemblance to aminoacyl tRNA. Puromycin enters the A site and gets incorporated into the growing peptide chain and causes its release. This antibiotic prevents protein synthesis in both prokaryotes and eukaryotes.

4. Chloramphenicol. It acts as a competitive inhibitor of the enzyme peptidyltransferase and thus interferes with elongation of peptide chain.

5. Erythromycin. It inhibits translocation by binding with 50S subunit of bacterial ribosome.

6. Diphtheria toxin. It prevents translocation in eukaryotic protein synthesis by inactivating elongation factor eEF_2.

IV. Post-translation modification of proteins

The proteins synthesized in translation are, as such, not functional. Many changes take place in the polypeptides after the initiation of their synthesis or, most frequently, **after the protein synthesis is completed**. These modifications include trimming by proteolytic degradation and covalent changes which are collectively known as post-translation modifications.

Proteolytic degradation

Many proteins are synthesized as the precursors which are much bigger in size than the functional proteins. Some portions of precursor molecules are removed by proteolysis to liberate active proteins. This process — commonly referred to as **trimming** — may occur in Golgi apparatus, secretory vesicles and, sometimes, after the secretion of proteins. The formation of insulin from preproinsulin, conversion of zymogens (inactive digestive enzymes e.g. trypsinogen) to the active enzymes are more examples of trimming.

The synthesis of the proteins as inactive precursors and their later conversion into active form, may be, to protect the functional protein unit from the environmental insults.

Covalent modification

The proteins synthesized in translation are subjected to many covalent changes. By three modifications, the proteins may be converted to active form or inactive form.

1. Phosphorylation. The hydroxyl group containing amino acids of proteins, namely serine, threonine and tyrosine are subjected to phosphorylation. The phosphorylation may either increase or decrease the activity of the proteins. A group of enzymes called protein kinases catalyse phosphorylation while protein phosphatases are responsible for dephosphorylation (removal of phosphate group).

2. Hydroxylation. During the formation of collagen, the amino acids proline and lysine are, respectively, converted to hydroxyproline and hydroxylysine. This hydroxylation occurs in the endoplasmic reticulum and requires vitamin C.

3. Glycosylation. The attachment of carbohydrate moiety is essential for some proteins to perform their functions. The complex carbohydrate moiety is

attached to the amino acids, serine and threonine (O-linked) or to asparagines (N-linked), leading to the synthesis of glycoproteins.

4. Vitamin K dependent carboxylation of glutamic acid residues in certain clotting factors is also a post-translational modification.

REGULATION OF GENE EXPRESSION

Based on genetic studies of the production of the enzymes involved in lactose metabolism, **Jobob** and **Monod** proposed the **operon model** to explain gene induction in prokaryotes. Although there are many operons in bacterial cells, the lactose (*lac*) operon discovered by Jacob and Monod in the classic example of all operons.

Among the observation that lead to their model was the finding that there were two kinds of genes.

1. Inducible genes. Some proteins are said to be inducible because they are produced only in significant amount when a specific inducing substance (i.e., an inducer) is present. For example, the production of the enzyme β-galactosidase is induced by the presence of its substrate, lactose, in the medium.

2. Constitutive genes refer to prokaryotic genes whose expression is not regulated. The products of these genes are produced at a constant, often low, rate. Such genes are called constitutive genes, and their expression is said to be constitutive.

Lactose metabolism in E.coli

In the absence of glucose, E.coli can use lactose, if present, as a source of carbon and energy. **β-Galactosidase** is the key enzyme in the metabolism of lactose by E.coli. In the **absence of lactose**, there are fewer than 10 molecules of β-galactosidase per cell. In the **presence of lactose**, and no other energy source, the number of β-galactosidase molecules can increase to 5000 molecules per cell within several minutes.

Lactose (lac) operon

An **operon** is a **group of coordinately regulated genes**, the products of which typically catalyze a multi-enzyme metabolic pathway and their controlling elements. The purpose of the *lac* operon is to make the enzymes necessary to metabolize lactose. **Two classes** of genes are needed to make a functional operon.

1. The products of operons are produced by **structural genes**. Structural genes code for products that may be enzymes or, in fact, truly structural in nature, such as transfer RNA (tRNA), ribosomal RNA (rRNA), or ribosomal proteins. Structural gene products are essential for the life of the cell.

2. Regulatory genes code for products that **regulate the level of expression of structural genes**. Although regulatory genes often are not considered part of operons because they can be located at sites remote from the structural genes they regulate, they are key elements of operons.

Basic regulation of lac operon expression

In the absence of lactose, the cell has no need for the production of β-galactosidase. A regulatory molecule, the **repressor**, **prevents expression of**

the *lac* operon in the absence of lactose. The repressor binds tightly to the **operator** (region lies adjacent to the promoter and spans the transcriptional initiation site) which **blocks initiation of transcription of the structural genes**. Upon binding of the **inducer** to the repressor, the repressor undergoes a conformational change to a shape that no longer binds the operator tightly. With the repressor no longer blocking the initiation site, **RNA polymerase initiates transcription**. As long as lactose is present, then inducer is present, and transcription of the *lac* operon continues. The result is the continued **production of the enzymes needed for the metabolism of lactose**. After the inducer is removed, expression of the *lac* operon stops quickly.

The lac operon and other operons can be positively controlled as well; that is, a regulatory protein can act to promote or enhance transcription. Other operons are **repressible**. In repressible operons, a metabolite produced by the action of the gene products of the operon **inhibits further expression** of the operon by acting as a **corepressor**.

Eukaryotes, and particularly mammals, are considerably more complex than prokaryotes. To maintain this complexity, there is a **need for greater gene** regulatory capacity. As a consequence of the increased complexity of gene regulation in eukaryotes, there is an increased number regulatory sites where disruption can lead to disease or medical problems.

MUTATIONS are **permanent changes in a DNA sequence**. Although germ line mutations are the driving force of evolution, it is undesirable to have a high rate of mutation, especially in somatic cells.

Causes of mutation

Mutations arise by a number of different means.

1. Errors in replication. If a base that is noncomplementary to the template base is added during replication, then a mispairing or mismatch occurs. This leads to a mutation during the next round of replication if the error is not repaired. **Postreplicative repair systems** also exist to correct base mispairing that occurs during replication and is not corrected by proofreading. In both prokaryotes and eukaryotes, mutations that arise from replication errors occur less than once for every **10^9 bases** replicated.

2. Errors that occur during recombination events. The DNA of living cells is surprisingly mobile and often is rearranged or recombined. Chromosomes sometimes cross over and recombine during meiosis. Many viruses are capable of moving their DNA in and out of their host cell's genomic DNA. Typically, some of these DNA rearrangements leave no changes or only a few small changes that can be repaired. However, **some of these DNA rearrangements may change the cell's DNA to such a great extent that it cannot be repaired.** This often leads to severe or even lethal mutations.

3. Chemical mutagens. Many chemicals alter DNA bases or the structure of DNA. These alterations often lead to mutations if they are not repaired. Types of chemical mutagens are:

a. Base analogs can become incorporated into DNA. Some lead to the inhibition of replication, whereas others are mutagenic because they lead to mispairing.

b. Chemical mutagens: nonalkylating agents (formaldehyde, hydroxylamine, nitrous acid), alkylating agents, intercalating agents.

4. Irradiation

a. Ultraviolet (UV) **light** (200—400 nm) induces dimerization of adjacent pyrimidines, particularly adjacent thymines. This direct mutation of DNA distorts the DNA structure, inhibits transcription, and disrupts replication until it is repaired.

b. Ionizing radiation, such as roentgen rays (x-rays) and gamma rays (γ-rays), can cause extensive damage to DNA including opening purine rings, which lead to depurination, and breaking phosphodiester bonds.

5. Spontaneous changes. DNA undergoes several spontaneous changes that lead to mutations if they are not repaired before a round of replication.

a. Deamination of cytosine (C) to form uracil (U) occurs spontaneously. If this is not repaired before a round of replication, an adenine (A) pairs with the template strand containing the uracil instead of a guanine (G).

b. Spontaneous depurination. Purines are less stable under normal cellular conditions than pyrimidines. The glycosidic bond that links purines to the sugar-phosphate backbone of DNA often is broken. If these purines are not replaced before a round of replication, any base may be added to complement the missing base during replication.

Types of mutations

Regardless of the method of formation, there are only a limited number of types of mutations.

1. Base substitution

a. Base substitutions are the **most common type of mutation,** and they can be classified into **two subtypes:**

(1)Transitions, in which one purine is replaced by another purine or one pyrimidine is replaced by another pyrimidine.

(2)Transversions, in which a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine

b. Missense, nonsense, or silent mutations are possible if base substitutions occur in the coding region of a gene.

(1). A missense mutation leads to a changed codon and results in an aminoacid change in the protein product of that gene. Depending on the amino acid that is changed, missense mutations can have either no effect or very serious consequences. The mistaken (or missense) amino acid may be **acceptable**, **partially acceptable** or **unacceptable** with regard to the function of protein molecule. **Clinical example: sickle cell anemia** is an example of a missense mutation with very serious health consequences.

(2). A nonsense mutation leads to the conversion of an amino acid codon to a stop codon. Translation of the RNA from a gene with a nonsense mutation is prematurely terminated. The protein products of nonsense mutations are usually nonfunctional. **Clinical example:** many **thalassemias** are the result of nonsense mutations.

(3)A silent mutation leads to the formation of a codon synonym and no change in the amino acid sequence of the gene product.

2. Deletion of one or more base pairs

a. A frameshift mutation results if a deletion of base pairs in a gene occurs that is not a multiple of three. That is, the reading frame of translation changes. This usually results in the production of a nonfunctional gene product.

b. If a deletion of three (or a multiple of three) base pairs occurs in a gene, then there is no change in the reading frame and only a deletion of one or more amino acids. Such a mutation is likely to be less severe than a frameshift mutation.

3. Insertion of one or more base pairs. As with deletions, insertions of base pairs into genes can lead to severe frameshift mutations or less severe additions of codons, depending on whether the insertions are multiples of three.

Mutagenesis and carcinogenesis

Most mutagens can **cause cancer** and, therefore, are said to be **carcinogenic** as well. However, many chemicals are not mutagenic in the form in which they enter the body, yet they are carcinogenic. Because of this apparent paradox, **carcinogens are classified into two types.**

1. Direct carcinogens exist in a mutagenic form when they enter the body. Unless they are rapidly inactivated before they can interact with DNA, they are potentially carcinogenic.

2. Indirect carcinogens are chemicals that are not mutagenic in the form in which they enter the body but are converted to mutagens on metabolism in the body. **The liver has a very active detoxification system that converts many inactive mutagens to active mutagens.** Many lipophilic chemicals accumulate in the body if they are not made water soluble. In the liver, there is a large number of endoplasmic reticulum (ER)-bound enzymes in the **cytochrome** P_{450} **oxidase system** that serve this purpose.

LECTURE 24

MECHANISM OF HORMONE ACTION. HORMONE OF HYPOTHALAMUS AND PITUITARY

The living body possesses a remarkable communication system to coordinate its biological functions. This is achieved by two distinctly organized functional systems

1. The **nervous system** coordinates the body functions through the transmission of electrochemical impulses.

2. The **endocrine system** acts through a wide range of chemical messengers known as hormones.

The term hormone (*Greek*: homo — to excite or arouse) was introduced in 1904 to describe **secretin**. Secretin is a substance produced in the upper small intestine which stimulates the formation of gastric juice and helps in digestion.

Hormones are conventionally defined as organic substances, produced in small amounts by specific tissues (endocrine glands), secreted into the blood stream to control the metabolic and biological activities in the target cells. Hormones may be regarded as the **chemical messengers** involved in the transmission of information from one tissue to another and from cell to cell.

Endocrine hormones are synthesized by endocrine glands and transported by the blood to their target cells.

Besides the endocrine hormones two other categories of hormones are now known

1. **Paracrine hormones**. They act on the cells adjacent/close to the cells from where they are released, e.g. prostaglandins and polypeptide growth factors. The latter are involved in the control of cell proliferation.

2. **Autocrine hormones.** They act on the same cells where they are synthesized, e.g. interleukin-2 produced by T-cells stimulates their proliferation.

There are yet two other classes of cellular mediators which are also involved in the message transmission of the body.

1. Neurotransmitters. They are released by the nerve cells and usually act on the adjacent cells. In fact, neurotransmitters are almost similar to hormones in their synthesis, transport and mechanism of action. The catecholamines (epinephrine and norepinephrine) function as neurotransmitters when secreted in the central nervous system, while they act as hormones when secreted from adrenal medulla.

2. Pheromones. They are transmitted between the cells in the organisms of opposite sex. The function of pheromones is to stimulate reproductive behaviour and, therefore, they serve as sex attractants.

CLASSIFICATION OF HORMONES

Hormones may be classified in many ways based on their characteristics and functions.

I. Based on the chemical nature

1. Proteins or peptides (e.g., insulin, glucagon), **which are synthesized as larger precursors that undergo processing and secretion.**

2. Amino acid derivatives (e.g., catecholamines, thyroid hormones).

3. Steroids hormones (e.g. glucocorticoids, mineralocorticoids, sex hormones)

4. Fatty acid derivatives (e.g., eicosanoids).

5. Gases (e.g., nitric oxide).

II. Based on the mechanism of action

Hormones are classified into two broad groups (I and II) based on the **location of the receptors** to which they bind and the signals used to mediate their action

1. Group I hormones. These hormones bind to **intracellular receptors** to form **receptor-hormone complexes** (the intracellular messengers) through which their biochemical functions are mediated. Group I hormones are **lipophilic** in nature and are steroid hormones (exception T_3 and T_4). They are found in circulation in association with **transport proteins** and possess relatively **longer half-lives** (hours or days), e.g. estrogens, androgens, glucocorticoids, calcitriol, T_3, T_4.

2. Group II hormones. These hormones bind to **cell surface** (plasma membrane) receptors and stimulate the release of certain molecules, namely the **second messengers** which, in turn, perform the biochemical functions. Thus, **hormones** themselves are the **first messengers**. These hormones are **hydrophilic in nature**, usually transported in the **free form** and **possess short half-lives** (in minutes).

Group II hormones are subdivided into **three categories** based on the chemical nature of the second messengers.

a. The second messenger is cAMP

b. The second messenger is **phosphatidyl/inositol/calcium**

c. The second messenger is **unknown**

MECHANISM OF ACTION OF GROUP I HORMONES

They enter the cell by diffusion through the cell membrane. Inside the cell, they interact with **intracellular receptors** located either in the cytosol or the nucleus. As a result of this interaction, a structural change occurs in the receptor, and the **hormone-receptor** complex induces a cellular response.

Lipophilic hormones are **slower** to act and have a **longer duration of action** than hydrophilic hormones. Their duration of action ranges from **hours to days.**

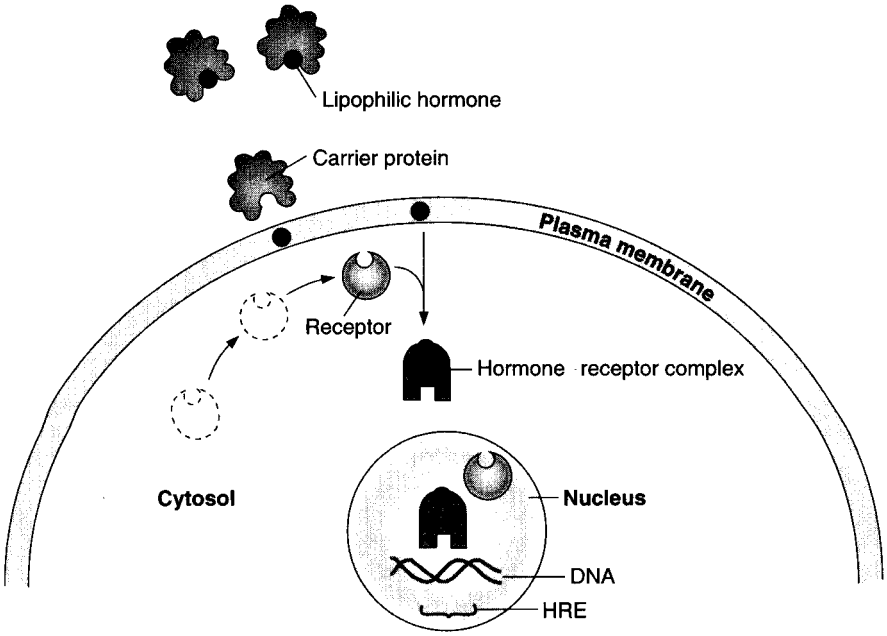


Figure 24—1. Mechanism action of lipophilic hormones (by Davidson V.L. et al., 1999).

Receptors for lipophilic hormones are proteins that consist of separate domains: one domain is responsible for binding to a specific sequence of DNA, and one domain binds to the specific hormone.

1. In the absence of the hormone, some receptors do not bind to their specific DNA sequences; only the hormone-receptor complex binds. Other receptors already may be bound to the DNA but only adopt an activating conformation when the hormone binds.

2. The specific DNA sequence that binds to a hormone-receptor complex is called the **hormone response element** (HRE). The response elements for different hormones are similar but sufficiently different to be specific for a particular hormone-receptor complex.

3. Binding the hormone-receptor complex to its response element results in the **stimulation of transcription of specific genes**. The specific genes that are transcribed depend on the target cell, presumably because other cell-type-specific proteins are also required for stimulation of transcription.

a. The first genes to be activated are called the primary response genes.

b. The primary response gene products may activate other genes — the secondary response genes.

MECHANISM OF ACTION OF GROUP II HORMONES

These hormones interact with receptors on the surface of a cell and elicit varied responses. These responses depend on the receptor type, and, for certain types of receptors, the receptor subtype. For example, receptor subtypes exist for epinephrine; the receptor subtypes bind to epinephrine, but the receptors are otherwise different and elicit different effects. Receptor subtypes also exist for many neurotransmitters.

Hormone-receptor interaction

1. Structure of receptor molecules. The receptor molecules for hydrophilic hormones are **large, integral membrane proteins** with specificity and high affinity for a given hormone.

2. The binding between hormone and receptor is reversible and the hormonal action declines as the plasma level of a hormone declines.

3. Group II hormones can initiate a response without entering the cell.

4. These hormones **tend to cause a more rapid response** and **have a shorter duration of action** than do group I hormones. **Their actions last from seconds to hours.**

cAMP — THE SECOND MESSENGER

Cycle AMP (cAMP, cyclic adenosine 3’,5’-monophosphate) is a ubiquitous nucleotide. It consists of adenine, ribose and a phosphate (linked by 3’,5’ lonkage). cAMP acts as a second messenger for a majority of polypeptide hormones.

The membrane-bound enzyme **adenylate cyclase** converts ATP to cycle AMP. cAMP is hydrolyzed by **phosphodiesterase.**

A series of events occur at the membrane level that influences the activity of adenylate cyclase leading to the synthesis of cAMP. This process is mediated by **G proteins** (Fig.24—2). G proteins are associated with **hormone receptors** on the **cytosolic side** of the cell membrane. The G protein is so named because it **binds guanine nucleotides.** Either guanosine triphosphate (GTP) or guanosine diphosphate (GDP) may be bound to the G protein.

a. The hormone binds to a receptor on the membrane which, in turn, stimulates the G-protein.

b. G proteins consist of **three subunits**: α, β, **and** γ. The α subunit binds GTP or GDP. The β and γ subunits do not bind nucleotides, they bind to the α subunit.

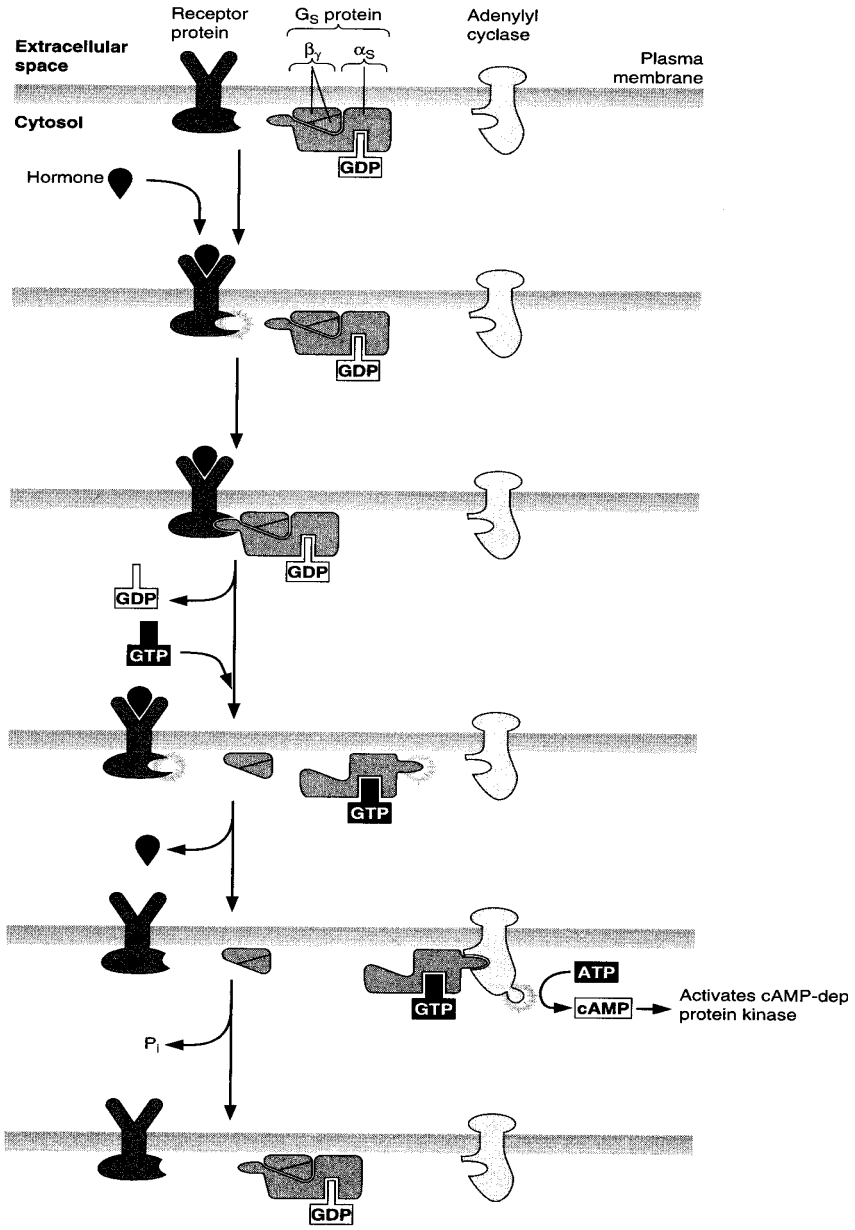


Figure 24—2. Activation of adenylate cyclase (by Davidson V.L. et al., 1999).

c. The **hormone-receptor complex** causes exchange of GDP with GTP on α subunit and dissociates it from $G_{\beta\gamma}$. The resultant α-subunit bound to GTP stimulates the adenylate cyclase.

d. ATP is converted to cAMP by the activated adenylate cyclase.

The effect of the active form (G_{α}-GTP) depends on the specific type of G protein. There are several different types of G proteins: $\mathbf{G_s}$ stimulates the enzyme adenylate cyclase, G_i inhibits the enzyme adenylate cyclase. The structure of β and γ subunits of G_i is similar to that of G protein with G_s.

However, α subunit ($G_{\alpha i}$) differs in structure.

GTP is short lived. The G_{α} subunit of all G proteins is a **GTPase.** It slowly hydrolyzes its bound GTP to GDP and thereby returns to its inactive, GDP-bound state. G_{α} then reassociates with $G_{\beta\gamma}$, where it remains until it is reactivated by a hormone-receptor complex.

Action of cAMP

1. cAMP affects cellular function by **activating a protein kinase**, which through phosphorylation activates specific cellular proteins. The protein kinase is specific for cAMP, and is thus called cAMP-dependent protein kinase.

2. The cAMP-dependent protein kinase is a tetramer having two types of subunits: two regulatory (R) subunits and two catalytic (C) subunits. The R_2C_2 tetramer is inactive. **Two molecules of cAMP** bind to each R subunit, whereupon the R_2C_2 complex disassociates into an R_2 subunit and two C units that are each catalytically active. The active protein kinase catalyzes phosphorylation of proteins. It is the phosphoprotein that ultimately causes the biochemical response.

3. The levels of cAMP are quickly reduced as a result of its hydrolysis to AMP by a cytoplasmic enzyme, cyclic nucleotide **phosphodiesterase**.

4. A group of enzymes called **protein phosphatases** hydrolyse and remove the phosphate group added to protein.

5. **Hormones that activate adenylate cyclase** include glucagon, ACTH, ADH, calcitonin, FSH, LH, PTH, TSH and epinephrine.

6. **Cholera toxin** is an enzyme produced by the bacterium *Vibrio cholerae.* Cholera toxin modifies the α subunit of G_s, which blocks the hydrolysis of GTP to GDP. This prevents the inactivation of G_s by this mechanism. The result is a persistently high level of cAMP, which causes the epithelial cells of the intestine to transport sodium ions and water into the intestinal lumen. This results in severe diarrhea.

7**. Pertussis toxin** is an enzyme that modifies the α subunit of G_i. The modification prevents G_i from exchanging GDP for GTP. Therefore, the modified G_i protein is unable to block the activation of adenylate cyclase. Pertussis toxin is produced by *Bordetella pertussis,* the bacterium that causes **whooping cough**.

PHOSPHATIDYL INOSITOL/CALCIUM SYSTEM AS SECOND MESSENGER

Many hormones and neurotransmitters act through the mediation of **phosphoinositide** system which generates two intracellular second messengers — diacylglycerols (DAG) and inositol-1,4,5-triphosphate (IP_3). Hormone-receptor complex, through G-protein (G_q), activates the membrane enzyme **phospholipase C.** This enzyme cleaves phosphatidyl inositol-4,5-bisphosphate to yield two products — **1,2-diacylglycerol** and inositol-**1,4,5-triphosphate** (Fig. 24—3).

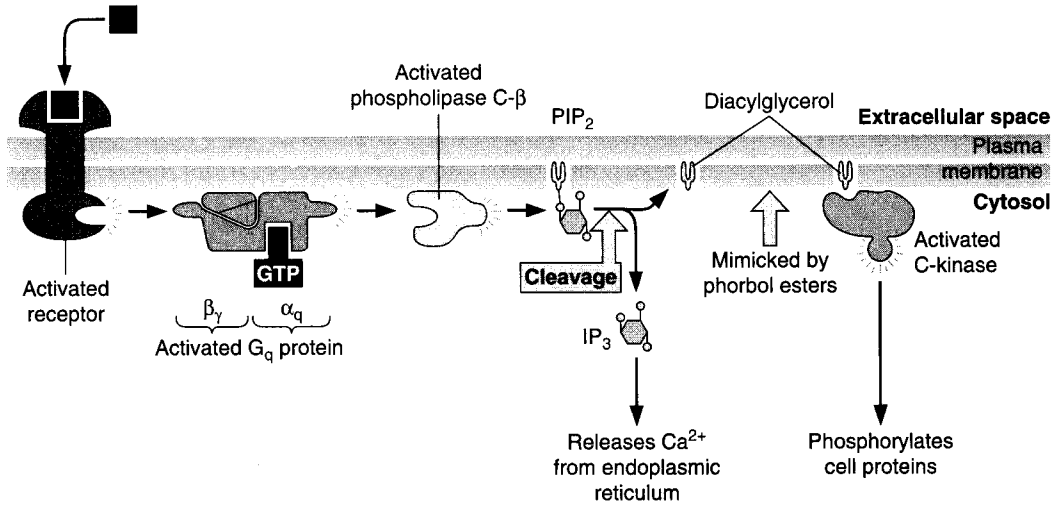


Figure 24—3. Activation of phospholipase C (by Davidson V.L. et al., 1999).

1. IP_3, a **water-soluble** molecule, diffuses into the cytosol and causes the release of **calcium ions** from endoplasmic reticulum. The Ca^{2+} in turn contributes to a variety of biochemical processes, predominantly through the mediation of **calmodulin.** Calmodulin has four Ca^{2+} binding sites, and full occupancy of these sites leads to a marked conformational change. This conformational change is presumably linked to calmodulin's ability to active or inactive enzymes. Calmodulin is often one numerous subunits of complex proteins. In addition to its effects on enzymes and ion transport, Ca^{2+}/calmodulin regulates the activity of many structural elements in cells. These include the actin-myosin complex of smooth muscle and various microfilament-mediated processes in noncontractile cells, including cell motility, conformation changes, mitosis, granule release, and endocytosis. IP_3 is rapidly inactivated by dephosphorylation.

2. DAG, a lipid-soluble molecule, diffuses laterally in the membrane and **activates protein kinase C, which is calcium-dependent**. Diacylglycerol greatly increases the affinity of protein kinase C to Ca^{2+}. DAG is rapidly inactivated by hydrolysis.

3. The function of the second messengers — inositol-1,4,5-triphosphate and diacylglycerol — is to phosphorylate the target proteins. They act synergis-

tically in this venture. The enzyme protein kinase C and calcium dependent calmodulin kinase bring about the protein phosphorylation.

4. Phorbol esters mimic the effects of DAG. They are **tumor promoters.** Although they do not cause tumors to form, they induce proliferation of cells. Unlike DAG, phorbol esters are not rapidly hydrolyzed. This results in prolonged activation of protein kinase C, which causes cell proliferation.

5. A hormone that activates phospholipase C is epinephrine at the α_1-adrenergic receptor subtype.

cGMP AS A MESSENGER

The synthesis of cyclic GMP is analogous to the formation of cAMP. The substrate GTP is acted upon by the membrane-bound enzyme **guanylate cyclase** to form cGMP. It was thought for sometime that cGMP acts as a functional antagonist of cAMP. It is now clear that cGMP has got own biochemical functions. Protein kinase G dependent on cGMP for protein phosphorylation is known. **Atriopeptins** are a small group of peptides synthesized by cardiac atrial tissue. They cause vasodilation, diuresis and inhibit aldosterone secretion. Atriopeptins exert their biochemical functions through cyclic GMP. cGMP is also involved in visual system, platelet aggregation and smooth muscle relaxation.

HYPOTHALAMUS HORMONES

Hypothalamus is a specialized center in the brain that functions as a **master coordinator** of **hormonal action**. In response to the stimuli of central nervous system, hypothalamus liberates certain releasing factors or hormones. These factors **stimulate** or **inhibit** the release of corresponding tropic hormones from the anterior pituitary. Tropic hormones stimulate the target endocrine tissues to secrete the hormones they synthesize. **The hormonal system is under feedback control**.

Hypothalamus produces at least six releasing factors or hormones.

1. Thyrotropin-releasing hormone (TRH). It is a tripeptide. TRH stimulates anterior pituitary to release thyroid-stimulating hormone (TSH or thyrotropin) which, in turn, stimulates the release of thyroid hormones (T_3 and T_4).

2. Corticotropin-releasing hormone (CRH). It stimulates anterior pituitary to release adrenocorticotropic hormone (ACTH) which in turn, acts on adrenal cortex to liberate adrenocorticosteroids. CRH contains 41 amino acids.

3. Gonatotropin-releasing hormone (GnRH). It is a decapeptide. GnRH stimulates anterior pituitary to release gonadotropins, namely luteinizing hormone (LH) and follicle stimulating hormone (FSH).

4. Growth hormone-releasing hormone (GRH) with 44 amino acids stimulates the release of growth hormone (GH or somatotropin) which promotes growth.

5. Growth hormone release-inhibiting hormone (GRIH). It contains 14 amino acids and is also known as somatostatin. GRIH inhibits the release of growth hormone from the anterior pituitary.

6. Prolactin release-inhibiting hormone (PRIH). It is believed to be a dopamine and/or a small peptide that inhibits the release of prolactin (PRL) from anterior pituitary.

It was originally thought that hypothalamic hormones act through cAMP for stimulating the release of anterior pituitary hormones. Recent studies, however, suggest that a calcium-phospholipid mediated mechanism is involved in the action of hypothalamic hormones.

PITUITARY HORMONES

The pituitary gland or hypophysis is located below the hypothalamus of the brain. It consists of two distinct parts — the anterior pituitary (adenohypophysis) and the posterior pituitary (neurohypophysis) connected by part intermedia. The latter is almost absent in humans, although found in lower organisms.

Anterior pituitary hormones

Anterior pituitary or adenohypophysis is truly the **master endocrine organ**. It produces several hormones that influence — either directly or indirectly — a variety of biochemical processes in the body. The hormones of adenohypophysis are broadly classified into three categories:

I. The growth hormone-prolactin group

II.The glycoprotein hormones

III. The pro-opiomelanocortin peptide family.

I. The growth hormone-prolactin group

1. Growth hormone (GH). The growth hormone (or **somatotropin**) is produced by somatotropes, a special group of acidophilic cells of anterior pituitary. Human growth hormone is a single polypeptide with 191 amino acids and a molecular weight of 22,000. Growth hormone promotes **growth** and also **influences the normal metabolisms** (protein, carbohydrate, lipid and mineral) in the body (details see lecture № 26).

2. Prolactin. Prolactin (PRL) is also called **lactogenic hormone**, luteotropic hormone, mammotropin or luteotropin.

PRL is produced by **lactotropes**, the **acidophilic cells** of anterior pituitary. It is a protein with a molecular weight of 23,000.

Regulation of PRL release. Prolactin release-inhibiting hormone (PRIH) of hypothalamus inhibits the secretion of prolactin. PRL release is increased during **pregnancy and lactation**. The hormones **estrogens** and oxytocin enhance prolactin secretion. Nipple stimulation, stress, sleep and sexual intercourse promote PRL release.

Biochemical functions of PRL. Prolactin is primarily concerned with the initiation and maintenance of **lactation in mammals**. PRL promotes transcription and translation in mammary glands. It increases the levels of several enzymes involved in carbohydrate and lipid metabolism. PRL **promotes pentose phosphate pathway, increases lipid biosynthesis** and **stimulates lactose production** in mammary glands.

Prolactin promotes the growth of corpus luteum (hence also known as luteotropic hormone) and stimulates the production of progesterone.

II. The glycoprotein hormones

1. Thyroid stimulating hormone (TSH). TSH is a dimer (αβ) glycoprotein with a molecular weigh-of about 30,000.

Regulation of TSH production. The release of TSH from anterior pituitary is controlled by feedback mechanism. This involves the hormone of thyroid gland (T_3 and T_4) and **thyrotropin releasing hormone** (TRH) of hypothalamus.

Functions of TSH. TSH binds with plasma membrane receptors and stimulates adenylate cyclase with a consequent increase in cAMP level. TSH, through the mediation of cAMP, exerts the following effects.

1. Promotes the uptake of iodide (iodide pump from the circulation by thyroid gland).

2. Enhances the conversion of iodide (I^-) to active iodide (I^+), a process known as organification.

3. Stimulates pentose phosphate pathway leading to increase formation of NADPH, required for H_2O_2 synthesis. NADPH and H_2O_2 are required for the synthesis of thyroid hormones.

4. Increases the proteolysis of thyroglobulin to release T_3 and T_4 into the circulation.

TSH increases the synthesis of proteins, nucleic acids and phospholipids in thyroid gland.

2. Gonadotropins. The follicle-stimulating hormone (FSH), luteinizing hormone (LH) and human chorionic gonadotropin (hCG) are commonly known as gonadotropins. All three are **glycoproteins.**

The release of FSH and LH from the anterior pituitary is controlled by **gonadotropin-releasin hormone** (GnRH) of hypothalamus.

Biochemical functions of FSH. In females, FSH stimulates follicular growth, increases the weight of the ovaries and enhances the production of estrogens.

In males, FSH stimulates testosterone production required for spermatogenesis. FSH also promotes growth of seminiferous tubules.

FSH binds with specific receptors on the follicular cells in the ovary and Sertoli cells in the testis. FSH activates adenylate cyclase and increases cAMP production through which the biochemical actions are exerted.

Biochemical functions of LH. Luteinizing hormone stimulates the **production of progesterone** from **corpus luteum cells in females** and **testosterone from Leydig cells in males**. The actions of LH are mediated through cAMP. LH and FSH are collectively responsible for the development and maintenance of secondary sexual characters in males.

3. Human chorionic gonadotropin (hCG). hCG is a glycoprotein (mol. wt. 100,000), produced by syncytiotrophoblast cells of placenta. The structure of

hCC closely resembles that of LH. The levels of hCG in plasma and urine increase almost immediately after the implantation of fertilized ovum. The detection of hCG in urine is conveniently used for the early detection (within a week after missing the menstrual cycle) of pregnancy.

III. The pro-opiomelanocortin (POMC) peptide family

This family consists of the hormones — **adrenocorticotropic hormone** (ACTH), **lipotropin** (LPH) and **melanocyte stimulating hormone** (MSH) and several (about 24) **neuromodulators** such as **endorphins** and **enkephalins.**

The synthesis of POMC family is very interesting. All the members of POMC are produced from a **single gene** of the anterior and intermediate lobes of pituitary. It is fascinating that a single polypeptide — pro-opiomelanocortin — is the precursor (approximately 285 amino acids) that contains multiple hormones. The name pro-opiomelanocortin is derived since it is a **pro**hormone to **opio**ids, **melano**cyte-stimulating hormone and **corti**cotropi**n**. POMC and its related products occur in several tissues — brain, lung, placenta, gastrointestinal tract etc. Due to differences in the cleavage of POMC, different products are obtained in different tissues.

1. Adrenocorticotropic hormone (ACTH). ACTH is a polypeptide with **39 amino acids** and a molecular weight of 4,500. This hormone is primarily concerned with the **growth and functions of adrenal cortex**.

Regulation of ACTH production. The release of ACTH from the anterior pituitary is under the regulation of hypothalamic hormone, namely **corticotropin releasing hormone** (CRH). The secretion of CRH is influenced by neurotransmitters such as serotonin, acetylcholine and norepinephrine.

Biochemical functions of ACTH

1. ACTH promotes the conversion of cholesterol to pregnenolone in the adrenal cortex. Pregnenolone in turn is the immediate precursor for the synthesis of all adrenocorticosteroids.

2. It enhances RNA and protein synthesis and thus promotes adrenocortical growth.

3. ACTH increases lipolysis by activating lipase of adipose tissue.

4. It stimulates the release of insulin from pancreas.

5. ACTH can act directly on melanocytes and cause increased pigmentation of the skin.

Overproduction of ACTH. Cushing's syndrome is caused by an excessive production of ACTH which may be due to a tumor. This syndrome is characterized by **hyperpigmentation** and **increased production of adrenocorticosteroids**. The associated symptoms include negative nitrogen balance, impaired glucose tolerance, hypertension, edema, muscle atrophy etc.

2. β-Lipotropin (β-LPH). β-LPH is derived from POMC and contains 93 carboxy terminal amino acids. This polypeptide consists of γ-LPH and β-endorphin from which β-MSH and γ-endorphin are, respectively, formed. γ-

Endorphin can be converted to α-endorphin and then to enkephalins (Fig. 24—4). β-**LPH** is found **only in the pituitary** and not in other tissues since it is rapidly degraded. The biochemical functions of β-LPH, as such, are limited. It **promotes lipolysis** and **increases the mobilization of fatty acids**. The most important function of β-LPH is its precursor role for the formation of β-endorphin and enkephalins.

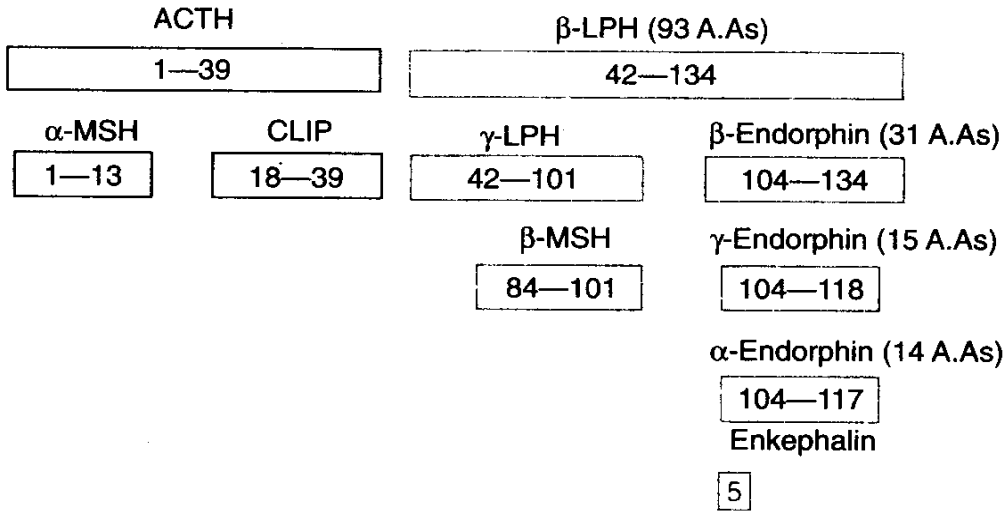


Figure 24—4. The members of the pro-opiomelanocortin family

Endorphins and enkephalins. These are the natural analgesics that control **pain and emotions.** They were discovered after an unexpected finding of opiate receptors in the human brain.

Synthesis. Endorphins and enkephalins are produced from β-endorphin which is derived from POMC. β-Endorphin has 31 amino acids while its modified products α and γ-endorphins have 15 and 14 amino acids, respectively.

Biochemical actions. Endorphins and enkephalins are peptide neurotransmitters that produce **opiate-like effects** on the central nervous system, hence they are also known as opioid-peptides. They bind to the same receptors as the morphine opiates and are believed to control the endogenous pain perception. Endorphins and enkephalins are more potent (20—30 times) than morphine in their function as analgesics.

It is **believed that the pain relief** through **acupuncture and placebos is mediated through opioid peptides.**

3. Melanocyte-stimulating hormone (MSH). Three types of MSH (α, β and γ) are present in the precursor POMC molecule. In humans, γ-**MSH** is important while in some animals α and β are functional. The activity of γ-MSH is contained in the molecule γ-LPH or its precursor β-LPH.

The functions of MSH have been clearly established in some animals. MSH **promotes the synthesis of skin pigment melanin** (melanogenesis) and disperses melanin granules that ultimately lead to darkening of the skin. In humans, MSH does not appear to play any role in melanin synthesis.

Posterior pituitary hormones

Two hormones namely **oxytocin** and **antidiuretic hormone** (ADH, vasopressin) are produced from the posterior pituitary (neurohypophysis). Both of them are nonapeptides (9 amino acids).

Oxytocin is primarily synthesized in the **paraventricular nucleus** whereas **ADH** is produced in **supraoptic nucleus** of the hypothalamus. Two carrier proteins — neurophysin I and II — are also synthesized along with these hormones. Oxytocin and ADH are transported through axons in combination with neurophysins and released into the posterior pituitary gland.

1. Oxytocin. The release of oxytocin from posterior pituitary gland is caused by the neural impulses of nipple stimulation. The other stimuli responsible for oxytocin release include vaginal and uterine distention.

Biochemical functions.

1. Effect on uterus: oxytocin causes the contraction of pregnant uterus (smooth muscles) and induces labor. This property of oxytocin is exploited and pharmacological doses of oxytocin are administered to induce labor.

2. Effect on milk ejection. In mammals, oxytocin causes contraction of myoepithelial cells (look like smooth muscle cells) of breast. This stimulates the squeezing effect, causing milk ejection from the breast.

3. Oxytocin synthesized in the ovary appears to **inhibit the synthesis of steroids.**

The mechanism of action of oxytocin is not clearly known. Membrane receptors for oxytocin are found in the uterus and mammary through which this hormone action is mediated.

2**. Antidiuretic hormone (ADH).** The release of ADH (also called **vasopressin**) mostly controlled by **osmoreceptors** (of thalamus) and baroreceptors (of heart), increase in the osmolarity of plasma stimulate ADH secretion. On the other hand, decreased plasma osmolarity depresses ADH release. **Physical** and **emotional stress** and **pharmacological** agents (e.g. morphine, nicotine) increase the synthesis and release of ADH. Epinephrine, on the other hand, inhibits ADH secretion.

Biochemical functions. ADH is primarily concerned with the regulation of water balance in the body. It stimulates kidneys to retain water and, thus, increases the blood pressure. In the absence of ADH, the urine output we be around 20 l/day. ADH acts on the distal convoluted tubules of kidneys and causes water reabsorption with a result that the urine output is around 0.5—1.5 l/day.

Mechanism of action. ADH specific receptors on the basal side of the collecting tube epithelial cells have been identified. ADH stimulates adenylate cyclase causing production of cAMP. Water reabsorption is promoted by cAMP. Inhibitors of adenylate cyclase (e.g. calcium) inhibit the activity of ADH. This support the view that ADH action is mostly mediated through cAMP.

LECTURE 25

HORMONAL REGULATION OF CARBOHYDRATES, LIPIDS AND PROTEIN METABOLISM: INSULIN, GLUCAGON, CATECHOLAMINES, GLUCOCORTICOIDS

INSULIN

Insulin is a **polypeptide hormone** produced by the **β-cells of islets** of **Langerhans of pancreas**. It has profound influence on the metabolism of **carbohydrate, fat and protein**. Insulin is considered as **anabolic hormone**, as it **promotes the synthesis of glycogen, triacylglycerols and proteins**.

Brief history

Insulin occupies a special place in the history of biochemistry as well as medicine. The islets of pancreas were identified by **Langerhans** in as early as **1860**. However, it was only in **1909** that **de Mayer** suggested an association between diabetes and the islets. **Banting and Best (1921)** extracted insulin from dog's pancreas and proved its association with diabetes.

Historically, insulin has several firsts to its credit.

1. Insulin was the first hormone to be isolated, purified, crystallized and synthesized.

2. The first protein detected to possess hormonal activity.

3. The first protein sequenced for amino acids to determine the structure.

4. The first protein estimated by radioimmunoassay.

5. The first protein produced by recombinant DNA technology.

Structure of insulin

Human insulin (mol. wt. 5,734) contains **51 amino acids**, arranged in **two polypeptide chains**. The chain **A** has **21 amino acids** while **B** has **30 amino acids**. Both are held together by two **interchain disulfide bridges**, connecting A_7 to B_7 and A_{20} to B_{19}. In addition, there is an intrachain disulfide link in chain A between the amino acids 6 and 11.

In most species, the structure of insulin **closely resembles** that of human with slight variations. For instance, pork (porcine) insulin differs from human insulin just by one amino acid — alanine in place of threonine at the C-terminal end of B-chain of human insulin. Variations are also common among the different species in positions 8, 9 and 10 of A chain of insulin. These three amino acids, however, do not determine the biological activity of insulin. In fact, **porcine** and **bovine insulin** were the standard therapy for diabetes in humans, until human insulin, in abundant quantity, was produced by genetic engineering.

Biosynthesis of insulin

Insulin is produced by the **β-cells of the islets of Langerhans of pancreas**. The gene for this protein synthesis is located on **chromosome 11**. The synthesis of insulin involves two precursors, namely **preproinsulin** with **108 amino acids** (mol. wt. 11,500) and **proinsulin with 86 amino acids** (mol. wt. 9,000). They

are sequentially degraded to form the active hormone insulin and a connecting peptide **(C-peptide).** Insulin and C-peptide are produced in **equimolar concentration**. C-peptide has no biological activity; however its estimation in the plasma serves as a **useful index** for the **endogenous production of insulin**.

In the β-cells, insulin (and also proinsulin) combines with **zinc** to form complexes. In this form, insulin is stored in the granules of the cytosol which is released in response to various stimuli by exocytosis.

Regulation of insulin secretion

About **40-50 units** of insulin are secreted daily by human pancreas. The normal insulin concentration in plasma is 20—30 μU/ml. The important factors that influence the release of insulin from the β-cells of pancreas are discussed hereunder.

1. Factors stimulating insulin secretion.

a. Glucose is the most important stimulus for insulin release. The effect is more predominant when glucose is administered orally. A **rise in blood glucose level** is a signal for insulin secretion. It is believed that glucose combines with a receptor and stimulates insulin release through the mediation of Ca^{2+} and cAMP.

b. Amino acids induce the secretion of insulin. This is particularly observed after the ingestion of protein-rich meal that causes transient rise in plasma amino acid concentration. Among the amino acids, **arginine** and **leucine** are potent stimulators of insulin release.

c. Gastrointestinal hormones (secretin, gastrin, pancreozymin) enhance the secretion of insulin. The GIT hormones are released after the ingestion of food. Oral glucose is more effective in promoting insulin secretion than that administered intravenously. This is attributed to the additional influence of GIT hormones.

d. Growth hormone, cortisol and estrogens also increase insulin secretion.

2. Factors inhibiting insulin secretion. **Epinephrine** is the most predominant inhibitor of insulin release. In emergency situations like stress, extreme exercise and trauma, the nervous system stimulates adrenal medulla to release epinephrine. Epinephrine suppresses insulin release and promotes energy metabolism by mobilizing energy-yielding compounds — glucose from liver and fatty acids from adipose tissue.

Degradation of insulin

In the plasma, insulin has a normal half-life of **4—5 minutes**. This short half-life permits rapid metabolic changes in accordance to the alterations in the circulating levels of insulin. A protease enzyme, namely **insulinase** (mainly found in liver and kidney), degrades insulin.

Metabolic effects of insulin

Insulin plays a key role in the regulation of **carbohydrate, lipid and protein** metabolisms. Insulin exerts anabolic and anticatabolic influences on the body metabolism.

1. Effects on carbohydrate metabolism. In a normal individual, about **50%** of the ingested glucose is utilized to meet the energy demands of the body (mainly through **glycolysis**). The other half is either converted to **fat** (~ 40%) or **glycogen** (-10%). Insulin influences glucose metabolism in many ways. The net effect is that insulin **lowers blood glucose level (hypoglycemic effect)** by promoting its utilization and storage and by inhibiting its production.

a. Effect on glucose uptake by tissues. Insulin is required for the uptake of glucose by **muscle** (skeletal, cardiac and smooth), **adipose tissue, leukocytes and mammary glands**. About 80% of glucose uptake in the body is not dependent on insulin. Tissues into which glucose can freely enter include **brain**, **kidney**, **erythrocytes**, **retina, nerve, blood vessels** and **intestinal mucosa**. As regards liver, glucose entry into hepatocytes does not require insulin. However, insulin stimulates glucose utilization in liver and, thus, indirectly promotes its uptake.

b. Effect on glucose utilization. Insulin **increases glycolysis** in **muscle** and **liver**. The activation as well as the amounts of certain key enzymes of glycolysis, namely **glucokinase** (not hexokinase), **phosphofructokinase** and **pyruvate kinase** are increased by insulin. Glycogen production is enhanced by insulin by increasing the activity of **glycogen synthase**. Insulin also stimulates pentose phosphate pathway by increasing the activity of **glucose 6-phosphate dehydrogenase**.

c. Effect on glucose production. Insulin **decreases gluconeogenesis** by suppressing the enzymes **pyruvate carboxylase, phosphoenol pyruvate carboxykinase** and **glucose 6-phosphatase**. Insulin also inhibits glycogenolysis by inactivating the enzyme glycogen phosphorylase.

2. Effects on lipid metabolism. The net effect of insulin on lipid metabolism is to **reduce the release of fatty acids** from the stored fat and **decrease the production of ketone bodies**. Among the tissues, adipose tissue is the most sensitive to the action of insulin.

a. Effect on lipogenesis. Insulin favours the synthesis of **triacylglycerols** from glucose by providing more **glycerol-3-phosphate** (from glycolysis) and **NADPH** (from pentose phosphate pathway). Insulin increases the activity of **acetyl CoA carboxylase**, a key enzyme in fatty acid synthesis.

b. Effect on lipolysis. Insulin **decreases** the activity of **hormone-sensitive lipase** and thus **reduces the release of fatty acids** from stored fat in adipose tissue. The mobilization of fatty acids from liver is also decreased by insulin. In this way, insulin keeps the circulating free fatty acids under a constant check.

c. Effect on ketogenesis. Insulin **reduces ketogenesis** by decreasing the activity of **HMG CoA synthetase**. Further, insulin promotes the **utilization of acetyl CoA** for oxidation (Krebs cycle) and **lipogenesis.** Therefore, the availability of acetyl CoA for ketogenesis, in the normal circumstances, is very low.

d. Effect on lipoprotein metabolism. It appears that insulin is required for the utilization of VLDL and LDL. The levels of VLDL and LDL and consequently the concentration of cholesterol are elevated in diabetics which has been implicated in the pathogenesis of atherosclerosis.

3. Effects on protein metabolism. Insulin is an **anabolic hormone**. It stimulates the entry of amino acids into the cells, enhances protein synthesis and reduces protein degradation.

4. Besides the metabolic effects described above, insulin promotes cell **growth and replication**. This is mediated through certain factors such as epidermal growth factor (ECF), platelet derived growth factor (PDGF) and prostaglandins.

Mechanism of insulin action

It is now recognized that insulin binds to specific plasma membrane receptors present on the target tissues, such as muscle and adipose. This results in a series of reactions ultimately leading to the biological action. Three distinct mechanisms of insulin action are known. One concerned with the **induction of transmembrane signals** (signal transduction), second with the **glucose transport across the membrane** and the **third with induction of enzyme synthesis.**

1. Insulin receptor. It is a tetramer consisting of 4 subunits of two types and is designated as $\alpha_2\beta_2$. The subunits are in the glycosylated form. Disulfide linkages hold them together. The α-subunit (mol. wt. 135,000) is extracellular and it contains insulin-binding site. The β-subunit (mol. wt. 95,000) is a transmembrane protein which is activated by insulin. The cytoplasmic domain of β-subunit has **tyrosine kinase activity**. The insulin receptor has a half-life of 6—12 hours. There are about 20,000 receptors per cell in mammals.

Signal transduction. As the hormone insulin binds to the receptor, a conformational change is induced in the α-subunits of insulin receptor. This results in the generation of signals which are transduced to β-subunits. The net effect is that insulin binding activates tyrosine kinase activity of intracellular β-subunit of insulin receptor. This causes the **autophosphorylation of tyrosine residues** on β-subunit. The phosphorylated β-subunits promote activation of other **protein kinases** and **phosphatases**, finally leading to biological action.

2. Insulin-mediated glucose transport. The binding of insulin to insulin receptors signals the translocation of glucose transporters from intracellular pool to the plasma membrane. The glucose transporters are responsible for the insulin-mediated uptake of glucose by the cells. As the insulin level falls, the glucose transporters move away from the membrane to the intracellular pool for storage and recycle.

3. Insulin mediated enzyme synthesis. Insulin **promotes the synthesis of enzymes** such as glucokinase, phosphofructokinase and pyruvate kinase. This is brought about by increased transcription (mRNA synthesis), followed by translation (protein synthesis).

Time course of insulin action

Insulin-mediated glucose transport is the most immediate that occurs within **seconds** after insulin binds to membrane. Signal transduction process may take **minutes** to **hours**, while increase in the enzyme synthesis requires hours to days.

DIABETES

Insulin has been implicated in the development **of diabetes mellitus**.

Diabetes mellitus is broadly divided into 2 groups, namely **insulin-dependent** diabetes mellitus (IDDM) and **non-insulin dependent diabetes** mellitus (NIDDM). This classification is mainly based on the requirement of insulin for treatment. (Note: IDDM and NIDDM represent the **primary type** of diabetes mellitus. In addition, there exists a secondary type of diabetes mellitus associated with certain pathological conditions e.g. pancreatitis, cystic fibrosis, acromegaly, Cushing's syndrome. Only the primary diabetes mellitus is discussed here).

Insulin-dependent diabetes mellitus (IDDM)

IDDM, also known as type **I diabetes** or (less frequently) **juvenile onset** diabetes, mainly occurs in **childhood** (particularly between 12—15 yrs age). IDDM accounts for about **10 to 20%** of the known diabetics. This disease is characterized by almost total deficiency of insulin due to destruction of β-cells of pancreas. The β-cell destruction may be caused by **drugs, viruses** or **autoimmunity**. Due to certain genetic variation, the β-cells are recognized as non-self and they are destroyed by immune mediated injury. Usually, the symptoms of diabetes appear when 80—90% of the β-cells have been destroyed. The pancreas ultimately fails to secrete insulin in response to glucose ingestion. The patients of IDDM require **insulin therapy**.

Non-insulin dependent diabetes mellitus (NIDDM)

NIDDM, also called **type II diabetes** or (less frequently) **adult-onset diabetes**, is the most common, accounting for **80 to 90%** of the diabetic population. NIDDM occurs in adults (usually above 35 years) and is less severe than IDDM. The causative factors of NIDDM include genetic and environmental. NIDDM more commonly occurs **in obese individuals.** Obesity acts as a **diabetogenic** factor in genetically predisposed individuals by increasing the resistance to the action of insulin. This is due to a **decrease** in insulin receptors on the **insulin responsive** (target) cells. The patients of NIDDM may have either **normal** or even **increased** insulin levels. It is suggested that over-eating causes increased insulin production but decreased synthesis of insulin receptors. This is based on the fact that weight reduction by diet control alone is often sufficient to correct NIDDM.

Metabolic changes in diabetes

Diabetes mellitus is associated with several **metabolic alterations**. Most important among them are **hyperglycemia**, **ketoacidosis** and **hypertriglyceridemia**.

1. Hyperglycemia. Elevation of blood glucose concentration is the **hallmark** of uncontrolled diabetes. Hyperglycemia is primarily due to reduced glu-

cose uptake by tissues and its increased production via gluconeogenesis and glycogenolysis. When the blood glucose level goes beyond the renal threshold, glucose is excreted into urine (glycosuria).

2. Ketoacidosis. Increased mobilization of fatty acids results in overproduction of ketone bodies which often leads to ketoacidosis.

3. Hypertriglyceridemia. Conversion of fatty acids to triacylglycerols and the secretion of VLDL and chylomicrons are comparatively higher in diabetics. Further, the activity of the enzyme lipoprotein lipase is low in diabetic patients. Consequently, the plasma levels of VLDL, chylomicrons and triacylglycerols are increased. Hypercholesterolemia is also frequently seen in diabetics.

Long-term effects of diabetes

Hyperglycemia is directly or indirectly associated with several complications. These include **atherosclerosis, retinopathy, nephropathy** and **neuropathy**. The biochemical basis of these complications is not clearly understood. It is believed that at least some they are related to **microvascular changes caused by glycation of proteins**.

Management of diabetes

Diet, exercise, drug and, finally, **insulin** are the management options in diabetics. Approximately, 50% of the new cases of diabetes can be adequately controlled by diet alone, 20—30% need oral hypoglycemic drugs while the remaining 20—30% require insulin.

Dietary management. A diabetic patient is advised to consume **low calories** (i.e. low carbohydrate and fat), **high protein** and **fiber rich diet**. Carbohydrates should be taken in the form of **starches** and **complex sugars**. Refined sugars (sucrose, glucose) should be avoided. **Fat** intake should be **drastically reduced** so as to meet the nutritional requirements of unsaturated fatty acids.

Hypoglycemic drugs. The oral hypoglycemic drugs are broadly of two categories sulfonylureas and biguanides. The latter are less commonly used these days due to side effects. **Sulfonylureas** such as acetohexamide, tolbuta-mide and gibenclamide are frequently used. They **promote** the secretion of **endogenous insulin** and thus help in reducing blood glucose level.

Management with insulin. Two types of insulin preparations are commercially available — **short acting** and **long acting**. The short acting insulins are unmodified and their action lasts for about 6 hours. The long acting insulins are modified ones (such as adsorption to protamine) and act for several hours, which depends on the type of preparation. The advent of genetic engineering is a boon to diabetic patients since bulk quantities of insulin can be produced in the laboratory.

Biochemical indices of diabetic control

1. Urine glucose detection and **blood glucose** estimations are traditionally followed in several laboratories. In recent years, more reliable and long-term biochemical indices of diabetic control are in use.

2. Glycated hemoglobin. Glycated or glycosylated hemoglobin refers to the glucose derived products of normal adult hemoglobin (HbA). Glycation is a post-translational, non-enzymatic addition of sugar residue to amino acids of proteins. Among the glycated hemoglobins, the most abundant form is HbA_{1c}. The rate of synthesis of HbA_{1c} is directly related to the exposure of red blood cells to glucose. Thus, the concentration of **HbA_{1c} serves as an indication of the blood glucose concentration over a period**, approximating to the half-life of RBC (hemoglobin) i.e. **6—8 weeks.** A close correlation between the blood glucose and HbA_{1c} concentrations have been observed when simultaneously monitored for several months.

3. Microalbuminuria is defined as the excretion of 30—300 mg of albumin in urine per day. It may be noted that microalbuminuria represents an intermediary stage between normal albumin excretion (2.5—30 mg/dl) and macroalbuminuria (>300 mg/dl). The small increase in albumin excretion predicts impairment in renal function in diabetic patients. Microalbuminuria serves as a signal of early reversible renal damage.

4. Determination of serum lipids (total cholesterol, HDL, triglycerides) serves as an index for overall metabolic control in diabetic patients. Hence, serum lipids should be frequently measured

GLUCAGON

Glucagon, secreted by **α-cells of the pancreas**, opposes the actions of insulin. It is a polypeptide hormone composed of **29 amino acids** (mol. wt. 3,500) in a single chain. Glucagon is actually synthesized as **proglucagon** (mol. wt. 9,000) which on sequential degradation releases active glucagon. Unlike insulin, the amino acid sequence of glucagon is the same in all mammalian species (so far studied). Glucagon has a shorter half-life in plasma i.e. about **5 minutes.**

Regulation of glucagon secretion

The secretion of glucagon is stimulated by **low blood glucose concentration**, **amino acids** derived from dietary protein and **low levels of epinephrine**. Increased blood glucose level markedly inhibits glucagon secretion.

Metabolic effects of glucagon

Glucagon influences **carbohydrate, lipid and protein metabolisms**. In general, the effects of this hormone **oppose that of insulin**.

1. Effects on carbohydrate metabolism. Glucagon is the most potent hormone that **enhances the blood glucose level (hyperglycemic).** Primarily, glucagon acts on liver to cause **increased synthesis of glucose (gluconeogenesis)** and **enhanced degradation of glycogen (glycogenolysis).** The actions of glucagon are mediated through cyclic AMP.

2. Effects on lipid metabolism. Glucagon **promotes fatty acid oxidation** resulting in energy production and **ketone body synthesis (ketogenesis).**

3. Effects on protein metabolism. Glucagon increases the amino acid uptake by liver which, in turn, promotes gluconeogenesis. Thus, glucagon **lowers plasma amino acids**.

HORMONES OF ADRENAL MEDULLA

Adrenal medulla is an extension of sympathetic nervous system. It produces two important hormones — **epinephrine** (formerly adrenaline) an **norepinephrine** (formerly noradrenaline). Both these hormones are catecholamines since they are amine derivatives of catechol nucleus (dihydroxylated phenyl ring). Epinephrine is a methyl derivative of norepinephrine. **Dopamine** is another catecholamine, produced as an intermediate during the synthesis of epinephrine. **Norepinephrine** and **dopamine** are important neurotransmitters **in the brain** and **autonomic nervous system**. Dopamine is predominantly synthesized in **substantia nigra** and **coerules of brain**.

Synthesis of catecholamines

The amino acid tyrosine is the precursor for the synthesis of catecholamines (Fig. 25—1). Catecholamines are produced response to **fight, fright and flight**. These include the emergencies like shock, cold, fatigue, emotional conditions like anger etc.

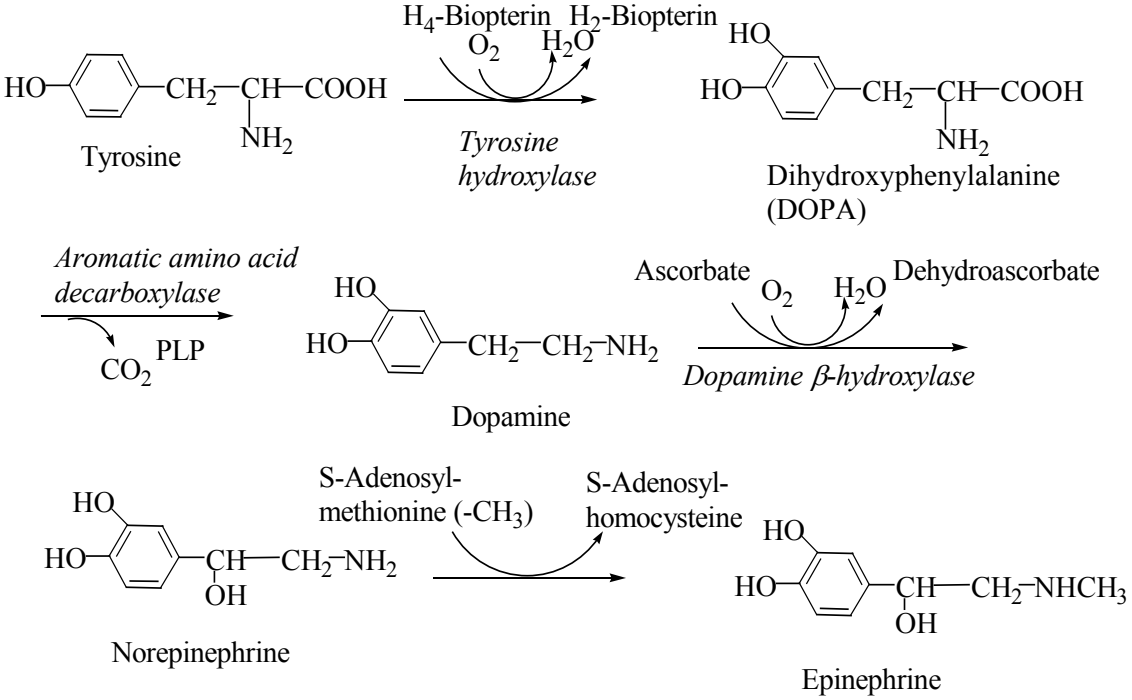


Figure 25—1. Synthesis of catecholamines.

Storage and release of catecholamine

Epinephrine constitutes about **80%** of the catecholamines present in the adrenal medulla (1—3 mg/g of epinephrine and 0.2—0.6 mg/g norepinephrine are found). **Chromaffin granules** of adrenal medulla are the organelles responsible for the synthesis, storage and release catecholamines. **Neural stimulation** of adrenal medulla causes the release of the hormones.

Catecholamines are found in plasma in loose association with **albumine**. Their half-lives are very short, less than **30 second**. The plasma concentration of norepinephrine is much higher than that of epinephrine.

Biochemical functions of catecholamines

Catecholamines cause diversified biochemical effects on the body. The ultimate goal of their action is to **mobilize energy resources** and **prepare** the **individuals to meet emergencies** (e.g. shock, cold, low blood glucose etc.).

1. Effects on carbohydrate metabolism. Epinephrine and norepinephrine in general increase the degradation of glycogen (glycogenolysis), synthesis of glucose (gluconeogenesis) and decrease glycogen formation (glycogenesis). By virtue of these actions, catecholamines promote the release of glucose from liver and decrease its utilization by muscle.

2. Epinephrine inhibits insulin secretion but **promotes glucagon secretion**. The overall effect of catecholamines is to **elevate blood glucose** levels and make it available for the brain and other tissues to meet the emergencies.

3. Effects on lipid metabolism. Both epinephrine and norepinephrine **enhance** the **breakdown of triacylglycerols** (lipolysis) in adipose tissue. This causes increase in the free fatty acids in the circulation which are effectively utilized by the heart and muscle as fuel source. The metabolic effects of catecholamines are mostly related to the increase in adenylate cyclase activity causing **elevation in cyclic AMP levels.**

4. Effects on physiological functions. In general, catecholamines (most predominantly epinephrine) increase **cardiac output**, **blood pressure** and **oxygen consumption**. They cause smooth muscle relaxation in bronchi, gastrointestinal tract and the blood vessels supplying skeletal muscle. On the other hand, catecholamines stimulate smooth muscle contraction of the blood vessels supplying skin and kidney. Platelet aggregation is inhibited by catecholamines.

Mechanism of action of catecholamines

Catecholamines act through two alpha (α_1 and α_2) and two beta (β_1 and β_2) receptors. The receptors are glycoprotein in nature. The study of receptors is based on the response to **agonists and antagonists.** Agonists are the substances that bind to receptors and evoke hormonal responses. On the other hand, antagonists are the substances that bind to receptors and block agonist actions. For instance, isoproterenol is an agonist that stimulates β-receptors while propranolol is an antagonist that blocks β-receptors. Phentolamine blocks α-receptors.

Epinephrine binds and activates both α and β receptors whereas norepinephrine mostly binds to α-receptors. The catecholamines act on β_1 and β_2 receptors to stimulate adenylate cyclase and increase the synthesis of cAMP. α-Receptors are not coupled to adenylate cyclase and the catecholamines are believed to act through increased influx of calcium.

Metabolism of catecholamines

Catecholamines are rapidly inactivated and metabolized. The enzymes **catechol-O-methyltransferase** and **monoamine oxidase** (MAO), found in many tissues act on catecholamines. The metabolic products metanephrine and vanillylmandelic acid (VMA) are excreted in urine.

Abnormalities of catecholamine production

Pheochromocytomas. These are the tumors of adrenal medulla. The diagnosis of pheochromocytoma is possible only when there is an excessive production of epinephrine and norepinephrine that causes severe hypertension. In the individuals affected by this disorder, the ratio of norepinephrine to epinephrine is increased. The measurement of urinary VMA (normal <8 mg/day) is helpful in the diagnosis of pheochromocytomas.

Parkinson's disease. Impairment in the synthesis of dopamine by the brain is believed to be the major causative factor of Parkinson's disease. Parkinson's disease is a common disorder in many elderly people, with about 1% of the population above 60 years being affected. It is characterized by muscular rigidity, tremors, expressionless face, lethargy, involuntary movement etc. Parkinson's disease is, however, linked with a **decreased production of dopamine** due to degeneration of certain parts of the brain (substantia nigra and coeruleus locus). **Treatment.** Dopamine cannot enter the brain, hence its administration is of no use. DOPA (levodopa or L-dopa) is used in the treatment of Parkinson's disease. In the brain, DOPA is decarboxylated to dopamine which alleviated the symptoms of this disorder. Unfortunately, dopamine synthesis occurs in various other tissues and results in side-effects such as nausea, vomiting, hypertension etc.

GLUCOCORTICOIDS

The adrenal glands are two small organs (each weighing about 10 g), located above the kidney. Each adrenal consists of two distinct tissues — an **outer cortex** (with 3 zones) and **inner medulla**. As many as 50 steroid hormones (namely **adrenocorticosteroids**), produced by adrenal cortex, have been identified. However, only a few of them possess biological activity. Adrenocorticosteroids are classified into three groups according to their dominant biological action: **glucocorticoids, mineralocorticoids**, **androgens** and **estrogens**.

Glucocorticoids are 21-carbon steroids, produced mostly by **zona fasciculate**. They affect glucose (hence the name), amino acid and fat metabolism in a manner that opposite to the action of insulin. **Cortisol** (also known as hydrocorticosteron) is the most important glucocorticoids in human. Corticosterone is **predominant** in rats.

Synthesis of corticosteroids

Cholesterol is the precursor for the synthesis of steroids hormones. Cholesterol undergoes cleavage with an elimination of 6-carbon fragment to form pregnenolone. **Pregnenolone is the common precursors for the synthesis of all steroids hormones** (Fig. 25—2). Conversion of cholesterol to pregnenolone is catalysed by cytochrome P_{450} side chain cleavage enzyme. This reaction is promoted by **ACTH**. The enzymes — hydroxylases, dehydrogenases/isomerases and lyases associated with mitochondria or endoplasmic reticulum — are responsible for the synthesis of steroid hormones.

Transport of corticosteroids

The steroid hormones, being **insoluble in water**, are bound to proteins and transported. They are mostly bound to a glycoprotein **transcortin** (cortisol bind-

ing protein) and, to a lesser extent, to albumin. Cortisol predominantly binds to transcortin while aldosterone is bound to albumin.

Biochemical functions of glucocorticoid hormones

1. The important glucocorticoids are cortisol, cortisone and corticosterone.

a. Effects on carbohydrate metabolism. Glucocorticoids promote the **synthesis of glucose** (gluconeogenesis). This is brought about by **increasing the substrates** (particularly amino acids) and **enhancing the synthesis of phosphoenolpyruvate carboxykinase**, the rate limiting enzyme in gluconeogenesis. Glucocorticoids **inhibit the uptake** and utilization of glucose by extrahepatic tissues.

These hormones promote **glycogen synthesis** in **liver** by increasing the synthase.

The overall influence of glucocorticoids on carbohydrate metabolism is to **increase blood glucose concentration**. This is more needed in situations like starvation where glucocorticoids effectively function. The biological actions of glucocorticoids generally **oppose that of insulin.**

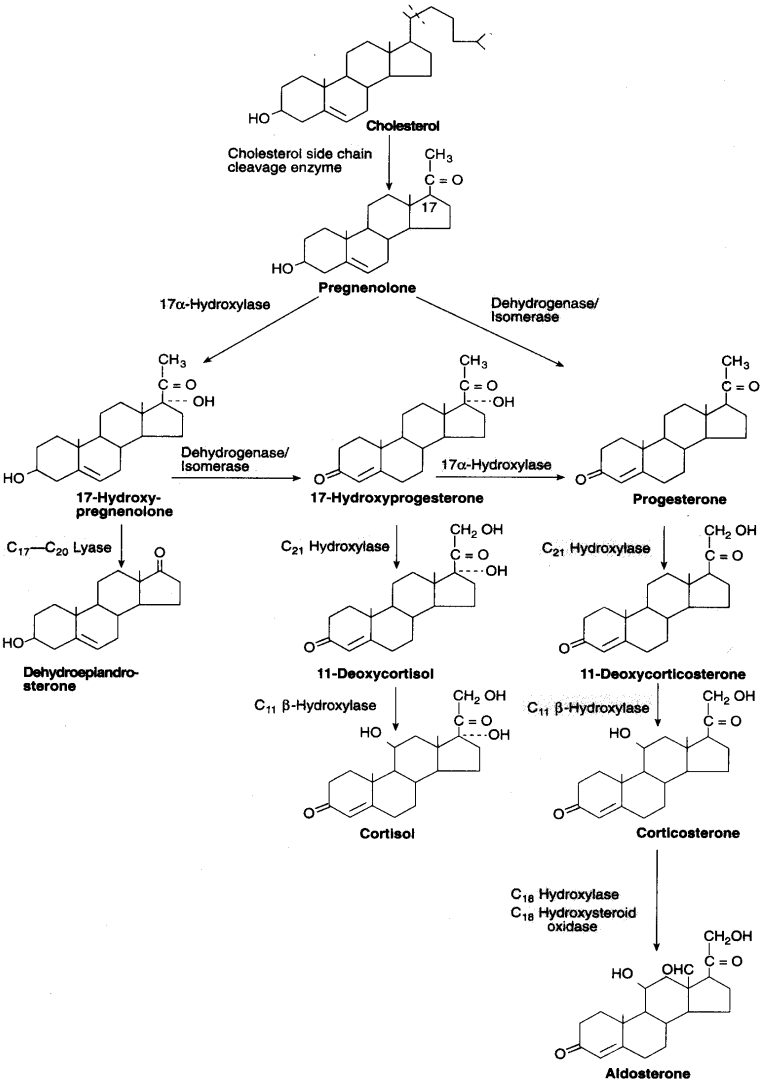


Figure 25—2. Biosynthesis of major adrenocorticosteroids (by U. Satyanarayana, 2002).

b. Effects on lipid metabolism. Glucocorticoids increase the circulating free fatty acids. This is caused by two mechanisms.

(1) Increased breakdown of **storage triacylglycerol** (lipolysis) in adipose tissue.

(2) Reduced utilization of plasma acids for the synthesis of triacylglycerols. This is mostly due to the insufficient availability of glycerol as a consequence of reduced glucose uptake by adipose tissue. Glycerol is mostly formed from glucose and is essentially required for the synthesis of triacylglycerols. Increased plasma levels of free fatty acids are often associated with ketosis. This is particularly observed in insulin deficient state.

(3) Effects on protein and nucleic acid metabolism. Glucocorticoids exhibit both catabolic and anabolic effects on protein and nucleic acid metabolism. They **promote transcription** (RNA synthesis) and protein biosynthesis in **liver**. These anabolic effects of glucocorticoids are caused by the stimulation of specific genes.

Glucocorticoids (particularly at high concentration) cause **catabolic effects in extrahepatic tissues** (e.g. muscle, adipose tissue, bone etc.). This results in **enhanced degradation of proteins**. Glucocorticoids inhibit protein and nucleic acid (DNA and RNA) synthesis. This leads to muscular weakness often accompanied by muscular atrophy.

(3)Effects on water and electrolyte metabolism. Deficiency of glucocorticoids causes increased production of antidiuretic hormone (ADH). ADH decreases glomerular filtration rate causing water retention in the body. Glucocorticoids possess some degree of mineralocorticoid activity. They promote sodium retention, potassium excretion and ultimately increase blood pressure.

(4)Effects on the immune system. Glucocorticoids (particularly cortisol), in high doses, **suppress the host immune response**. The steroid hormones act at different levels — damaging lymphocytes, impairment of antibody synthesis, suppression of inflammatory response etc. The visible effect of glucocorticoid administration is increased susceptibility to infection, delayed healing etc. The adverse effects of glucocorticoids on immune system are related to high doses. The role of these hormones at physiological concentration still remains unclear.

(5) Other physiological effects of glucocorticoids. Glucocorticoids are involved in several physiological functions: stimulate the fight and flight response (to face sudden emergencies) of catecholamines; increase the production of gastric HCI and pepsinogen; inhibit the bone formation, hence the subjects are at a risk for osteoporosis; required to maintain cardiac output and normal blood pressure; decrease the formation of collagen, causing development of thin skin.

Mechanism of action of glucocorticoids

Glucocorticoids bind to specific receptors on the target cells and bring about the action. These hormones mostly act at the **transcription** level and control the **protein synthesis**. Recent observations also suggest that glucocorticoids are involved in **post-transcriptional** and **post-translational modifications**. All these actions are ultimately connected with the formation of biologically active proteins.

LECTURE 26

HORMONAL REGULATION OF ANABOLIC PROCESSES, GROWTH AND DEVELOPMENT

THYROID HORMONES

Thyroid gland (weighs about 30 g in adults) is located on either side of the trachea below the larynx. It produces two principal hormones — **thyroxine** (T_4; 3, 5, 3', 5'-tetraiodothyronine) and **3, 5, 3'-triiodothyronine** (T_3) — which regulate the metabolic rate of the body (Fig. 26—1).

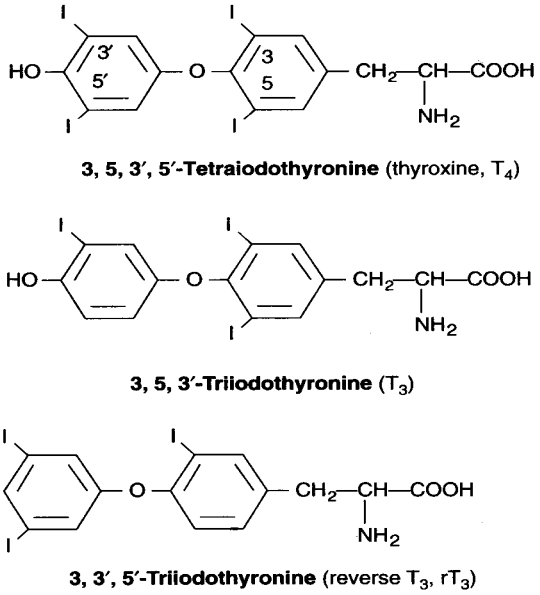


Figure 26—1. Structures of thyroid hormones.

Biosynthesis of thyroid hormones

Iodine is essential for the synthesis of thyroid hormones. More than half of the body's total iodine content is found in the thyroid gland.

1. Uptake of iodide. The uptake of iodide by the thyroid gland occurs **against a** concentration gradient (about 20:1). It is an **energy** requiring process and is linked to the ATPase dependent Na^+-K^+ pump. Iodide uptake is primarily controlled by TSH. Antithyroid agents such as thiocyanate and perchlorate inhibit iodide transport.

2. Formation of active iodine. The conversion of iodide (I^-) to active iodine (I^+) is an **essential step** for its incorporation into thyroid hormones. Thyroid is the only tissue that can oxidize I^- to a higher valence state I^+. This reaction requires $\mathbf{H_2O_2}$ and is catalyzed by the enzyme **thyroperoxidase** (mol. wt. 60,000). An NADPH dependent system supplies H_2O_2. TSH promotes the oxidation of iodide to active iodine while the antithyroid drugs (thiourea, thiouracil, methinazole) inhibit.

3. Thyroglobulin (mol. wt. 660,000) is a **glycoprotein** and **precursor** for the synthesis of $\mathbf{T_3}$ and $\mathbf{T_4}$**.** Thyroglobulin contains about 140 **tyrosine residues** which can serve as substrates for iodine for the formation of thyroid hormones.

4. Tyrosine (of thyroglobulin) is first iodinated at position 3 to form **monoiodotyrosine** (MIT) and then at position 5 to form **diiodotyrosine** (DIT). Two molecules of DIT couple to form thyroxine (T_4). One molecule of MIT, when coupled with one molecule of DIT, **triiodothyronine** (T_3) is produced. The mechanism of coupling is not well understood. A diagrammatic representation is depicted in Fig. 26—2.

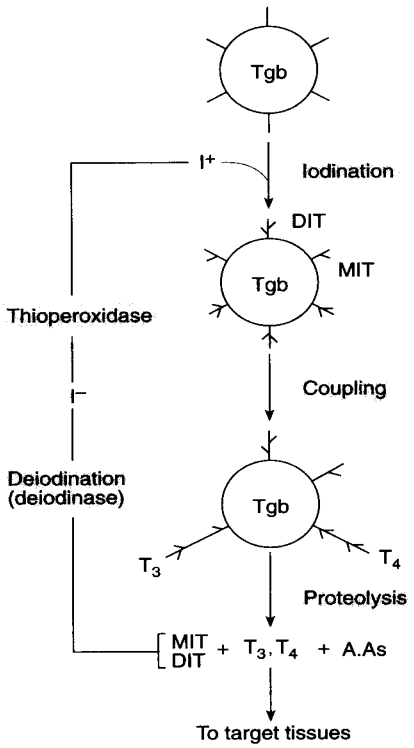


Figure 26—2. Biosynthesis of thyroid hormones (by U. Satyanarayana, 2002).

As the process of iodination is completed each molecule of thyroglobulin contains about 6—8 molecules of thyroxine (T_4). The ratio of T_3 to T_4 in thyroglobulin is usually around 1:10.

Storage and release of thyroid hormones

Thyroglobulin containing T_4 and T_3 can be stored for **several months** in the thyroid gland. It is estimated that the stored thyroid hormones can meet the body requirement for 1-3 months. Thyroglobulin is digested by **lysosomal proteolytic enzymes** in the thyroid gland. The free hormones thyroxine (90%) and triiodothyronin (10%) are released into the blood, a process stimulated by TSH. MIT and DIT produced in the thyroid gland undergo deiodination by the enzyme deiodinase and the iodine thus liberated can be reutilized.

Transport of T_4 and T_3

Two specific binding proteins — **thyroxine binding globulin** (TBC) and **thyroxine binding prealbumin** (TBPA) — are responsible for the transport of thyroid hormones. Both T_4 and T_3 are more predominantly bound to TBG. A small fraction of **free hormones are biologically active**. T_4 has a half-life of 4—7 days while T_3 has about one day.

Biochemical functions of thyroid hormones

Triiodothyronine (T_3) is about **four times more active** in its biological functions than thyroxine (T_4). About 80% of the circulatory T_4 is converted (on deiodination at 5' position) to T_3 on entering the peripheral tissues. Some amount of reverse T_3 (rT_3; 3, 3', 5'-triiodothyronine) with a negligible biological activity is also produced. Some workers consider T_4 as a prohormone and T_3 as the active hormone. Thyroid hormones bind to the specific receptors on the target cell nuclei and bring about the biochemical functions. T_3 is nearly ten times more active than T_4 in binding to the receptors. The following are the biochemical functions attributed to thyroid hormones (T_3 and T_4).

1. Influence on the metabolic rate. Thyroid hormones **stimulate the metabolic activities** and **increase** the **oxygen consumption** in most of the tissues of the body (exception — brain, lungs, testes and retina). These hormones increase the activity of mitochondrial α-glycerophosphate dehydrogenase which may partly explain the increased oxygen consumption (due to oxidation of NADH in ETC). Administration of high concentration of T_4 **uncouples** oxidative phosphorylation, blocks ATP synthesis, causes high consumption of O_2 and liberates heat. The physiological significance of such observation is unclear.

2. Na^+-K^+ ATP pump. This is an energy dependent process which consumes a major share of cellular ATP. Na^+-K^+ ATPase activity is directly correlated to thyroid hormones and this, in turn, with ATP utilization. ATP synthesis predominantly occurs by oxidative phosphorylation coupled with electron transport chain which consumes oxygen. This is now believed to be the mechanism of action of thyroid hormones to explain the increased metabolic rate. Obesity in some individuals is attributed to a decreased energy utilization and heat production due to diminished Na^+-K^+ ATPase activity.

3. Effect on protein synthesis. Thyroid hormones act like steroid hormones in promoting protein synthesis by acting at the transcriptional level (activate DNA to produce RNA). In this process, the thyroid hormones may **directly increase the protein synthesis** in general or **act through promoting the synthesis of growth hormone**. The latter enhances the biosynthesis of proteins. Thyroid hormones, thus, function as **anabolic hormones** and **cause positive nitrogen balance** and **promote growth and development**. However, high concentration of T_3 inhibits protein synthesis and leads to negative nitrogen balance. Therefore, thyroid hormones are catabolic in hyperthyroid state.

4. Influence on carbohydrate metabolism. Thyroid hormones promote **intestinal absorption of glucose** and its **utilization**. These hormones increase gluconeogenesis and glycogenolysis, with an overall effect of enhancing blood glucose level (hyperglycemia).

5. Effect on lipid metabolism. Lipid turnover and utilization are **stimulated** by thyroid hormones. Hypothyroidism is associated with elevated plasma cholesterol levels which can be reversed by thyroid hormone administration.

6. Influence on water and electrolyte balance. Impairment of thyroid function is associated with **retention of water** and **electrolytes** which can be reversed by hormonal administration. This clearly indicates that thyroid hormones regulate water and electrolyte metabolism.

Regulation of T_3 and T_4 synthesis

The synthesis of thyroid hormones is controlled by feedback regulation (Fig. 26—3). T_3 appears to be more actively involved than T_4 in the regulation process. The production of thyroid stimulating hormone (TSH) by pituitary and **thyrotropin releasing hormone** (TRH) by hypothalamus are inhibited by T_3 and, to a lesser degree, by T_4. The increased synthesis of TSH and TRH occurs in response to decreased circulatory levels of T_3 and T_4. As already discussed, the body has sufficient stores of hormones to last for several weeks. Hence it takes some months to observe thyroid functional deficiency.

Metabolic fate of T_3 and T_4

Thyroid hormones undergo **deiodination** in the peripheral tissues. The iodine liberated may be reutilized by the thyroid. T_3 and T_4 may get conjugated with glucuronic acid or sulfate in the liver and excreted through bile. Thyroid hormones are also subjected to **deamination** to produce tetraiodothyroacetic acid (from T_4) and triiodothyroacetic acid (from T_3) which may then undergo conjugation and excretion.

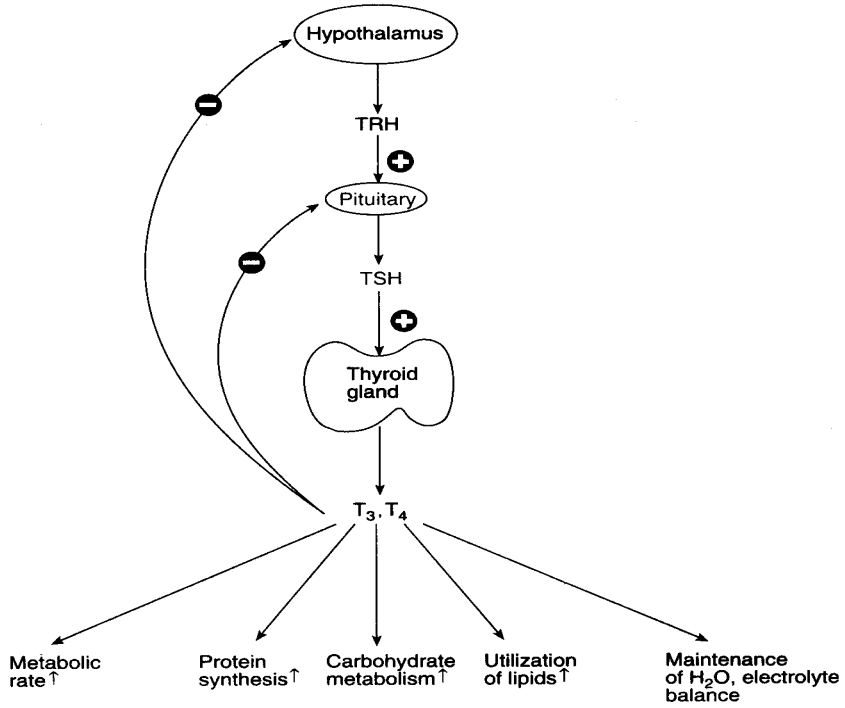


Figure 26—3. Regulation of synthesis and functions of thyroid hormones (by U. Satyanarayana, 2002).

Abnormalities of thyroid function

Among the endocrine glands, **thyroid is the most susceptible for hypo- or hyperfunction.**

Three abnormalities associated with thyroid functions are known.

1. Goiter. Any abnormal increase in the **size** of the thyroid gland is known **as goiter**. Enlargement of thyroid gland is mostly to compensate the decreased synthesis of thyroid hormones and is associated with elevated TSH. Goiter is primarily due to a failure in the autoregulation of T_3 and T_4 synthesis. This may be caused by deficiency or excess of iodide.

2. Goitrogenic substances (goitrogens). These are the substances that interfere with the production of thyroid hormones. These include **thiocyanates, nitrates and perchlorates** and the drugs such as **thiourea, thiouracil, thiocarbamide** etc. Certain plant foods — cabbage, cauliflower and turnip — contain goitrogenic factors (mostly thiocyanates).

3. Simple endemic goiter. This is due to iodine deficiency in the diet. It is mostly found in the geographical regions away from sea coast where the water and soil are low in iodine content. Consumption of iodized salt is advocated to overcome the problem of endemic goiter. In certain cases, administration of thyroid hormone is also employed.

Hyperthyroidism. This is also known as **thyrotoxicosis** and is associated with **overproduction** of thyroid hormones. Hyperthyroidism is characterized by **increased metabolic rate**, **nervousness**, **irritability**, **anxiety, rapid heart rate, loss of weight despite increased appetite, weakness, diarrhea, sweating,** sensitivity to heat and often protrusion of eyeballs (**exopthalmos**). Hyperthyroidism is caused by **Grave's disease** (particularly in the developed countries) or due to increased intake of thyroid hormones.

The treatment includes administration of antithyroid drugs. In severe cases, thyroid gland is surgically removed.

Hypothyroidism. This is due to an impairment in the function of thyroid gland that often causes decreased circulatory levels of T_3 and T_4. Disorders of pituitary or hypothalamus also contribute to hypothyroidism. Women are more susceptible than men. Hypothyroidism is characterized by slow heart rate, weight gain, sluggish behaviour, constipation, sensitivity to cold, dry skin etc.

Hypothyroidism in children is associated with physical and mental retardation, collectively known as **cretinism.** Early diagnosis and proper treatment are essential. Hypothyroidism in adult causes **myxoedema,** characterized by **bagginess under the eyes, puffiness of face, slowness in physical and mental activities.**

Thyroid hormonal administration is employed to treat hypothyroidism.

Laboratory diagnosis of thyroid function

In recent years, more sensitive and reliable tests have been developed to assess thyroid activity. The concentration of free T_3 and T_4, and TSH are measured by RIA or ELISA. Radioactive iodine uptake (RAID) and scanning of thyroid gland are also used for diagnosis.

GROWTH HORMONE (GH)

The growth hormone (or **somatotropin**) is produced by **somatotropes**, a special group of acidophilic cells of **anterior pituitary**. Human growth hormone is a single polypeptide with **191** amino acids and a **molecular weight** of **22,000.**

Regulation of GH release. Two hypothalamic factors play a prominent role in the release of growth hormones. These are the **growth hormone-releasing hormone (CRH)** that **stimulates and the growth hormone release-inhibiting** hormone (GRIH, **somatostatin**) that **inhibits.** This, in turn, is regulated by a feedback mechanism. The release of growth hormone is also controlled by a variety of other agents which include dopamine, serotonin, estrogens, glucagon and gut hormones.

Growth hormone production is influenced by many factors such as **sleep, stress** (pain, cold, surgery), **exercise, food intake** etc. It is observed that the largest increase in the production of GH occurs after the onset of sleep. This supports the **adage "If you don't sleep, you won't grow."**

Biochemical functions of GH. Growth hormone **promotes growth** and also influences the normal metabolisms (protein, carbohydrate, lipid and mineral) in the body.

1. **Effects on growth.** As is obvious from the name, GH is essential for the growth. The growth-related effects of GH are mediated through insulin like growth factor I (**IGF—I**) which is also known as **somatomedin C** (formerly sulfation factor), produced by liver. The structure of IGF-I (with 70 amino acids) is similar to proinsulin. Another less important, **IGF—II** (with 67 amino acids), has been identified in human plasma. However, IGF-I is directly related to growth hormone function.
2. **Effects on protein metabolism.** Growth hormone has an **anabolic effect** on protein metabolism. It promotes the **uptake of amino acids** into the tissues and increases the protein synthesis. GH also stimulates **transcription** (RNA synthesis from DNA). The overall effect of GH is a **positive nitrogen balance** that leads to increase in body weight. The actions of GH on protein metabolism and the growth promoting process resemble that of insulin.
3. **Effects on carbohydrate metabolism.** Growth hormone is **antagonistic** to **insulin** and causes **hyperglycemia**. GH **increases gluconeogenesis**, **decreases glucose utilization**, **impairs glycolysis** and **reduces the tissue uptake of glucose**. Inhibition of glycolysis by GH is mediated through the free fatty acids as a consequence of their increased mobilization.
4. **Effects on lipid metabolism.** Growth hormone promotes lipolysis in the adipose tissue and increases **the circulatory levels of free fatty acids** and their **oxidation**. It increases ketogenesis, particularly in diabetes. These effects are mostly observed after the administration of GH. It appears that the influence of GH on carbohydrate and lipid metabolism is not mediated through IGF-I.
5. **Effects on mineral metabolism.** Growth hormone **promotes bone mineralization and its growth**, as clearly observed in the growing children. GH, probably through the mediation of IGF—I, causes positive balance of the minerals calcium, phosphate and magnesium. It also promotes the retention of Na^+, K^+ and Ch in the body.

6. Effect on lactation. Growth hormone enhances milk production in lactating animals. Here, GH acts like prolactin and hence this action is referred to as prolactin like effect.

Abnormalities of GH production

Deficiency of GH. Impairment in the secretion of growth hormone in the growing age causes **dwarfism.** The other deficiency metabolic effects are not that serious in nature. GH deficiency may occur either due to **panhypopituitarism** — a generalized undersecretion of pituitary hormones or due to a defect in GH secretion alone. Dwarfs due to GH deficiency respond to GH administration. GH acts through the mediation of IGF—I.

Overproduction of GH. Excessive production of GH causes **gigantism in children and acromegaly in adults.** This usually occurs in the acidophil tumor of pituitary gland. Gigantism is characterized by increased growth of long bones and this is observed before the epiphyseal plates close. Acromegaly occurs after epiphyseal closure and is characterized by increase in the size of hands, facial changes (enlarged nose, protruding jaw), excessive hair, thickening of skin etc. Diabetes mellitus, related to overproduction of GH, has been identified.

HORMONES OF GONADS

The gonads (testes in males, ovaries in females) perform closely related dual functions.

1. Synthesize sex hormones

2. Produce germ cells

The steroid sex hormones are responsible for **growth, development**, **maintenance** and **regulation of reproductive system**. Sex hormones are essentially required for the development of germ cells.

The sex hormones are categorized into three groups

1. Androgens or male sex hormones which are C—19 steroids.

2. Estrogens or female sex hormones which are C—18 steroids. Ring A of steroid nucleus is phenolic in nature and is devoid of C—19 methyl group.

3. Progesterone is a C—21 steroid produced during the luteal phase of menstrual cycle and also during pregnancy.

ANDROGENS

The male sex hormones or androgens are produced by the **Leydig cells** of the **testes** and to a minor extent by the **adrenal glands** in both the sexes. Ovaries also produce small amounts of androgens.

Biosynthesis of androgens

Cholesterol is the precursor for the synthesis of androgens. It is first converted to pregnenolone which then forms androstenedione by two pathways — either through progesterone or through 17-hydroxypregnenolone (Fig.26—4).

Testosterone is produced from androstenedione. The production of androgens is under the control of **LH** and **FSH**.

Active form of androgen. The primary product of testes is testosterone. However, the active hormone in many tissues is not testosterone but it metabolite **dihydrotestosterone** (DHT). Testosterone, on reduction by the enzyme 5 α-reductase forms DHT. This conversion mostly occurs in the **peripheral tissues**. Some workers consider testosterone as a prohormone and dihydrotestosterone, the more potent form as the hormone

Transport of androgens. In the plasma, testosterone and DHT bind to two proteins — **sex hormone-binding globulin** and/or **testosterone-estrogen binding globulin.** Both these proteins are synthesized in the liver. The plasma testosterone level in normal men is about 0.7 μg/dl while in women it is <0.1 μg/dl.

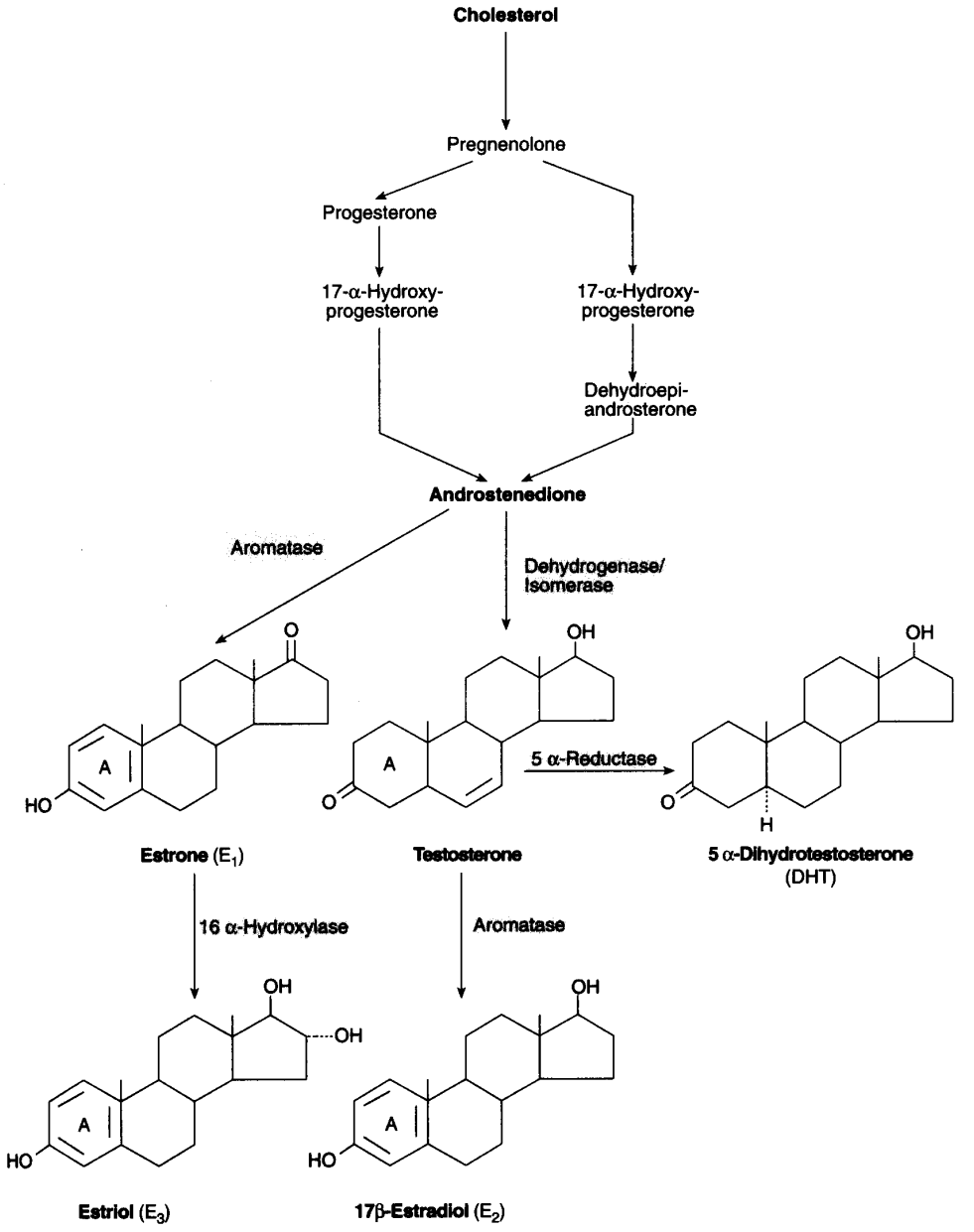


Fig. 26—4. Biosynthesis of steroid sex hormones from cholesterol (by U. Satyanarayana, 2002).

Metabolism of androgens

The major end products of androgen metabolism are 17-ketosteroids which are excreted in urine. However, 17-ketosteroids derived from adrenocorticosteroids are excreted in greater quantity (2/3 of total). For this reason, estimation of urinary 17-ketosteroids is more reliable in assessing adrenocortical function than gonadal activity.

Physiological and biochemical functions of androgens

1. Sex related physiological functions. The androgens, primarily DHT and testosterone, influence:

a. Growth, development and maintenance of male reproductive organs.

b. Sexual differentiation and secondary sexual characteristics.

c. Spermatogenesis.

d. Male pattern of aggressive behavior.

The effect of androgens is widespread throughout the body. It is rather difficult to differentiate a target and a non-target tissue in so far as androgen action is concerned.

2. Biochemical functions. Many specific biochemical effects of androgens that ultimately influence the physiological functions stated above are identified. Androgens are anabolic in nature.

a. Effects on protein metabolism. Androgens **promote RNA synthesis** (transcription) and **protein synthesis** (translation). They are involved in the rapid **growth of musculoskeletal system** associated with puberty. Androgens cause positive nitrogen balance and increase the muscle mass.

b. Effects on carbohydrate and fat metabolisms. Androgens increase **glycolysis** by increasing the synthesis of certain enzymes (e.g. aldolase). The production of D-fructose from D-glucose by seminal vesicles is enhanced by androgens. Androgens increase **fatty acid synthesis** and **citric acid cycle**.

c. Effects on mineral metabolism. Androgens promote mineral deposition and bone growth before the closure of epiphyseal cartilage. These hormones are responsible for bone maturation. Androgens enhance the kidney reabsorption of Na^+, Cl^- and water.

Mechanism of action of androgens

Both testosterone and dihydrotestosterone are believed to bind to a single class of receptors on the target tissue. The affinity of DHT for the receptor, however, is much higher compared to testosterone. Receptor sites for androgens are found in muscle, brain and other target tissues where androgens exert their influence.

Abnormalities associated with male sex hormones

Hypogonadism is a disorder characterized by a defect in testosterone synthesis. It may be of two types.

1. Primary hypogonadism is caused by a failure of testes to produce testosterone.

2. Secondary hypogonadism is due to an impairment in the release of gonadotropins.

ESTROGENS

Estrogens are predominantly **ovarian hormones**, synthesized by the **follicles** and **corpus luteum** of ovary. These hormones are responsible for maintenance of menstrual cycle and reproductive process in women.

Synthesis of estrogens

Estrogen synthesis occurs from the precursor cholesterol (Fig. 26—4). Estrogens are produced by **aromatization** (formation of aromatic ring) of **androgens**. The **ovary** produces **estradiol** (E_2) and **estrone** (E_1) while the **placenta** synthesizes these two steroid hormones and **estriol** (E_3). The synthesis of estrogens is under the control of LH and FSH.

Transport of estrogens

Estrogens are bound to SHBG and transported in plasma. Estradiol has less affinity to bind to SHBG compared to testosterone or DHT.

Metabolism of estrogens

Estradiol is the predominant estrogen in plasma. The liver converts estradiol and estrone to estriol. All these three estrogens can undergo **conjugation** with **glucuronide or sulfate**. The conjugated estrogens are water soluble and **excreted into urine**.

Physiological and biochemical functions of estrogens

1. Sex-related physiological functions. The estrogens are primarily concerned with

a. Growth, development and maintenance of female reproductive organs.

b. Maintenance of menstrual cycles.

c. Development of female sexual characteristics.

2. Biochemical functions. Estrogens are involved in many metabolic functions.

a. Lipogenic effect. Estrogens increase lipogenesis in adipose tissue and, for this reason, women have relatively more fat (about 5%) than men.

b. Hypocholesterolemic effect. Estrogens lower the plasma total cholesterol. The LDL fraction of lipoproteins is decreased while the HDL fraction is increased. This explains the low incidence of atherosclerosis and coronary heart diseases in the women during reproductive age.

c. Anabolic effect. Estrogens in general promote transcription and translation. The synthesis of many proteins in liver is elevated e.g. transferrin, ceruloplasmin.

d. Effect on bone growth. Estrogens like androgens promote calcification and bone growth. It is believed that decalcification of bone in the postmenopausal women leading to osteoporosis is due to lack of estrogens.

e. Effect on transhydrogenase. Transhydrogenase is an enzyme activated by estrogen. It is capable of transferring reducing equivalents from NADPH to NAD^+. The NADH so formed can be oxidized. It is explained that in the women after menopause, due to deficiency of estrogens, the transhydrogenase activity is low. This results in the diversion of NADPH towards lipogenesis — causing obesity.

f. Estrogen 'priming effect' for progesterone action. Estrogens induce the synthesis of progesterone receptors in the uterus and mammary glands. This is called priming effect and is essential for progesterone to bind to the target tissues.

PROGESTERONE

Progesterone is synthesized and secreted by corpus luteum and placenta (structure see Fig. 25—2). Progesterone, as such, is an intermediate in the formation of steroid hormones from cholesterol. LH controls the production of progesterone.

Metabolism of progesterone

Progesterone is present in the plasma in a bound form to protein transcortin. Progesterone is converted to pregnanediol which is excreted in urine as glucuronide conjugate.

Biochemical functions of progesterone

1. Progesterone is essentially required for the implantation of fertilized ovum and maintenance of pregnancy.

2. It promotes the growth of glandular tissue in uterus and mammary gland.

3. Progesterone increases the body temperature by 0.5—1.5 F°. The exact mechanism of this thermogenic effect is not clearly known. The measurement of temperature was used as an indicator for ovulation.

Mechanism of action of estrogens and progestins

Estrogen and progestin bind to the specific receptors on the target tissue. They act on the specific genes and promote transcription. The hormones-estrogens and progesterones have been found to perform receptor-independent functions. For instance, estradiol directly affects histamine release and prostaglandin synthesis.

Abnormalities associated with female sex hormones

1. Primary hypogonadism. This occurs due to deficiency in ovarian function causing decreased ovulation and/or insufficient hormone production.

2. Secondary hypogonadism. This is caused by impairment in pituitary gonadotropin function.

3. Turner's syndrome (gonadal dysgenesis). This is a frequently seen genetic disorder and is characterized by XO karyotype, associated with delayed puberty and developmental abnormalities.

The menstrual cycle

The occurrence of menstrual cycle is a good example of coordination among the hormonal functions. In humans, the menstrual cycle is under the control of FSH, LH, estrogens and progesterone. The cycle normally varies between 25 and 35 days in length, with a mean of 28 days. The menstrual cycle can be divided into two phases — follicular phase and luteal phase (Fig. 26—5).

1. Follicular phase. Follicular stimulating hormone (FSH) causes the development and maturation of ovarian follicles. As the follicle enlarges, estradiol

progressively rises and reaches its peak value 24 hours before LH and FSH attain their respective maximum levels. LH surge or peak initiates ovulation-release of ovum from the ruptured follicles. The levels of progesterone are low during follicular phase

2. Luteal phase. After the ovulation occurs, the ruptured follicles form corpus luteum and start producing progesterone and estradiol. The predominant hormone of luteal phase is progesterone which prepares the endometrium of uterus for implantation of the fertilized ovum. LH maintains the corpus for a few days. In the absence of implantation, the corpus luteum regresses and sheds endometrium causing menstruation. And another new cycle begins.

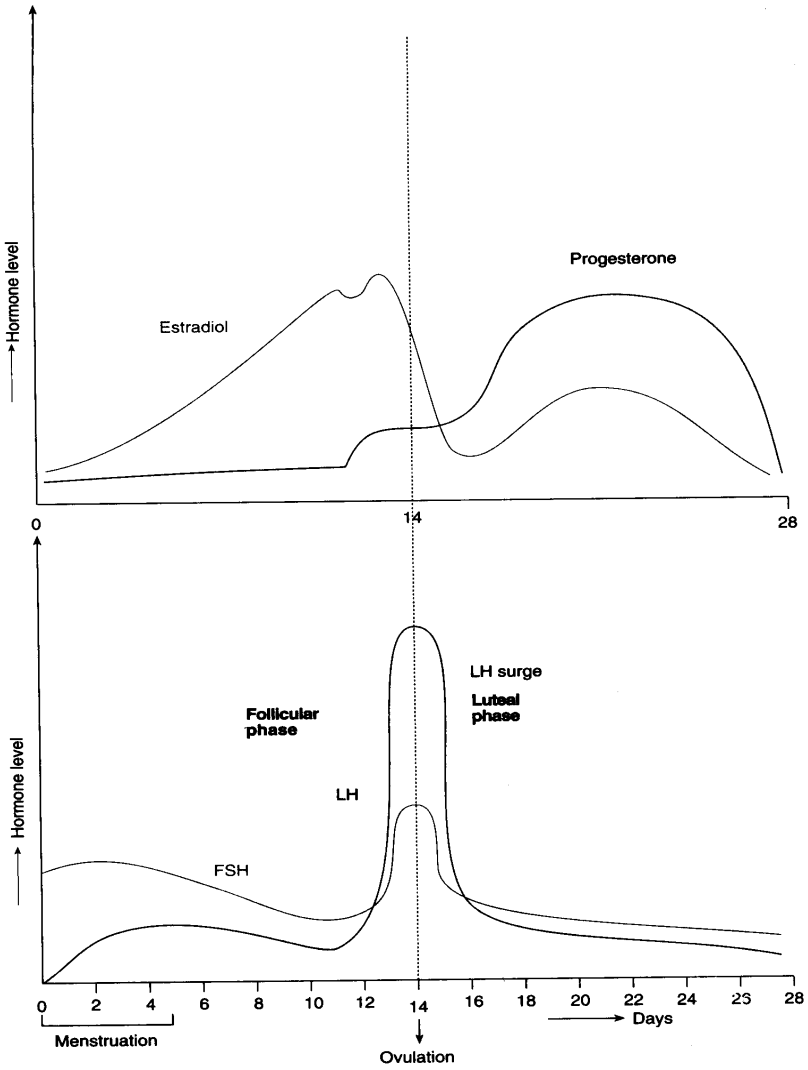


Figure 26—5. Hormonal pattern in women during menstrual cycle (by U. Satyanarayana, 2002).

The luteal phase is always fixed, with 14 ± 2 days in length. The observed variations in the length of menstrual cycle are due to changes in the follicular phase. In case of implantation of the fertilized ovum, human chorionic gonadotropin (hCC) is produced by the cells of implanted early embryo. hCG stimulates corpus luteum to synthesize progesterone. This continues till the plancenta starts making high quantities of progesterone.

LECTURE 27

MINERALS. METABOLISM AND REGULATION OF CALCIUM AND PHOSPHOROUS METABOLISM. REGULATION OF ELECTROLYTE AND WATER BALANCE

The **mineral** (inorganic) elements constitute only a small proportion of the body weight. There is a wide variation in their body content. For instance, calcium constitutes about 2% of body weight while cobalt about 0.00004%.

General functions

Minerals perform several vital functions which are absolutely **essential** for the very existence of the organism. These include **1) calcification of bone, 2) blood coagulation, 3) neuromuscular irritability, 4) acid-base equilibrium, 5) fluid balance and 6) osmotic regulation.**

Certain minerals are **integral components** of **biologically important compounds** such as hemoglobin (Fe), thyroxine (I), insulin (Zn) and vitamin B_{12} (Co). Sulfur is present in thiamine, biotin, lipoic acid and coenzyme A. Several minerals participate as **cofactors** for enzymes in metabolism (e.g. Mg, Mn, Cu, Zn, K). Some elements are essential constituents of certain enzymes (e.g. Co, Mo, Se).

Classification

The minerals are classified as **principal elements** and **trace elements**.

The seven **principal elements** constitute 60—80% of the body's inorganic material. These are **calcium, phosphorus, magnesium, sodium, potassium, chloride and sulfur.**

The trace elements are subdivided into three categories

1. **Essential trace elements**: iron, copper, iodine, manganese, zinc, molybdenum, cobalt, fluorine, selenium and chromium.
2. **Possibly essential trace elements**: nickel, vanadium, cadmium and barium.
3. **Non-essential trace elements**: aluminium, lead, mercury, boron, silver, bismuth etc.

CALCIUM

Calcium is the most abundant among the minerals in the body. The total content of calcium in an adult man is about **1 to 1.5 kg.** As much as **99%** of it is present in the **bones** and **teeth.** A small fraction (**1%**) of the calcium, found **outside the skeletal tissue**, performs a wide variety of functions.

Biochemical functions

1. **Development of bones and teeth.** Calcium, along with phosphate, is required for the formation (of **hydroxyapatite**) and physical strength of skeletal tissue. **Osteoblasts** are responsible for bone formation while **osteoclasts** result in demineralization. Most of the calcium in bone is not freely exchangeable with **extracellular fluid calcium**. Thus, in addition to its mechanical role, bone serves as a large reservoir of calcium. About **1%** of skeletal Ca^{2+} is in a **freely exchangeable pool** and this, with another **1%** of the total found in the periostal space, constitutes the **miscible pool** of Ca^{2+}. The hormones regulate the amount

of calcium in the extracellular fluid by influencing the transport of calcium across the membrane that separates the extracellular fluid space from the periosteal fluid space.

2. Muscle contraction. Ca^{2+} interacts with troponin C to trigger muscle contraction. Calcium also activates ATPase, increases the interaction between actin and myosin.

3. Blood coagulation. Several reactions in the cascade of blood clotting process are dependent on Ca^{2+}(factor IV).

4. Nerve transmission. Ca^{2+} is necessary for the transmission of nerve impulse.

5. Membrane integrity and permeability. Ca^{2+} influences the membrane structure and transport of water and several ions across it.

6. Activation of enzymes. Ca^{2+} is needed for the direct activation of enzymes such as lipase (pancreatic), ATPase and succinate dehydrogenase.

7. Calmodulin mediated action of Ca^{2+}. Calmodulin (mol. wt. 17,000) is a calcium binding regulatory protein. Ca-calmodulin complex activates certain enzymes e.g. adenylate cyclase, Ca^{2+} dependent protein kinases.

8. Calcium as intracellular messenger. Certain hormones exert their action through the mediation of Ca^{2+} (instead of cAMP). Calcium is regarded as a **second messenger** for such hormonal action e.g. epinephrine in liver glycogenolysis. Calcium serves as a third messenger for some hormones e.g. antidiuretic hormone (ADH) acts through cAMP, and then Ca^{2+}.

9. Release of hormones. The release of certain hormones (insulin, PTH, calcitonin) from the endocrine glands is facilitated by Ca^{2+}.

10. Secretory processes. Ca^{2+} regulates microfilament and microtubule mediated processes such as endocytosis, exocytosis and cell motility.

11. Contact inhibition. Calcium is believed to be involved in cell to cell contact and adhesion of cells in a tissue. The cell to cell communication may also require Ca^{2+}.

12.Action on heart. Ca^{2+} acts on myocardium and prolongs systole.

Dietary requirements. Adult men and women **800 mg/day**, women during pregnancy, lactation and post-menopause **1.5 g/day.**

Sources. Best sources — milk and milk products. Good sources — beans, leafy vegetables, fish, cabbage, egg yolk.

Absorption. The absorption of calcium mostly occurs in the duodenum by an energy dependent active process. It is influenced by several factors.

Factors promoting Ca absorption

1. Vitamin D (through its active form calcitriol) induces the synthesis of calcium binding protein in the intestinal epithelial cells and promotes Ca absorption.

2. Parathyroid hormone enhances Ca^{2+} absorption through the increased synthesis of calcitriol.

3. Acidity (low pH) is more favourable for Ca^{2+} absorption.

4. Lactose promotes calcium uptake by intestinal cells.

5. The amino acids lysine and arginine facilitate Ca^{2+} absorption.

Factors inhibiting Ca absorption

1. Phytates and **oxalates** form insoluble salts and interfere with Ca absorption.

2. High content of dietary phosphate results in the formation of insoluble calcium phosphate and prevents Ca^{2+} uptake. The dietary ratio of Ca and P between 1:2 and 2:1 — is ideal for optimum Ca^{2+} absorption by intestinal cells.

3. The **free fatty acids** react with Ca^{2+} to form insoluble calcium soaps. This is particularly observed when the fat absorption is impaired.

4. Alkaline condition (high pH) is unfavourable for Ca^{2+} absorption.

5. High content of dietary fiber interferes with Ca^{2+} absorption.

Plasma calcium. Most of the blood Ca^{2+} is present in the plasma since the blood cells contain very little of it. The normal concentration of plasma or serum Ca^{2+} is 9—11 mg/dl (4.5—5.5 mEq/l). About **half** of this (5 mg/dl) is in the **ionized** form which is functionally the most active**.** At least 1 mg/dl serum Ca^{2+} is found in **association with citrate** and/or **phosphate**. The **other half** of serum Ca (4-5mg/dl) is **bound to proteins**, mostly albumin and, to a lesser extent, globulin. Ionized and citrate (or phosphate) bound Ca^{2+} is diffusible from blood to the tissues while protein bound Ca is non-diffusible. In the usual laboratory determination of serum Ca^{2+}, all the three fractions are measured together.

Excretion of calcium. Calcium is excreted partly through the **kidneys** and mostly through the **intestine**. The renal threshold for serum Ca^{2+} is 10 mg/dl. Calcium gets excreted into urine beyond this concentration. Ingestion of excess protein causes increased calcium excretion in urine. This is mainly due to an increase in the acidity of urine as a result of high protein diet. Excretion of Ca^{2+} into the feces is a continuous process and this is increased in vitamin D deficiency.

PHOSPHORUS

An adult body contains about **1 kg** phosphate and it is found in every cell of the body. Most of it (about **80%**) occurs in **combination with Ca^{2+}** in the **bones and teeth**. About **10%** of body P is found in **muscles** and **blood** in **association with proteins, carbohydrates and lipids**. The remaining 10% is widely distributed in various chemical compounds.

Biochemical functions

1. Phosphorus is essential for the **development of bones and teeth**.

2. It plays a central role for the **formation** and **utilization** of **high-energy phosphate compounds** e.g. ATP, GTP, creatine phosphate etc.

3. Phosphorus is required for the **formation of phospholipids**, **phosphoproteins and nucleic acids** (DNA and RNA).

4. It is an essential component of several **nucleotide coenzymes** e.g. NAD^+, $NADP^+$, pyridoxal phosphate, ADP, AMP.

5. Several proteins and enzymes are activated by **phosphorylation**.

6. Phosphate buffer system is important for the maintenance of pH in the blood as well as in the cells.

7. Phosphate is necessary for the **absorption** and **metabolism** of carbohydrates.

Dietary requirements. The recommended dietary allowance of phosphate is based on the intake of calcium. The ratio Ca:P of **1:1** is recommended (i.e. 800 mg/day) for an adult. For infants, however, the ratio is around 2:1, which is based on the ratio found in human milk. Calcium and phosphate are distributed in the majority of natural foods in 1:1 ratio. Therefore, adequate intake of Ca generally takes care of the P requirement also.

Sources. Milk, cereals, leafy vegetables, meat, eggs.

Absorption. Phosphate absorption occurs from jejunum

1. Calcitriol promotes phosphate uptake along with calcium.

2. Absorption of phosphorus and calcium is optimum when the dietary Ca:P is between 1:2 and 2:1.

3. Acidity favours while phytate decreases phosphate uptake by intestinal cells.

Serum phosphate. The phosphate level of the whole blood is around 40 mg/dl while serum contains about 3—4 mg/dl. This is because the red blood cells and white blood cells have very high content of phosphate. The serum phosphate may exist as **free ions** (40%) or in a **complex form** (50%) with **cations** such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}. About 10% of serum phosphate is bound to proteins. It is interesting to note that the **fasting serum phosphate** levels are **higher** than the post-prandial. This is attributed to the fact that following the ingestion of carbohydrate (glucose), the phosphate from the serum is drawn by the cells for metabolism (phosphorylation reactions).

Excretion. About 500 mg phosphate is excreted in urine per day. The renal threshold is 2 mg/dl. The reabsorption of phosphate by renal tubules is inhibited by PTH.

REGULATION OF CALCIUM HOMEOSTASIS

The hormones — **calcitriol, parathyroid hormone** (PTH) and **calcitonin** are the major factors that regulate the plasma calcium and (**homeostasis of Ca**) within a narrow range (9—11 mg/dl).

Calcitriol

The physiologically active form of vitamin D is a hormone, namely calcitriol or 1, 25-dihydroxy-cholecalciferol (1, 25 DHCC).

Calcitriol induces the synthesis of a specific calcium binding protein in the intestinal cells. This protein increases the intestinal absorption of calcium as well as phosphate. Thus blood Ca level is increased by calcitriol (the active vitamin D). Furthermore, calcitriol **stimulates calcium uptake by osteoblasts of bone and promotes calcification** or mineralization (deposition of calcium phosphate) and remodeling.

Parathyroid hormone

Parathyroid hormone (PTH) is secreted by two pairs of **parathyroid glands** that are closely associated with thyroid glands. Parathyroid hormone

(mol. wt. 95,000) is a single chain polypeptide, containing **84 amino acids**. It is originally synthesized as preproPTH which is degraded to proPTH and, finally, to active PTH. The rate of formation (by degradation of proPTH) and the secretion of PTH are promoted by low Ca^{2+} concentration. Thus, the release of PTH from parathyroid glands is under the negative feedback regulation of serum Ca^{2+}.

Mechanism of action of PTH. PTH binds to a membrane receptor protein on the target cell and activates adenylate cyclase to liberate cAMP. This, in turn, increases intracellular calcium that promotes the phosphorylation of proteins (by kinases) which, finally brings about the biological actions. PTH has 3 independent tissues — bone, kidneys and intestine — to exert its action. The **prime function of PTH is to elevate serum calcium level.**

Action on the bone. PTH causes decalcification or **demineralization of bone,** a process carried out by **osteoclasts.** This is brought out by PTH stimulated increased activity of the enzymes pyrophosphatase and collagenase. These enzymes result in **bone resorption**. Demineralization ultimately leads to an increase in the blood Ca^{2+} level. The action of PTH on bone is quantitatively very significant to maintain Ca^{2+} homeostasis. It must, however, be noted that this is being done at the expense of loss of Ca^{2+} from bone, particularly in dietary Ca^{2+} deficiency.

Action on the kidney. PTH increases the **Ca^{2+} reabsorption by kidney tubules**. This is the most rapid action of PTH to elevate blood Ca^{2+} levels. However, quantitatively, this is less important compared to the action of PTH on bone. PTH promotes the production of calcitriol in the kidney by stimulating 1-hydroxylation of 25-hydroxycholecalciferol.

Action on the intestine. The action of PTH on the intestine is **indirect**. It increases the intestinal absorbtion of Ca^{2+} by promoting the synthesis of calcitriol.

Effect of PTH on phosphate homeostasis. The usual counter-ion for Ca^{2+} is phosphate, and the hydroxyapatite crystal in bone consists of calcium phosphate. Phosphate is released with calcium from bone whenever PTH increases dissolution of the mineral matrix. PTH increases renal phosphate clearance; thus, the net effect of PTH on bone and kidney is to increases extracellular calcium concentration and decrease the extracellular fluid phosphate concentration. Importantly, this prevents the development of a supersaturated concentration of calcium and phosphate in plasma.

Calcitonin

Calcitonin (CT) was reported in 1962 by Hirsch. Within the next 7 years CT was isolated, sequenced and synthesized in the laboratory. However, the biochemical action of CT is not yet clearly known.

Calcitonin is a **peptide** containing **32 amino acids**. It is secreted by **parafollicular cells of thyroid gland**. The action of CT on calcium metabolism is antagonistic to that of PTH. Thus, calcitonin **promotes calcification** by increasing the activity of osteoblasts. Further, calcitonin decreases bone resorption and

increases the excretion of Ca into urine. CT, therefore, has a **decreasing influence on blood calcium.**

Importance of Ca:P ratio. The ratio of Ca:P is important for calcification of bones. The product of Ca × P (in mg/dl) in children is around 50 and in adults around 40. This product is less than 30 in rickets.

Disease states

The blood Ca level is maintained within narrow range by the homeostatic control, most predominantly by PTH. Hence abnormalities in Ca metabolism are mainly associated with alterations in PTH.

Hypercalcemia

The serum Ca level is elevated to in **hypercalcemia**. Hypercalcemia is associated with **hyperparathyroidism** caused by increased activity of parathyroid glands. Decrease in serum phosphate (due to increased renal losses) and increase alkaline phosphatase activity are also found hyperparathyroidism. Elevation in the urine excretion of Ca and P, often resulting in the formation of urinary calculi, is also observed these patients.

Hypocalcemia

Hypocalcemia is a more **serious** and **life threatening condition**. It is characterized by a fall in the serum Ca to below 7 mg/dl, causing **tetany.** The symptoms of tetany include neuromuscular irritability, spasms and convulsions. Hypocalcemia is mostly due to **hypoparathyroidism**. This may happen after an accidental surgical removal of parathyroid glands or due to an autoimmune disease. Hypoparathyroidism is associated with a decrease in serum Ca and an increase in serum phosphate, besides the reduced urinary excretion of both Ca and P.

Osteoporosis

Osteoporosis is characterized by **demineralization of bone** resulting in the **progressive loss of bone mass**. The elderly people (over 60 yr.) of both sexes are at risk for osteoporosis. However, it more predominantly occurs in the postmenopausal women. Osteoporosis results in frequent bone fractures which are a major cause of disability among the elderly.

SODIUM

Sodium is the **chief cation in the extracellular fluid**. About **50%** of body sodium is present in the **bones**, **40%** in the **extracellular fluid** and the remaining (**10%**) in the **soft tissues**.

Biochemical functions

1. In association with chloride and bicarbonate, sodium regulates the body's **acid-base balance**.

2. Sodium is required for the **maintenance of osmotic pressure and fluid balance.**

3. It is necessary for the **normal muscle irritability** and **cell permeability**.

4. Sodium is involved in the **intestinal absorption** of glucose, galactose and amino acids.

5. It is necessary for initiating and maintaining heart beat.

Dietary requirements. For normal individuals, the requirement of sodium is about **5—10 g/day** which is mainly consumed as **NaCl**. For persons with a family history of hypertension, the daily NaCl intake should be less than 5 g. For patients of hypertension, around 1 g/day is recommended. It may be noted that 10 g of NaCl contains 4 g of sodium. The daily consumption of Na is generally higher than required due to its flavour.

Sources. The common salt (NaCl) used in the cooking medium is the major source of sodium. The ingested foods also contribute to sodium. The good sources of sodium include bread, whole grains, leafy vegetables, nuts, eggs and milk.

Absorption. Sodium is readily absorbed in the gastrointestinal tract and, therefore, very little of it (<2%) is normally found in feces. However, in diarrhea, a large quantity of sodium is lost in feces.

Plasma sodium. In the plasma (serum), the normal concentration of sodium is 135—145 mEq/l. Sodium is an **extracellular cation,** therefore, the blood cells contain much less (35 mEq/l).

Excretion. Kidney is the major route of sodium excretion from the body. As much as 800 g Na/day is filtered by the glomeruli, 99% of this is reabsorbed by the renal tubules by an active process.

POTASSIUM

Potassium is the principal **intracellular cation.** It is equally important in the extracellular fluid for specific functions.

Biochemical functions

1. Potassium maintains **intracellular osmotic pressure**.

2. It is required for the regulation of **acid-base balance** and **water balance** in the cells.

3. The enzyme **pyruvate kinase** (of glycolysis) is dependent on K^+ for optimal activity.

4. Potassium is required for the **transmission of nerve impulse**.

5. Adequate intracellular concentration K^+ is necessary for proper **biosynthesis of proteins by ribosomes**.

6. Extracellular K^+ influences **cardiac muscle activity**.

Dietary requirements. About 3—4 g/day.

Sources. Banana, orange, pineapple, potato, beans, chicken, liver. Tender coconut water is a rich source of potassium.

Absorption. The absorption of K^+ from the gastrointestinal tract is very efficient (90%) and very little is lost through feces. However, in subjects with diarrhea, a good proportion of K^+ is lost in the feces.

Plasma potassium. The plasma (serum) concentration of potassium is 3.4—5.0 mEq/l. The whole blood contains much higher level of K^+ (50 mEq/l), since it is predominantly an intracellular cation. Care should, therefore, be taken to avoid hemolysis of red blood cells the estimation of serum K^+.

Excretion. Potassium is mainly excreted through urine. The maintenance of body acid-base balance influences K^+ excretion.

WATER BALANCE

Water is the **solvent** of life. Undoubtedly, water is more important than any other single compound to life. It is involved in several body functions.

Functions of water

1. Water provides the aqueous medium to the organism which is essential for the various biochemical reactions to occur.

2. Water directly participates as a reactant in several metabolic reactions.

3. It serves as a vehicle for transport of solutes.

4. Water is closely associated with the regulation of body temperature.

Distribution of water

Water is the major body constituent. An adult human contains about **60%** water (men 55—70%, women 45—60%). The women and obese individuals have relatively less water which is due to the higher content of stored fat in an anhydrous form. A 70 kg normal man contains about **42 litres** of water. This is distributed in **intracellular** (inside the cells 28 l) and **extracellular** (outside the cells 14 l) compartments, respectively known as **intracellular fluid** and **extracellular fluid**. The extracellular fluid is further divided into **interstitial fluid** (10.5 l) and **plasma** (3.51).

Water turnover and balance

The body possesses tremendous capacity to regulate its water content. In a healthy individual, this is achieved by balancing the daily water intake and water output.

Water intake

Water is supplied to the body by **exogenous and endogenous** sources.

Exogenous water. Ingested water and beverages, water content of solid foods-constitute the exogenous source of water. Water intake is highly variable which may range from 0.5—5 litres. It largely depends on the social habits and climate. In general, people living in hot climate drink more water. Ingestion of water is mainly controlled by a **thirst centre** located in the **hypothalamus.** Increase in the osmolality of plasma causes increased water intake by stimulating thirst centre.

Endogenous water. The **metabolic water** produced within the body is the endogenous water. This water (300—350 ml/day) is derived from the oxidation of foodstuffs. It is estimated that 1 g each of carbohydrate, protein and fat, respectively, yield 0.6 ml, 0.4 ml and 1.1 ml of water. On an average, about 125 ml of water is generated for 1,000 Cal consumed by the body.

Water output

Water losses from the body are variable. There are four distinct routes for the elimination of water from the body — urine, skin, lungs and feces.

Urine. This is the major route for water loss from the body. In a healthy individual, the urine output is about 1—2 l/day. Water loss through kidneys al-

though highly variable, is well regulated to meet the body demands — to get rid of water or to retain

Skin. Loss of water (450 ml/day) occurs through the body surface by perspiration. This is an unregulated process by the body which mostly depends on the atmospheric temperature and humidity. The loss is more in hot climate. Fever causes increased water loss through the skin. It is estimated that for every 1°C rise in body temperature, about 15% increase is observed in the loss of water (through skin).

Lungs. During respiration, some amount of water (about 400 ml/day) is lost through the expired air. The latter is saturated with water and expelled from the body. In hot climates and/or when the person is suffering from fever, the water loss through lungs is increased. The loss of water by perspiration (via skin) and respiration (via lungs) is collectively referred to as **insensible water loss.**

Feces. Most of the water entering the gastrointestinal tract is reabsorbed by the intestine. About 150 ml/day is lost through feces in a healthy individual. Fecal loss of water is tremendously.

REGULATION OF ELECTROLYTE AND WATER BALANCE

Electrolyte and water balance are regulated **together** and the **kidney play role** a predominant role in this regards. The regulation is mostly achieved through the **rennin-angiotensin-aldosterone system**, **vasopressin** (antidiuretic hormone) and **atrial natriuretic peptide**.

Renin-angiotensin-aldosterone system

1. Any combination of factors that **decreases fluid volume** (dehydration, decreased blood pressure, fluid or blood loss) or **decreases NaCl** concentration stimulates rennin release. **Renin** is enzyme produced in the **juxtaglomerular cells** of the renal afferent arteriole. The position of these cells makes them particularly sensitive to **blood pressure** changes, changes of Na^+ and Cl^- concentration.

Angiotensinogen is a α_2-globulin synthesized in liver and it is substrate for renin. Angiotensinogen is cleaved by renin to liberate a **10-amino acid** peptide **angiotensin I** which, in turn, is acted upon by an **angiotensin-converting enzyme** to produce **angiotensin II** (**8 amino acid** peptide). It is a **potent vasoconstrictor** that **raises blood pressure**. Various nonapeptide analogs of angiotensin I and other compounds acts as competitive inhibitors of converting enzyme and are used to treat **rennin-dependent hypertension.**

Anigiotensin II is converted to **angiotensin III** (**7-amino acid** peptide) by **aminopeptidase**. Angiotensin II stimulates **aldosterone** production of **adrenal cortex** and **inhibits renin** release from kidney. In humans, angiotensin II is about four times more active than angiotensin III, in stimulating aldosterone production. However, in some animals, both of them are equally effective. Angiotensin II stimulates the formation of pregnenolone from cholesterol and the conversion of corticosterone to **aldosterone**. Angiotensin II and III are rapidly inactivated by **angiotensinases.**

2. Mechanism of aldosterone action. Aldosterone acts like other steroid hormones. (Fig. 27—1)**.**

It binds with specific receptors on the target tissue and promotes transcription and translation. The mechanism of action of aldosterone on stimulating the active transport and absorption of Na^+ by the kidney is not clear.

Na^+ from the luminal fluid bathing the apical surface of the renal cell enters passively through Na^+ channels. Na^+ is then transported into the interstitial fluid through the serosal side of the cell by the Na^+-K^+-dependent ATPase pump. ATP provides the energy required for this active process.

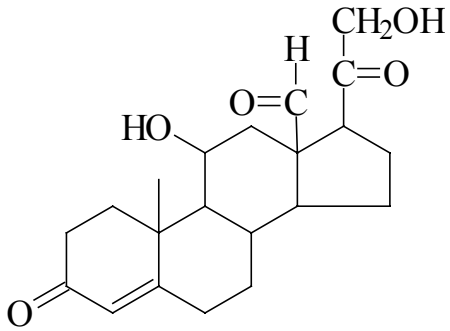


Figure 27—1. Structure of aldosterone

Aldosterone **increases the number of apical membrane Na^+ channels**, and this presumably increases intracellular Na^+. Aldosterone also **increases the activity of several mitochondrial enzymes** (citrate synthase), and this could result in the generation of the ATP required to drive the serosal membrane Na^+-K^+ pump.

3. Potassium. Any small increase in plasma potassium level immediately stimulates aldosterone production. On the other hand, decreased plasma potassium reduces aldosterone synthesis. The mechanism of action of K^+ on aldosterone synthesis is not clearly known. It is believed that K^+ like angiotensin II acts at the enzymatic level to increase aldosterone synthesis.

4. Sodium. Low plasma Na^+ increases while high plasma Na^+ decreases aldosterone level. These effects of sodium are mediated through renin-angiotensin mechanism and direct synthesis of aldosterone.

5. ACTH. The deficiency of ACTH over a long period reduces the effects of regulators (angiotensin II, K^+, Na^+) on aldosterone production.

Vasopressin (antidiuretic hormone, ADH)

Increased osmolality of plasma is the primary physiologic stimulus. Plasma osmolality is largely dependent on the sodium concentration, hence **sodium** indirectly controls the amount of water in the body. This is mediated by **osmoreceptors** located in the hypothalamus and by **baroreceptors** located in the heart and other regions of the vascular system. Hemodilution (decreased osmolality) has opposite effect. Other stimuli include emotional and physical stress and pharmacologic agents including acetylcholine, nicotine, and mor-

phine. Most of these effects involve increased synthesis of ADH and neurophsin II, since the depletion of stored hormone is not associated with this action.

The most important physiologic target cells of ADH in mammals are those of the distal convoluted tubules and collecting structures of the kidney. The receptors for ADH are linked to adenylate cyclese, and cAMP is thought to mediate the effects of ADH in the renal tubule. This physiologic action is the basis of the name “antidiuretic hormone”. cAMP and inhibitors of phosphodiesterase activity (caffeine, for example) mimic the action of ADH. ADH **increases the permeability** of the cells to water and permits osmotic equilibration of the collecting tubule urine with the hypertonic interstitium, resulting in urine volumes in the range of 0.5—1 L/d.

All extrarenal ADH receptors are receptors causes activation of phospholipase C, which results in the generation of IP_3 and diacylglycerol. This results in an increase of intracellular Ca^{2+} and activation of proteinkinase C. A major effect of these receptors is vasoconstriction and increased peripheral vascular resistance — hence the name vasopressin that is also used to denote this hormone. **Diabetes insipidus** is a disorder characterized by the deficiency of ADH which results in an increased loss of water from the body. Primary diabetes insipidus is usually due to destruction of the hypothalamus-hypophysial tract from a basal skull fracture, tumor, or infection, but it can be hereditary. In hereditary nephrogenic diabetes insipidus, ADH is secreted normally but the target cell is incapable of responding, presumably because of a receptor defect.

Atrial natriuretic peptide (ANP)

This is a polypeptide hormone secreted by the **right atrium of the heart**. Atrial natriuretic peptide **reduces tubular sodium reabsorbtion**, **inhibits of renin** and **aldosterone secretion** and **results in systemic vasodilatation**. The significance of this hormone, however, is not clear.

Abnormalities of adrenocortical function

Addison's disease. Impairment in adrenocortical function results in Addison's disease. This disorder is characterized by decreased blood glucose level (hypoglycemia), loss of weight, loss of appetite (anorexia), muscle weakness, impaired cardiac function, low blood pressure, decreased Na^+ and increased K^+ level in serum, increased susceptibility to stress etc. Such patients often show **increased pigmentation of skin and mucous** membrane because of the exaggerated compensatory secretion of ACTH. **Secondary adrenal insufficiency** is due to a deficiency of ACTH resulting from tumor, infarction, or infection. Administration of glucocorticoids and mineralocorticoids is advised to save the victims from death.

Cushing’s syndrome. Hyperfunction of adrenal cortex may be due to **long term pharmacological** use of **steroids** or **tumor** of adrenal cortex or **tumor of pituitary**. Cushing's syndrome is characterized by hyperglycemia (due to increased gluconeogenesis), fatigue, muscle wasting, edema, osteoporosis, nega-

tive nitrogen balance, hypertension. There is a peculiar redistribution of fat, with truncal obesity and the typical "buffalo hump" (moon-face). Resistance to infections and inflammatory responses is impaired, as is wound healing.

Aldosteronism. Small adenomas of the glomerulosa cells results in **primary aldosteronism** (**Conn's syndrome**), the classic manifestation of which include hypertension, hypokalemia, hypernatremia, and alkalosis. Patients with primary aldosteronism do not have evidence of glucocorticoid hormone excess, and plasma rennin and angiotensin II levels are suppressed. Renal artery stenosis, with the attendant decrease in perfusion pressure, can lead to hyperplasia and hyperfunction of the juxtaglomerular cells and cause elevated levels of rennin and angiotensin II. This action results in **secondary aldosteronism.**

Assessment of adrenocortical function

The adrenocortical function can be assessed by measuring plasma cortisol (5—25 μg/dl at 9.00 AM), plasma ACTH, urinary 17-ketosteroids etc.

LECTURE 28

INTEGRATION OF METABOLISM

Metabolism is a continuous process, with thousands of reactions, simultaneously occurring in the living cell. However, biochemists prefer to present metabolism in the form of reactions and metabolic pathways. This is done for the sake of convenience in the presentation and understanding.

The organisms possess variable energy demands, hence the supply (input) is also equally variable. The consumed metabolic fuel may be **burnt** (oxidized to CO_2 and H_2O) or **stored** to meet the energy requirements as per the body needs. **ATP** serves as the **energy currency of the cell** in this process.

Metabolic fuels are substances used by the body as **sources of carbon** or **sources of free energy**, which are used for anabolic processes and cellular functions.

Caloric value of metabolic fuels is expressed in terms of kilocalories (kcal) per gram. The approximately caloric values of the major types of metabolic fuels are:

1. **Carbohydrates** (e.g. glucose) = 4 kcal/g
2. **Amino acids** = 4 kcal/g
3. **Ketone bodies** = 4 kcal/g
4. **Fatty acids** = 9 kcal/g.

Body stores of metabolic fuels

1. **Glucose** circulating in the blood is a major metabolic fuel.

2. Carbohydrates is the stored primarily as glycogen in the liver and skeletal muscle.

3. Triacylglycerol are stored primarily in the adipose tissue. They are source of fatty acids and glycerol, the latter of which is a substrate for gluconeogenesis.

4. Body proteins also may be considered a source of fuel because amino acids may be converted to either glucose or ketone bodies.

Feeding-fasting cycle

Any consideration of metabolism and the use of metabolic fuels must take into account the fact that humans are intermittent feeders.

1. The **fed (postprandial) state** occurs during and just after a meal. Plasma substrate levels are elevated above fasting levels, and the metabolic fuels used by tissues may be derived directly from the ingested, digested, and absorbed food molecules.

2. The **fasting (postabsorptive) state** occurs several hours after eating. Metabolic fuels used by tissues are derived from mobilized stores of fuel molecules.

3. Starvation occurs after extended fasting (i.e., 2 or 3 days without food).

The humans possess enormous capacity for food consumption. Obesity, a disorder of overnutrition mostly prevalent in affluent societies, is primarily a consequence of overconsumption.

INTEGRATION OF MAJOR METABOLIC PATHWAYS OF ENERGY METABOLISM

An overview of the interrelationship between the important metabolic pathways, concerned with fuel metabolism depicted in Fig. 28-1.

1. Glycolysis. The degradation of glucose to puruvate (lactate under anaerobic condition) generates 8 ATP. Pyruvate is converted to acetyl CoA.

2. Fatty acid oxidation. Fatty acids undergo sequential degradation with release of 2-carbon fragment, namely acetyl CoA. The energy is trapped in the form of NADH and $FADH_2$.

3. Degradation of amino acids. Amino acids, particularly when consumed in excess than required for protein synthesis, are degraded and utilized to meet the fuel demands of the body. The **glucogenic amino acids** can serve as precursors for the synthesis of glucose via formation of pyruvate or intermediates of citric acid cycle. The **ketogenic amino acids** are the precursors for acetyl CoA.

4. Citric acid cycle. Acetyl CoA is the key and common metabolite, produced from different fuel sources (carbohydrates, lipids, amino acids). **Acetyl CoA** enters citric acid cycle and gets oxidized to CO_2. Thus, **citric acid cycle is the final common metabolic pathway** for the oxidation of all foodstuffs. Most of the energy is trapped in the form of NADH and $FADH_2$.

5. Oxidative phosphorylation. The NADH and $FADH_2$, produced in different metabolic pathways, are finally oxidized in the electron transport chain (ETC). The ETC is coupled with oxidative phosphorylation to **generate ATP**.

6. Pentose phosphate pathway. The pathway is primary concerned with the liberation of NADPH and ribose sugar. NADPH is utilized for the biosynthesis of several compounds, including fatty acids. Ribose is an essential component of nucleotides and nucleic acids (note – DNA contains deoxyribose).

7. Gluconeogenesis. The synthesis of glucose from non-carbohydrate sources constitutes gluconeogenesis. Several compounds (e.g. pyruvate, glycerol, amino acids) can serve as precursors for gluconeogenesis.

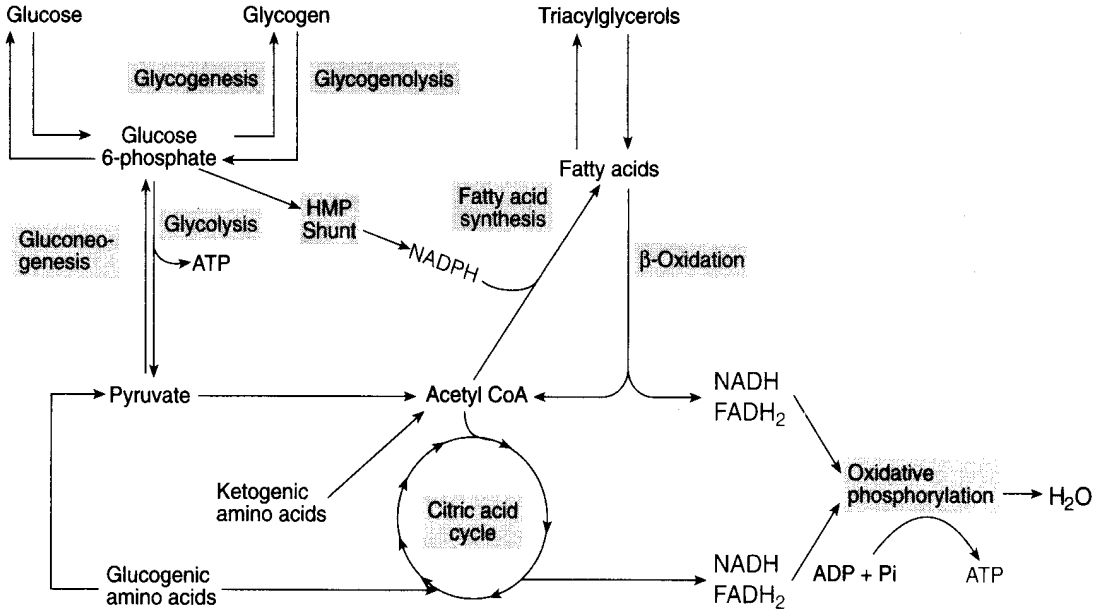


Figure 28—1. An overview of integration of metabolic pathways of energy metabolism (by U. Satyanarayana, 2002).

8. Glycogen metabolism. Glycogen is the storage form of glucose, mostly found in liver and muscle. It is degraded (glycogenolysis) and synthesized (glycogenesis) by independent pathways. Glycogen effectively serves as a **fuel reserve** to meet body needs, **for a brief period** (between meals).

Regulation of metabolic pathways

The metabolic pathways, in general, are controlled by four different mechanisms

1. The availability of substrates.

2. Covalent modification of enzymes.

3. Allosteric regulation.

4. Regulation of enzyme synthesis.

The details of these regulatory processes are discussed under the individual metabolic pathways.

Hormonal regulation of metabolic pathways

Certain hormones exert direct and indirect effects that regulate the flow of metabolites through certain pathways.

1. Insulin signals the fed state through the following actions.

a. Insulin stimulates the synthesis of glycogen, fat, and proteins.

b. Insulin inhibits the degradation of glycogen, fat, and proteins.

2. Glucagon and epinephrine signal the fasting state through the following actions.

a. Glucagon and epinephrine inhibit the synthesis of glycogen, fat, proteins.

b. Glucagon and epinephrine stimulate the degradation of glycogen, fat, and proteins.

3. Epinephrine also signals stressful states when mobilization of fuel is required

ORGAN SPECIALIZATION AND METABOLIC INTEGRATION

The various tissues and organs of the body work in a well coordinated manner to meet its metabolic demands. The major organs along with their most important metabolic functions, in a well-fed absorptive state (**usually 2—4 hours after food consumptions**) are described.

Liver

The liver is specialized to serve as the body's **central metabolic clearing house**. It **possesses** and **distributes** the **nutrients** to **different tissues** for utilization. After a meal, the liver takes up the carbohydrates, lipids and most of the amino acids, processes them and routes to other tissues. The major metabolic functions of liver, in an absorptive state, are:

1. Carbohydrate metabolism. Increased glycolysis, glyconeogenesis and pentose phosphate pathway and decreased gluconeogenesis.

2. Lipid metabolism. Increased synthesis of fatty acids and triacylglycerols.

3. Protein metabolism. Increased degradation of amino acids and protein synthesis.

Adipose tissue

Adipose tissue is regarded as the **energy storage tissue**. As much as 15 kg of triacylglycerol is stored in a normal adult man. The major metabolic functions of adipose tissue in an absorptive state are listed here.

1. Carbohydrate metabolism. The uptake of glucose is increased. This follows an increase in glycolysis and pentose phosphate pathway.

2. Lipid metabolism. The synthesis of fatty acids and triacylglycerols is increased. The degradation of triacylglycerols is inhibited.

Skeletal muscle

The **metabolism** of skeletal muscle is rather **variable** depending on its needs. For instance, the resting muscle of the body utilizes about 30% of body's oxygen consumption. However, during strenuous exercise, this may be as high as 90%. The important metabolic functions of skeletal muscle in an absorptive state are:

1. Carbohydrate metabolism. The uptake of glucose is higher and glycogen synthesis is increased.

2. Lipids metabolism. Fatty acids taken up from the circulation are also important fuel sources for the skeletal muscle.

3. Protein metabolism. Incorporation of amino acids into proteins is higher.

Brain

The human brain constitutes about 2% of the body's weight. But it utilizes as much as **20% of the oxygen** consumed by the body. Being a vital organ, special priority is given to the metabolic needs of the brain.

1. Carbohydrate metabolism. In an absorptive state, **glucose is the only fuel source** to the brain. About 120 g of glucose is utilized per day by an adult brain. This constitutes about 60% of the glucose consumed by the body at rest. It is estimated that about 50% of the energy consumed by brain is utilized by plasma membrane Na^+-K^+ ATPase to maintain membrane potential required for nerve impulse transmission.

2. Lipid metabolism. The **free fatty acids cannot cross the blood-brain barrier**, hence their contribution for the supply of energy to the brain is insignificant. Further, in a fed state, ketone bodies are almost negligible as fuel source to the brain. However, **brain predominantly depends on ketone bodies during prolonged starvation**.

The metabolic interrelationship among the major tissues in an absorptive state are given in Fig. 28-2.

METABOLISM IN STARVATION

Starvation may be due to food scarcity or the desire to rapidly lose weight or certain clinical conditions (e.g. surgery, burns etc.). Starvation is a metabolic stress which imposes certain metabolic compulsions of the organism. The metabolism is reorganized to meet the new demands of starvation.

Glucose is the fuel of choice for brain and muscle. Unfortunately, the carbohydrate reserve of the body is so low that it cannot meet the energy requirements even for a day. **Triacylglycerol** (fat) of adipose tissue is the predominant energy reserve of the body. The survival time of an individual on starvation is mostly dependent on his/her fat stores. And for this reason, obese individuals can survive longer than lean individuals without consuming food.

Protein is basically a structural constituent, mostly present in the muscle. However, during starvation, protein can also meet the fuel demands of the body. It is estimated that about $1/3^{rd}$ of the body's protein can be utilized towards energy needs without compromising the vital functions.

Liver in starvation

1. Carbohydrate metabolism. An important function of liver is to act as a **blood glucose buffering organ**. The action of liver is to suit the metabolic needs of the body. During starvation, increased gluconeogenesis and elevated glycogen degradation furnish glucose to the needy tissues (mostly brain).

2. Lipid metabolism. Fatty acid oxidation is increased with an elevated synthesis of ketone bodies. This is due to the fact that TCA cycle cannot cope up with the excess production of acetyl CoA, hence the latter is diverted for ketone body synthesis.

Ketone bodies (primarily β-hydroxybutyrate) effectively serve as fuel source for the peripheral tissues. The brain slowly adapts itself to use ketone bodies. Thus, after 3-days fast, about 1/3rd of the brain's fuel demands are met by ketone bodies, while, after 40 day's starvation, they contribute to about 70% of energy needs.

Adipose tissue in starvation

1. Carbohydrate metabolism. Glucose uptake and its metabolism are lowered.

2. Lipid metabolism. The degradation of triacylglycerols is elevated, leading to an increased release of fatty acids from the adipose tissue which serve as fuel source for various tissues (brain is an exception). The glycerol liberated in lipolysis serves as precursor for glucose synthesis by liver. The synthesis of fatty acids and triacylglycerols is totally stopped in adipose tissue.

Skeletal muscle in starvation

1. Carbohydrate metabolism. Glucose uptake and its metabolism are very much depressed.

2. Lipid metabolism. Both fatty acids and ketone bodies are utilized by the muscle as fuel source. However, on prolonged starvation beyond 3 week, the muscle adapts to exclusively utilize fatty acids. The further increases the level of ketone bodies in the circulation.

3. Protein metabolism. During the early period of starvation, muscle proteins are degraded to liberate the amino acids which are effectively utilized by the liver for glucose synthesis (gluconeogenesis). On prolonged starvation, however, protein breakdown is reduced.

Brain in starvation

As already stated, glucose is the preferred fuel source by brain. During the first 2 weeks of starvation, the brain is mostly dependent on glucose, supplied by liver gluconeogenesis. This, in turn, is dependent on the amino acids released from the muscle protein degradation. Starvation beyond 3 weeks generally results in a marked increase in the plasma ketone bodies. By this time, the brain adapts itself to depend on ketone bodies for the energy needs.

The metabolic interrelationship among the major organs in starvation are depicted in Fig. 28—3. **The biochemical changes that occur during starvation are such that an adequate supply of fuel molecules is maintained to various tissues to meet the energy demands**. This is natural adaptation for the survival of the organism.

LECTURE 29

OVERVIEW OF VITAMINS. FAT-SOLUBLE VITAMINS

Vitamins may be regarded as organic compounds required in the diet in small amounts to perform specific biological functions for normal maintenance of optimum growth and health of the organism. Vitamins are required for normal growth, development, and maintenance of health.

1. They **cannot be synthesized by human tissues** but must be included in the diet.

2. When intake in the diet is below the needed level, deficiency symptoms appear. The time of onset of symptoms depends on the size and the daily flux of body reserves.

History

In the beginning of this century, it was clearly understood that the diets containing purified carbohydrates, protein, fat and minerals were not adequate to maintain the growth and health of experimental rats, which the natural foods (such as milk) could do.

Hopkins coined the term "accessory factors" to unknown and essential nutrients present in the natural foods. Funk (1913) isolated an active principle (an amine) from rice polishings and, later, in yeast, which could cure beri-beri in pigeons. He coined the term vitamin (*Greek:* vita — life) to the accessory factors with a belief that all of them were amines. It was later realized that only few of them are amines. The term vitamin, however, is continued without the final letter "e".

The usage of A, B and C to vitamins was introduced in 1915 by McCollum and Davis. They first felt there were only two vitamins — a fat soluble A and a water soluble B (anti-beriberi factor). Soon another water soluble anti-scurvy factor named vitamin C was described. Vitamin A was later found to possess two components-one that prevents night blindness (vitamin A) and another anti-ricket factor named as vitamin D. A fat soluble factor called vitamin E, in the absence of which rats failed to reproduce properly, was discovered. Yet another fat soluble vitamin concerned with coagulation was discovered in mid 1930s. It was named as vitamin K. In the sequence of alphabets it should have been F, but K was preferred to reflect its function.

As regards the water soluble factors, vitamin C was identified as a pure substance and named as ascorbic acid. Vitamin B was found to be a complex mixture and nomenclature also

become complex. B_1 was clearly identified as anti-beri factor. Many investigators carried out intensive research between 1920 and 1930 and went on naming them as the water soluble vitamins B_2, B_3, B_4, B_5, B_6, B_7, B_8, B_9, B_{10}, B_{11} and B_{12}. Some of them were found to be mixtures of already known vitamins. And for this reason, a few members of B-complex series disappeared from the scene. Except for B_1, B_2, B_6 and B_{12} names are more commonly used for other B-complex vitamins.

Classification

Vitamins are classified as water soluble or fat-soluble.

1. Water-soluble vitamins include B vitamins, folic acid, niacin, pantothenic acid, biotin, and vitamin C.

2. Fat-soluble vitamins include vitamins A, D, E, and K.

Sources

1. All vitamins **can be supplied by foods**, but no single food is a rich source of all vitamins.

2. Some vitamins also can be synthesized by **intestinal microorganisms**, but these microorganisms may not provide humans with the total requirement.

Enzyme cofactors

1. Vitamins and their derivatives often serve as cofactors for enzymes. Vitamin cofactors are referred to as **coenzymes.**

2. To be activated, some enzymes depend on conjugation of the protein portion of the enzyme (i.e., the apoenzyme) with a cofactor such that:

Apoenzyme + Cofactor = Holoenzyme

3. Cofactors that remain tightly bound to the enzyme and do not dissociate from it are called **prosthetic groups.**

Deficiency

Most vitamin deficiencies present a variety of nonspecific symptoms (e.g., anemia, dermatologic problems, neurologic disorders), which are not easily attributed to a specific vitamin and are not easily distinguished from many other types of metabolic disorders. In the United States and in developed countries, vitamin deficiencies are relatively rare. Causes of deficiency include:

1. Inadequate dietary intake

2. Inadequate absorption, which may result from:

a. Biliary obstruction (lack of bile leads to decreased absorption of the fat-soluble vitamins)

b. Intestinal diseases or disorders

c. Pernicious anemia, owing to lack of the intrinsic factor

3. Inadequate use, which may result from:

a. Lack of a transport protein for a particular vitamin in the serum

b. Failure to convert a vitamin to its activated (i.e., coenzyme) form

4. Increased requirements. In some instances, an increased caloric requirement can unmask a borderline vitamin deficiency. Increased requirements occur during:

a. Growth

b. Pregnancy
c. Lactation
d. Wound healing and convalescence
5. Increased excretion (e.g., blood loss, diarrhea)
6. Drug-induced deficiency, such as:
a. Loss of microbial vitamin synthesis in the intestine because of antibiotic therapy
b. Alcoholism may increase certain vitamin requirements.
c. Drug-nutrient interactions may increase certain vitamin requirements.

FAT-SOLUBLE VITAMINS (Tabl.29—1)

Table 29-1.

Fat-soluble vitamins

Vitamin		Function	Deficiency disease
A	Retinol, β-carotene	Visual pigments in the retina; regulation of gene expression and cell differentiation; β-carotene is an antioxidant	Night blindness, xerophthalmia; keratinization of skin
D	Calciferol	Maintenance of calcium balance; enhances intestinal absorption of Ca^{2+} and mobilizes bone mineral	Rickets = pool mineralization of bone; osteomalacia = bone demineralization
E	Tocopherols, tocotrienols	Antioxidant, especially in cell membranes	Extremely rare – serious neurologic dysfunction
K	Phylloquinone, menaquinones	Coenzyme in formation of γ-carboxyglutamate in enzymes of blood clotting and bone matrix	Impaired blood clotting, hemorrhagic disease

General properties of lipid-soluble vitamins

1. The lipid-soluble (fat-soluble) vitamins are **apolar hydrophobic molecules** which are all **isoprene derivatives**.

2. They can only be absorbed efficiently when normal fat absorbed is taking place.

3. Once absorbed, they must be transported in the blood in **lipoproteins** or attached to **specific binding proteins**.

VITAMIN A (retinol)

1. Structure. Vitamin A is a polyisoprenoid compound containing a β-**ionone ring.** Vitamin A is not destroyed by heat. Due to presence of double bonds, it is susceptible to oxidation. In the body, the main functions of vitamin A are carried out by **retinol** and its two derivatives **retinal** and **retinoic acid.** In vetetables, vitamin A exist as a provitamin in the form of the yellow pigment **β-**

carotine, which consists of two molecules of retinal joined at the aldehyde end of their carbon chains (Fig. 29—1).

2. Sources include green leafy and yellow vegetables, liver, egg yolk, cheese butterfat, and fortified milk. Fish (cod or shark) liver oils are very rich in vitamin A.

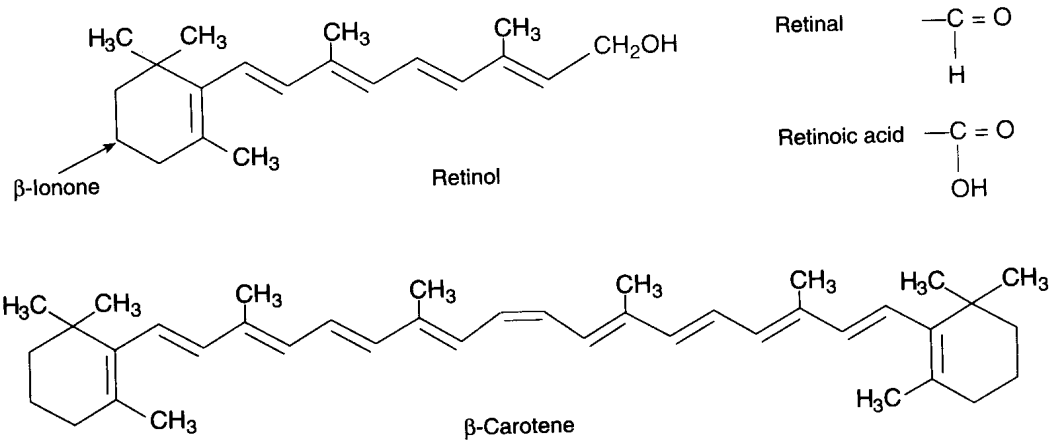


Figure 29—1. β-Carotine and the major vitamin A vitamers.

3. Metabolism

a. Carotenoids are converted to retinal and retinol, which are the forms used by the body.

b. Carotenoids may be directly absorbed, but most are cleaved to retinal and then converted to retinol, which is absorbed.

c. Retinol esters dissolved in the fat of the diet are dispersed in bile droplets and hydrolyzed in the intestinal lumen, and retinol is absorbed in the upper intestine.

d. In the intestinal mucosal cells, retinol is re-esterified with palmitate, incorporated into lymph chylomicrons, which enter the bloodstream. There are converted to chylomicron remnant, which are taken up by the liver together with their content of retinol.

e. In the liver, vitamin A is stored as an ester in the lypocytes (stellate cells).

f. For transport to the tissues, it is hydrolyzed and the retinol bound to **plasma retinol-binding protein**. Retinoic acid is transported in plasma bound to albumin. Once inside extrahepatic cells, retinol is bound by a **cellular retinol-binding protein**.

g. Retinol and retinal are interconverted in the presence of reductase, present in many tissue. Retinoic acid cannot be converted back to retinal or to retinol. Thus, retinoic acid can support growth and differentiation but cannot replace retinal in its role in vision or retinolin its support of the reproductive system.

4. Functions

a. Vitamin A (retinol and retinoic acid) behaves similarly to **steroid hormones**. When retinol is taken up into cellular retinol-binding protein it is trans-

ported around the cell and bind to nuclear proteins, where it is probably involved in the control of the **expression** of certain **genes**. They regulate the protein synthesis and thus are involved in the cell growth and differentiation.

b. Role in vision

(1) On entering the retina, retinol is esterified to a fatty acid, providing a means of concentrating the retinol within the cell.

(2) The fatty acid esters are hydrolyzed, and the retinol is oxidized to retinal by a specific NAD^+-linked dehydrogenase.

(3) The retinal forms complex with proteins called **opsins** in the rods and cones.

(4) Opsins preferentially bind the 11-*cis* isomer rather than all-trans retinal.

(5) The absorption of light triggers a conformational change in opsin and conversion of the 11-*cis* isomer of retinal to the all-trans form, which is only weakly bound to opsin. This reaction isaccompanied by a conformational change that induces a calcium ion channel in the membrane of the rod cell. The rapid influx of calcium ions triggers a nerve impulse, allowing light to be perceived by the brain.

c. Vitamin A also appears necessary for **growth** of **epithelial tissue**, **reproduction**, and **bone growth**. This is due to the fact that retinol and retinoic acid are required to prevent keratin synthesis (responsible for horny surface) Further, retinyl phosphate is essential for the formation of **mucopolysaccharides**, compounds of mucus secreted by epithelial cells to maintain moist surface.

d. Retinoic acid participates in **glycoprotein synthesis**, which are required for growth and mucus secretion.

e.Retinoids and β-carotene are an **antioxidants** and may play a role in trapping peroxy free radicals in tissues at low partial pressures of oxygen. The antioxidant properties of vitamin A may well account for their possible **anticancer** activity.

f. Retinol and retinoic acid are involved in the **synthesis of transferrin**, the iron transport protein.

g.Vitamin A is considered to be essential for the maintenance of proper **immune system** to flight against various infections.

5. Deficiency may cause the following:

a. Defects in vision, progressing from **night blindness** to total blindness, which occurs when liver stores are nearly exhausted. Further depletion leds to keratinization of epithelial tissues of the eye, lungs, gastrointestinal, and genitourinary tracts, coupled with reduction in mucous secretion. Deterioration in the tissues of the eye, **xerophthalmia**, leads to blindness.

b. Skin lesions such as follicular hyperkeratosis.

c. Gonadal dysfunction in males and miscarriage in females.

6. There is only a limited capacity to metabolize vitamin A, and excessive intakes lead to accumulation beyond the capacity of binding proteins, so that

unbound vitamin A causes tissue damage. Symptoms of toxicity affect the central nervous system (headache, nausea, ataxia, and anorexia, all associated with increased cerebrospinal fluif pressure), the liver (hepatomegaly with histologic changes and hyperlipidemia), calcium homeostasis (thickening of the long bones, hypercalcemia and calcification of soft tissues), and the skin (excessive dryness, desquamation, and alopecia).

7. Recommended dietary allowance. The daily requirement of vitamin A is expressed as retinol equivalent (RE) rather than International Units (IU). 1 retinol equivalent = 1 μg retinol; = 6 μg of β-carotene; = 12 μg other carotenoids; = 3.33 IU of vitamin A activity from retinol; = 10 IU of vitamin A activity from β-carotene. The RDA of vitamin A for adults is around 1000 retinol equivalents (5000 IU) for man and 800 retinol equivalents (4000 IU) for women. One International Unit equals to 0.3 μg of retinol.

VITAMIN D

1. Structure. Ergosterol occurs in plants and 7-dehydrocholesterol in animals (Fig.29—2). Ergosterol differs from 7-dehydrocholesterol only in its side chain, which is unsaturated and contains an extra methyl group. Ultraviolet irradiation cleaves the B ring of both compounds. Ergocalciferol (vitamin D_2) may be made commercially from plants in this way, whereas in animals, **cholecalciferol** (vitamin D_3) is formed from 7-dehydrocholesterol in exposed skin. Both vitamin D_2 and D_3 are of equal potency.

2. Sources. Vitamin D is required only as an accessory food factor when humans are deprived of sunlight. It is synthesized in the skin by the action of ultraviolet light. Dietary sources include liver, eggs, and fortified milk.

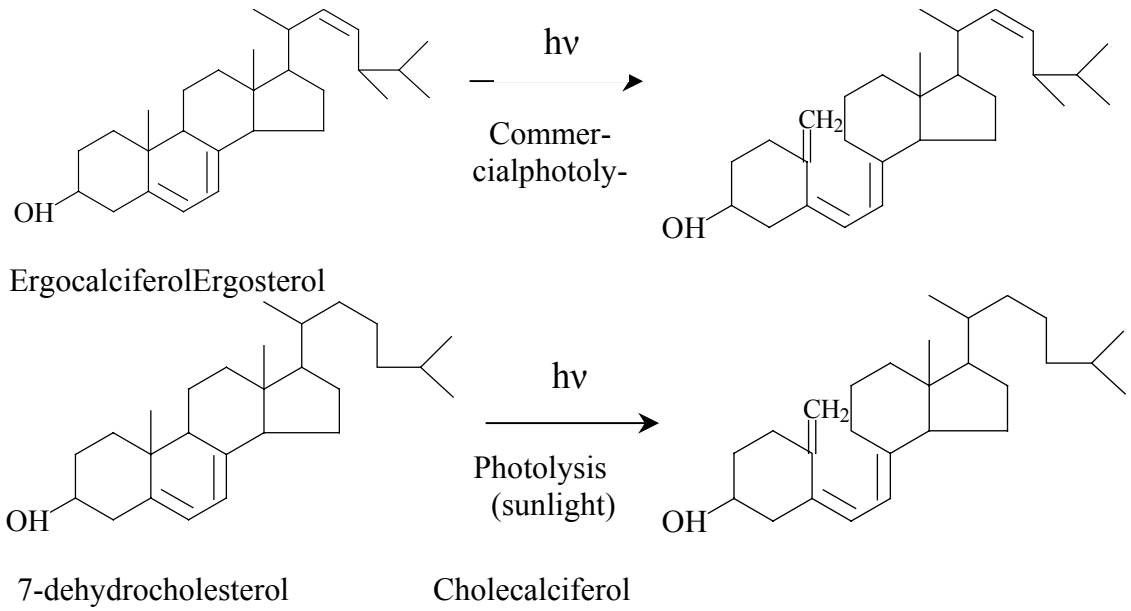


Figure 29—2. Ergosterol and 7-dehydrocholesterol and their convention by photolysis to ergocalciferol and cholecalciferol.

3. Metabolism. Vitamin D is absorbed in the small intestine for which bile is essential. Through lymph, vitamin D esters the circulation bound to plasma α_2 globulin and is distributed throughout the body. Liver and other tissues store small amount of vitamin D. In the **liver**, cholecalciferol, which has been synthesized in the skin or derived from food, is **hydroxylated** to form the **25-hydroxycholecalciferol**. This is released into the circulation bound to a vitamin D-binding globulin which is the main storage form of the vitamin. In the **kidney**, calcidiol undergoes either 1-hydroxylation to yield the active metabolite **1,25-dihydroxycholecalciferol (calcitriol)** or 24-hydroxylation to yield an inactive metabolite, 24,25-dihydroxyvitamin D. Ergocalciferol from fortified foofs undergoes similar hydroxylation to yield **ercalcitriol**. Both the hydroxylase enzymes (of liver and kidney) require cytochrome P_{450}, NADPH and molecular oxygen for the hydroxylation process.

4. Function. The main function of vitamin D is in the control of **calcium** homeostasis, and in turn vitamin D metabolism is regulated by factors that respond to plasma concebtrations of calcium and phosphate.

a. Its principal function is to maintain the plasma calcium concentration.

b. Calcitriol achieves this in **three ways**: it increases intestinal absorption of calcium, reduces excretion of calcium (by stimulating resorption in the distal renal tubules), and mobilized bone mineral (see hormones).

c. In addition, calcitriol is involved in **insulin secretion, synthesis and secretion of parathyroid and thyroid hormones.**

d. In its actions, it behaves like a **steroid hormone**, binding to a nuclear receptor protein and the complex acts on DNA to stimulate the synthesis of calcium binding protein.

5. Deficiency.

a. In children, vitamin D deficiency leads to a condition known as **rickets,** which manifests as a malformation of the long bones.

b. In adults, there is an increased radiolucency of bones and tendency for fractures to occur. The condition is known as **osteomalacia.**

6. Some infants are sensitive to intakes of vitamin D as low as 50 μg/d, resulting in an elevated plasma concentration of calcium. This can lead to contraction of blood vessels, high blood pressure, and **calcinosis** – the calcification of soft tissues. Although excess dietary vitamin D is toxic, excessive exposure to sunlight does not lead to vitamin D poisoning because there is a limited capacity to form the precursor 7-dehydrocholesterol and to take up cholecalciferol from the skin.

7. Recommended dietary allowance. The daily requirement of vitamin D is 400 IU or 10 μg of cholecalciferol. In countries with good sunlight, the RDA for vitamin D is 200 IU.

VITAMIN E (tocopherol)

1. Structure. Vitamin E is the name given to a group of tocopherols, possessing vitamin E activity. About eight tocopherols have been identified — α, β, γ, δ etc. Among these, α-tocopherol is the most active and it is usual to express vitamin E intake in milligrams of **D-α-tocopherol equivalents**. The tocopherols are derivative of **6-hydroxychromane (tocol) ring** with isoprenoid (3 units) side chain (Fig. 29—3).

2. Sources include vegetable oils are rich sources of vitamin E. Wheat germ oil, cotton seed oil, peanut oil, corn oil and sunflower oil are the good sources of this vitamin. It is also present in meat, milk, butter and eggs.

3. Metabolism. Vitamin E apparently is unmetabolized in the body. Impaired fat absorption leads to vitamin E deficiency because tocopherol is found dissolved in the fat of the diet and is liberated and absorbed during fat digestion. It is transported in the blood by lipoproteins – first, by incorporation into chylomicrons, which distribute the vitamin to the tissues containing lipoprotein lipase and then to the liver in the chylomicron remnants; and second, by export from the liver in very low density lipoproteins. It is **stored** in **adipose tissue, liver and muscle**.

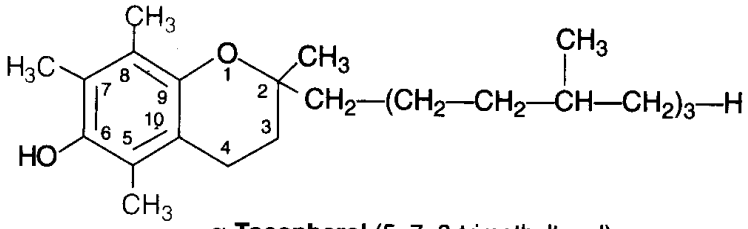


Figure 29—3. Structure of α-tocopherol

4. Function.

a.Its role appears to be primarily that of a **scavenger of potentially damaging free radicals.** This antioxidant action of tocopherol is effective at **high oxygen concentrations** (the erythrocyte membrane, the membranes of the respiratory tree, and the retina). It prevents oxidation and peroxidation of polyunsaturated fatty acids, thus preventing membrane dysfunction and altered lipoprotein metabolism.

b. Tocopherol and **selenium** reinforce each other in their actions against lipid peroxides. Selenium is required for normal pancreatic function, which is necessary for the digestion and absorption of lipids, including vitamin E. Conversely, vitamin E reduces selenium requirements by preventing loss of selenium from the body or maintaining it in an active form.

c. It is required for cellular respiration-through electron transport chain (believed to stabilize coenzyme Q).

5. Deficiency of vitamin E in humans is associated with hemolysis of erythrocytes, because of a lack of protection against peroxides, and with creatinuria due to increased muscle breakdown. Vitamin E deficiency may be found in situations associated with dysfunction of the absorbtion or transport of vitamin (e.g. in chronic steatorrhea, abetalipoproteinemia, cholestatic liver disease, cystic fibrosis, and in patients who have undergone intestinal resection).

In **experimental animals**, vitamin E deficiency results in resorption of fetuses and testicular atrophy.

6. Recommended dietary allowance. Since the vitamin E deficiency symptoms are rare in human, it is rather difficult to find out its requirements precisely. A daily consumption of about 8- 12 mg (12—15 IU) of α-tocopherol is recommended. One mg of α-tocopherol is equal to 1,5 IU.

VITAMIN K

1. Structure. Three compounds have the biologic activity of vitamin K (Fig. 29-4): **phylloquinone** (vitamin K_1), the normal dietary source, found in green vegetables; **menaquinones** (vitamin K_2), synthesized by intestinal bacteria, with differing lengths of side-chain; **menadione** (Vitamin K_3), synthetic compounds that can be metabolized to phylloquinone. All the three vitamins (K_1, K_2, K_3) are **naphthoquinone derivaties**. Isoprenoid side chain is present in vitamin K_1 and K_2.

2. Sources. Vitamin K is widely distributed in plant and animal tissues used as food and synthesized by intestinal bacteria. Cabbage, cauliflower, tomatoes, spinach and other green vegetables are good sources. It is present in egg yolk, meat, liver, cheese and dairy products.

3. Metabolism. Vitamin K is taken in the diet or synthesized by the intestinal bacteria. Its absorption takes place along with fat (chylomicrons) and is dependent on bile salts. Vitamin K is transported along with LDL and is stored mainly in liver and, to lesser extent, in other tissue.

4. Functions. Vitamin K plays a role in blood coagulation.

a. Vitamin K has been shown to be involved in the maintenance of normal levels of **blood clotting factors II, VII, IX, and X**, all of which are synthesized in the liver initially as **inactive precursor proteins**.

b. Generation of the biologically active clotting factors involves the **post-translational modification** of **glutamate** (Glu) residues of the precursor by a specific vitamin K dependent **carboxylase**. It involves the conversion of glutamate to **γ-carboxyglutamate.** The γ-carboxyglutamic acid residues of clotting factors are negatively charged (COO^-) and they combine with positively charged calcium ions (Ca^{2+}) to form complex.

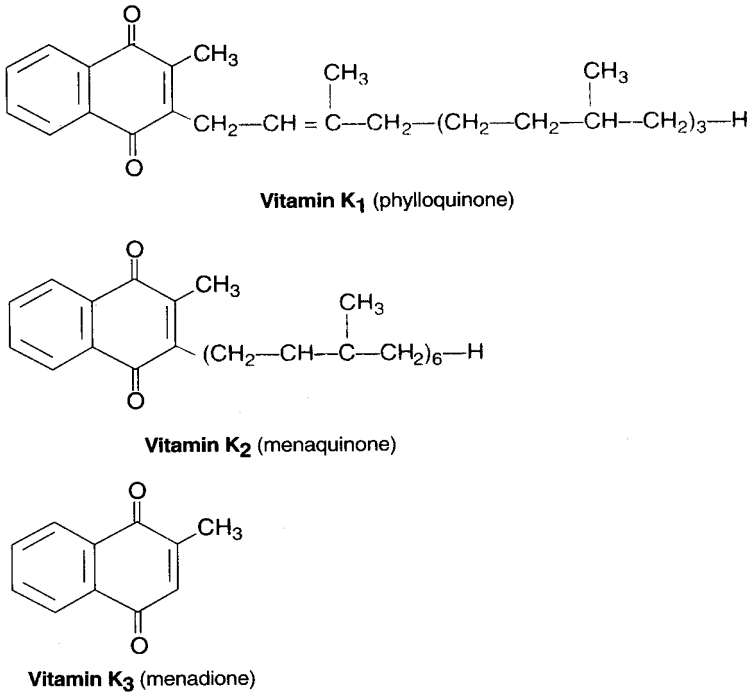


Figure 29—4. Structures of vitamin K

c. The vitamin K-dependent carboxylase reaction occurs in the endoplasmic reticulum of many tissues and requires molecular oxygen, cabon dioxide, and the **hydroquinone** (reduced) form of vitamin K. In the endoplasmic reticulum of liver there exist a vitamin K cycle (Fig.29—5) in which the **2,3-epoxide** product of the carboxylation reaction is converted by 2,3-epoxide reductase to the quinone form of vitamin K. This reaction is sensitive to inhibition by dicumarol type of anticoagulant, such as **warfarin**.

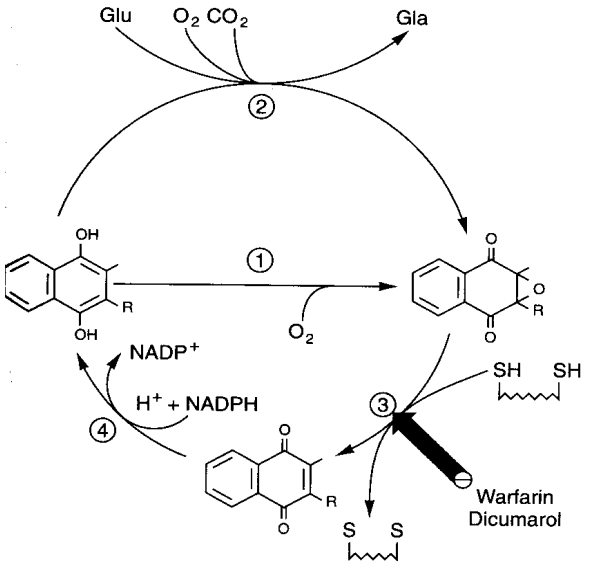


Figure 29—5. Vitamin K cycle in liver. 1 monooxygenase; 2 carboxylase; 3 2,3 — epoxide reductase; 4 reductase (by Murray R.K. et al., 1996).

d. Vitamin K is structurally related to ubiquinones, the components of mitochondrial electron transport chain. Vitamin K is believed to be involved in electron transport chain and oxidative phosphorylation.

3. Coumarins act by inhibiting the vitamin K-dependent γ-carboxylation of carboxylated glutamate residues in several of the clotting factors.

a. Because coumarins can be taken orally, they are prophylactically used in patients with prosthetic implants that are in direct contact with blood.

b. Coumarins reduce the chance of clot formation on the abnormal surfaces of the prosthesis.

4. An important therapeutic use of vitamin K is as an **antidote** to poisoning by dicumarol-type drugs.

5. Deficiency.

a. Dietary **deficiency** does not occur in adults. Newborn infants are vulnerable to the deficiency, because the placenta does not pass the vitamin to the fetus efficiently and the gut is sterile immediately after birth.

b. Vitamin K deficiency can be caused by fat malabsorption, which may be associated with pancreatic dysfunction, biliary disease, atrophy of the intestinal mucosa, or any cause of steatorrhea.

c. Sterilisation of the large intestine by antibiotics can results in deficiency when dietary intake is limited.

6. Recommended dietary allowance. Strictly speaking, there is no RDA for vitamin K, since it can be adequately synthesized in the gut. Accordingly, the suggested RDA for an adults is 70—140 μg/day.

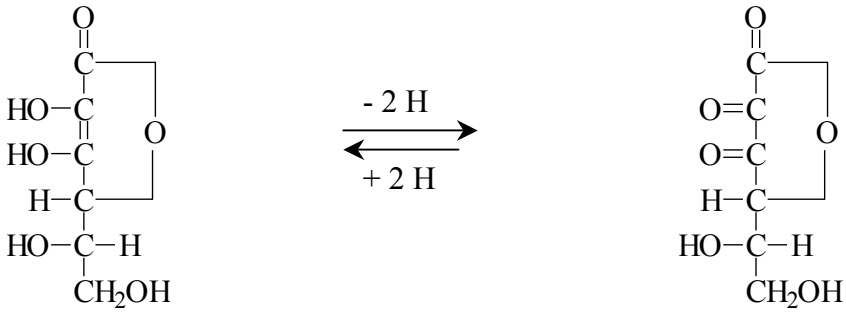
LECTURE 30

WATER-SOLUBLE VITAMINS: C, B_1, B_2 ,B_6, niacin

The water-soluble vitamins are, in most cases, cofactors in enzyme systems or precursors of cofactors. Table 30—1 lists the water-soluble vitamins, the cofactors to which they are converted in the body, some major enzyme systems that require each cofactor, and diseases associated with deficiencies.

VITAMIN C (ascorbic acid)

1. Structure. Ascorbic acid is a hexose derivative and closely resembles monosaccharides in structure (Fig. 30-1). The acidic property of vitamin C is due to the enolic hydroxyl groups. It is a strong reducing agent. L—Ascorbic acid undergoes oxidation to form dehydroascorbic acid and this reaction is reversible. Both **ascorbic** and **dehydroascorbic** acid are **biologically active**. The plasma and tissues predominantly contain ascorbic acid in the reduced form. On hydration, dehydroascorbic acid is irreversibly converted to 2,3-diketogulonic acid which is inactive. Hydration reaction is almost spontaneous, in alkaline or neutral solution. Oxidation of ascorbic acid is rapid in the presence of copper. Hence vitamin C becomes inactive if the foods are prepared in copper vessels.



L-Ascorbic acid Dehydroascorbic acid

Figure 30—1. Structures of vitamin C

2. Sources include citrus fruits and their juices, strawberries, gooseberry (alma), guava, green vegetable (cabbage, spinach), tomatoes, potatoes, cantaloupes, and raw or minimally cooked vegetables.

3. Metabolism. Vitamin C is rapidly absorbed from the intestine. It is not stored in the body to significant extent. Ii is not metabolized but functions as a cofactor in this form. Ascorbic acid is excreted in urine.

4. Functions

a. Ascorbic acid is a reducing agent with a hydrogen potential of + 0.08 V, making it capable of reducing such compounds as molecular oxygen, nitrate, and cyrochromes *a* and *c*.

b. Vitamin C is required for the **hydroxylation** of proline and lysine residues during **biosynthesis of collagen**. The hydroxylation reaction is catalyzed by **lysyl hydroxylase** and **prolyl hydroxylase.** Hydroxyproline and hydroxylysine are essential for the collagen cross-linking and the strength of the fiber. **Bone** tissues possess an organic matrix, collagen and inorganic calcium, phosphate etc. Vitamin C is required for bone formation.

c. Tyrosine metabolism. In the syntheses of the catecholamines **norepinephrine** and **epinephrine** from tyrosine in the adrenal medulla and central nervous system it is required at the dopamine β-hydroxylase. During hydroxylation, the Cu^{+} is oxidized to Cu^{2+}; reduction back to Cu^{+} specifically requires ascorbate. Ascorbic acid is required for the oxidation of *p*-hydroxyphenylpyruvate to homogentisate in tyrosine metabolism.

d. Tryptophan metabolism. Vitamin C is essential for the hydroxylation of tryptophan to hydroxytryptophan in the synthesis of **serotonin.**

e. Cholesterol metabolism. The synthesis of **bile acid** from cholesterol is enhanced by ascorbic acid.

f. Synthesis of corticosteroid hormones. The **adrenal cortex** contains high levels of vitamin C, particularly in periods of stress. It is believed that vitamin C is necessary for the hydroxylation reactions in the synthesis of corticosteroid hormones.

g. Iron and hemoglobin metabolism. Ascorbic acid enhances iron absorption by keeping it in the ferrous form. This is due to the reducing property of

vitamin C. It helps in the formation of ferritin (storage form of iron) and mobilization of iron from ferritin. Vitamin C is useful in the reconversion of methemoglobin to hemoglobin. The degradation of hemoglobin to bile pigments requires ascorbic acid.

h. Folic acid metabolism. The active form of the vitamin folic acid is tetrahydrofolate (FH_4). Vitamin C is needed for the formation of FH_4. Further, in association with FH_4, ascorbic acid is involved in the maturation of erythrocytes.

k. Vitamin C is a general water-soluble **antioxidant** that protects cells against free radical damage. It spares vitamin A, vitamin E, and some B-complex vitamins from oxidation.

5. Deficiency leads to the condition known as **scurvy** in humans. It arises in bottle-fed babies whose milk is not supplemented with vitamin C and in people who are on fad diets that are deficient in vitamin C.

a. Symptoms include: abnormal bone development in infants and children, easy bruising and bleeding due to fragile capillaries, loosening of teeth and swollen gums, poor wound healing, osteoporosis

b. Molecular basis of the disorder. Without the hydroxylation of lysine and proline, proper aligned stable helices of the α chains are not formed, so the procollagen that is formed is unstable and degraded.

c. Prevention and treatment. Daily ingestion of vitamin C from fresh fruit and vegetables should prevent scurvy.

6. Recommended dietary allowance. About 60—70 mg vitamin C intake per day will meet the adult requirement. Additional intakes (20—40% increase) are recommended for women during pregnancy and lactation.

VITAMIN B_1 (thiamin) has a central role in energy-yielding metabolism, and especially the metabolism of **carbohydrate**.

1. Structure. Thiamin consists of a substituted pyrimidine joined by a methylene bridge to a substituted thiazole (Fig. 30-2).

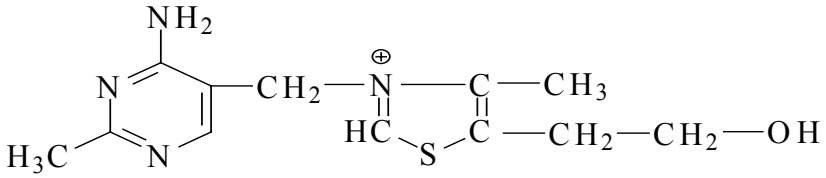


Figure 30—2. Structure of thiamin

2. Sources include pork, whole-grain cereals, liver, heart, kidney, milk, legumes, and enriched grain products. In the parboiled and milled rice, thiamin is not lost in polishing. Storage is limited, and the liver stores can be depleted in 12—14 days.

3. Metabolism. Thiamin is the precursor of **thiamin pyrophosphate (TPP),** which is formed by reaction with adenosine triphosphate (ATP).

4. Functions.

a. Thiamin pyrophosphate is the coenzyme for three multi-enzyme complexes that catalyze oxidative decarboxylation reactions: **pyruvate dehydrogenase** in carbohydrate metabolism, **α-ketoglutarate dehydrogenase** in the citric acid cycle and branched-chain **keto-acid dehydrogenase** involved in the metabolism of leucine, isoleucine, and valine.

b. It is also coenzyme of **transketolase** in the pentose phosphate pathway. This pathway is concerned with the production of ribose and NADPH, respectively required for nucleic acid and lipid synthesis.

c. TPP plays an important role in the transmission of **nerve impulse.** It is believed that TPP is required for acetylcholine synthesis and the ion translocation of neural tissue.

5. Deficiency of vitamin B_1 can lead to disturbances in carbohydrate metabolism and to decreased transketolase activity, particularly in erythrocytes and leukocytes. Clinically, deficiency can lead to cardiovascular and neurologic lesions, as well as to emotional disturbances. Erythrocyte transketolase activity is used as a measure of thiamin deficiency, as are thiamin excretion in urine and blood thiamin concentration.

a. "Dry" beriberi develops when the diet chronically contains slightly less thiamin than required. Symptoms include peripheral neuropathy, fatigue, and impaired capacity to work.

b. "Wet" beriberi develops with a severe deficiency. In addition to neurologic manifestations, cardiovascular symptoms are more apparent, including heart enlargement and tachycardia. Cardiac failure is common after stress, and edema and anorexia are characteristic, as well.

c. Wernicke-Korsakoff syndrome develops in the most acute deficiencies and is seen primarily in alcoholics because of their poor nutrition coupled with impaired absorption of the vitamin. Clinical manifestations of the syndrome include weakness or paralysis and impaired mental function.

d. Certain **raw fish** contain a heat-labile enzyme (thiaminase) that destroys thiamin, but this is not considered to be critical in human nutrition.

e. The central role of thiamin diphosphate in pyruvate dehydrogenase means that in deficiency there is impaired conversion of **pyruvate to acetyl CoA**. In subjects on a relatively high carbohydrate diet, this results in increased plasma concentrations of lactate and pyruvate, which may cause life-threatening lactic acidosis. In thiamin deficiency, an alteration occurs in the blood-brain barrier permitting the pyruvate to enter the brain directly. It is believed that pyruvate accumulation in brain results in disturbed metabolism that may be responsible for polyneuritis.

6. Recommended dietary allowance. The daily requirement of thiamin depends on the intake of carbohydrates. A dietary supply of 1—1.5 mg/day is recommended for adults. The requirement marginally increases in pregnancy and lactation (2 mg/day), old age and alcoholism.

VITAMIN B_2 (riboflavin) has a central role in a variety of cellular oxidation-reduction reactions.

1. Structure. Ribiflavin consists of a 6,7-dimethyl isoalloxazine ring attached to the D-ribitol by a nitrogen atom (Fig. 30—3). It is a colored, fluorescent pigment that is relatively heat-stable but decomposes in the presence of visible light.

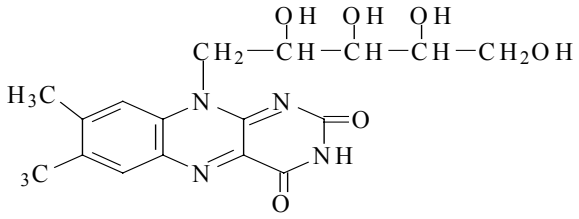


Figure 30—3. Structure of riboflavin.

2. Sources include dairy products, eggs, kidney, meats, yeast, liver, vegetables and whole grain foods. The requirement for riboflavin is related to protein use and is increased during growth, pregnancy, lactation, and wound healing.

3. Metabolism. Riboflavin is converted by ATP-dependent phosphorylation to flavin mononucleotide (FMN) in the intestinal mucosal cells and then to flavin adenine dinucleotide (FAD) in the liver.

4. Functions. The coenzymes **FAD** and **FMN** are required by several oxidative enzymes. These enzymes are known as **flavoproteins**. The prosthetic groups are usually tighly but not covalently bound to their apoproteins. The functional unit of both the coenzymes is **isoalloxazine** ring which serves as an acceptor of two hydrogen atoms (with electron). FMN or FAD undergo identical

reversible reactions accepting two hydrogen atoms forming $FMNH_2$ and $FADH_2$. Flavoprotein enzymes are widespread and are represented by several important oxidoreductases in metabolism:

(1) L-Amino acid oxidase in amino acid deamination

(2) Xanthine oxidase in purine degradation

(3) Aldehyde dehydrogenase in the degradation of aldehydes

(4) Succinate dehydrogenase in the citric acid cycle

(5) Acyl-CoA dehydrogenase and the electron-transferring flavoprotein in fatty acid oxidation

(6) Dihydrolipoyl dehydrogenase in the oxidative decarboxylation of pyruvate and α-ketoglutarate

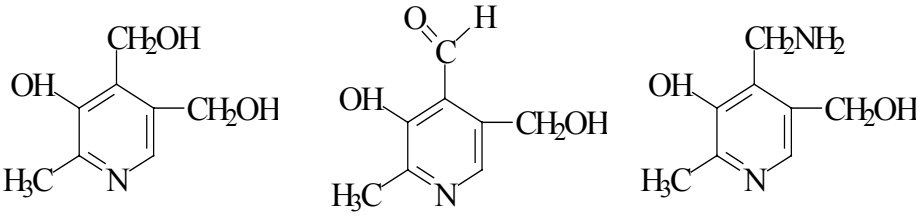
(7) NADH dehydrogenase is a major component of the respiratory chain in mitochondria

5. Deficiency of vitamin B_2 causes **ariboflavinosis,** which is characterized by vascularization of the cornea, lesions of the lips, mouth, skin, and genitalia, especially angular **stomatitis, cheilosis, glossitis, and seborrheic dermatitis**. Because of its light sensitivity, riboflavin deficiency may occur in **newborn** infants with hyperbilirubinemnia who are treated by phototherapy. Although riboflavin is fundamentally involved in metabolism, and deficiencies are found in most countries, it is not fatal as there is very efficient conservation of tissue riboflavin.

6. Recommended dietary allowance. The daily requirement of riboflavin for an adult is 1.2—1.7 mg.

VITAMIN B_6 (pyridoxine) is important in amino acid, glycogen metabolism and in steroid hormone action.

1. Structure. Vitamin B_6 consists of three closely related pyridine derivatives: **pyridoxine, pyridoxal, and pyridoxamine** (Fig.30—4). They differ from each other in the structure of a functional group attached to 4^{th} carbon in the pyridine ring. Pyridoxine can be converted to pyridoxal and pyridoxamine, but the latter two cannot form pyridoxine.



Pyridoxine Pyridoxal Pyridoxamine

Figure 30— 4. Vitamin B_6 and its derivatives

2. Sources include meat, eggs, fish, poultry, whole-grain cereals, and certain vegetables, avocados, bananas.The requirements increase during pregnancy and lactation.

3. Metabolism. All forms of vitamin B_6 are absorbed from the intestine, but some hydrolysis of the phosphate esters occurs during digestion. Pyridoxal phosphate is the major form transported in plasma. Most tissues contain the enzyme **pyridoxal kinase,** which is able to catalyze the phosphorylation by ATP of the unphosphorylated forms of the vitamin to their respective phosphate esters. While **pyridoxal phosphate (PLP)** is the major coenzyme expressing vitamin B_6 activity, pyridoxamine phosphate may also act as an active form. Approximately 80% of the body's total vitamin B_6 is present as pyridoxal phosphate in muscle.

4. Functions

a. PLP serves as a cofactor for many enzymes that use amino acids as substrates. **PLP enzymes form covalent Schiff-base intermediates** between the aldehyde portion of PLP and the α-amino group of amino acids. A wide range of amino acid transformations are catalyzed by PLP enzymes after formation of this reaction intermediate. These include:

(1) Transamination with α-keto acids. Pyridoxal phosphate is involved in the transamination reaction converting amino acids to keto acids. The keto acids enter the citric acid cycle and get oxidized to generate energy. Thus B_6 is an energy releasing vitamin. It integrates carbohydrate and amino acid metabolism.

(2) Decarboxylation to form the respective amines. This is carried out by a group of enzymes called **decarboxylases**.

(3) Aldol cleavages

(4) Deamination

(5) PLP plays an important role in the metabolism of sulfur containing amino acids.

b. PLP also is a cofactor for **glycogen phosphorylase** where it is needed for glycogen metabolism.

c. Vitamin B_6 is important in **steroid hormone action** where it removes the hormone-receptors complex from DNA binding, terminating the action of the hormones. In vitamin B_6 deficiency, this results in increased sensitivity to the actions of low concentrations of estrogens, androgens, cortisol, and vitamin D

4. Deficiency of vitamin B_6 is rare but may occur as a result of interaction with certain drugs, most notably **isoniazid**, which is used for the treatment of **tuberculosis** and is an antagonist of PLP. Clinical symptoms include:

a. Lesions of the skin and mucosa

b. Sideroblastic anemia

c. Neurological symptoms such as depression, irritability, nervousness and mental confusion. Convulsions and peripheral neuropathy are observed in severe deficiency. These symptoms are related to the decreased synthesis of biogenic amines (serotonin, norepinephrine and epinephrine).

A possibility of deficiency is recognized in **nursing infants** whose mothers are depleted of the vitamin owing to long-term use of oral contraceptives.

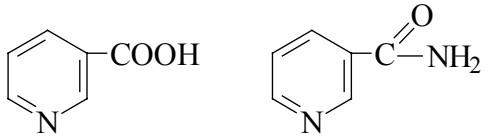
Alcoholics may also be deficient owing to metabolism of ethanol to acetaldehyde, which stimulates hydrolysis of the phosphate of the coenzyme.

Increased sensitivity to steroid hormone action may be important in the development of **hormone-dependent cancer** of the breast, uterus, and prostate, and vitamin B_6 status may affect the prognosis.

6. Recommended dietary allowance. The requirement of pyridoxine for an adult is 2—2.2 mg/day.

NIACIN (nicotinic acid). Niacin or nicotinic acid is also known as pellagra preventive (P.P) factor of Goldberg.

1. Structure. Niacin is the generic name for nicotinic acid and nicotinamide, either of which may act as a source of vitamin in the diet. Nicotinic acid is a monocarboxylic acid derivative of pyridine (Fig. 30—5).



Nicotinic acid Nicotinamide

Figure 30—5. Niacin (nicotinic acid and nicotinamide)

2. Sources

a. Niacin is formed in humans during the catabolism of tryptophan. About 60 mg of tryptophan is equivalent to 1 mg of dietary niacin. However, this provides only approximately 10% of the requirement. The remainder must come from the diet.

b. Dietary sources include cereals, liver, yeast, whole grain, pulses like beans, legumes, and meats.

3. Metabolism and function. Two compounds, **nicotinic acid** and **nicotinamide**, have the biologic activity of niacin.

a.Niacin is the precursor for biosynthesis of nicotinamide adenine dinucleotide (**NAD^+**) and its phosphate (**$NADP^+$**).

b. The nicotinamide nucleotides play a widespread role as coenzyme to many dehydrogenase enzyme occurring both in the cytosol and within the mitochondria. They are therefore key components of many metabolic pathways affecting **carbohydrate**, **lipid**, and **amino acid** metabolism. Generally, **NAD-linked dehydrogenases** catalyze oxidoreduction reactions in **oxidative** pathways; **NADP-linked dehydrogenases** or reductases are often found in pathways concerned with reductive **synthesis**.

c.In addition to its coenzyme role, NAD is the source of ADP-ribose for the **ADP-ribosylation** of proteins and polyADP-ribosilation of nucleoproteins involved in the **DNA repair mechanism.**

d. 4. Deficiency of niacin results in **pellagra.** The symptoms of pellagra are commonly referred to as **three D:** photosensitive **dermatitis**, **diarrhea** from chronic inflammation of the intestinal mucosa and **dementia**.

a. In maize (corn), niacin is in fact present, but it is in a bound unavailable form, niacin, from which niacin can be release by pretreatment with alkali.

b. Dependence on sorghum is also pellagragenic not because of low tryptophan but because of sorghum's high leucine content. It inhibits a key enzyme in the conversion of tryptophan to NAD^+.

c. Vitamin B_6 deficiency can potentiate a deficiency in niacin because pyridoxal phosphate is involved as a cofactor in the pathways of synthesis of NAD^+ from pryptophan.

d. A number of **genetic** diseases that result in defects of tryptophan metabolism are associated with the development of pellagra dispite an apparently adequate intake of both tryptophan and niacin

(1) Hartnup disease is a rare genetic condition in which there is a defect of the membrane transport mechanism for tryptophan, resulting in large losses due to intestinal malabsorption and failure of the renal resorption mechanism.

(2) In **carcinoid syndrome** there is metastasis of a primary liver tumor of enterochromaffin cells which synthesize serotonin. Overproduction of serotonin may account for as much as 60% of the body's tryptophan metabolism, causing pellagra because of the diversion away from NAD synthesis.

6. Recommended dietary allowance. The daily requirement of niacin for adult is 15—20 mg and for children, around 10—15 mg. Very often, the term niacin equivalents (NE) is used while expressing its RDA. One NE = 1 mg niacin or 60 mg of tryptophan. Instead of mg, the daily requirements are known as niacin equivalents.

LECTURE 31

WATER-SOLUBLE VITAMINS: biotin, folic acid, B_{12}, pantothenic acid

BIOTIN

1. Structure. Biotin is a heterocyclic sulfur containing monocarboxic acid. The structure is formed by fusion of **imidazole** and **thiophene** rings with a **valeric** acid side chain (Fig. 31—1). Biotin is widely distributed in many foods as biocytin (ε-amino-biotinyl lysine), which is released on proteolysis.

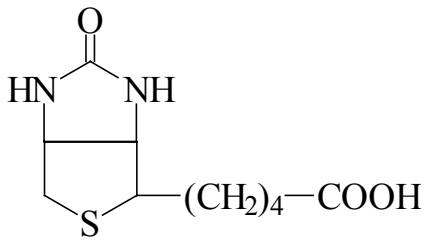


Figure 31—1. Structure of biotin

2. Sources. Biotin is synthesized by **intestinal microorganisms** in sufficient quantities that a dietary source is normally not necessary. Dietary sources include liver and eggs.

3. Metabolism

a. Biotin is not modified but **must be covalently attached to the enzymes** that use it as a prosthetic group.

b. Biotin holocarboxylase synthetase covalently links the free carboxyl group of biotin to a specific lysine residue of the enzyme. Biotin-enzyme reacts with CO_2 in presence ATP (provides energy) to form a **carboxybiotin-enzyme complex**.

c. Biotinidase catalyzes the removal of biotin from enzymes during protein turnover, which allows biotin to be recycled in the body.

4. Functions. Biotin acts as a coenzyme in **carboxylation reactions,** where it is a carrier of carbon dioxide. Four carboxylase enzymes in the body require biotin:

a. Pyruvate carboxylase. The oxaloacetate is produced. It is essential for synthesis of glucose from non-carbohydrate sources (**gluconeogenesis**). Oxaloacetate so formed is also required for the continuous operation of **citric acid cycle**.

b. Acetyl CoA carboxylase (commits acetyl units to fatty acid synthesis by forming malonyl CoA).

c. Propionyl CoA carboxylate (converts propionyl CoA to D-methylmalonyl CoA in the pathway of conversion of propionate to succinate, which can then enter citric acid cycle).

d. β-Methylcrotonyl CoA carboxylase (catabolized leucine and certain isoprenoid compounds).

5. Deficiency

a. Biotin deficiency due to inadequate dietary intake is very rare unless accompanied by other factors, such as the following.

(1) Antibiotics that inhibit the growth of intestinal bacteria eliminate this source of biotin.

(2) Ingestion of unusually large amounts of **avidin,** a protein present in raw egg whites, prevents biotin absorption because it has a very high affinity for biotin.

(3) Among people maintained for many month on parenteral nutrition.

b. Multiple carboxylase deficiency results from a biotin deficiency or a defect in biotin holocarboxylase synthetase, which prevents the attachment of biotin to biotin-dependent enzymes. Symptoms include seborrheic dermatitis, anorexia, nausea, hair loss, erythematous rash and muscular pain.

6. Treatment. In some cases in which the defect in holocarboxylase synthetase reduced its affinity for biotin, multiple carboxylase deficiency can be treated with therapeutic doses of biotin. Multiple carboxylase deficiency caused by biotinase deficiency also can be treated with therapeutic biotin supplementation.

7. Recommended dietary allowance. A daily intake of about 100-300 μg is recommended for adults. In fact, biotin is normally synthesized by intestinal bacteria.

PANTOTHENIC ACID (pantothenate)

1. Structure. Pantothenic acid is formed by combination of pantoic acid and β-alanine (Fig. 31—2).

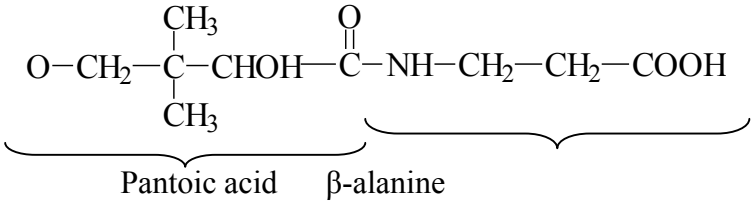


Figure 31—2. Structure of pantothenic acid.

2. Sources. Pantothenate is widely distributed in foods and is synthesized by some intestinal bacteria.

3. Metabolism and function.

a.Pantothenic acid is absorbed readily in the intestines and subsequently phosphorylated by ATP to form 4'-phosphopantothenate. Addition of cysteine and removal of its carboxyl group results in the net addition of thioethanolamine, generating **4'-phosphopantetheine**, the prosthetic group of **coenzyme A** and **acyl carrier protein (ACP).**

b. The thiol group acts as a **carrier of acyl radicals** in both CoA and ACP. This occurs with CoA in reaction of the **citric acid cycle, fatty acid synthesis and oxidation**, **acetylation reactions** (e.g. of drugs), and **cholesterol synthesis**. ACP participates in reactions concerned with **fatty acid synthesis**. Coenzyme A may be regarded as a **coenzyme of metabolic integration**, since acetyl CoA is a central molecule for a wide variety of biochemical reactions.

4. Deficiency. The vitamin is widely distributed in all foodstuffs, and deficiency has not been unequivocally reported in human being excepted in specific depletion studies.

5. Recommended dietary allowance. The requirement of pantothenic acid for humans is not clearly known. A daily intake of about 5—10 mg for adults.

FOLIC ACID (folate)

It is important for one carbon metabolism and is required for the synthesis of certain amino acids, purines and the pyrimidine-thymine.

1. Structure. Folic acid consists of the base **pteridine** attached to one molecule each of ***p*-aminobenzoic acid (PABA)** and **glutamic acid** (Fig.31—3).

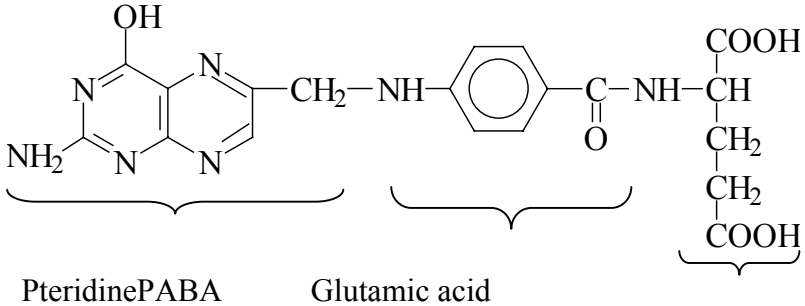


Figure 31—3. Structure of folic acid.

2. Sources include synthesis by intestinal bacteria, as well as liver and green vegetables in the diet. Folate is easily destroyed by cooking.

3. Metabolism. Folate in food is primarily present in a **polyglutamate** form. The extra glutamate residues are removed by an intestinal **conjugase** before absorption, primarily in the small intestine. The active form of folic acid is **tetrahydrofolate** (THF, FH_4). It is synthesized from folic acid by the enzyme **dihydrofolate reductase** which uses 2 moles of NADPH as donor of reducing equivalent. The hydrogen atoms are present at position 5, 6, 7 and 8 of THF.

4. Functions

a. Folate coenzymes (e.g., tetrahydrofolate) act as carriers of one-carbon fragments at different levels of oxidation: **methyl (–CH_3)**, **methylene (=CH_2)**, **methenyl (≡CH)**, **formyl (–CHO)** and **formimino (–CH=NH)**. The one carbon units bind with THF at position N^5 or N^{10} or on both N^5 and N^{10} of pteroyl structure. The attachment of formyl at position 5 of THF gives N^5-formyl tetrahydrofolate which is commonly known as **folinic acid** or **citrovorum factor.**

The major point of entry for one-carbon fragments into substituted folate is **methylene** tetrahydrofolate, which is formed by the reaction of glycine, serine, and choline with tetrahydrofolate.

b. Folate is necessary for proper **purine** (carbon 2, 8) nucleotide and **deoxythymidylate synthesis**. N^5,N^{10}-Methylen-tetrahydrofolate provides the methyl group in the formation of thymidilate, a necessary precursor of DNA synthesis and erythrocyte formation. **Aminopterin and amethopterin** (also called as **methotrexate**) an anolog of 10-methyl-tetrahydrofolate, inhibits **dihydrofolate reductase** and has been eploited as an **anticancer drug**. The dihydrofolate reductases of some bacteris (gram-negative) and parasites differ from the human enzyme; inhibitors of these enzymes can be used as **antibacterial drugs**, e.g. **trimethoprim**, and **antimalarial drugs**, e.g. **pyrimethamine**.

c. Glycine, serine, ethanolamine are produced in one carbon metabolism.

d. N-Formylmethionine, the initiator of protein biosynthesis is formed.

e. Tetrahydrofolate is mostly trapped as N^5-methyl THF in which form it is present in the circulation. Vitamin B_{12} is needed for the conversion of N^5-methyl THF to THF, in a reaction wherein homocystein is converted to methionine. This step is essential for the liberation of free THF and for its repeated use in one carbon metabolism. In B_{12} deficiency, conversion of N^5-methyl THF to THF is blocked.

5. Deficiency. In folic acid deficiency, decreased production of purine and dTMP is observed which impairs DNA synthesis. Due to a block in DNA synthesis, the maturation of erythrocytes is slowed down leading to macrocytic red blood cells. The rapidly dividing cells of bone marrow are seriously affected. The **macrocytic anemia** associated with megaloblastic changes in bone marrow is a characteristic feature of folate deficiency.

Supplementation with 400 μg of folic acid per day during the periconceptual period can markedly reduce the incidence of neural tube defects such as spina bifida. It is also important to maintain adequate folic acid supplementation in the later stages of pregnancy and beyond, when many women a low plane of nutrition suffer from megaloblastic change.

Elevated blood homocysteine is an associated risk factor for **atherosclerosis**, **thrombosis**, and **hypertension**.

6. Recommended dietary allowance. The daily requirement of folic acid is around 100 μg.

VITAMIN B_{12} (cobalamin)

Vitamin B_{12} is also known as **anti-pernicious anemia vitamin.**

1. Structure. Cobalamin is composed in part of a **corrin ring** with an atom of **cobalt** present at its active site (Fig. 31—4).

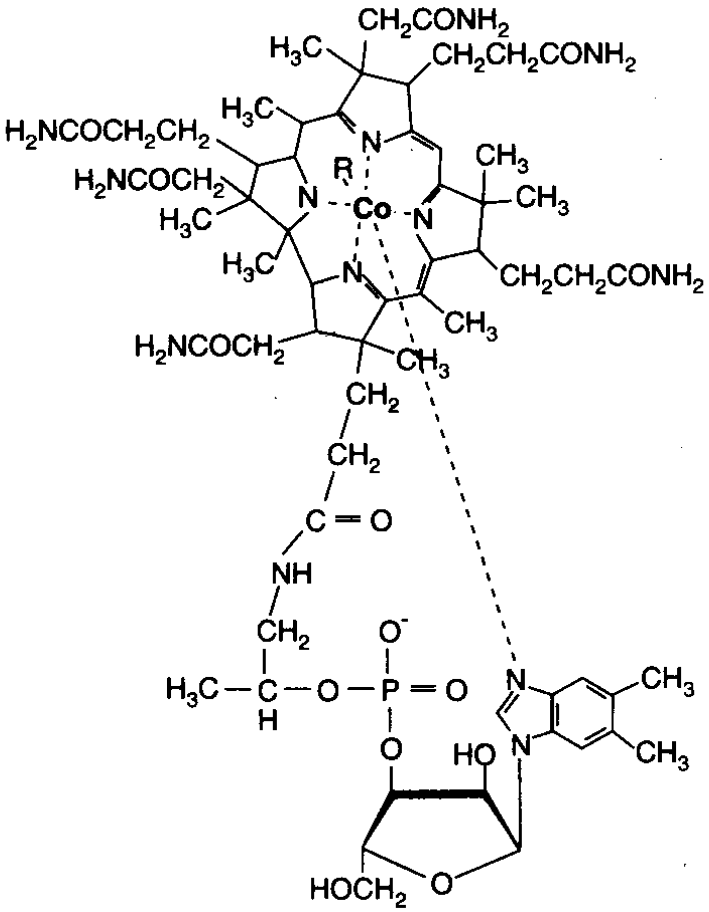


Figure 31—4. Vitamin B_{12}. R=CN^- in cyanocobalamin; R= OH^- in hydroxycobalamin; R=5'-deoxyadenosyl in 5'-deoxyadenosylcobalamin; R=H_2O in aquocobalamin; R= CH_3 in methylcobalamin.

In commercial preparations, cyanide serves as a ligand to cobalt. In the liver this must be changed to generate the two active cofactor forms of the vitamin, **methylcobalamin and 5-deoxyadenosylcobalamin.**

2. Sources. Microorganisms are the sole source of cobalamin in nature. Plants are devoid of vitamin B_{12}, but it is present in most animal and dairy products. The small amounts of the vitamin formed by bacteria on the surface of fruits may be adequate to meet requirments. Liver is a good source of the vitamin, as is yeast.

3. Metabolism

a. The vitamin B_{12} is present in the diet in a bound form to proteins. B_{12} is liberated by the enzymes (acid hydrolases) in the stomach. The dietary source of B_{12} is known as **extrinsic factor of Castle**. Vitamin B_{12} is absorbed in the ileum as a complex with **intrinsic factor,** a glycoprotein secreted by parietal cells of the gastric mucosa. Gastric acid and pepsin release the vitamin from protein binding in food and make it available to bind **cobalophilin**, a binding protein secreted in the saliva. In the duodenum, cobalophilin is hydrolyzed, releasing the vitamin for binding to intrinsic factor. **Pancreatic insufficiency** can therefore be a factor in the development of vitamin B_{12} deficiency, resulting in the excretion of cobalophilin-bound vitamin B_{12}. Vitamin B_{12} is absorbed from the distal third of the ileum via receptors that bind the intrinsic factor-vitamin B_{12} complex but not free intrinsic factor or free vitamin.

b. The vitamin is transported in serum bound to proteins namely **transcobalamins** (TC—I, TC—II) and converted to **methylcobalamin and 5-deoxyadenosylcobalamin** in the liver, bone marrow cells, and reticulocytes. It is believed that TC-I acts as a **repository of B_{12},** while TC-II mediates the tissue **uptake of B_{12}**. After transport in the blood, free cobalamin is released into the cytosol of cells as hydroxycobalamin. It is either converted in the **cytosol** to methycobalamin or it enters **mitochondria** for conversion to 5-deoxyadenosylcobalamin. It is believed that liver can store about 4—5 mg, an amount sufficient to meet the body requirement of B_{12} for 4—6 — years.

4. Functions

a. Only two human enzymes require cobalamin (Fig. 31—5).

(1)Methylmalonyl coenzyme A (methylmalonyl CoA) mutase requires 5-deoxyadenosylcobalamin as its cofactor. Methylmalonyl CoA is formed as an intermediate in the catabolism of valine and by the carboxylation of propionyl CoA arising in the catabolism of isoleucine, cholesterol, and, rarely, fatty acids with an add number of carbon atoms — or directly from propionate, a major product of microbial fermentation in ruminants. It undergoes vitamin B_{12}-dependent rearrangement to succinyl-CoA.

(2)Homocysteine methyltransferase, which catalyzes the methylation of homocysteine to methionine, **requires methylcobalamin** as its cofactor. The cobalamine then removes the methyl group from N^5-methyltetrahydrofolate to form tetrahydrofolate. The metabolic benefits of this reaction are that stores of methinone are maintained and tetrahydrofolate is made available to participate in purine, pyrimidine, and nucleic acid synthesis.

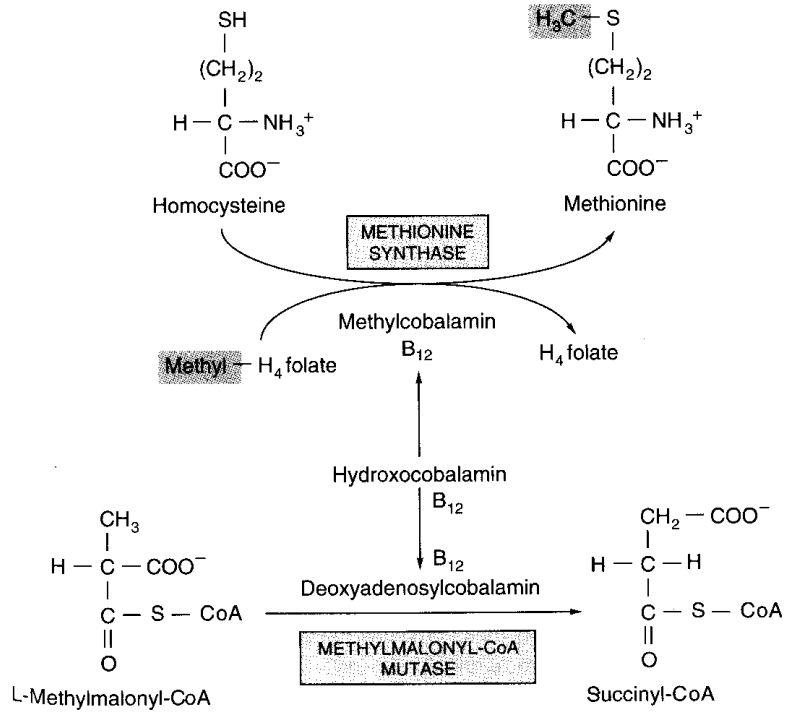


Figure 31—5. The two important reactions catalyzed by vitamin B_{12} (by Murray R.K. et al., 2003).

b. Vitamin B_{12} function is related to folate metabolism; lack of methylcobalamin leads to a deficiency in the folate coenzyme pool.

5. Deficiency

a. The enterohepatic circulation of vitamin B_{12} provides almost total conservation of the vitamin, and a deficiency would take several years to develop if the vitamin were removed from the diet. However, damage to the stomach or the ileum causes deficiency to occur more rapidly.

b. Pernicious anemia is the consequence of vitamin B_{12} deficiency.

(1) It is due to an absence of intrinsic factor.

(2) Consequences include macrocytic anemia, megaloblastosis of bone marrow, degeneration of axis cylinders of spinal cord neurons, lesions of mucous surfaces, glossitis, and methylmalonic aciduria. Anemia results from impaired DNA synthsis, preventing cell division and formation of the nucleus of new erythrocytes with consequent accumulation in the bone marrow of megaloblasts. The impaired purine and pyrimidine synthesis resulting from tetrahydrofolate deficiency is a consequence of folate being trapped as methyltetrahydrofolate (known as the **"folate trap" or methyl trap**).

c. A long-term, strict vegetarian diet may also lead to a deficiency of vitamin B_{12}.

6. Recommended dietary allowance. A daily intake of about 3 μg of vitamin B_{12} is adequate to meet the adult requirements.

VITAMINE LIKE COMPOUNDS

Besides the vitamins describe above, there are many other compounds present in food as accessory factors. Earlier workers have described these factors sometime or the other, as essential to higher animals. However, their essential nature and requirement in humans has not been established. Although not essential in the diet, they perform many important functions in the body.

CHOLINE

Choline is **trimethylhydroxy ethylammonium hydroxide**.

Best and Huntsman (1934) found that choline deficiency in rats produced fatty liver and established its nutritional importance. However, in a strict sense, choline is not a vitamin, since, since it is adequately synthesized in the body (from serine). It is also available from many dietary sources (e.g. milk, eggs, liver, cereals etc).

Biochemical function

1. Choline, as a **component of phospholipids** (lecithins) is involved in membrane structure and lipid transport.

2. Choline prevents the accumulation of fat in liver (as **lipotropic factor**). It promotes the synthesis of phospholipids and lipoproteins and the disposal of **triacylglycerols** from liver.

3. Due to the presence of tree methyl groups (one carbon fragments), choline is actively involved in **one carbon metabolism**.

4. Choline is a precursor for the synthesis of acetylcholine which is required for transmission of nerve impulse.

INOSITOL

Inositol is **hexahydroxy-cyclohexane.** It is also known as myo-inositol or meso-inositol.

Biochemical functions

1. Inositol is required for the synthesis of **phosphatidylinositol** which is a constituent of cell membrane.

2. Its acts as **lipotropic factor** (along with choline) and prevents the accumulation of fat in liver.

3. For some hormones, inositol acts as a **second messenger** at the membrane level for the release of Ca^{+} ions.

4. Inositol concentration in the heart muscle is high, the significance of which however, is not known.

LIPOIC ACID

Lipoic acid (thioctic acid) is a sulfur containing fatty acid (6,8-dithiooctanoic acid). It exists in an oxidized and reduced form. Lipoic acid is fat as well as water soluble.

Biochemical functions

Lipoic acid is involved in the decarboxylation reactions along with other vitamins (thiamine, niacin, ricoflavin and pantothenic acid). The conversion of pyruvate to acetyl CoA and α-ketoglutarate to succinyl CoA require lipoic acid.

PARA AMINOBENZOIC ACID

Para aminobenzoic acid (PABA) is a structural constituent of folic acid. PABA may be regarded as a **vitamin in another vitamin** (folic acid).

The deficiency of PABA was first found to be associated with failure of lactation and graying of black hair in rats. The specific functions of PABA in human, except that it is a component of folic acid, have not been identified.

PABA is synthesized by the bacteria and is essential for their growth. The sulfa drug sulfanilamide (p-amino benzene sulfanilamide) is a structural analogue of PABA. Sulfanilamide competes with PABA and acts as a bacteriostatic agent. Ingestion of large doses of PABA will compete with the action of drugs and therefore should be avoided during sulfanilamide therapy.

BIOFLAVONOIDS

Szent-Gyorgy and his associates (1936) observed that flavonoids, isolated from lemon peel (known as citrin) were responsible for maintenance of normal capillary permeability. The term vitamin P (P fro permeability) was used to this group of substances. However, they are commonly known as bioflavonoid.

Bioflavonoids act as antioxidants and protect ascorbic acid from being destroyed. It is suggested that this antioxidant property may be responsible for maintenance of capillary permeability. Bioflavonoids have been used to correct the vascular abnormality in human.

Bioflavonoids are found in peel and pulp of citrus fruits, tobacco leaves and many vegetables. The requirement of these compounds in humans has not been established.

ANTIVITAMINS

Antivitamins are antagonist to (oppose and block) the action of vitamins. They usually have structural similarities with vitamins. Administration of antivitamins causes vitamin deficiencies.

LECTURE 32

METABOLISM OF IRON AND COPPER. BLOOD PROTEINS. HEMOGLOBIN

IRON

The total content of iron in an adult body is **3—5 g**. About **70%** of this occurs in the erythrocytes of blood as a constituent of **hemoglobin.** At least **5%** of body iron is present in **myoglobin of muscle**. Heme is the most predominant iron-containing substance. It is a constituent of several proteins/enzymes **(hemoproteins) —** hemoglobin, myoglobin, cytochromes, xanthine oxidase, catalase, peroxidase. Certain other proteins contain non-heme iron e.g. transferring, ferritin, hemosiderin.

Biochemical functions

1. Iron mainly exerts its functions through the compounds in which it is present. Hemoglobin and myoglobin are required for the transport of O_2 and CO_2**.**

2. Cytochromes and certain non-heme proteins are necessary for electron transport chain and oxidative phosphorylation.

3. Peroxidase, the lysosomal enzyme, is required for phagocytosis and killing of bacteria by neutrophils.

4. Iron is associated with effective immunocompetence of the body.

Dietary requirements. Adult man — 10 mg/day, women — 18 mg/day, pregnant and lactating woman — 40 mg/day.

Sources. Rich sources — organ meats (liver, heart, kidney). Good sources — leafy vegetables, pulses, cereals, fish, apples, dried fruits, molasses. Poor sources — milk, wheat, polished rice.

Absorption, transport and storage. Iron is mainly absorbed in the stomach and duodenum. In normal people, about 10% of dietary iron is usually absorbed. However, in iron deficient (anemic) individuals and growing children, a much higher proportion of dietary iron is absorbed to meet the increased body demands.

Iron is mostly found in the foods in ferric form (Fe^{3+}), bound to proteins or organic acids. In the acid medium provided by gastric HCI, the Fe^{3+} is released from foods. Reducing substances such as ascorbic acid (vitamin C) and cysteine convert ferric iron (Fe^{3+}) to ferrous form (Fe^{2+}). Iron in the **ferrous form is soluble and readily absorbed.**

Factors affecting Fe absorption

1. Acidity, ascorbic acid and cysteine promote iron absorption.

2. Small peptides and amino acids favour iron uptake.

3. Phytate (found in cereals) and oxalate (found in leafy vegetables) interfere with Fe absorption.

4. A diet with high phosphate content decreases Fe absorption while low phosphate promotes.

5. Tea and eggs decrease iron absorption to a limited extent.

6. Iron absorption is diminished in copper deficiency.

7. Impaired absorption of iron is observed malabsorption syndromes such as steatorrhea.

8. Administration of alkali decreases iron absorption.

9. In patients with partial or total surgical removal of stomach and/or intestine, iron absorption is severely impaired.

Iron in the mucosal cells. The iron (Fe^{2+}) entering the mucosal cells by absorption is oxidized to ferric form (Fe^{3+}) by the enzyme ferroxidase. Fe^{3+} then combines with **apoferritin** to form **ferritin** which is the **temporary storage form of iron**. From the mucosal cells, iron may enter the blood stream (which mainly depends on the body needs) or lost when the cells are desquamated.

Transport of Fe in the plasma. The iron liberated from the ferritin of mucosal cells enters the plasma in **ferrous state**. Here, it is oxidized to **ferric form** by a copper-containing protein, **ceruloplasmin** which possesses ferroxidase activity. Another cuproprotein **ferroxidase II** also helps for the conversion of Fe^{2+} to Fe^{3+}. Ferric iron then binds with a specific iron-binding protein, namely **transferrin** or **siderophilin** (a glycoprotein with M.W. 90,000). Each transferrin molecule can bind with **two atoms** of ferric iron (Fe^{3+}). The plasma transferrin can bind with 400 μg of iron/dl plasma. This is known as **total iron binding capacity (TIBC) of plasma.**

Storage of iron. Iron is stored in **liver, spleen and bone marrow** in the form of **ferritin.** In the mucosal cells, ferritin is the temporary storage form of iron. A molecule of apoferritin (M.W. 500,000) can combine with 4,000 atoms of iron. The maximum iron content of ferritin on weight basis is around 25%. **Hemosiderin** is another iron storage protein which can hold about 35% of iron by weight. Hemosiderin accumulates in the body (spleen, liver) when the supply of iron is in excess of body demands.

Iron is a one-way substance

Iron metabolism is unique as it operates in a **closed system**. It is very efficiently utilized and reutilized by the body. Further, iron losses from the body are minimal (<1 mg/day) which may occur through **bile, sweat, hair loss** etc. Iron is **not excreted into urine**. Thus, iron differs from the vitamins or other organic and inorganic substances which are either inactivated or excreted during the course of metabolic function. Hence, iron is appropriately regarded as a one-way substance.

Thus the periodical blood loss in menstruating women increases its requirements. Increased iron demands are also observed in pregnancy, lactation, and in growing children.

Overview of iron metabolism

A general overview of iron metabolism is depicted in Fig. 32—2. It shows the distribution of iron in the body and its efficient reutilization. It may be noted that about 1-2 mg of iron is absorbed per day to replace the loss.

Disease states

1. Iron deficiency anemia. Several factors may contribute to iron deficiency anemia. These include inadequate intake or defective absorption of iron, chronic blood loss, repeated pregnancies and hookworm infections. Iron deficiency anemia mostly occurs in growing children, adolescent girls, pregnant and lactating women. It is characterized by **microcytic hypochromic anemia** with reduced blood hemoglobin levels (<12 g/dl). The other manifestations include apathy (dull and inactive), sluggish metabolic activities, retarded growth and loss of appetite.

2. Hemosiderosis: This is a less common disorder and is due to **excessive** iron in the body. It is commonly observed in subjects receiving repeated blood

transfusions over the years, e.g. patients of hemolytic anemia, hemophilia. As already stated, iron is a one-way compound, once it enters the body, it cannot escape. Excessive iron is deposited as ferritin and hemosiderin. Hemosiderin deposits in cells can be observed under the microscope with suitable staining while ferritin cannot be identified.

3. Hemochromatosis. This is a rare disease in which iron is directly deposited in the tissues (liver, spleen, pancreas and skin). Hemosiderosis is sometimes accompanied by hemochromatosis. Bronzed-pigmentation of the skin, cirrhosis of liver, pancreatic fibrosis are the manifestations of this disorder. Hemochromatosis causes a condition known as **bronze diabetes.**

COPPER

The body contains about **100 mg copper** distributed in different organs. It is involved in several important functions.

Biochemical functions

1. Copper is an essential constituent of several enzymes. These include cytochrome oxidase, catalase, tyrosinase, superoxide dismutase, monoamine oxidase, ascorbic acid oxidase. Due to its presence in a wide variety of enzymes, copper is involved in many metabolic reactions.

2. Copper is necessary for the synthesis of hemoglobin (Cu is a constituent of ALA synthase).

3. Lysyl oxidase (a copper-containing enzyme) is required for the conversion of certain lysine residues of collagen and elastin to allysine which are necessary for cross-linking these structural proteins.

4. Ceruloplasmin serves as ferroxidase and is involved in the conversion of iron from Fe^{2+} to Fe^{3+} in which form iron (transferrin) is transported in plasma.

5. Copper is necessary for the synthesis of melanin and phospholipids.

6. Development of bone and nervous system (myelin) requires Cu.

7. Certain copper-containing non-enzymatic proteins have been identified, although their functions are not clearly known. These include hepatocuprein (storage form in liver), cerebrocuprein (in brain) and hemocuprein (in RBC).

8. Hemocyanin, a copper protein complex in invertebrates, functions like hemoglobin for O_2 transport.

Dietary requirements. Adults — 2—3 mg/day, infants and children — 0.5—2 mg/day

Sources. Liver, kidney, meat, egg yolk, cereals, nuts and green leafy vegetables. Milk is a poor source.

Absorption. About 10% of dietary copper is absorbed, mainly in the duodenum. **Metallothionein** is a transport protein that facilitates copper absorption. Phytate, zinc and molybdenum decrease copper uptake.

Plasma copper. The copper concentration of plasma is about 100—200 μg/dl. Most of this (95%) is tightly bound to **ceruloplasmin** while a small fraction (5%) is loosely held to albumin. Normal concentration of serum **ceruloplasmin**

is 25—50 mg/dl. It contains about 0.34% copper (6—8 atoms of Cu per molecule, half in Cu^{2+} state and the other half in Cu^{+} state). Ceruloplasmin is not a transport protein since this copper is not readily exchangeable with other molecules. The RBC contains erythrocuprein (superoxide dismutase).

Disease states

1. Copper deficiency. Severe deficiency of copper causes demineralization of bones, demyelination of neural tissue, anemia, fragility of arteries, myocardial fibrosis, hypopigmentation of skin, greying of hair.

2. Menke's disease. This disorder is due to a defect in the intestinal absorption of copper. It is possible that copper may be trapped by metallothionein in the intestinal cells. The symptoms of Menke's disease include decreased copper in plasma and urine, anemia and depigmentation of hair.

3. Wilson's disease (hepatolenticular degeneration). It is a rare disorder of abnormal copper metabolism and is characterized by the following manifestations. Copper is deposited in abnormal amounts in liver and lenticular nucleus of brain. This may lead to hepatic cirrhosis and brain necrosis. Low levels of copper and ceruloplasmin in plasma with increased excretion of copper in urine. Copper deposition in kidney causes renal damage. This leads to increased excretion of amino acids, glucose, peptides and hemoglobin in urine. Intestinal absorption of copper is very high, about 4—6 times higher than normal.

Treatment. Administration of pencillamine, a naturally occurring copper chelating agent, is used for the treatment of Wilson's disease.

BLOOD

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** separated 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 in an adult is around 4.5 to 5 liters. Blood performs several diversified functions. They 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 protein is about **65—85 g/L**. **Electrophoresis** is the most commonly employed analytical technique for the separation of plasma (serum) protein. Paper or agar gel electrophoresis with vernal buffer (pH — 8.6) separates plasma proteins into 5 distinct bands namely **albumin**, **α_1, α_2, β and γ globulins.**

ALBUMIN is the major constituent (55%—60%) of the total plasma proteins with a concentration of 35—50 g/L. Human albumin has a molecular weight of 69,000 and consists of a single polypeptide chain of 585 amino acids with 17 disulfide bonds.

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 disease). Liver produced about 12 g albumin per day which represents 25% of the total hepatic protein synthesis. Albumin has a half-life of 20 days.

Function. Albumin is a multifunctional protein.

1. Transport. Because of albumin's ability to bind to many diverse molecules, it serves as a **low-specificity transport protein**. These include **metal ions** (calcium and copper); **free fatty acids; bilirubin** is bound very tightly by albumin (this protects from the toxic side effects of unconjugated bilirubin); **bile acids; hormones** (thyroid hormones and the steroid hormones).

2. Maintenance of osmotic pressure. Due to its high concentration and low molecular weight, albumin contributes to 75—80%of the total plasma osmotic pressure. 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 of protein-energy malnutrition, is attributed to a drastic reduction in plasma albumin level.

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 system.

Clinical aspects

1. Albumin binds to a wide array of different drugs and strongly affects the turnover or pharmacokinetics of these drugs.

2. The antimicrobial sulfonamides, by competitive binding, cause the release of unconjugated bilirubin from albumin. If given to infants, sulfonamides may lead to kernicterus.

GLOBULINS

Globulins constitute several proteins that are separated into four distinct bands (α_1, α_2, β, γ) on electrophoresis. The total globulin concentration is **25—35 g/L**. Globulins, in general are bigger in size than albumin. They perform a variety of functions. The most important globulins are:

1. α_1-Antitrypsin is a glycoprotein with 934 amino acids and a molecular weight of 54,000. It is a major constituent of **α_1-globulin** fraction of plasma proteins. α_1-Antitrypsin is a serine **protease inhibitor**. It **combines** with trypsin, elastase and other protease enzymes and **inhibits** their activity. α_1-Antitrypsin

deficiency causes liver damage (hepatitis) followed by accumulation of collagen resulting in fibrosis.

2. 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 **nephritic syndrome**.

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

4. Ceruloplasmin is a blue coloured, copper-containing α_2-globulin (See above).

5. Transferrin is a glycoprotein with a molecular weight of 75,000. It is associated with β-globulin fraction.

6. Immunoglobulins. Immunoglobulins, a specialized group of proteins are mostly associated with **γ-globulin** fraction of plasma protein. Immunoglobulins protect the body against the invasion of foreign substances- a virus, a bacterium or a protein.

7. Complement system. The complement system is composed of about 20 plasma proteins that "complement" the function of antibodies in defending the body from the invading antigens.

8. Proteins of blood clotting. The proteins participate in **hemostasis**. The term hemostasis is applied to the sequence of physiological responses to stop bleeding (loss of blood after an injury).

Albumin/globulin (A/G) ratio. The normal A/G ration is 1.2 to 1.5:1. The A/G is lowered either due to decrease in albumin or increase in globulin, 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.

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 function concerned with respiration:

1. Delivery of O_2 from the lungs to the tissues

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

Structure of hemoglobin

Hemoglobin (M.W. 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**.

1. 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 dimmers — $(\alpha\beta)_1$ 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 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.

2. 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.

The iron atom is in ferrous (Fe^{2+}) state in the heme of functional hemoglobin. It can form six coordinated bonds. Iron is held at the centre of the heme by the four nitrogens of porphyrin ring. The other two bonds are formed on either side of the planar porphyrin ring. On one side, iron binds with the amino acid histidine of the globin. On the other side, the coordinated position of Fe^{2+} is avaible to bind to oxygen. This happens when Hb is converted to oxyhemoglobin.

Biosynthesis of heme

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 stage.

1. Formation of δ-aminolevulinate. **Glycine**, a non-essential amino acid and **succinylCoA**, an intermediate in the citric acid cycle, are the starting materials for porphyrin synthesis. Glycine combines with succinylCoA to form **δ-aminolevulinate** (ALA) (Fig. 32—1). 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 (in the cytosol).This reaction is catalyzed by a Zn-containing enzyme **ALA dehydratase**. It is sensitive to inhibition by heavy metals such as lead.

3. Synthesis of uroporphyrinogen. The interaction of two enzymes results in condensation of porphobilinogen followed by ring closure and isomerization to produce **uroporphyrinogen**.

4. Uroporphyrinogen is converted to form **protoporphyrin IX**.

5. Synthesis of heme from protoporphyrin IX. The incorporation of ferrous iron (Fe^{2+}) into protoporphyrin IX is catalyzed by the enzyme 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.

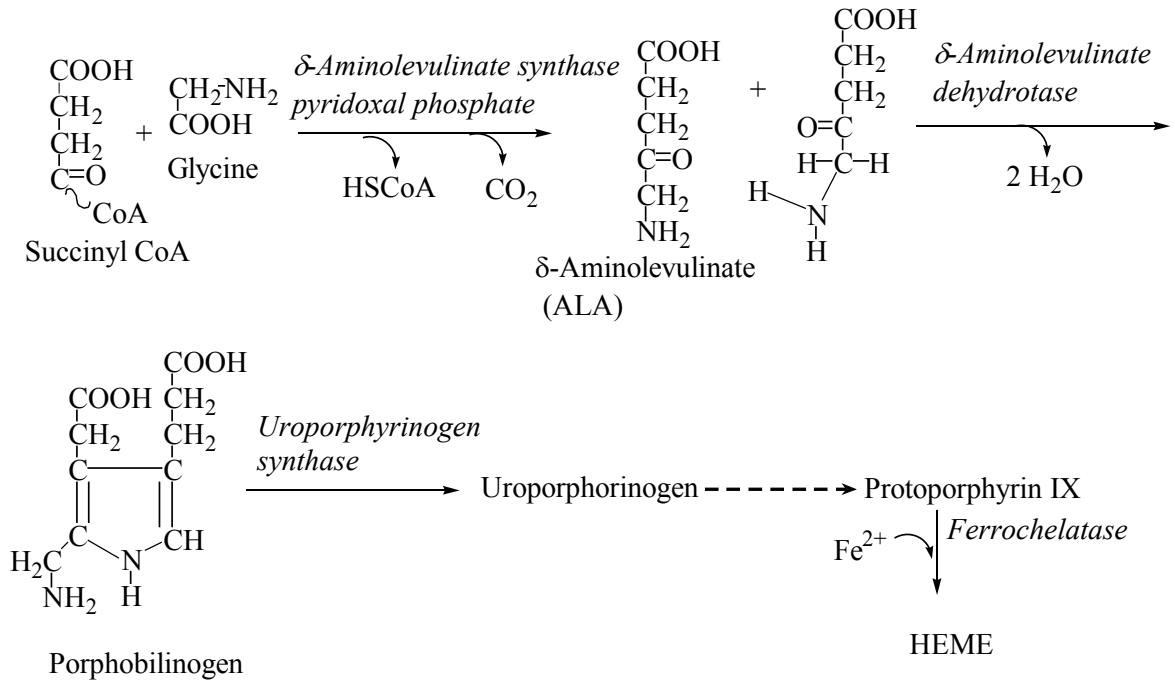


Figure 32—1. Biosynthesis of heme

Regulation of heme synthesis

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

a. Feedback inhibition

b. Repression of ALA synthase.

c. Inhibition of transport of ALA synthase from cytosol to mitochondria.

2. Effect of drug on ALA synthase activity. The activity of ALA synthase is markedly increased by the administration of a large number of drug e.g. **phenobarbital**, **insecticides**, **carcinogens** etc. 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 of ALA synthase to meet the cellular demands.

3. Regulation in the erythroid cells. Uroporphyrinogen synthase and ferrochelatase mostly regulate heme formation in these cells. Further, the cellular uptake of iron also influences heme synthesis. Ii is observed that **heme stimulates globin synthesis**. This ensures that heme and globin synthesis occur in the **right proportion** to finally form hemoglobin.

Hemoglobin derivatives

Hemoglobin (specifically heme) combines with different ligands and forms hemoglobin derivatives. The normal blood contains oxyHb and deoxyHb.

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

2. Carboxyhemoglobin (COHb). Carbon monoxide is a toxic compound 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, breathlessness, vomiting and irritability. Administration of O_2 through oxygen masks will help to reverse the manifestation of CO toxicity.

Structural diversity of hemoglobin

Several different forms of hemoglobin can be found in adult humans and during early human development. The early developmental forms are particularly suited for the oxygen transport needs at the stage of development in which they are expressed. The various hemoglobin forms differ in the primary structure of the subunits that form the hemoglobin tetramer.

Genetic variation. The different subunits of various hemoglobins are products of different globin genes.

1. Hemoglobin A_1 is the major (98%) form found in human adults.

2. Hemoglobin A_2 is a minor (2%) form found in human adults. It is a tetramer of two α subunits and two delta (δ) subunits ($\alpha_2\delta_2$).

3. Fetal hemoglobins. The first hemoglobin formed during embryogenesis is a tetramer of two zeta (£) subunits, which are evolutionary similar to α subunits, and two epsilon (ε) subunits ($£_2\varepsilon_2$). Through the first 6 months of development, the £ subunits are replaced by α subunits, and the ε subunits are replaced by the γ subunits forming hemoglobin F ($\alpha_2\gamma_2$). Through later embryonic development and just after birth, the γ- subunits are replaced by β subunits. **Fetal hemoglobin** differs from adult hemoglobin by having a **much higher affinity for oxygen.** The fetus must pick up oxygen at the lower PO_2 of the placenta, so its hemoglobin must be different from the maternal hemoglobin that releases it in the placenta.

Clinical aspects of hemoglobin

1. Glycosylation of HbA_1 and diabetes mellitus.

a. Hb A_1 reacts with glucose to form a derivative known as **hemoglobin A_{1c}** (HbA_{1c}).

b. Normally the concentration of HbA_{1c} in blood is **very low**, but in patients with diabetes mellitus, in whom blood sugar levels may be high, the concentration of HbA_{1c} may rich 12% or more of the total hemoglobin.

c. Because the average life of an red blood cells is 120 days, the amount of HbA_{1c} becomes a good indicator of blood glucose levels over a 2—4-month period. For example, determination of the amount of HbA_{1c} can tell a physician if patients

have maintained their blood glucose levels over the preceding months or have or have lowered their glucose levels just before their clinical examination.

2. Hemoglobinopathies are genetic diseases in which the globin subunits of hemoglobin are mutated. Hundreds of hemoglobinopathies have been described. In some there are no symptoms or impairment, whereas in other there may be severe impairment. Major hemoglobinopathies include the following.

Sickle cell anemia

1. Sickle cell anemia, or **hemoglobin S (HbS)** was one of the first hemoglobinophaties to be described. It occurs when **valine** replaces **glutamic acid** in the six position of the **β chains**. The α chains are normal.

2. 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 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 black (almost 1 in 10 are affected). The individuals of sickle-cell trait lead a **normal life** and do not usually show clinical symptoms.

3. Sickle-cell anemia is characterized by the following **abnormalities**. The sickled erythrocytes are fragile and their continuous breakdown leads to **lifelong anemia**. 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**. Hemolysis and tissue damage are accompanied by **increased susceptibility** to **infection and disease**.

4. Sickle-cell trait provides resistance to **malaria** which is a major cause of death in tropical areas. 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.

Hemoglobin C disease (HbC) is characterized by substitution of glutamate by lysine in the sixth position of β-chain. HbC disease occurs only in blacks. Both homozygous and heterozygous individuals of HbC are known. This disease is characterized by mild hemolytic anemia.

Hemoglobin M (Hb M). A number of rare hemoglobinopathies lead to a high percentage of **methemoglobins** in red blood cells. These usually arise due to mutations in either the proximal or distal histidines of either α or β chains, which bond with the iron in the heme group. These mutations stabilize the iron in the ferric form (Fe^{3+}), which cannot bind oxygen. Only patients who are heterozygous for these mutations have been found. Presumably, homozygosity is lethal.

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.

FEATURES OF ERYTHROCYTES METABOLISM

Two main processes are occurred in the erythrocytes: glycolysis and pentose phosphate pathway.

Glycolysis and erythrocyte metabolism

1. Mature erythrocytes contain no mitochondria, so they are totally dependent on glycolysis for ATP production.

2. ATP is required for the activity of the sodium- and potassium-stimulated ATPase-ion transport system, which is necessary to maintain the proper biconcave shape of the erythrocyte membrane.

3. Disorder of glycolysis typically present as disorders of erythrocyte metabolism.

4. 2,3-Bisphosphoglycerate (2,3-BPG) is the most abundant organic phosphate in the erythrocytes. Its molar concentration is approximately equivalent to that of hemoglobin. 2,3-Bisphosphglycerate is produced in the erythrocytes from intermediate (1,2-bisphosphoglycerate) of glycolysis. 2,3-BPG regulated the binding of O_2 to hemoglobin. It specially binds to deoxyhemoglobin (and not to oxyhemoglobin) and decreases the O_2 affinity to Hb.

a. 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. However, within a couple of days (24—48 hrs.) the erythrocytes restore the normal 2,3-BPG levels and the tissues O_2 demands are adequately met. Addition of **inosine** (hypoxantine-ribose) to the stored blood prevents the decrease of 2,3-BPG. The ribose moiety of inosine gets phosphorylated and enters the pentose phosphate pathway and finally gets converted to 2,3-BPG.

b. 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.

Role of pentose phosphate pathway in erythrocytes. NADPH produced in erythrocytes has special functions to perform. It maintains the concentration of reduced glutathione which is essentially required to preserve the **integrity of the red blood cell membrane.** NADPH is also necessary to keep the ferrous iron (Fe^{2+}) of hemoglobin in the reduced state so that accumulation of methemoglobin (Fe^{3+}) is prevented.

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.

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 catalyzed by a NADPH dependent soluble enzyme**, biliverdin reductase. 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 in liver. Bilirubin is **lipophilic** and therefore insoluble in aqueous solution. Bilirubin is transported in the plasma in a bound (noncovalently) 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 per 100 ml of plasma. The rest of the bilirubin binds loosely (at the low affinity site) 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 nervous**.

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, catalyzed by **bilirubin glucuronyltransferase** (of smooth endoplasmic reticulum) results in the formation of a water-soluble bilirubin diglucuronide. When bilirubin is inexcess, 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 hydrolyzed in the intestine by specific bacterial enzymes namely **β-glucuronidases** to liberate bilirubin. The latter is then converted to **urobilinogen** (colourless compounds), a small part of which may be reabsorbed into the circulation. Urobilinogen can be converted to **urobilin** (an yellow colours compounds) 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 0.8 mg/dl. Of this, about 0.2—0.6 mg/dl is **uncojugated** while 0 to 0.2 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 cause 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.

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 is 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 formation of urobilinogen and stercobilinogen. Hemolytic jaundice is characterized by

a. Elevation in the serum unconjugated bilirubin.

b. Increased excretion of urobilinogen in urine.

c. Dark brown colour of feces due to high content of stercobilinogen.

2. Hepatic (hepatocellular) jaundice. This type of jaundice is cause 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 the liver cells. Hepatic jaundice is characterized by

a. Increased levels of conjugated and unconjugated bilirubin in the serum.o

b. Dark coloured urine due to the excessive excretion of bilirubin and urobilinogen.

c. Increased activities of **alanine transaminase** and **aspartate transaminase** released intocirculation due to damage to hepatocytes.

d. The patients pass pale, clay coloured stools due to the absence of stercobilinogen.

e. 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

a. Increased concentration of conjugated bilirubin in serum.

b. Serum alkaline phosphatase is elevated as it is released from the cells of the damaged bile duct.

c. Dark coloured urine due to elevated excretion of bilirubin and clay coloured feces due to absence of stercobilinogen.

d. Feces contain excess fat indicating impairment in fat digestion and absorption in the absence of bile (specifically bile salts).

e. The patients experience nausea and gastrointestinal pain,

Jaundice due to genetic defects

There are certain hereditary abnormalities that cause jaundice.

1. Neonatal-physiologic jaundice. This 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-glucuronyltransferase is low in the newborn**. Further, there is a limitation in the availability of the substrate UDP-glucuronic acid for conjugation. The net defect is the serum unconjugated bilirubin is highly elevated (may go beyond 25 mg/ml), which can cross the blood-brain barrier and cause damage to the brain leading to mental retardation.

2. 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.

3. 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 level concentration is usually less than 20 mg/dl and this is less dangerous than type I.

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

a. A defect in the uptake of bilirubin by liver cells.

b. An impairment in conjugation due to reduced activity of UDP-glucuronyltransferase.

c. Decreases hepatic clearance of bilirubin.

LECTURE 33

EXTRACELLULAR MATRIX

Cells are the basic units of life. Most mammalian cells are located in tissues, where they are surrounded by a complex **extracellular matrix**, often referred to as "**connective tissue**". This matrix has a variety of important functions apart from acting as supporting scaffolding for the cells it surrounds. Extracellular matrix contains three major classes of biomolecules: 1) the **structural proteins**, collagen, elastin, and fibrillin; 2) certain **specialized proteins**, such as fibrillin, fibronectin, and laminin, which have specific functions in the extracellular matrix; 3) **proteoglycans**, which consist of long chains of repeating disaccharides (glycosaminoglycans, formerly called mucopolysaccharides) attached to specific core proteins.

COLLAGEN

Collagen, the major component of most connective tissues, constitutes approximately 25% of the protein of mammals. About 19 distinct types of collagen made up of about 30 distinct polypeptide chains have been identified in human tissues. Although several of these are present only in small proportions, they may play important roles in determining the physical properties of the tissues. **Collagen I** and **II** are the major collagens of **skin** and **bone** and of **cartilage**, respectively.

All collagen types have a **triple helical structure**. In some collagens, the entire molecule is triple helical, whereas in other the triple helix may involve only a fraction of the structure.

Mature collagen type I contains approximately 1000 amino acids. Each polypeptide subunit or **α chain** is twisted into a **left-handed helix of three residues per turn**. Three of these α chains are then wound into a right-handed **superhelix**, forming a rod-like molecule 1.4 nm in diameter and about 300 nm long.

A striking characteristic of collagen is the occurrence of **glycine residues** at **every third position** of the triple helical portion of the α chain. This is neces-

sary because glycine is the only amino acid small enough to be accommodated in the limited space available down the central core of the triple helix. This repeating structure, represented as $(Gly\text{-}X—Y)_n$, is an absolute requirement for the formation of the triple helix. While X and Y can be any other amino acids, about 100 of the **X** positions are **proline** and about 100 of **Y** positions are **hydroxyproline**. Proline and hydroxyproline confer **rigidity** on the collagen molecule. Hydroxyproline is formed by the **posttranslational hydroxylation** of peptide-bound proline residues catalyzed by the enzyme **prolyl hydroxylase**, whose cofactors are **ascorbic acid** (vitamin C) and **α-ketoglutarate.** Lysines in the Y position may also be posttranslationally modified to hydroxylisine through the action of lysyl hydroxylase, an enzyme with similar cofactors. Some of these hydroxylysines may be further modified by the addition of galactose or galactosyl-glucose through an *O*-glycosidic linkage, a glycosylation site that is unique to collagen.

Collagen types that form long rod-like fibers in tissues are assembled by lateral association of these triple helical units into a **"quarter staggered"** alignment such that each is displaced longitudinally from its neighbor by slightly less than one-quarter of its length. This arrangement is responsible for the banded appearance of these fibers in connective tissues. Collagen fibers are further stabilized by the formation of covalent cross-links, both within and between the triple helical units. These cross-links form through the action of **lysyl oxidase**, a copper-dependent enzyme.

Several collagen types do not form fibrils in tissues. They are characterized by interruptions of the triple helix with stretches of protein lacking Gly-X—Y repeat sequences. These non-Gly-X—Y sequences result in areas of globular structure interspersed in the triple helical structure.

Posttranslational modification of collagen

Newly synthesized collagen undergoes extensive posttranslational modification before becoming part of a mature, extracellular collagen fiber. Like most secreted proteins, collagen is synthesized on ribosomes in a precursor form, preprocollagen, which contains a leader or signal sequence that directs the polypeptide chain into the vesicular space of the endoplasmic reticulum. As it enters the endoplasmic reticulum, this leader sequence is enzymatically removed. Hydroxylation of proline and lysine residues and glycosylation of hydroxylysine in this **procollagen** molecule also take place at this site. The procollagen molecule contains polypeptide extensions of 20—35 kDa at both its amino and carboxyl terminal ends, neither of which is present in mature collagen. Both extension peptides contain cysteine residues. While the amino terminal propeptide forms only intrachain disulfide bonds, the carboxyl terminal propeptides form both intrachain and interchain disulfide bonds. Formation of these disulfide bonds assists in the registration of the three collagen molecules to form the triple helix, winding from the carboxyl terminal end. After formation of the triple helix, no

further hydroxylation of proline or lysine or glycosylation of hydroxylysines can take place. Self-assembly is a cardinal principle in the biosynthesis of collagen.

Following secretion from the cell by way of the Golgi apparatus, extracellular enzymes called **procollagen aminoproteinase** and **procollagen carboxyproteinase** remove the extension peptides at the amino and carboxyl terminal ends, respectively. Once the propeptides are removed, the triple helical collagen molecules, containing approximately 1000 amino acids per chain, spontaneously assemble into collagen fibers. These are further stabilized by the formation of inter- and intrachain cross-links through the action of lysyl oxidase, as described previously.

The same cells that secrete collagen also secrete **fibronectin**, a large **glycoprotein** present on **cell surfaces**, in the **extracellular matrix**, and in **blood**. Fibronectin binds to aggregating precollagen fibers and alters the kinetics of fiber formation in the pericellular matrix. Associated with fibronectin and procollagen in this matrix are the **proteoglycans heparan sulfate** and **chondroitin sulfate** (see below). In fact, type IX collagen, a minor collagen type from cartilage, contains attached proteoglycan chains. Such interactions may serve to regulate the formation of collagen fibers and to determine their orientation n tissues.

Once formed, collagen is relatively **metabolically stable**. However, its breakdown is increased during **starvation** and **various inflammatory states**. Excessive production of collagen occurs in a number of conditions, e.g. hepatic cirrhosis.

Genetic diseases of collagen synthesis

About 30 genes encode collagen, and its pathway of biosynthesis is complex, involving at least eight enzyme-catalyzed posttranslational steps. The diseases are due to **mutations in collagen genes** or in **genes encoding some of the enzymes** involved in these posttranslational modifications.

Ehlers-Danlos syndrome comprises a group of inherited disorders whose principal clinical features are **hyperextensibility** of the **skin**, **abnormal tissue fragility**, and **increased joint mobility**. The clinical picture is variable, reflecting underlying extensive genetic heterogeneity. At least 11 types have been recognized, most of which reflect a variety of lesions n the synthesis of collagen. Type IV is the most serious because of its tendency for spontaneous rupture of arteries or the bowel, reflecting abnormalities in type III collagen. Patients with type VI, due to a deficiency of lysyl hydroxylase, exhibit marked joint hypermobility and a tendency to ocular rupture. A deficiency of procollagen N-proteinase, causing formation of abnormal thin, irregular collagen fibrils, results in type VIIC Ehlers-Danlos syndrome, manifested by marked joint hypermobility and soft skin.

Alport's syndrome refers to a number of genetic disorders (both X-linked and autosomal) affecting the structure of type IV collagen fibers, the major collagen found in the basement membranes of the renal glomeruli. The presenting

sign is **hematuria**, and patients may eventually develop end-stage renal disease. Electron microscopy reveals characteristic abnormalities of the structure of the basement membrane and lamina densa.

In **epidermolysis bullosa**, the skin breaks and blisters as a result of minor trauma. The dystrophic form is due to mutations in COL7A1, affecting the structure of type VII collagen. This collagen forms delicate fibrils that anchor the basal lamina to collagen fibrils in the dermis. These anchoring fibrils have been shown to be markedly reduced in this form of the disease, probably resulting in the blistering.

Scurvy affects the structure of collagen. However, it is due to a deficiency of ascorbic acid and is not a genetic disease. Its major signs are bleeding gums, subcutaneous hemorrhages, and poor wound healing. These signs reflect impaired synthesis of collagen due to deficiencies of prolyl and lysyl hydroxylases, both of which require ascorbic acid as a cofactor.

ELASTIN

Elastin is a **connective tissue protein** that is responsible for properties of extensibility and elastic recoil in tissues. Although not as widespread as collagen, elastin is present in large amounts, particularly in tissues that require these physical properties, e.g. **lung, large arterial blood vessels**, and **some elastic ligaments**. Smaller quantities of elastin are also found in **skin**, **ear cartilage**, and several other tissues. In contrast to collagen, there appears to be only **one genetic type of elastin**, although variants arise by differential processing of the hnRNA for elastin. Elastin is synthesized as a soluble monomer of 70 kDa called “tropoelastin”. Some of the prolines of tropoelastin are hydroxylated to **hydroxyproline** by prolyl hydroxylase, though hydroxylysine and glycosylated hydroxylysine are not present. Unlike Collagen, tropoelastin is not synthesized in a proform with extension peptides. Furthermore, elastin does not contain repeat Gly-X—Y sequences, triple helical structure, or carbohydrate moieties.

After secretion from the cell, certain lysyl residues of tropoelastin are oxidatevely deaminated to aldehydes by lysyl oxidase, the same enzyme involved in this process in collagen. However, the major cross-links formed in elastin are the **desmosines**, which result from the condensation of three of these lysine-derived aldehydes with an unmodified lysine to form a tetrafunctional cross-link unique to elastin. Once cross-linked in its mature, extracellular form, elastin is highly insoluble and extremely stable and has a very low turnover rate. Elastin exhibits a variety of random coil conformations that permit the protein to stretch and subsequently recoil during the performance of its physiologic functions.

Deletions in the elastin gene have been found in approximately 90% of subjects with **Williams syndrome**, a developmental disorder affecting connective tissue and the central nervous system. The mutations, by affecting syn-

thesis of elastin, probably play a causative role in the supravalvular aortic stenosis often found in this condition. A number of skin diseases (e.g. scleroderma) are associated with accumulation of elastin.

Marfan's syndrome

Marfan's syndrome is a relatively frequent inherited disease affecting connective tissue; it is inherited as an autosomal dominant train. It affects the eyes (e.g. causing dislocation of the lens, known as ectopia lentis), the skeletal system (most patients ate tall and exhibit long digits [arachnodactyly] and hyperxtensibility of the joins), and the cardiovascular system (e.g. causing weakness of the aortic media, leading to dilation of the ascending aorta). Abraham Linkoln may have had this condition.

FIBRILLIN

Fibrillin is a large glycoprotein (about 350 kDa) that is a structural component of **microfibrils,** 10 — to 120nm fibers found in many tissues. Fibrillin is secreted (subsequent to a proteolytic cleavage) into the extracellular matrix by fibroblasts and becomes incorporated into the insoluble microfibrils, which appear to provide a scaffold for deposition of elastin. Of special relevance to Marfan's syndrome, fibrillin is found in the zonular fibers of the lens, in the periosteum, and associated with elastin fibers in the aorta (and elsewhere); these locations respectively explain the ectopia lentis, arachnodactyly, and cardiovascular problems found in the syndrome. Other proteins are also present in these microfibrils, and it appears likely that abnormalities of them may cause other connective tissue disorders.

FIBRONECTIN

Fibronectin is a major glycoprotein of the extracellular matrix, also found in a soluble form in **plasma**. It consists of two identical subunits, each of about 230 kDa, joined by two disulfide bridges near their carboxyl terminals. Fibronectin contains three types of repeating motifs (I, II and III), which are organized into functional domains (at least seven); functions of these domains include binding **heparin** and fibrin, collagen, DNA, and cell surfaces.

The fibronectin receptor interacts indirectly with actin microfilaments present in the cytosol. A number of proteins, collectively known as attachment proteins, are involved; these include **talin, vinculin**, an **actin-filament capping protein**, and **α-actinin**. Talin interacts with the receptor and vinculin, whereas the latter two interact with actin. The interaction of fibronectin with its receptor provides one route whereby the exterior of the cell can communicate with the interior and thus affect cell behavior. Via the interaction with its cell receptor, **fibronectin plays an important role in the adhesion of cells to the extracellular matrix**. It is also involved in cell migration, by providing a binding site for cells and thus helping them to steer their way through the extracellular matrix. The amount of fibronectin around many transformed cells is sharply reduced, partly explaining their faulty interaction with the extracellular matrix.

LAMININ

Basal laminae are specialized areas of the extracellular matrix that surround epithelial and some other cells (e.g. muscle cells); here we discuss only the laminae found in the **renal glomerulus.** In that structure, the basal lamina is contributed by two separate sheets of cells (one endothelial and one epithelial), each disposed on opposite sides of the lamina; these three layers make up the **glomerular membrane.** The primary components of the basal lamina are three proteins — laminin, entactin, and type IV collagen — and the GAG **heparin or heparan sulfate.** These components are synthesized by the underlying cells.

Laminin (about 850 kDa, 70 nm long) consists of three distinct elongated polypeptide chains (A, B_1, and B_2) linked together to form an elongated cruciform shape. It has binding sites for type IV collagen, heparin, and integrins on cell surfaces. The collagen interacts with laminin (rather than directly with the cell surface), which in turn interacts with integrins or other laminin receptor proteins, thus anchoring the lamina to the cells. **Entactin,** also known as "nidogen", is a glycoprotein; it binds to laminin and is a major cell **attachment factor**. The relatively thick basal lamina of the renal glomerulus has an important role in **glomerular filtration,** regulating the passage of large molecules (most plasma proteins) across the glomerulus into the renal tubule. The glomerular membrane allows small molecules, such as inulin (5.2 kDa), to pass through as easily as water. On the other hand, only a small amount of the protein albumin (69 kDa), the major plasma protein, passes through the normal glomerulus. This is explained by two sets of facts: (1) The pores in the glomerular membrane are large enough to allow molecules up to about 8 nm to pass through. (2) Albumin is smaller than this pore size, but it is prevented from passing through easily by the negative charges of heparan sulfate and of certain sialic acid-containing glycoproteins present in the lamina. These negative charges repel albumin and most plasma proteins, which are negatively charged at the pH of blood. The normal structure of the glomerulus may be severely damaged in certain types of **glomerulonephritis** (eg, caused by antibodies directed against various components of the glomerular membrane). This alters the pores and the amounts and dispositions of the negatively charged macromolecules referred to above, and relatively massive amounts of albumin (and of certain other plasma proteins) can pass through into the urine, resulting in severe **albuminuria.**

PROTEOGLYCANS AND GLYCOSAMINOGLYCANS

Proteoglycans are proteins that contain covalently linked glycosaminoglycans. They vary in tissue distribution, nature of the core protein, attached glycosaminoglycans, and function. The proteins bound covalently to glycosaminoglycans are called "core proteins". The amount of carbohydrate in a proteoglycan is usually much greater than is found in a glycoprotein and may comprise up to 95% of its weight. **Aggrecan**, the major type found in cartilage. It is very large (about 2×10^3 kDa), with its overall structure resembling that of a

bottle brush. It contains a long strand of hyaluronic acid (one type of GAG) to which link proteins are attached noncovalently. In turn, these latter interact noncovalently with core protein molecules from which chains of other GAGs (keratan sulfate and chondroitin sulfate in this case) project.

There are at least seven **glycosaminoglycans (GAGs): hyaluronic acid**, **chondroitin sulfate**, **keratan sulfates I** and **II**, **heparin**, **heparan sulfate**, and **dermatan sulfate**. A GAG is an **unbranched polysaccharide** made up of **repeating disaccharides**, one component of which is always an **amino sugar** (hence the name GAG), either **D-glucosamine** or **D-galactosamine**. The other component of the repeating disaccharide (except in the case of keratan sulfate) is a **uronic acid**, either **L-glucuronic acid** (GlcUA) or its 5'-epimer, **L-iduronic acid** (IdUA). With the exception of hyaluronic acid, all the GAGs contain sulfate groups, either as *O*-esters or as *N*-sulfate (in heparin and heparan sulfate). Hyaluronic acid affords another exception because there is no clear evidence that it is attached covalently to protein, as the definition of a proteoglycan given above specifies.

Biosynthesis of glycosaminoglycans

1. Attachment to core proteins. The linkage between GAGs and their core proteins is occurred. The synthesis of the core proteins occurs in the endoplasmic reticulum, and formation of at least some of the above linkages also occurs there. Most of the later steps in the biosynthesis of GAG chains and their subsequent modifications occur in the Golgi apparatus.

2. Chain elongation. Appropriate nucleotide sugars and highly specific Golgi-located glycosyltransferases are employed to synthesize the oligosaccharide chains of GAGs. The “one enzyme, one linkage” relationship appears to hold here, as in the case of certain types of linkages found in glycoproteins. The enzyme systems involved in chain elongation are capable of high fidelity reproduction of complex GAGs.

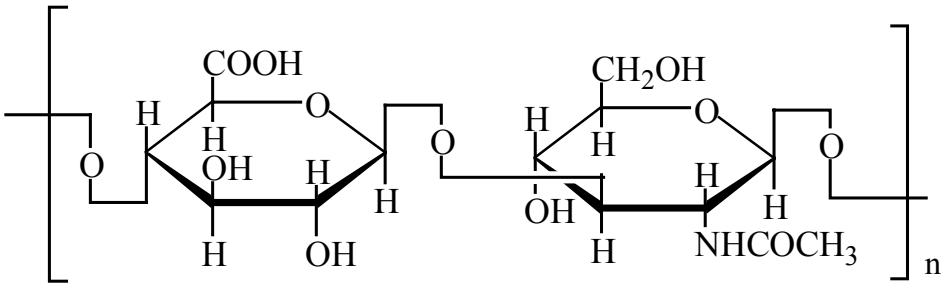
3. Chain termination. This appears to result from (1) sulfation, particulary at certain positions of the sugars, and (2) the progression of the growing GAG chain away from the membrane site where catalysis occurs.

4. Further modification. After formation of the GAG chain, numerous chemical modifications occur, such as the introduction of sulfate groups onto N-acetylgalactosamine (GalNAc) and other moieties and the epimerization of L-glucuronic acid to L-iduronic acid residues. The enzymes catalyzing sulfation are designated sulfotrabsferases and use 3’-phosphoadenosine-5’-phosphosulfate (PAPS, active sulfate) as the sulfate donors. These Golgi-located enzymes are highly specific, and distinct enzymes catalyze sulfation at different positions (e.g. carbon 2, 3, 4, and 6) on the acceptor sugars. An **epimerase** catalyzes conversions of glycuronyl to iduronyl residues.

Characteristic of glycosaminoglycans

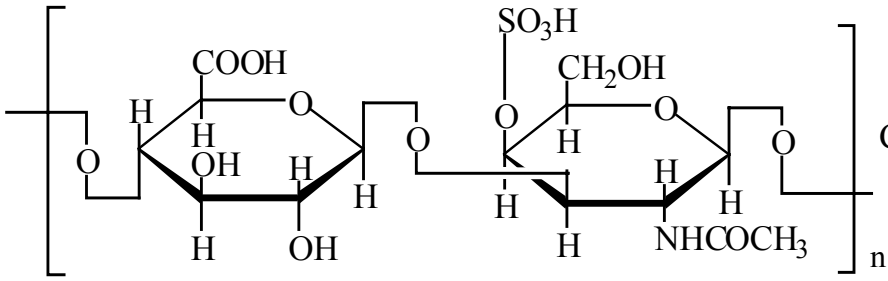
The seven GAGs named above differ from each other in a number of the following properties: amino sugar composition, uronic acid composition, linkages between these components, chain length of the disaccharides, the presence or absence of sulfate groups and their positions of attachment to the constituent sugars, the nature of the core proteins to which they are attached, the nature of the linkage to core protein, their tissue and subcellular distribution, and their biologic functions.

1. Hyaluronic acid. Hyaluronic acid consists of an unbranched chain of repeating disaccharide units containing D-glucuronic acid and N-acetylglucosamine. Hyaluronic acid is present in bacteria and is widely distributed among various animals and tissues, including synovial fluid, the vitreous body of the eye, cartilage, and loose connective tissues.



D-Glucuronic acid N-acetylglucosamine

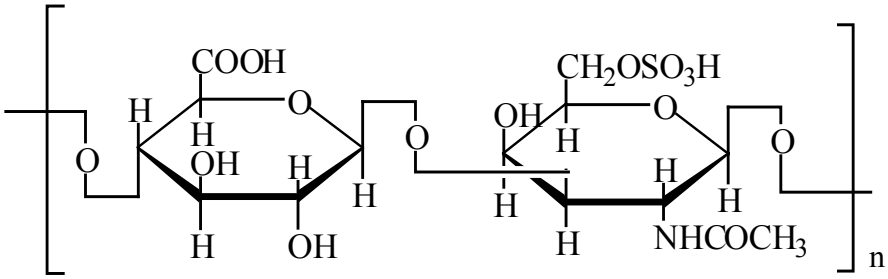
2. Chondroitin sulfates (chondroitin-4-sulfate and chondroitin-6-sulfate). Proteoglycans linked to chondroitin by the xylose-serine-*O*-glycosidic bond are prominent components of cartilage. The repeating disaccharide is similar to that found in hyaluronic acid, containing L-glucuronic acid but with N-acetylgalactosamine replacing N-acetylglucosamine. The N-acetylgalactosamine is substituted with sulfate at either its 4’ or its 6’ position, with approximately one sulfate being present per disaccharide unit.



Chondroitin-4-sulfate

D-Glucuronic acid

N-acetylgalactosamine-4-sulfate

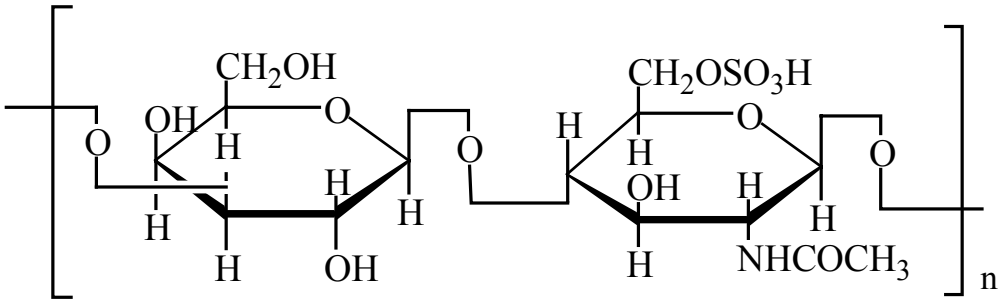


Chondroitin-6-sulfate

D-Glucuronic acid

N-acetylgalactosamine-6-sulfate

3. Keratan sulfates I and II. The keratan sulfates consist of repeating galactose-N-acetylglucosamine disaccharide units containing sulfate attached to the 6’ position of N-acetylglucosamine or occasionally of galactose.



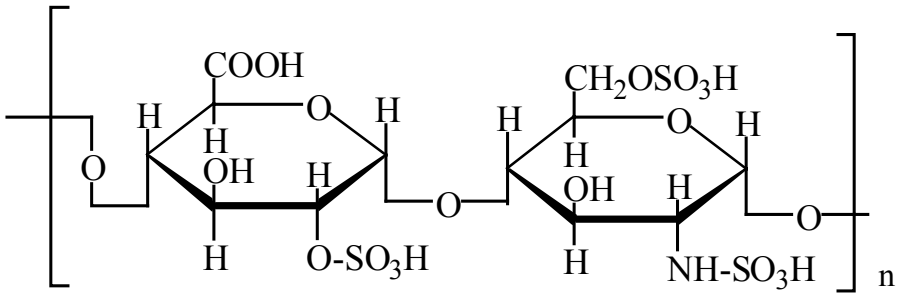
Galactose

N-acetylgalactosamine-6'-sulfate

Type I is abundant in cornea, and type II is found along with chondroitin sulfate attached to hyaluronic acid in loose connective tissue. Types I and II have different attachments to protein.

4. Heparin. The repeating disaccharide contains glucosamine and either of the two uronic acids. Most of the amino groups of the glucosamine residues are N-sulfated, but a few are acetylated. The glucosamine also carries a C_6 sulfate ester. Approximately 90% of the uronic acid residues are L-iduronic acid. Initially, all of the uronic acids are L-glucuronic acid, but a 5’-epimerase converts approximately 90% of the L-glucuronic acid residues to L-iduronic acid after the polysaccharide chain is formed. The protein molecule of the heparin proteogly-

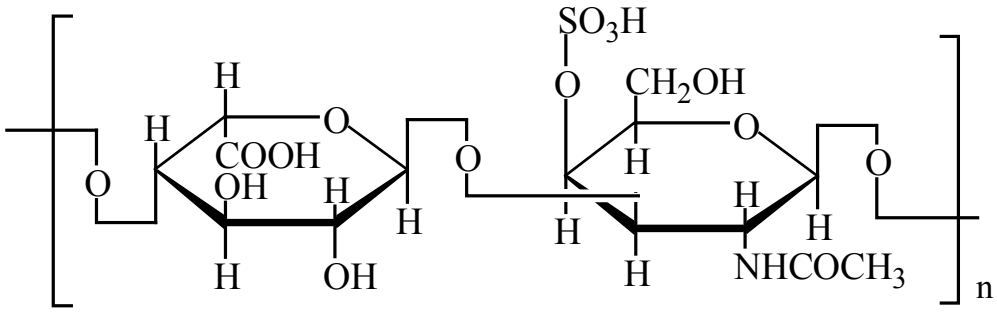
can is unique, consisting exclusively of serine and glycine residues. Approximately two-thirds of the serine residues contain GAG chains, usually of 5—15 kDa but occasionally much larger. Heparin is found in the granules of mast cells and also in liver, lung, and skin.



D-Glucuronate-2-sulfate N-sulfoglucosamine-6-sulfate

5. Heparan sulfate. This molecule is present on many cell surfaces as a proteoglycans and is extracellular. It contains N-acetylglucosamine fewer N-sulfates than heparine, and unlike heparin, its predominant uronic acid is L-glucuronic acid.

6. Dermatan sulfate. This substance is widely distributed in animal tissues. Its structure is similar to that of chondroitin sulfate, except that in place of a L-glucuronic acid in β-1,3 linkage to N-acetylgalactosamine, it contains an L-iduronic acid in an α-1,3 linkage to N-acetylgalactosamine. Formation of the L-iduronic acid occurs, as in heparin and heparin sulfate, by 5'-epimerization of L-glucuronic acid. Because this is regulated by the degree of sulfation, and sulfation is incomplete, dermatan sulfate contains both L-iduronic acid-N-acetylgalactosamine and L-glucuronic acid-N-acetylgalactosamin disaccharides.



L-iduronic acid N-acetylgalactosamine-4'-sulfate

Mucopolysaccharidoses

Both exo- and endoglycosidases degrade GAGs. Like most other biomolecules, GAGs are subject to turnover, being both synthesized and degraded. In adult tissues, GAGs generally exhibit relatively slow turnover, their half-lives being in the order of days to weeks. If deficiencies of specific enzymes of GAGs

degradation are occurred, these inborn errors are called **mucopolysaccharidoses**. Degradation of GAGs is carried out by a battery of lysosomal hydrolyses. These include certain endoglycosidases, various exoglycosidases, and sulfatases, generally acting in sequence to degrade the various GAGs. The mucopolysaccharidoses share a common mechanism of causation: mutation(s) in a gene encoding a lysosomal hydrolase involved in the degradation of one or more GAGs → defective lysosomal hydrolase → accumulation of substrate in various tissues, including liver, spleen, bone, skin, and central nervous system.

Specific laboratory investigations of help in their diagnosis are urine testing for the presence of increased amount of GAGs and assay of suspected enzymes in white cells, fibroblasts, or sometimes in serum. In certain cases, a tissue biopsy is performed and the GAG that has accumulated can be determined by electrophoresis. DNA tests are increasingly available. Prenatal diagnosis can be made using amniotic cells or chorionic villus biopsy.

The term "**mucolipidosis**" was introduced to denote diseases that combined features common to both mucopolysaccharidoses and sphingolipidoses.

Hyaluronidase is one important enzyme involved in the catabolism of certain GAGs that has not been implicated in any mucopolysaccharidosis. It is a widely distributed endoglycosidase that cleaves hexosaminidic linkages.

Function of proteoglycans

1. As indicated above, proteoglycans are remarkably complex molecules and are found in every tissue of the body, mainly in the extracellular matrix or "ground substance". There are associated with each other and also with the other major structural components of the matrix, collagen and elastin, in quite specific manners. Some proteoglycans bind to collagen and other to elastin. These interactions are important in determining the structural organization of the matrix.

2. The GAGs present in the proteoglycans are polyanions and hence **bind polycations** and **cations** such as $\mathbf{Na}^+$ and $\mathbf{K}^+$. This latter ability attracts water by osmotic pressure into the extracellular matrix and contributes to its turgor.

3. GAGs also **gel** at relatively low concentrations. Because of the long extended nature of the polysaccharides chains of GAGs and their ability to gel, the proteoglycans can act as **sieves**, **restricting the passage of large macromolecules** into the extracellular matrix but **allowing relatively free diffusion of small molecules**. Again, because of their extended structures and the huge macromolecular aggregates they often form, they occupy a large volume of the matrix relative to proteins.

4. Hyaluronic acid is especially high in concentration in embryonic tissues and is thought to play an important role in **permitting cell migration during morphogenesis and wound repair**. Its ability to attract water into the extracellular matrix and thereby "loosen it up" may be important in this regard. The high concentration of hyaluronic acid and chondroitin sulfates present in cartilage contribute to its compressibility.

5. Chondroitin sulfates are located at sites of **calcification in endochondral bone** and are also found in **cartilage**. They are also located inside certain neurons and may provide an **endoskeletal structure**, helping to maintain their shape. Both **keratan sulfate I** and **dermatan sulfate** are present in the cornea. They lie between collagen fibrils and play a critical role in cornea transparency. Changes in proteoglycan composition found in corneal scars disappear when the cornea heals. The presence of dermatan sulfate in the sclera may also play a role in maintaining the overall shape of the eye. Keratan sulfate I is also present in cartilage.

6. Heparin is am important anticoagulant. Heparin can also bind specifically to lipoprotein lipase present in capillary walls, causing a release of this enzyme into the circulation.

LECTURE 34

MUSCLE

Structure of the muscle

Both **skeletal** and **cardiac muscle**s appear striated upon microscopic observation; smooth muscle is **nonstriated**. Skeletal muscle is under **voluntary** nervous control, the control of both cardiac and smooth muscle is **involuntary**.

Striated muscle is composed of multinucleated **muscle fiber** cells surrounded by an electrically excitable membrane, the **sarcolemma.** An individual fiber cell, which may extend the entire length of the muscle, contains a bundle of many myofibrils arranged in parallel, embedded in intracellular fluid termed **sarcoplasm.** Within this fluid is contained glycogen, high-energy compounds ATP and phosphocreatine, and the enzymes of glycolysis.

When the myofibril is examined by electron microscopy, alternating **dark** and **light** bands (anisotropic bands meaning birefringent in polarized light; and isotropic bands, meaning not altered by polarized light can be observed. These bands are thus referred to as **A** and **I** bands, respectively. The central region of the A band (the **H** band, or H zone) appears less dense than the rest of the band. The I band is bisected by a very dense and narrow **Z** line (Fig. 34—1). The **sarcomere** is defined as the region between two Z lines (Fig. 34—1) and is repeated along the axis of a fibril at distances of 1500—2300 nm, depending upon the state of contraction.

When myofibrils are examined by electron microscopy, it appears that each one is constructed of two types of longitudinal filaments. One type, the **thick filament**, confined to the A band, contains chiefly the protein **myosin**. These filaments are about 16 nm in diameter and arranged in cross section as a hexagonal array. The other filaments, the **thin filament**, lies in the I band and extends also into the A band but not into its H zone (Fig.34—1 and 34—2).

The thin filaments contain the proteins **actin, tropomyosin**, and **troponin**. In the A band, the thin filaments are arranged around the thick (myosin) filament as a secondary hexagonal array. Each thin filament lies symmetrically between three thick filaments (Fig. 34—3), and each thick filament is surrounded symmetrically by 6 thin filaments.

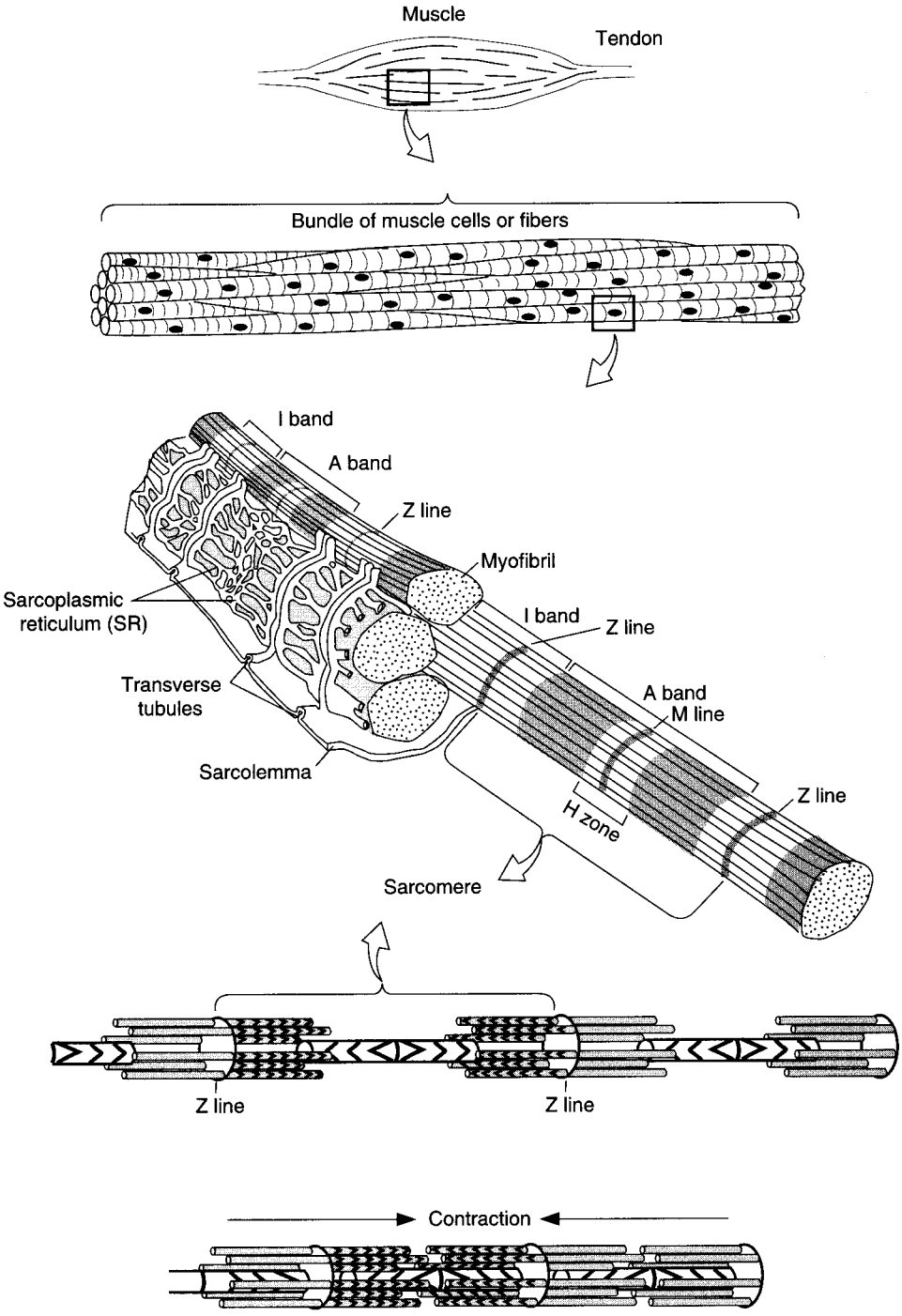


Figure 34—1. Muscle structure and organization showing the sliding filament model of contraction (by Davidson V.L. et al., 1999).

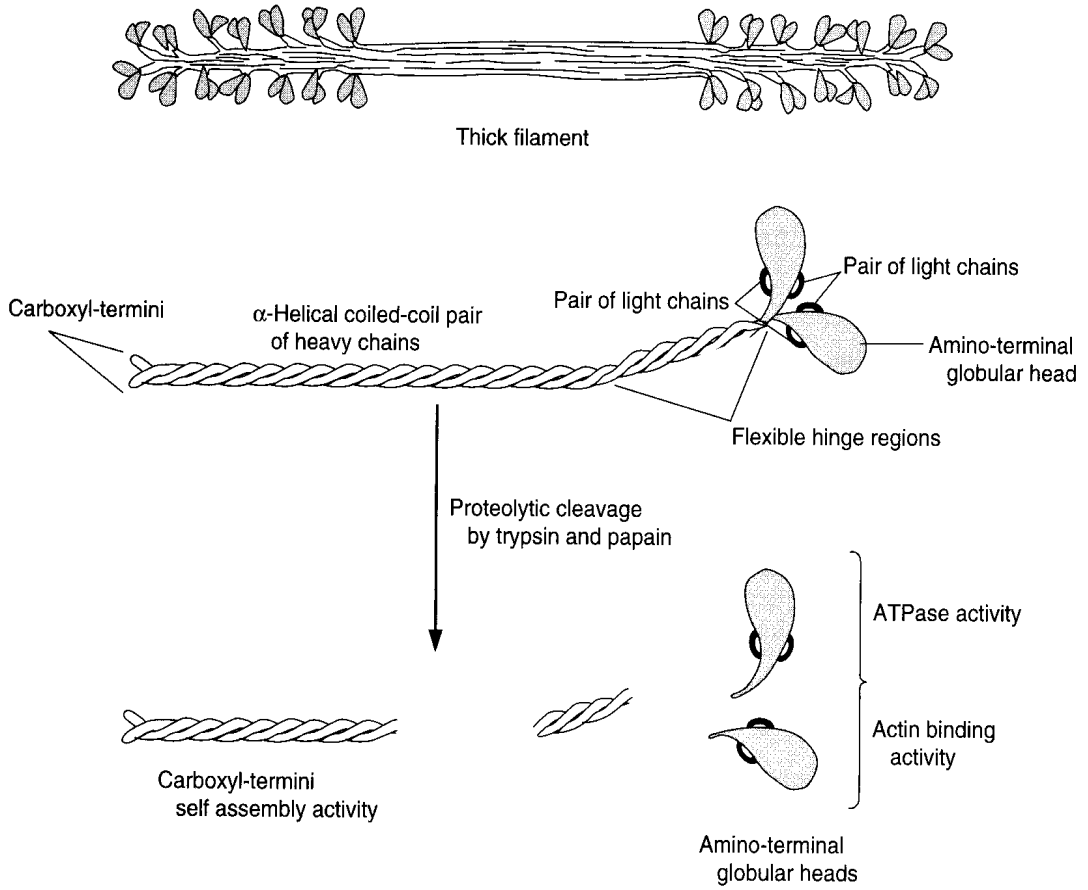


Figure 34—2. Structure and regions of myosine (by Davidson V.L. et al., 1999).

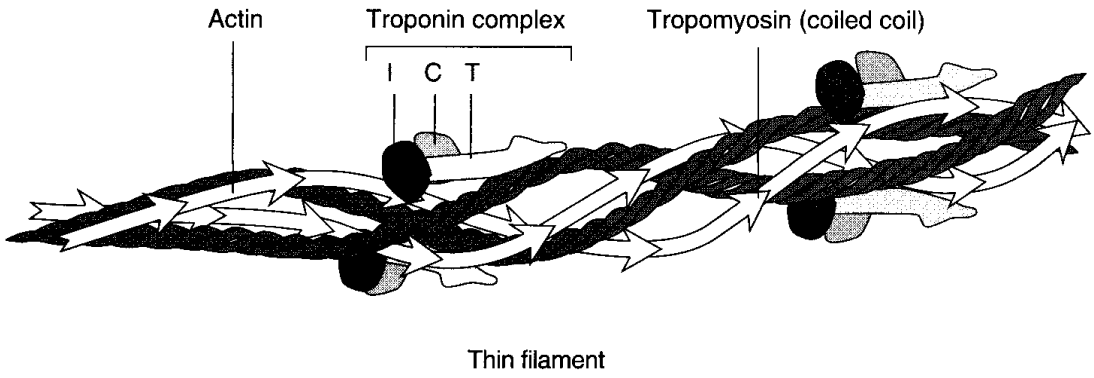


Figure 34—3. Organization of thin filaments (by Davidson V.L. et al., 1999)

The thick and thin filaments interact via cross-bridges that emerge at intervals of 14 nm along the thick filaments. The cross-bridges (or **arrowheads**) on the thick filaments have opposite polarities at the two ends of the filaments. The two poles of the thick filaments are separated by a 150-nm segment (the M band) that is free of projections.

Proteins of muscle

The mass of a fresh muscle fibril is made up of 75% water and more than 20% protein. The two major muscle proteins are actin and myosin.

1. Monomeric G-actin (43 kDa; G, globular) makes up 25% of muscle protein by weigh. At physiologic ionic strength and in the presence of Mg^{2+}, G-actin **polymerizes** noncovalently to form an insoluble double helical fragment called F-actin. The F-actin fiber is 6—7 nm thick and has a pitch or repeating structure every 35.5 nm. Neither G- nor F-actin exhibits any catalytic activity.

2. Myosin contributes 55% of muscle protein by weigh and forms the thick filaments. It is an asymmetric hexamer with a molecular weigh of 460 kDa. Myosin has a **fibrous tail** consisting of two intertwined helices. Each helix has a **globular head** portion attached at one end. The hexamer consists of one pair of heavy (**H**) chains each approximately 200 kDa molecular mass, and two pairs of light (**L**) chains each with a molecular mass of approximately 20 kDa. The L chains differ, one being called the essential light chain and the other regulatory light chain. Skeletal muscle myosin binds actin to form actomyosin and its intrinsic ATPase activity is markedly enhanced in this complex.

In striated muscle, there are two other proteins that are minor in terms of their mass but important in terms of their function.

1. Tropomyosin is a fibrous molecule that consists of two chains, α and β, that attach to F-actin in the groove between its filaments. Tropomyosin is present in all muscular and muscle-like structure.

2. Troponin T (TpT) binds to tropomyosin as well as to the other two troponin components. **Troponin I** (TpI) prevents binding of the myosin head to its F-actin attachment site either by altering the conformation of F-actin via the tropomyosin molecules or by simply rolling tropomyosin into a position that directly blocks the site on F-actin to which the myosin heads attach. **Troponin C** (TpC) is a calcium-binding polypeptide that is structurally and functionally analogous to calmodulin. Four molecules of calcium ion are bound per molecule of troponin C.

Muscle contraction

In resting muscle sarcoplasm, the concentration of Ca^{2+} is 10^{-7} to 10^{-8} mol/L. The resting state is achieved because Ca^{2+} is pumped into the sarcoplasmic reticulum through the action of an active transport system, called the Ca^{2+} ATPase, initiating relaxation. Inside the sarcoplasmic reticulum, Ca^{2+} is bound to a specific Ca^{2+}-binding protein designated **calsequestrin**. The sarcomere is surrounded by an excitable membrane (the T tubule system) composed of transverse (T) channels closely associate with the sarcoplasmic reticulum.

When the sarcolemme is excited by a nerve impulse, the signal is transmitted into the T tubule system and a **Ca^{2+}release channel** in the nearby sarcoplasmic reticulum opens, releasing Ca^{2+} from the sarcoplasmic reticulum into the sarcoplasm. The concentration of Ca^{2+} in the sarcoplasm rises rapidly to 10^{-5} mol/L. The Ca^{2+}-binding sites on troponin C in the thin filament are quickly occupied by Ca^{2+}. The $TpC{\cdot}4Ca^{2+}$ interacts with TpI and TpT to alter their interaction with tropomyosin. Accordingly, tropomyosin moves out of the way or alters

the conformation of F-actin so that the myosin head-ADP-Pi can interact with F-actin to start the contraction cycle.

Contraction cycle

1. In the relaxation phase of muscle contraction, the head of myosin hydrolyzed ATP to ADP and Pi, but these products remain bound. The resultant ADP-Pi-myosin complex has been energized and is in a so-called high-energy conformation.

2. When contraction of muscle is stimulated actin becomes accessible and the head of myosin finds it, binds it, and forms the actin-myosin-ADP-Pi complex indicated.

3. Formation of this complex promotes the release of Pi, which initiates the power stroke. This is followed by release of ADP and is accompanied by a large conformational change in the head of myosin in relation to its tail, pulling actin about 10 nm toward the center of the sarcomere. This is the power stroke. The myosin is now in a so-called low-energy state, indicated as actin-myosin.

4. Another molecule of ATP binds to the head, forming an actin-myosin-ATP complex.

5. Myosin-ATP has a low affinity for actin, and actin is thus released. This last step is a key component of relaxation and its dependent upon the binding of ATP to the actin-myosin complex.

6. Another cycle then commences with the hydrolysis of ATP, re-forming the high-energy conformation.

Relaxation occurs when (1) sarcoplasmic Ca^{2+} falls below 10^{-7} mol/L owing to its resequestration into the sarcoplasmic reticulum by the Ca^{2+} ATPase; (2) TpC· $4Ca^{2+}$ loses its Ca^{2+}; (3) troponin, via its interaction with tropomyosin, inhibits further myosin head-F-actin interaction; (4) in the presence of ATP, the myosin head detaches from the F-actin.

Sources of energy in muscle. The ATP required as the constant energy source for the contraction-relaxation cycle of muscle can be generated (1) by glycolysis, using blood glucose or muscle glycogen, (2) by oxidative phosphorylation, (3) from creatine phosphate, and (4) from two molecules of ADP in a reaction catalyzed by adenylyl kinase.

The amount of ATP in skeletal muscle is only sufficient to provide energy for contraction for 1-2 seconds, so that ATP must be constantly renewed from one or more of the above sources, depending upon metabolic conditions.

1. The sarcoplasm of skeletal muscle contains large stores of glycogen, located in granules close to the I bands. The release of glucose from glycogen is dependent on a specific muscle glycogen phosphorylase, which can be activated by Ca^{2+}, epinephrine, and AMP. Ca^{2+}promotes the activation of phosphorylase b kinase by phosphorylation. Thus, Ca^{2+} both initiates muscle contraction and activates a pathway to provide necessary energy. The hormone epinephrine also activates glycogenesis in muscle. AMP, produced by breakdown of ADP during

muscular exercise, can also activate phosphorylase b without causing phosphorylation.

2. Synthesis of ATP via oxidative phosphorylation requires a supply of oxygen. Muscle that have a high demand for oxygen as a result of sustained contraction store it attached to the heme moiety of myoglobin. Because of the moiety, muscles containing myoglobin are red, whereas muscles with little or no myoglobin are white. Glucose, derived from the blood glucose or from endogenous glycogen, and fatty acids derived from the triacylglycerols of adipose tissue, are the principal substrates used for aerobic metabolism in muscle.

3. Creatine phosphate prevents the rapid depletion of ATP by providing a readily available high-energy phosphate, which can be used to regenerate ATP from ADP. Creatine phosphate is formed from ATP and creatine at time when the muscle is relaxed and demands for ATP are not so great. The enzyme catalyzing the phosphorylation of cratine is creatine kinase, a muscle-specific enzyme with clinical utility in the detection of acute or chronic disease of muscle.

4. Adenylyl kinase catalyzed formation of one molecule of ATP and one of AMP from two molecules of ADP. This reaction is coupled with the hydrolysis of ATP by myosin ATPase during muscle contraction.

Types of skeletal muscle fibers

There are two types of fiber: type I (slow twitch, oxidative) and type II (fast twitch, glycolytic). The type I fibers are red because they contain myoglobin and mitochondria; their metabolism is aerobic, and they maintain relatively sustained contractions. The type II fibers, lacking myoglobin and containing few mitochondria, are white: they derive their energy from anaerobic glycolysis and exhibit relatively short durations of contraction. The proportion of these two types of fiber varies among the muscles of the body, depending on function. The proportion also varies with training; for example, the number of type I fibers in certain leg muscle increases in athletes training for marathons, whereas the number of type II fibers increases in sprinters.

LECTURE 35

CANCEROGENESIS. ONCOGENES. GROWTH FACTORS

In the normal circumstances, the proliferation of body cells is under strict control. The cells differentiate, divide and die in a sequential manner in a healthy organism. Cancer is characterized by **loss of control of cellular growth and development leading to excessive proliferation and spread of cells.** Cancer is derived from a *Latin* word meaning *crab.* It is presumed that the word cancer originated from the character of cancerous cells which can migrate and adhere and cause pain (like a crab) to any part of the body.

Neoplasia literally means new growth. Uncontrolled growth of cells results in tumors (a word originally used to represent swelling). Oncology *(Greek:* oncos—tumor) deals with the study of tumors.

The tumors are of two types:

1. Benign tumors. They usually grow by expansion and remain encapsulated in a layer of connective tissue. Normally benign tumors are not life-threatening e.g. moles, warts. These types of benign tumors are not considered as cancers.

2. **Malignant tumors or cancers.** They are characterized by uncontrolled proliferation and spread of cells to various parts of the body, a process referred to as **metastasis.** Malignant tumors are invariably life-threatening e.g. lung cancer, leukemia.

About 100 different types of human cancers have been recognized. Cancers arising from **epithelial cells** are referred to as **carcinomas** while that from **connective tissues** are known as **sarcomas**. Methods for the early detection and treatment of cancers have been developed. However, little is known about the biochemical basis of cancer.

Incidence

Cancer is the **second largest killer disease** (the first being coronary heart disease) in the developed countries. It is estimated that cancer accounts for more than 20% of the deaths in United States. Based on the current rate of incidence, it is believed that one in every 3 persons will develop cancer at sometime during his life. Although humans of all age develop cancer, the incidence increases with advancement of age. More than 70% of the new cancer cases occur in persons over 60 years. Surprisingly, cancer is a leading cause of death in children in the age group 3—13 years, half of them die due to leukemia.

Etiology

In general, cancers are multifactorial in origin. The causative agents include **physical, chemical, genetic and environmental factors.** A survey in USA has shown that about 90% of all cancer deaths are due to avoidable factors such as tobacco, pollution, occupation, alcohol and diet. Most of the cancers are caused by chemical carcinogens, radiation energy and viruses. These agents may damage DNA or interfere with its replication or repair.

Chemical carcinogens. It is estimated that almost 80% of the human cancers are caused by chemical carcinogens in nature. The chemicals may be organic (e.g. dimethylbenzanthracene, benzo (a) pyrene, dimethyl nitrosamine) or inorganic (arsenic, cadmium) in nature. Entry of the chemicals into the body may occur by one of the following mechanisms.

1. Occupation e.g. asbestos, benzene.

2. Diet e.g. aflatoxin B produced by fungus *(Aspergillus flavus)* contamination of foodstuffs, particularly peanuts.

3. Drugs — certain therapeutic drugs can be carcinogenic e.g. diethylstibesterol.

4. Life style e.g. cigarette smoking.

Mechanism of action

Although a few of the chemicals are directly carcinogenic, majority of them require prior metabolism to become carcinogenic. The enzymes such as

cytochrome P_{450} responsible for the metabolism of xenobiotics are involved in dealing with the chemical carcinogens. In general, a chemically non-reactive procarcinogen is converted to an ultimate carcinogen by a series of reactions.

The carcinogens can covalently bind to purines, pyrimidines and phosphodiester bonds of DNA, often causing unrepairable damage. The chemical carcinogens frequently cause mutations (a change in the nucleotide sequence of DNA) which may finally lead to the development of cancer, hence they are regarded as mutagens.

Ames assay

This is a laboratory test to check the carcinogenecity of chemicals. Ames assay employs the use of a special mutant strain of bacterium, namely *Salmonella typhimurium* (His^-). This organism cannot synthesize histidine; hence the same should be supplied in the medium for its growth. Addition of chemical carcinogens causes mutations (reverse mutation) restoring the ability of the bacteria to synthesize histidine (His^+). By detecting the strain of *Salmonella* (His^+) in the colonies of agar plates, the chemical mutagens can be identified. The Ames assay can detect about 90% of the chemical carcinogens. This test is regarded as a preliminary screening procedure. Animal experiments are conducted for the final assessment of carcinogenecity.

Promoters of carcinogenesis

Some of the chemicals on their own are not carcinogenic. Certain substances known as promoting agents make them carcinogenic. The application of benzo(a)pyrene to the skin, as such, does not cause tumor development. However, if this is followed by the application of croton oil, tumors will develop. In this case, benzo(a)pyrene is the initiating agent while croton oil acts as a promoting agent or promoter. Several compounds that act as promoting agents in various organs of the body have been identified. These include saccharin and phenobarbital.

Radiation energy

Ultraviolet rays, X-rays and γ-rays been proved to be mutagenic in nature causing cancers. These rays damage DNA which is the basic mechanism to explain the carcinogenicity of radiation energy. For instance, exposure to UV rays results in the formation of pyrimidine dimers in DNA while X-rays cause the production of free radicals. This type of molecular damages is responsible for the carcinogenic effects of radiations.

Carcinogenic viruses

The involvement of viruses in the etiology of cancer was first reported by Rouse in 1911. He demonstrated that the cell-free filtrates from certain chicken sarcomas (tumors of connective tissues) promote new sarcomas in chickens. Unfortunately, this epoch-making discovery of Rouse was ignored for several years. This is evident from the fact that Rous was awarded the Nobel Prize in 1966 at the age of 85 for his discovery in 1911!

The presence of viral particles and the enzyme reverse transcriptase, besides the occurrence of base sequence in the DNA of malignant cells, complementary to tumor viruses indicate the involvement of viruses in cancer. The viruses involved in the development of cancer, commonly known as **oncogenic viruses,** may contain either DNA (adenovirus, herpersvirus, parovirus) or RNA (retrovirus type B, retrovirus type C).

DNA — the ultimate in carcinogenesis

DNA is the ultimate critical macromolecule in carcinogenesis. This fact is supported by several evidences.

1. Cancers are transmitted from mother to daughter cells. In other words, cancer cells beget cancer cells.

2. Chromosomal abnormalities are observed in many tumor cells.

3. Damage to DNA caused by mutations often results in carcinogenesis.

4. Laboratory experiments have proved that purified oncogenes can transform normal cells into cancer cells.

Molecular basis of cancer

Cancer is caused by a genetic change in a single cell resulting in its uncontrolled multiplication. Thus, tumors are monoclonal. Two type of regulation genes — **oncogenes** and **antioncogenes** are involved in the development of cancer (carcinogenesis). In recent years, a third category of genes that control the cell death or **apoptosis** are also believed to be involved in carcinogenesis.

Oncogenes

The genes capable of causing cancer are known as **oncogenes** (Creek: oncos — tumor or mass). Oncogenes were originally discovered in tumor causing viruses. These viral oncogenes were found to be closely similar to certain genes present in the normal host cells which are referred to as proto-oncogenes. Now, about 40 viral and cellular proto oncogenes have been identified. Protooncogenes encode for growth-regulating proteins. The activation of protooncogenes to oncogenes is an important step in the causation of cancer.

Activation of protooncogenes to oncogenes

There are several mechanisms for converting the protooncogenes to oncogenes, some of the important ones are described hereunder.

1. Viral insertion into chromosome. When certain retroviruses (genetic material RNA) infect cells, a complementary DNA (cDNA) is made fro their RNA by the enzyme reverse transcriptase. The cDNA so produced gets inserted into the host genome. The integrated double-stranded cDNA is referred to as provirus. This pro-viral DNA takes over the control of the transcription of cellular chromosomal DNA and transforms the cells. Activation of protooncogene *myc* to oncogene by viral insertion ultimately causing carcinogenesis is well known (e.g. avian leukemia). Some DNA viruses also get inserted into the host chromosome and activate the protooncogenes.

2. Chromosomal translocation. Some of the tumors exhibit chromosomal abnormalities. This is due to the rearrangement of genetic material (DNA) by chromosomal translocation i.e. splitting off a small fragment of chromosome which is joined to another chromosome. Chromosomal translocation usually results in overexpression of protooncogenes. Burkitt's lymphoma, a cancer of human B-lymphocytes, is a good example of chromosomal translocation. In this case, a fragment from chromosome 8 is split off and joined to chromosome 14 containing *myc* gene. This results in the activation of inactive *myc* gene leading to the increased synthesis of certain proteins which make the cell malignant.

3. Gene amplification. Severalfold amplifications of certain DNA sequences are observed in some cancers. Administration of anticancer drugs methotrexate (an inhibitor of the enzyme dihydrofolate reductase) is associated with gene amplification. The drug becomes inactive due to gene amplification resulting in a severalfold (about 400) increase in the activity of dihydrofolate reductase.

4. Point mutation. The ras protooncogene is the best example of activation by point mutation (change in a single base in the DNA). The mutated *ras* oncogene produces a protein (GTPase) which differs in structure by a single amino acid. This alteration diminishes the activity of GTPase, a key enzyme involved in the control of cell growth (details described later). The presence of *ras* mutations is detected in several human tumors — 90% of pancreatic, 50% of colon and 30% of lung. However, *ras* mutations have not been detected in the breast cancer.

Mechanism of action of oncogenes

Oncogenes encode for certain proteins, namely **oncoproteins.** These proteins are the altered versions of their normal counterparts and are involved in the transformation and multiplication of cells. Some of the products of oncogenes are discussed below.

Growth factors

Several growth factors stimulating the proliferation of normal cells are known. They regulate cell division by transmitting the message across the plasma membrane to the interior of the cell (transmembrane signal transduction). It is believed that growth factors play a key role in carcinogenesis. The cell proliferation is stimulated by growth factors. In general, a growth factor binds to a protein receptor on the plasma membrane. This binding activates cytoplasmic protein kinases leading to the phosphorylation of intracellular target proteins. The phosphorylated proteins, in turn, act as intracellular messengers to stimulate cell division the mechanism of which is not clearly known.

Transforming growth factor (TGF-α) is a protein synthesized and required for the growth epithelial cells. TGF-α is produced in high concentration in individuals suffering from psoriasis, disease characterized by excessive proliferation epidermal cells.

Growth factor receptors. Some oncogenes encoding growth factor receptors have be identified. Overexpression and/or structural alterations in growth factor receptors are associated with carcinogenesis. For instance, the overexpression of gene *erb*-B, encoding EGF-receptor observed in lung cancer.

GTP-binding proteins. These are a group of signal transducing proteins. Guanosine triphosphate (GTP)-binding proteins are found in about 30% of human cancers. The mutation of *ras* protooncogene is the single-most dominant cause many human tumors.

The inactive *ras* is in a bound state with GDP. When the cells are stimulated by growth factors, *ras* P_{21} gets activated by exchanging GDP for GTP. This exchange process is catalysed by guanine nucleotide releasing factor (GRF). The active *ras* P_{21} stimulates regulators such as cytoplasmic kinases, ultimately causing DNA replication and cell division. In normal cells, the activity of *ras* P_{21} is shortlived. The GTPase activity, which is an integral part (intrinsic) of *ras* P_{21} hydrolyses GTP to GDP, reverting *ras* 21 to the original state. There are certain proteins, namely GTPase activating proteins (GAP), which accelerate the hydrolysis of GTP of *ras* P_{21}. Thus, in normal cells, the activity of *ras* P_{21} is well regulated. Point mutations in *ras* gene result in the production of altered *ras* P_{21}, lacking GTPase activity. This leads to the occurrence of *ras* P_{21} in a permanently activated state, causing uncontrolled multiplication of cells.

Non-receptor tyrosine kinases. These proteins are found on the interior of the inner plasma membrane. They phosphorylate the cellular target proteins (involved in cell division) in response to external growth stimuli. Mutations in the proto-oncogenes (e.g. *abl)* encoding non-receptor tyrosine kinases increase the kinase activity and, in turn, phosphorylation of target proteins causing unlimited cell multiplication.

Antioncogenes

A special category of genes, namely **cancer suppressor genes** or, more commonly, antioncogenes, have been identified. The products of these genes apply breaks and regulate cell proliferation. The loss of these suppressor genes removes the growth control of cells and is believed to be a key factor in the development of several tumors, e.g. retinoblastoma, one type of breast cancer, carcinoma of lung, Wilms' kidney tumor.

With the rapid advances in the field of genetic engineering, introducing antioncogenes to a normal chromosome to correct the altered growth rate of cells may soon become a reality.

Genes that regulate apoptosis

A new category of genes that regulate **programmed cell death** (apoptosis) have been discovered. These genes are also important in the development of tumors.

The gene, namely *bcl—2,* causes B-cell lymphoma by preventing programmed cell death. It is believed that over-expression of *bcl—2* allows other mutations of protooncogenes that, ultimately, leads to cancer.

Unified hypothesis of carcinogenesis

The multifactorial origin of cancer can be suitably explained by oncogenes. The physical and chemical agents, viruses and mutations all lead to the activation of oncogenes causing carcinogenesis. The antioncogenes and the genes regulating apoptosis are intimately involved in development of cancer.

Tumor markers

The biochemical indicators employed to **detect the presence of cancers** are collectively referred to as tumor markers. These are the abnormally produced molecules of tumor cells such as surface antigens, cytoplasmic proteins, enzymes and hormones. Tumor markers can be measured in serum (or plasma). In theory, the tumor markers must ideally be useful for screening the population to detect cancers. In practice, however, this has not been totally true. As such, the tumor markers support the diagnosis of cancers, besides being useful for monitoring the response to therapy and for the early detection of recurrence.

1. Carcinoembryonic antigen (CEA). This is a complex glycoprotein, normally produced by the embryonic tissue of liver, gut and pancreas. The presence of CEA in serum is detected in several cancers (colon, pancreas, stomach, lung). In about 67% of the patients with colorectal cancer, CEA can be identified. Unfortunately, serum CEA is also detected in several other disorders such as alcoholic cirrhosis (70%), emphysema (57%) and diabetes mellitus (38%). Due to this, CEA lacks specificity for cancer detection. However, in established cancer patients (particularly of colon and breast), the serum level of CEA is a useful indicator to detect the burden of tumor mass, besides **monitoring the treatment.**

2. Alpha-fetoprotein (AFP). It is chemically a glycoprotein, normally synthesized by yolk sac in early fetal life. Elevation in serum levels of AFP mainly indicates the cancers of liver and germ cells of testis and, to some extent, carcinomas of lung, pancreas and colon. As is the case with CEA, alpha-fetoprotein is not specific for the detection of cancers. Elevated levels of AFP are observed in cirrhosis, hepatitis and pregnancy. However, measurement of serum AFP provides a sensitive index for tumor therapy and detection of recurrence.

Characteristics of growing tumor cells

Knowledge on the alterations in the biochemical profile of tumor cells guides in the selection of chemotherapy of cancers.

1. General and morphological changes

a. Shape of cells. The tumor cells are much rounder in shape compared to normal cells.

b. Alterations in cell structures. The cytoskeletal structure of the tumor cells with regard to actin filaments is different.

c. Loss of contact inhibition. The normal cells are characterized by contact inhibition i.e. they form monolayers. Further, they cannot move away from each other. The cancer cells form multilayers due to loss of contact inhibition.

As a result, the cancer cells freely move and get deposited in any part of the body, a property referred to as **metastasis.**

d. Loss of anchorage dependence. The cancer cells can grow without attachment to the surface. This is in contrast to the normal cells which firmly adhere to the surface. Alteration in the structure of a protein, namely **vinculin,** is said to be responsible for the loss of anchorage property in cancer cells.

e. Alteration in permeability properties. The tumor cells have altered permeability and transport across the membranes.

2. Biochemical changes

a. Increased replication and transcription. The synthesis of DNA and RNA is increased in cancer cells, indicating an increase in anabolic processes.

b. Increased glycolysis. The, fast growing tumor cells are characterized by elevation in aerobic and anaerobic glycolysis. This truly reflects the increased energy demands of multiplying cells.

c. Decreased pyrimidine metabolism. A reduction in the catabolic reactions such as degradation of pyrimidines is observed in tumor cells.

d. Enzyme alterations. The activities of certain enzymes are changed e.g. proteases.

e. Reduced requirement of growth factors. The tumor cells require much less quantities of growth factors. Despite this fact, there is an increased production of growth factors by these cells.

f. Synthesis of fetal proteins. During fetal life, certain genes are active, leading to the synthesis of specific proteins. These genes are suppressed in adult cells. However, the tumor cells synthesize the fetal proteins e.g. carcinoembryonic antigen, alfa fetoprotein.

g. Alterations in the structure of molecules. Changes in the structure of glycoproteins and glycolipids are observed.

h. Reduced synthesis of certain molecules. A diminished synthesis of specialized proteins is seen in tumor cells.

i. Changes in isoenzymes. The isoenzyme profile of cancer cells is close to the fetal pattern.

j. Alterations in antigens. A loss of regularly occurring antigens coupled with the appearance of new antigens in tumor cell is reported.

Metastasis

Metastasis refers to the spread of cancer cell from the primary site of origin to other tissues of the body where they get deposited and grow as secondary tumors. Metastasis is the major cause c cancer related morbidity and mortality. The biochemical basis of metastasis is not clearly known. It is believed that the morphologic; changes in tumor cells, loss of contact inhibition, loss of anchorage dependence and alterations in the structure of certain macromolecules are among the important factors responsible for metastasis.

LECTURE 36

METABOLISM OF XENOBIOTICS. LIVER FUNCTION TESTS. RENAL FUNCTION TESTS

Man is continuously exposed to several foreign compounds such as drugs, pollutants, food additives, cosmetics, pesticides etc. Certain unwanted compounds are produced in the large intestine by the bacteria which enter the circulation. These include indole from tryptophan, cadaverine from lysine, tyramine from tyrosine, phenol from phenylalanine etc. In the normal metabolism of the body, certain waste compounds (e.g. bilirubin) are formed. A vast majority of the foreign compounds or the unwanted substances, produced in the body, are toxic and, therefore, they should be quickly eliminated from the body.

The term **detoxication or detoxification** refers to **the series of biochemical reactions occurring in the body to convert the foreign (often toxic) compounds to non-toxic or less toxic and more easily excretable forms**.

Detoxication is rather misleading, since sometimes a detoxified product is more toxic than the original substance. It appears that the body tries to get rid of a foreign substance by converting it into a more soluble (often polar) and easily excretable compound, and this may be sometimes associated with increased toxicity (e.g. conversion of methanol to formaldehyde).

In recent years, the term detoxification is replaced by **biotransformation** or metabolism of xenobiotics (Greek: xenos — strange, foreign) or simple **metabolism of foreign compounds**.

Site of detoxification

The detoxification reactions are carried out mainly in the liver which is equipped with the enzyme machinery. Kidney and other organs may sometimes be involved. The products formed by detoxification are mostly excreted by the **kidney**, less frequently excreted via **feces** or expired **air**.

MECHANISM OF DETOXIFICATION

The metabolism of xenobiotics may be divided into two phases which may occur together or separately.

Phase I. The reactions of phase I are **oxidation**, **reduction** and **hydrolysis**.

Phase II. These are the conjugation reactions, involving compounds such as glucuronic acid, amino acids (glycine), glutathione, sulfate, acetate and methyl group.

Generally, detoxification of a compound involves phase I as well as phase II reactions. For instance, oxidation followed by conjugation is the most frequent process in the metabolism of xenobiotics.

Oxidation

A large number of foreign substances are detoxified by oxidation. These include alcohols, aldehydes, amines, aromatic hydrocarbons and sulfur compounds. In general, **aliphatic compounds are more easily oxidized than aromatic**.

Role of cytochrome P_{450}

Most of the oxidation reactions of detoxification are catalyzed by monooxygenase or cytochrome P_{450}. This enzyme, also called **mixed function oxidase**, is associated with the microsomes. The usage P_{450} refers to the absorption peak (at 450 nm), exhibited by the enzyme when exposed to carbon monoxide.

Most of the reactions of cytochrome P_{450} involve the addition of a hydroxyl group to aliphatic or aromatic compounds which may be represented as $RH+O_2+NADPH \rightarrow ROH+H_2O+NADP^+$

Salient features of cytochrome P_{450}

1. Multiple forms of cytochrome P_{450} are believed to exist, ranging from 20 to 200. At least 6 species have been isolated and worked in detail.

2. There are all hemoproteins, containing heme as the prosthetic group.

3. Cytochrome P_{450} species are found in the highest concentration in the microsomes of liver. In the adrenal gland, they occur in mitochondria.

4. The mechanism of action of cytochrom P_{450} is complex and is dependent on NADPH (see lecture № 10).

5. The phospholipids-phosphatidylcholine is a constituent of cytochrome P_{450} system which is necessary for the action of this enzyme.

6. Cytochrome P_{450} is an inducible enzyme. Its synthesis is increased by the administration of drugs such as phenobarbitol.

Hydrolysis

The hydrolysis of the bonds such as ester, glycoside and amide is important in the metabolism of xenobiotics. Several compounds undergo hydrolysis during the course of their detoxification. These include aspirin, acetanilide, diisopropylfluorophosphate, atropine and procaine.

Conjugation

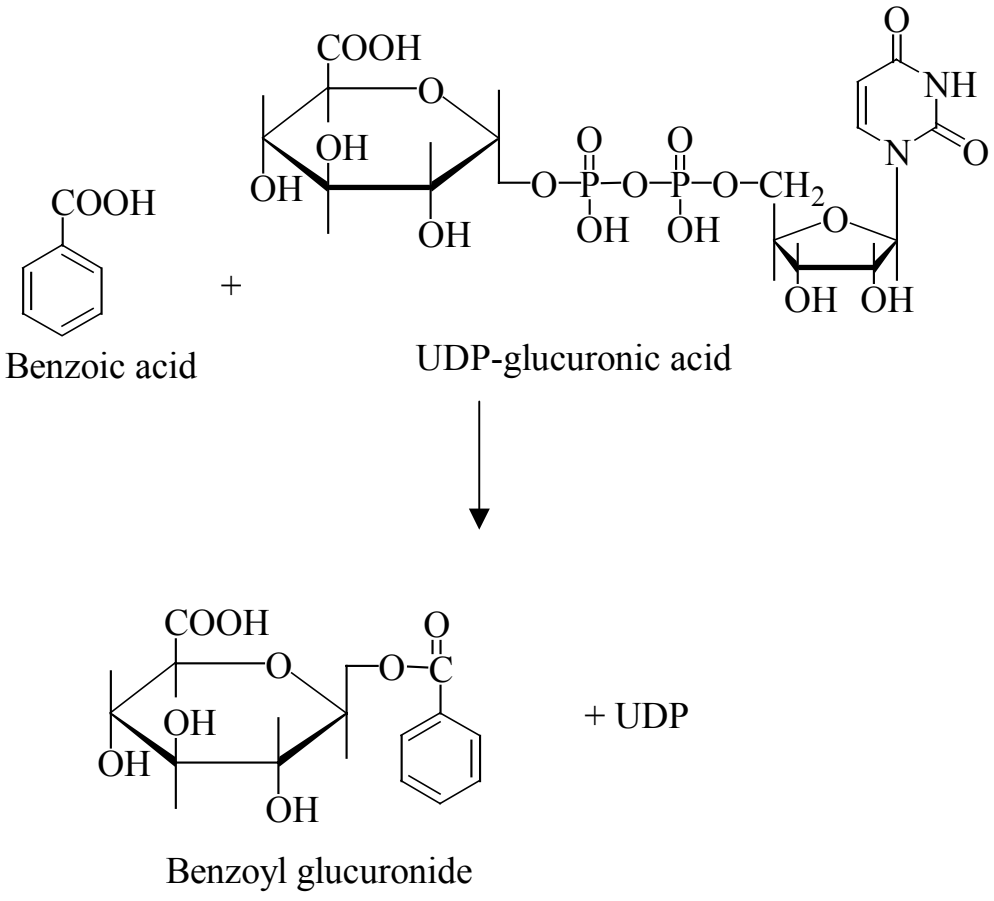
Several xenobiotics undergo detoxification by conjugation to produce less toxic and/or more easily excretable compounds. Conjugation is the process in which a foreign compounds combines with a substance produced in the body. The process of conjugation may occur either directly or after the phase I reactions. At least 8 different conjugating agents have been identified in the body. There are glucuronic acid, glucine, cyateine, glutamine, methyl group, sulfate, acetic acid and thiosulfate.

1. Glucuronic acid. Conjugation with glucuronic acid is the most common. The active form of glucuronic acid is **UDP-glucuronic** acid produced in the uronic acid. The microsomal enzymes **UDP-glucuronyl transferases** participate in glucuronide formation. A general reaction of glucuronide conjugation is shown below (X-OH represents xenobiotic).

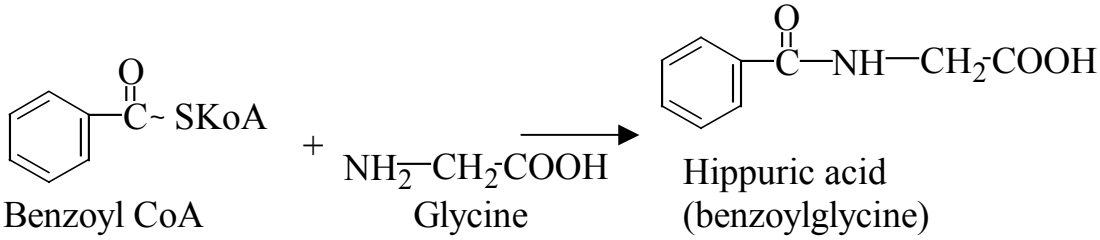
X-OH + UDP~glucuronic acid → X-O-glucuronide + UDP

Certain drugs (e.g. barbiturates) when administered induce glucuronyltarnsferase and this increases the glucuronide formation.

Glucuronic acid conjugation may occur with compounds containing hydroxyl, carbonyl, sulfhydryl or amino groups. A few example



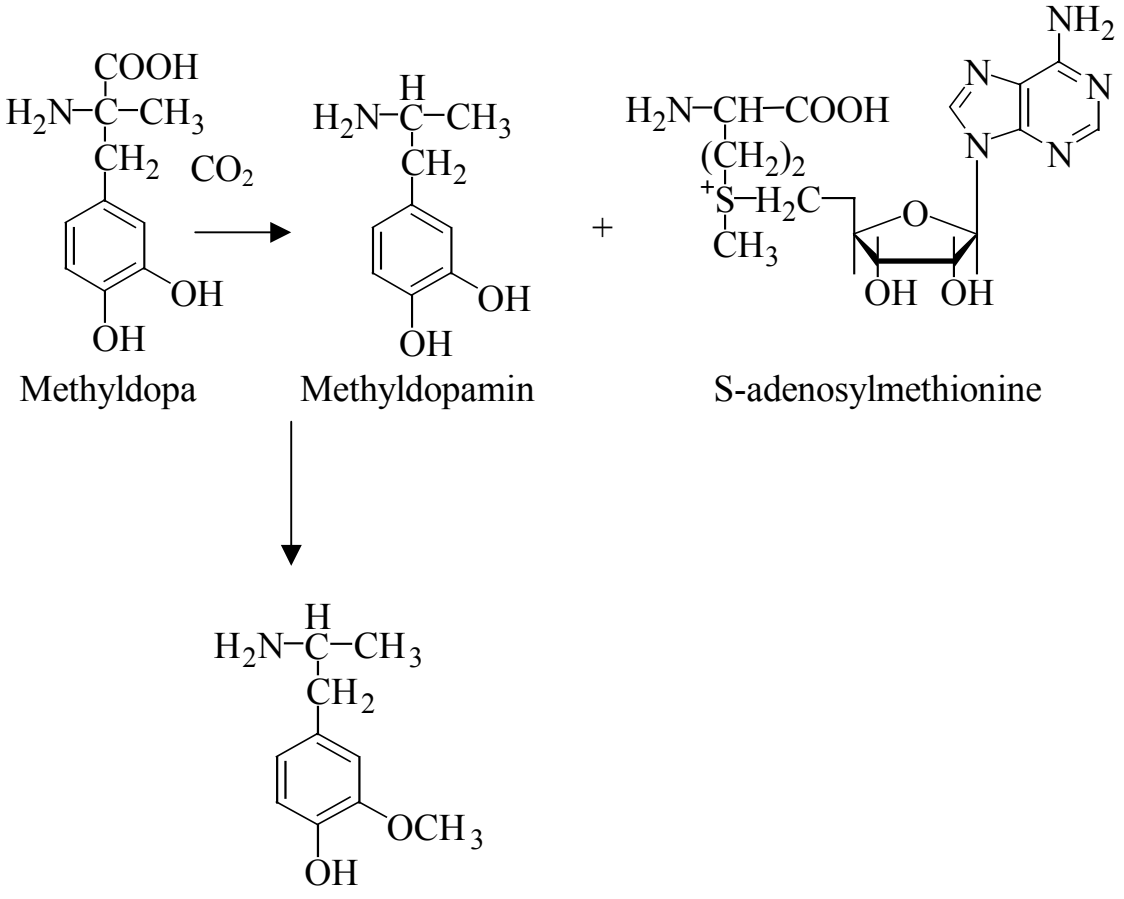
2. Glycine. Many aromatic carboxylic acids (e.g. benzoic acid, phenylacetic acid) are conjugated with glycine. **Hippuric acid** is formed when glycine is conjugated with benzyl CoA. The excretion of hippuric acid (*Greek*: hippos — horse) was first reported in 1829 in the urine of cows and horses.



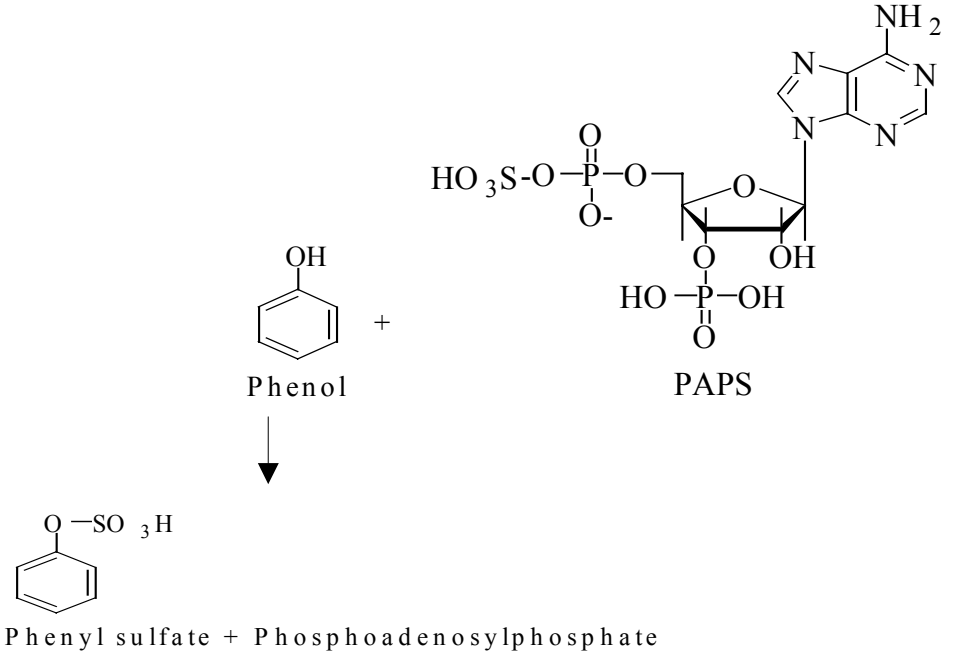
3. Cysteine. Cysteine, required for conjugation process, is derived from the tripeptide glutathione (Glu-Cys-Gly), which is the active conjugating agent. A wide range of organic compounds such as alkyl or aryl halides, alkene, nitro compounds and epoxides get conjugated with cysteine of glutathione.

4. Glutamine. Phenylacetatic acid is conjugated with glutamine to form phenylacetyl glutamine. Conjugation with glutamine is, however, relatively less important.

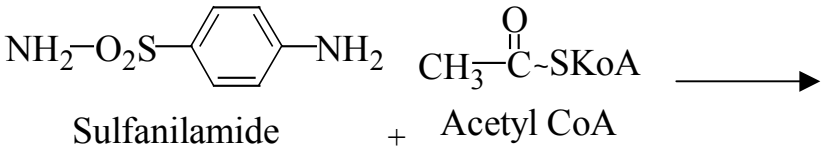
5. Methyl group. The methyl group ($-CH_3$) of S-adenosylmethionine is frequently used to methylate certain xenobiotics. This is catalyzed by the enzyme **methyltransferase**.



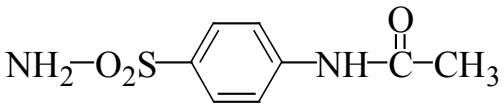
6. Sulfate. The active form of sulfate — 3'-phosphoadenosine-5-phosphosulfate (PAPS) — participates in conjugation reactions and the enzyme **sulfotransferase** is involved in this process. Several aliphatic and aromatic compounds undergo sulfation.



7. Acetic acid. Acetyl CoA is the active form of acetic acid that takes part in conjugating reactions.



Sulfanilamide + Acetyl CoA



Acetyl sulfanilamide

8. Thiosulfate. The highly toxic cyanides are conjugated with thiosulfate to form less toxic thiocyanide.

ORGAN FUNCTION TESTS

Each organ of the body has to perform its biochemical functions to keep the body, as a whole, in a healthy state. This is possible only when the cells of the organ are intact in structure and function. Any abnormality in the tissue, caused by exogenous or endogenous factors, will seriously impair the organ function which, in turn, influences the health of the organism.

Based on the functional capabilities of the organs, **specific biochemical investigations** have been developed **in the laboratory**, to assess their function.

LIVER FUNCTION TESTS

Liver performs several diversified functions. It is the central organ of body's metabolism.

Functions of liver

1. Metabolic functions. Liver actively participates in carbohydrates, lipid, protein, mineral and vitamin metabolism.

2. Excretory functions. Bile pigments, bile salts and cholesterol are excreted in the bile into intestine.

3. Protective functions and detoxification. Kupffer cells of liver perform phagocytosis to eliminate foreign compounds. Ammonia is detoxified to urea. Liver is responsible for the metabolism of xenobiotics.

4. Hematological functions. Liver participates in the formation of blood (particularly in the embryo), synthesis of plasma proteins (including blood clotting factors) and destruction of erythrocytes.

5. Storage functions. Glycogen, vitamin A, D and B_{12} and trace element iron are stored in liver.

Test to assess liver function

The liver function tests (LFT) are the biochemical investigations to assess the capacity of the liver to carry out any of the functions it performs. LFT will help to detect the abnormalities and the extent of liver damage.

Two important facts should be borne in mind while carrying out LFT.

1. Liver is a large-size factory of safety. Therefore, it can perform many of its functions almost normally, despite the damage.

2. Selection of the right test is important in LFT. This is due to the fact that since liver participates in several functions, the function that is measured in LFT may not be the one that is adversely affected.

The major liver function tests may be classified as follows

1. Test based on excretory functions — measurement of bile pigments, bile salts, bromosulphthalein.

2. Tests based on serum enzymes derived from liver — determination of transaminases, alkaline phosphatase, 5'-nucleotidase, γ-glutamyltranspeptidase.

3. Tests based on synthetic functions — prothrombin time, serum albumin.

4. Tests based on detoxification — hippuric acid synthesis.

Serum enzymes derived from liver

Liver cells contain several enzymes which may be released into the circulation in liver damage. Measurement of selected enzymes in serum is often used to assess the liver function. It must, however, be noted that there is no single enzyme that is absolutely specific to liver alone. Despite this fact, serum enzymes provide valuable information for LFT.

1. Transaminases or aminotransferases. The activities of two enzymes — namely serum glutamate pyruvate transaminase (recently called as **alanine transaminase** — ALT) and serum glutamate oxaloacetate transaminase (recently known as aspartate transaminase — AST) — are widely used to assess the liver function. ALT is a cytoplasmic enzyme while AST is found in both cytoplasm and mitochondria. The activity of these enzymes is low in normal serum (ALT 5—25 U/L; AST 5—35 U/L). Serum ALT and AST are increased in liver damage. However, alanine transaminase is more sensitive and reliable for the assessment of LFT. Estimation of serum transaminases cannot identify the **causes (etiology)** of **hepatic damage**. Further, they do not have much prognostic value.

2. Alkaline phosphatase. Alkaline phosphatase (ALP) is mainly derived from bone and liver (the cell lining the bile canaliculi). A rise in serum ALP usually associated with elevated serum bilirubin is an indicator of biliary obstruction (obstructive jaundice or cholestasis). ALP is also elevated in cirrhosis of liver and hepatic tumors.

3. γ-Glutamyl transpeptidase. This is a microsomal enzyme widely distributed in body tissues, including liver. Measurement of γ-glutamyl transpeptidase (GGT) activity provides a sensitive index to asses liver abnormality. The activity of this enzyme almost parallels that of transaminases in hepatic damage. Serum GGT is highly elevated (normal 10—15 U/L) in biliary obstruction and alcoholism. Further, several drugs (e.g. phenytoin) induce (liver synthesis) and increase this enzyme in circulation.

4. 5'-nucleotidase. The serum activity of 5'-nucleotidase (normal 2—15 U/L) is elevated in hepatobiliary disease and this parallel ALP. The advantage with 5'-nucleotidase is that it is not altered in bone disease.

5. Serum insocitrate dehydrogenase and **isoenzymes of lactate dehydrogenase** (LDH_4 and LDH_5) are also useful in LFT.

KIDNEY (RENAL) FUNCTION TESTS

The kidneys are the vital organs of the body, performing the following major functions.

1. Maintenance of homeostasis. The kidneys are largely responsible for the regulation of water, electrolyte and acid-base balance in the body.

2. Excretion of metabolic waste products. The end products of protein and nucleic acid metabolism are eliminated from the body. These include urea, creatinine, creatine, uric acid, sulfate and phosphate.

3. Retention of substances vital to body. The kidneys reabsorb and retain several substances of biochemical importance in the body e.g. glucose, amino acids etc.

4. Hormonal functions. The kidneys also function as endocrine organs by producing hormones.

a. Erythropoeietin, a peptide hormone, stimulates hemoglobin synthesis and formation of erythrocytes.

b. 1,25-Dihydroxycholecalciferol **(calcitriol)** —the biochemically active form of vitamin D — is finally produced in the kidney. It regulates calcium absorption from the gut.

c. Renin, a proteolytic enzyme liberated by kidney, stimulates the formation of angiotensin II which, in turn, leads to aldosteron production. Angiotensin II and aldosteron are the hormones involved in the regulation of electrolyte balance.

The formation of urine

Nephron is the functional unit of kidney. Each kidney is composed of approximately one million nephrons. The structure of a nephrone, as depicted in Fig. 36—1, consists of a Bowman's capsule (with blood capillaries), proximal convoluted tubule (PCT), loop of Henle, distal convoluted tubule (DCT) and collecting tubule.

The blood supply to kidneys is relatively large. About 1200 ml of blood (650 ml plasma) passes through the kidneys, every minute. From this, about **120—125 ml is filtered per minute by the kidneys** and this referred to as **glomerular filtration rate (GFR)**. With a normal GFR (120—125 ml/min), the glomerular filtrate formed in an adult is about 175—180 litres per day, out of which only 1.5 litres is excreted as urine. Thus, more than 99% of the glomerular filtrate is reabsorbed by the kidney.

The process of urine formation basically involves two steps — glomerular filtration and tubular reabsorption.

1. Glomerular filtration. This is a passive process that results in the formation of ultrafiltrate of blood. All the (unbound) constituents of plasma, with a molecular weight less than about 70000, are passed into the filtrate. Therefore, the **glomerular filtrate is almost similar in composition to plasma.**

2. Tubular reabsorption. The renal tubules (PCT, DCT and collecting tubules) retain water and most of the soluble constituents of the glomerular filtrate by reabsorption. This may occur either by passive or active process. The excreted urine has an entirely different composition compared to glomerular filtrate from which it is derived.

Renal threshold substances

There are certain substances in the blood whose excretion in urine is dependent on their concentration. Such substances are referred to as **renal threshold substances**. At the normal concentration in the blood, they are completely reabsorbed by the kidney, with a result that their excretion in urine is almost negligible.

The renal threshold of **a substance** is defined as its **concentration in blood (or plasma) beyond** which it is **excreted into urine**. The renal threshold for glucose is 180 mg/dl; for ketone bodies 3 mg/dl; for calcium 10 mg/dl and for bicarbonate 30 mEq/l. While calculating the renal threshold of a particular compound, it is assumed that both the kidneys are optimally functioning, without any abnormality. But this is not always true — in which case the renal threshold is altered. For instance, renal glycosuria is associated with reduced threshold for glucose due to its diminished tubular reabsorption.

The term tubular maximum (Tm) is used to indicate the maximum capacity of the kidneys to absorb a particular substance. For instance, tubular maximum for glucose (TmG) is 350 mg/min.

Test for assess renal function

In view of the important and sensitive functions the kidney performs, it is essential that the abnormalities (renal damage), if any, must be detected at the earliest. Several tests are employed in the laboratory to assess kidney (renal) function. It must, however, be remembered that about two-thirds of the renal tissue must be functionally damaged to show any abnormality by these tests. The kidney function tests may be divided into four groups.

1. Glomerular function tests. All the clearance tests (inulin, creatinine, urea) are included in this group.

2. Tubular function tests. Urine concentration of dilution test, urine acidification test.

3. Analysis of blood/serum. Estimation of blood urea, serum creatinine, protein and electrolyte are often useful to assess renal function.

4. Urine examination. Simple routine examination of urine for volume, pH, specific gravity, osmolality and presence of certain abnormal constituents (proteins, blood, ketone bodies, glucose etc.) also help, of course to a limited degree, to assess kidney functioning.

Clearance tests

The clearance tests, measuring the glomerular filtration rate (GFR) are the most useful in assessing the renal function. The excretion of a substance can be expressed quantitatively by using the concept of clearance.

Clearance, in general, is defined as the **volume of plasma that would be completely cleared of a substance per minute**. In other words, **clearance of a substance refers to the milliliters of plasma which contains the amount of that substance excreted by kidney per minute**. Clearance (C), expressed as ml/minute, can be calculated by using the formula

$$C = \frac{U \times V}{P}$$

Where U = Concentration of the substance in urine

V = Volume of urine in ml excreted per minute

P = Concentration of the substance in plasma

Care should be taken to express the concentrations of plasma and urine in the same units (mmol/l or mg/dl).

The clearance of a given substance is determined by its mode of excretion. The maximum rate at which the plasma can be cleared of any substance is equal to the GFR. This can be easily calculated by measuring the clearance of a plasma compound which is freely filtered by the glomerulus and is neither absorbed nor secreted in the tubule. **Inulin** (a plant carbohydrate, composed of fructose units) satisfies this criteria. Inulin is intravenously administered to measure GFR.

In practice, however, measurement of clearance for the substances already present in the blood is preferred. The two compounds, namely **creatinine** and **urea**, are commonly employed for this purpose. Creatinine clearance (~ 145 ml/min) is marginally higher than the GFR as it is secreted by the tubules. On the other hand, urea clearance (~ 75 ml/min) is less than the GFR, since it is partially reabsorbed by the tubules.

Diodrast (diidopyridone acetic acid) is used as a contrast medium to take urinary tract X-rays. Diodrast and para amino hippuric acid (PAH) are peculiar substances as they are entirely excreted by a single passage of blood through the kidneys. It is partly filtered by the glomerulus and mostly excreted by the tubules.

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