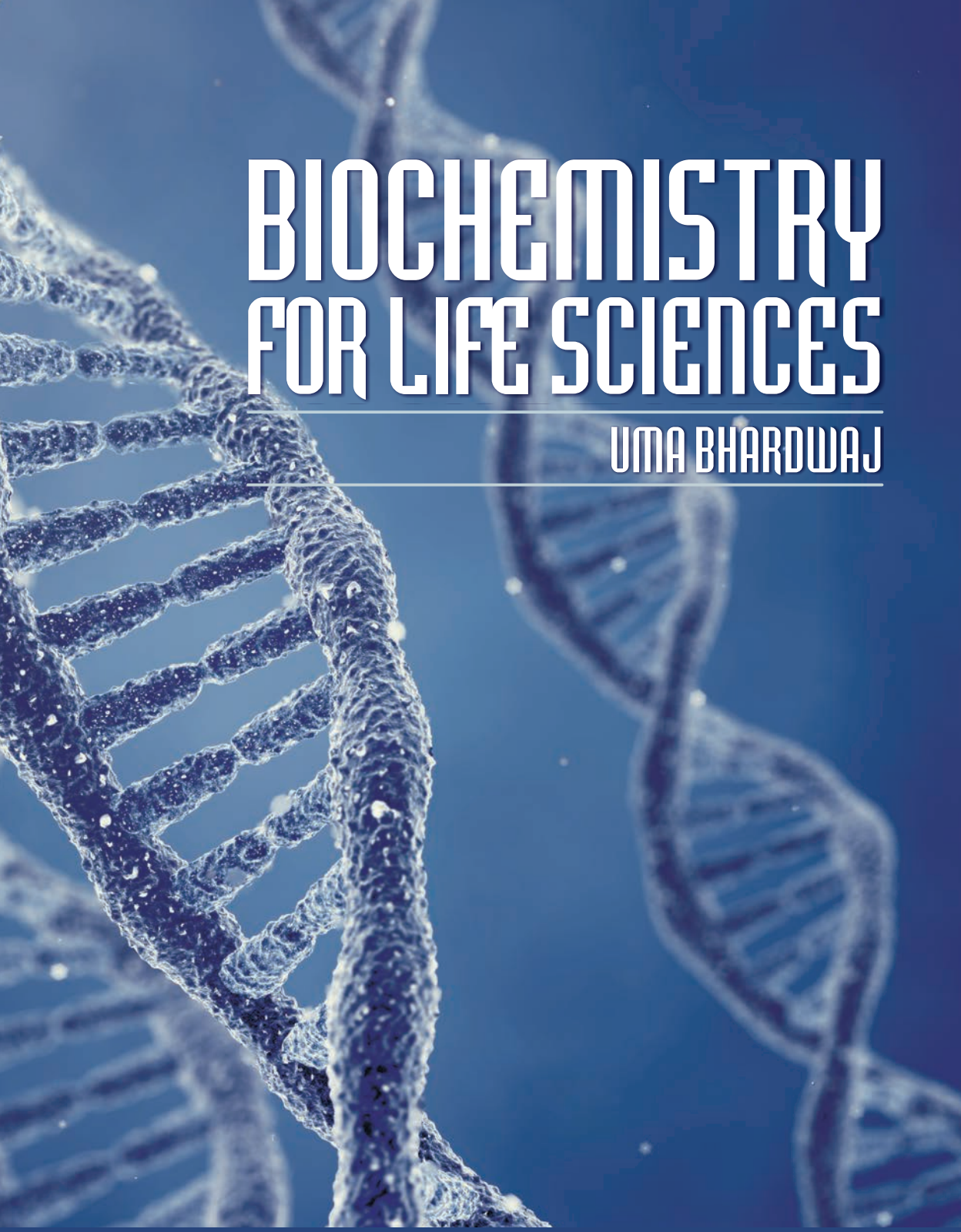

BIOCHEMISTRY FOR LIFE SCIENCES

UMA BHARDWAJ

ALWAYS LEARNING

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Biochemistry for Life Sciences

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Biochemistry for Life Sciences

Dr Uma Bhardwaj
Professor of Biochemistry

PEARSON

Delhi • Chennai

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Dedicated to

My Father Dr P.L. Dwivedi and Mother Mrs Laxmi Dwivedi

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Preface

Biochemistry as a subject is more than 50 years old and deals with physico-chemical principles of life processes. The aim of this book is to make sure that the students first learn the necessary chemical and physical background and then the basic principles and observation of biochemistry and molecular biology. The biochemistry is intended to furnish students with a great understanding of the chemical and molecular events involved in biological processes. Biochemistry is the investigation of the molecular basis of life. It provides a basis for careers in, biotechnology, molecular biology or delve into in all branches of the biological sciences. Their success is confirmed in the mammoth impact that the biochemical approach has had on the life sciences. At the commencement of the twenty-first century, the profundity and wideness of this influence is astounding. The progress in our understanding of living organisms, already huge by the beginning 1990s, is now being exceeded, because of the magnificent advances in DNA-based technologies that are founded on biochemical research.

The subject is vast as the processes and mechanisms are being discovered and reshaped. The genomic revolution is continuously upgrading the knowledge field. The complete medical fraternity has to be armed with the biochemical basis, molecular basis and genomic basis of the disease and their symptoms and identification. Therefore, this book incorporates the biochemical basis and symptoms of diseases.

The most important device that teachers can offer the students is a reasoned understanding of biochemistry. This edition of *Biochemistry for Life Sciences* has to provide a rational and coherent introduction to biochemistry and Molecular Biology.

Organization and Approach This textbook is designed for life science, Biochemistry, Pharmacy, Medical, Microbiology and biotechnology students. To ensure that all students can acquires a evocative understanding of biochemistry, the first three chapters review the principles of relevant topics, such as, Important properties and chemistry of water, pH and Buffers, Bioenergetics and Biological Oxidation, Structure, Composition and functions of Cell and Cell Membrane. Several themes are introduced in these beginning chapters and continued throughout the book. The book is highlighting the relationship between molecular construction and the functional properties of biomolecules. This book also covers the major Biotechniques.

The salient features of the book are listed below:

- First and the only book which covers the topics and content as prescribed by the different universities of India of B.Sc. Zoology, Botany, Microbiology, Biotechnology and Biochemistry. The book covers almost all the topics of Biochemistry of M.Sc. Biochemistry, Microbiology, and Biotechnology.

- This book covers some of the important topics of Molecular Biology which are the part of the curriculum of all B.Sc. and all M.Sc. courses of biological sciences.
- Language of the book is lucid and simple.
- Illustrated with numerous diagrams. The diagrams are simple for better understanding of the students. Some of the color leaflets of the important diagrams are also provided in the book.
- Interpretation and investigation part is described in detail.
- The metabolic disorders are described in detail.

Organization

The book is primarily targeted for B.Sc. and M.Sc., Biochemistry, Microbiology, Biotechnology, Botany and Zoology. Also for B.Tech. Biotechnology and B. Pharmacy students.

Thus, as per the requirements of B.Sc. and M.Sc., of biological sciences syllabus, the book has been divided into seventeen chapters.

Chapter 1 deals with Important Properties and Chemistry of Water, pH and Buffers.

Chapter 2 deals with Bioenergetics and Biological Oxidation.

Chapter 3 deals with Structure, Composition and Functions of Cell and Cell membrane.

Chapter 4 deals with Composition of Carbohydrates.

Chapter 5 deals with Composition of Lipids.

Chapter 6 deals with Composition of Amino Acids.

Chapter 7 deals with Proteins and Peptides.

Chapter 8 deals with Composition of Nucleic Acids.

Chapter 9 deals with Carbohydrate Metabolism.

Chapter 10 deals with Metabolism of Fatty Acids.

Chapter 11 deals with Metabolism of Amino Acids and Proteins.

Chapter 12 deals with Metabolism of Nucleic Acids.

Chapter 13 deals with Enzymes and Co-Enzymes.

Chapter 14 deals with Immunology.

Chapter 15 deals with Composition of Vitamins and Minerals.

Chapter 16 deals with Photosynthesis.

Chapter 17 deals with Harmones.

ACKNOWLEDGEMENTS

A lot of effort has gone into bringing this book into the present shape. The text is based on the lectures to my students during the past 18 years. The language of the book has been kept simple and self-explanatory. Lot of efforts put in to simplified the diagrams so as to interests the students to understand the subject. Both my father and mother have inspired me to write this book, they were my great inner strength otherwise it would not have been possible for me to complete the book. The constant support of my daughter and my husband helped me in completing the book.

The following who have in one way or the other contributed to this book deserves my special appreciation and thanks for supporting me.

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1 Important Properties and Chemistry of Water, pH and Buffers

CHAPTER OBJECTIVES

1.1 Introduction
1.2 Chemistry of Water
1.3 The Law of Mass Action
1.4 Bronsted Acids
1.5 Dissociation of Strong Electrolytes
1.6 Ionization of Weak Acids
1.7 Ionization of Weak Bases
1.8 The Henderson–Hasselbalch Equation
1.9 Titration Curves
1.10 Determination of pK_a
1.11 Buffers
1.12 Physiological Buffers

1.1 INTRODUCTION

Living cells contain carbohydrates, lipids, amino acids, proteins, nucleic acids, nucleotides and related compounds in varying amounts. Although these compounds have an almost infinite number of chemical structures, the mass of these compounds is accounted for almost entirely by only six elements namely carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P) and sulphur (S). Moreover, two of the elements, hydrogen and oxygen, combine to make the most abundant cellular component, H_2O, which does not fall into any of the categories listed above. Over 90 per cent of blood plasma is H_2O; muscle contains about 80 per cent H_2O, and H_2O constitutes more than half of most other plant or animal tissues.

While H_2O is the most abundant cell component, it is also an indispensable compound for life. The nutrients which a cell consumes, the oxygen it uses in oxidation of those nutrients and the waste products it produces are all transported by H_2O. It is useful therefore to note that this familiar, important chemical has a number of exceptional properties that make it peculiarly well suited for its job as the solvent of life.

1.2 CHEMISTRY OF WATER

Many of the physical properties of H_2O are uniquely different. Consider, for example, the group of compounds listed in Table 1.1. These compounds may be compared with H_2O either because they have good solvent properties or because they have the same number of electrons (isoelectronic). As can be seen, H_2O has the highest boiling point, the highest specific heat of vaporization and by far the highest melting point of all these compounds. Pauling has expressed the anomalous behaviour of H_2O in another way by comparing it with the hydrides of other elements in Group VI of the periodic table—H_2S, H_2Se and H_2Te. When this is done, we would predict that H_2O should have a boiling point of –100°C instead of +100°C which it possesses.

Table 1.1 Some Physical Properties of Water and Other Compounds

Substance	Melting point (°C)	Boiling point (°C)	Heat of vaporization (cal/g)	Heat capacity (cal/g)	Heat of fusion (cal/g)
H_2O	0	100	540	1.000	80
Ethanol	–114	78	204	0.581	24.9
Methanol	–98	65	263	0.600	22
Acetone	–95	56	125	0.528	23
Ethyl acetate	–84	77	102	0.459	—
Chloroform	–63	61	59	0.226	—
NH_3	–78	–33	327	1.120	84
H_2S	–83	–60	132	—	16.7
HF	–92	19	360	—	54.7

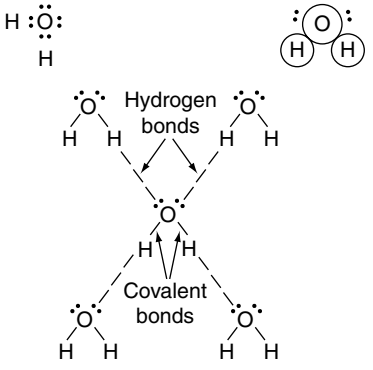


Figure 1.1 Hydrogen bonding in water

The water molecule is highly polarized because the electronegative oxygen atom tends to draw electrons away from the hydrogen atoms, leaving a net positive charge surrounding the proton. Because of this polarization, water molecules behave like dipoles since they can be oriented toward both positive and negative ions. This property in turn accounts for the unusual ability of water to act as a solvent. Positive or negative ions in a crystal lattice can be approached by dipolar water molecules and brought into solution. Once in solution, ions of both positive and negative charge will be surrounded by protective layers of water molecules and further interaction between those ions of opposite charge will be subsequently decreased (Figure 1.1). Glucose is soluble in water as shown in Figure 1.2.

The high boiling and melting points of H_2O and its high heat of vapourization are the result of an interaction between adjacent water molecules known as hydrogen bonding. Briefly put, the

term hydrogen bond refers to the interaction of an hydrogen atom that is covalently bonded to one electronegative atom with a second electronegative atom. There is a tendency for the hydrogen atom to associate with the second electronegative atom by sharing the nonbonded electron pair of that atom, and a weak bond of ~4.5 kcal/mole can exist. (In biological material, the two atoms most commonly involved in hydrogen bonding are nitrogen (N_2) and oxygen (O_2).) In liquid water, small transient chains of water molecules will occur due to this interaction.

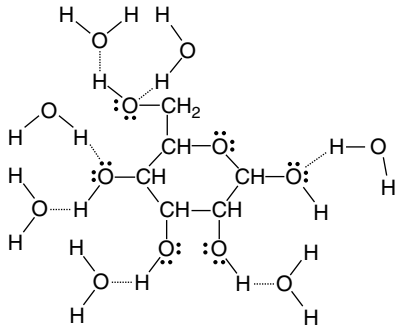


Figure 1.2 Structure of glucose soluble in water

The energy necessary to disrupt the hydrogen bond (4–10 kcal/mole) is much less than that required to break an O–H covalent bond, and in solution hydrogen bonds are broken and formed readily. The additive effect with hydrogen bonding of water is a major factor in explaining many of the unusual properties of H_2O. Thus, the extra energy required to boil water and melt ice may be attributed largely to extensive hydrogen bonding.

Other unusual properties of water make it an ideal medium for living organisms. Thus, the water specific heat capacity required 1.0 calories to raise the temperature of 1 g of water from 15 to 16°C, and is unusually high among several of the solvents just considered (ethanol, 0.58; methanol, 0.6; acetone, 0.53; chloroform, 0.23 and ethyl acetate, 0.46).

Only liquid ammonia is higher at 1.12. The higher the specific heat of a substance, the less the change in temperature which results when a given amount of heat is absorbed by that substance. Thus, H_2O is well designed for keeping the temperature of a living organism relatively constant. It is this property of water that also made the oceans of the earth an ideal environment for the origin of life and evolution of the primeval forms.

The heat of vaporization of H_2O, as already mentioned, is unusually high. Expressed as the specific heat of vaporization (calories absorbed per gram vaporized) the value for water is 540 at its boiling point and even higher at lower temperatures. This high value is very useful in helping the living organism to keep its temperature constant, since a large amount of heat can be dissipated by vaporization of H_2O.

The high heat of fusion of H_2O (80 cal/g compared with 25 for ethanol, 22 for methanol, 17 for H_2S and 23 for acetone) is also of significance in stabilizing the biological environment. While cellular water rarely freezes in higher living forms, the heat released by H_2O on freezing is a major factor in decreasing the actual lowering of the temperature of a body of water during the winter. Thus, a gram of H_2O must give up 80 times as much heat in freezing at 0°C as it does in being lowered from 1 to 0°C just before freezing.

One final example of a property of H_2O that is of biological significance may be cited. This is the fact that H_2O passes through its maximum density at 4°C. That is, H_2O expands on solidifying and ice is less dense. This phenomenon is rare, but its importance in biology has long been recognized. If ice were heavier than liquid H_2O, it would sink to the bottom of the container on freezing. This would mean that oceans, lakes and streams would freeze from the bottom to the top and once frozen would be extremely difficult to melt. Such a situation would obviously be incompatible with those bodies of H_2O serving as the habitat of many living forms as they do.

As it is, however, the warmer, liquid H_2O falls to the bottom of any lake and the ice floats on top where heat from the external environment can reach it and melt it.

Additional properties of water such as high surface tension and a high dielectric constant have significance in biology. Now, we review the law of mass action and the ion product of water.

1.3 THE LAW OF MASS ACTION

For the reaction:

$$A + B \rightleftharpoons C + D \tag{1.1}$$

in which two reactions A and B interact to form two products C and D, we may write the expression

$$K_{eq} = \frac{C_C \cdot C_D}{C_A \cdot C_B} \tag{1.2}$$

This is an expression of the law of mass action, applied to reaction (1.1), which states that at equilibrium, the products of the concentrations of the substances formed in a chemical reaction divided by the product of the concentrations of the reactants in that reaction is a constant known as the equilibrium constant, K_{eq}. This constant is fixed for any given temperature. If the concentration of any single component of the reaction is varied, it follows that the concentration of at least one other component must also change in order to meet the conditions of the equilibrium as defined by K_{eq}.

To be precise we should distinguish between the concentration of the reactants and products in this reaction and the activity or effective concentration of these reactants. It was recognized early that the concentration of a substance did not always accurately describe its reactivity in a chemical reaction. Moreover, these discrepancies in behaviour were appreciable when the concentration of reactants was large. Under these conditions the individual particles of the reactants may exert a mutual attraction on each other or exhibit interactions with the solvent in which the reaction occurs. On the other hand, in dilute solution or low concentration, the interactions are considerably less if not negligible. In order to correct for the difference between concentration and effective concentration, the activity coefficient γ was introduced. Thus

$$a_A = C_A \times \gamma \tag{1.3}$$

where a_A refers to the activity and C_A to the concentration of the substance. The activity coefficient is not a fixed quantity but varies in value depending on the situation under concentration. In very dilute concentrations the activity coefficient approaches unity, because there is little if any solute–solute interaction. At infinite dilution, the activity and the concentration are the same. This is not a serious deviation from accuracy, since the reactants in many biochemical reactions are quite low in concentration. In addition, the H^+ concentration in most biological tissues is approximately 10^{-7} mole/litre, at which concentration the activity coefficient would be unity.

1.3.1 Dissociation of Water and its Ion Product, k_w

Water is a weak electrolyte which dissociates only slightly to form H^+ and OH^- ions:

$$H_2O \rightleftharpoons H^+ + OH^- \tag{1.4}$$

The equilibrium constant for this dissociation reaction has been accurately measured and at 25°C it has the value 1.8×10^{-16} mole/litre. That is

$$K_{eq} = \frac{C_{H^+} C_{OH^-}}{C_{H_2O}} = 1.8 \times 10^{-16}$$

The concentration of H_2O (C_{H_2O}) in pure water may be calculated to be 1000/18 or 55.5 moles/litre. Since the concentration of H_2O in dilute aqueous solution is essentially unchanged from that in pure H_2O, this value may be taken as a constant. It is, in fact, usually incorporated into the expression for the dissociation of water to give

$$\begin{aligned} C_{H^+} C_{OH^-} &= 1.8 \times 10^{-16} \times 55.5 = 1.01 \times 10^{-14} \\ &= K_w = 1.01 \times 10^{-14} \end{aligned} \tag{1.5}$$

at 25°C.

This new constant K_w, termed the ion product of water, expresses the relation between the concentration of H^+ and OH^- ions in aqueous solutions; for example, this relation may be used to calculate the concentration of H^+ in pure water. To do this, let x equal the concentration of H^+. Since in pure water one OH^- is produced for every H^+ formed on dissociation of a molecule of H_2O, x must also equal the concentration of H^-. Substituting in equation (1.5), we have

$$\begin{aligned} x \times x &= 1.01 \times 10^{-14} \\ x^2 &= 1.01 \times 10^{-14} \\ x = C_{H^+} &= C_{OH^-} = 1.0 \times 10^{-7} \text{ mole/litre} \end{aligned}$$

1.3.2 pH

pH may be defined as

$$pH = \log \frac{1}{a_{H+}} = -\log a_{H+} \tag{1.6}$$

where a_{H+} is defined as the activity of H^+. In this text no distinction is made between activities and concentrations, and so

$$pH = \log \frac{1}{[H^+]} = -\log [H^+] \tag{1.7}$$

Moreover, to indicate that we are dealing with concentrations, we use brackets [ ] to indicate them. Thus, the concentration of H^+ (C_{H^+}) is represented as $[H^+]$. We may point out the difference between activities and concentrations by the following example: The pH of 0.1 M HCI when measured with a pH meter is 1.09. This value can be substituted in equation (1.6), as the pH meter measures activities and not concentrations.

$$\begin{aligned} 1.09 &= \log \frac{1}{a_{H+}} \\ a_{H+} &= 10^{-1.09} \\ a_{H+} &= \text{anti} \log \bar{2}.91 \\ a_{H+} &= 8.1 \times 10^{-2} \text{ mole/litre} \end{aligned}$$

Since the concentration of H^+ on 0.1 M HCl is 0.1 mole/litre, the activity coefficient γ may be calculated:

$$\gamma = \frac{a_{H+}}{[H^+]}$$
$$= \frac{0.081}{0.1} = 0.81$$

It is important to stress that the pH is a logarithmic function; thus, when the pH of a solution is decreased one unit from 5 to 4, the H^+ concentration has increased tenfold from 10^{-5} M to 10^{-4} M. When the pH has increased three-tenths of a unit from 6 to 6.3, the H^+ concentration has decreased from 10^{-6} M to 5×10^{-7} M.

If we now apply the term of pH to the ion product expression for pure water, we obtain another useful expression

$$[H^+] \times [OH^-] = 1.0 \times 10^{-14}$$

We take the logarithms of this equation

$$\log [H^+] + \log [OH^-] = \log(1.0 \times 10^{-14})$$
$$= -14$$

Now, we multiply by −1

$$-\log [H^+] - \log[OH^-] = 14$$

If we now define $-\log[OH^-]$ as pOH, a definition similar to that of pH, we have an expression relating the pH and pOH in any aqueous solution

$$pH + pOH = 14 \tag{1.8}$$

1.4 BRONSTED ACIDS

A most useful definition of acids and bases in biochemistry is that proposed by Bronsted. He defined an acid as any substance that can donate a proton and a base as a substance that can accept a proton. Although other definitions of acids, notably one proposed by G. N. Lewis, are even more general, the Bronsted concept should be thoroughly understood by students of biochemistry.

The following substances are examples of Bronsted acids:

$$HCI \rightarrow H^+ + CI^-$$
$$CH_3COOH \rightarrow H^+ + CH_3COO^-$$
$$NH_4^+ \rightarrow NH_3 + H^+$$

And the generalized expression would be

$$HA \rightarrow H^+ + A^-$$

The corresponding bases are now shown reacting with a proton

$$Cl^- + H^+ \rightarrow HCl$$
$$CH_3COO^- + H^+ \rightarrow CH_3COOH$$

$$NH_3 + H^+ \rightarrow NH_4^+$$

The corresponding base for the generalized weak acid HA is

$$A^- + H^+ \rightarrow HA$$

It is customary to refer to the acid–base pair as follows: HA is the Bronsted acid because it can furnish a proton; the anion A^- is called the conjugate base because it can accept the proton to form the acid HA.

1.5 DISSOCIATION OF STRONG ELECTROLYTES

Strong electrolytes are substances that in aqueous solution are dissociated almost completely into charged particles known as ions. Sodium chloride, even in its solid, crystalline form exists as Na^+ ions and Cl^- ions. We may represent the dissociation of NaCl as being complete

$$Na^+Cl^- \rightarrow Na^+ + Cl^-$$

Strong acids and bases are electrolytes that are almost completely dissociated into their corresponding ions in aqueous solution. Thus, hydrochloric acid (HCl), a familiar mineral acid, is completely dissociated in H_2O:

$$HCl \rightarrow H^+ + Cl^- \tag{1.9}$$

We should, however, represent the reaction of HCl in H_2O more accurately as an ionization,

$$HCl + H_2O \rightarrow H_3O^+ + Cl^-$$

in which the electrically neutral HCl has reacted with H_2O to form Cl^- anion and the hydrated proton or hydronium ion, H_3O^+. In the terminology of Bronsted, the Bronsted acid HCl, on ionization, has contributed a proton to the conjugate base H_2O to form a new Bronsted acid (H_3O^+) and the conjugate base of HCl, the chloride ion Cl^-

$$\underset{(\text{Conjugate acid})_1}{HCl} + \underset{(\text{Conjugate base})_2}{H_2O} \longrightarrow \underset{(\text{Conjugate acid})_2}{H_3O^+} + \underset{(\text{Conjugate acid})_1}{Cl^-}$$

It should also be remembered that not only is the proton contributed by HCl hydrated to form the hydronium ion (H_3O^+), but also that the Cl^- is hydrated. It is a common practice to omit the water of hydration in chemical reactions and to represent the ionization of a strong acid like HCl as a simple dissociation according to reaction (1.9).

1.6 IONIZATION OF WEAK ACIDS

A weak acid, in contrast to a strong acid, is only partially ionized in an aqueous solution. Consider the ionization of the generalized weak acid, HA:

$$\underset{(\text{Conjugate acid})_1}{HA} + \underset{(\text{Conjugate base})_2}{H_2O} \rightleftharpoons \underset{(\text{Conjugate acid})_2}{H_3O^+} + \underset{(\text{Conjugate acid})_1}{A^-} \tag{1.10}$$

The proton donated by HA is accepted by H_2O to form the hydronium ion, H_3O^+. The equilibrium constant for this ionization reaction is known as an ionization constant, K_{ion}

$$K_{eq} = K_{ion} = \frac{[H_2O^+][A^-]}{[HA][H_2O]} \tag{1.11}$$

Because, as we have already seen, the concentration of H_2O in aqueous solution is itself a constant, 55.5 moles/litre, we can combine K_{ion} and $[H_2O]$ to obtain a new constant, K_a

$$K_a = K_{100}[H_2O] = [H_3O^+][A^-]/[HA] \tag{1.12}$$

Moreover, because $[H_3O^+]$ is the same as the hydrogen ion concentration, we see that K_a becomes

$$K_a = \frac{[H^+][A^+]}{[HA]} \tag{1.13}$$

This expression in turn is identical with the equilibrium constant we would have written if HA is considered as a weak acid that partially dissociates to yield protons and A^- anions

$$HA \rightleftharpoons H^+ + A^-$$

$$K_{eq} = \frac{[H^+][A^-]}{[HA]} \tag{1.14}$$

1.7 IONIZATION OF WEAK BASES

The ionization of a weak base, defined in the chemical sense as a substance that furnishes OH^- ions on dissociation, can be represented as

$$BOH \rightleftharpoons B^+ + OH^-$$

$$K_{eq} = K_b = \frac{[B^+][OH^-]}{[BOH]} \tag{1.15}$$

For ammonium hydroxide (NH_4OH), the K_b is given in chemical handbooks as 1.8×10^{-5}. It is therefore important to realize that the extent of dissociation of NH_4OH is identical with that of acetic acid (CH_3COOH; $K_a = 1.8 \times 10^{-5}$). The important difference, of course, is that NH_4OH dissociated to form hydroxyl ions (OH^-), whereas CH_3COOH dissociated to form protons (H^+), and that the pH of 0.1 M solutions of these two substances is by no means similar.

One of the most common types of weak base encountered in biochemistry is the group called organic amines (e.g., the amino groups of amino acids). Such compounds, when represented with the general formula R-NH_2, do not contain hydroxyl groups that can dissociate as in reaction (1.15). On the other hand, such compounds can ionize in H_2O to produce hydroxyl ions

$$\underset{(\text{Conjugate acid})_1}{RNH_2} + \underset{(\text{Conjugate base})_2}{H_2O} \rightleftharpoons \underset{(\text{Conjugate acid})_1}{RNH_3^+} + \underset{(\text{Conjugate acid})_2}{OH^-} \tag{1.16}$$

In this reaction, H_2O serves as an acid to contribute a proton to the base RNH_2.

Using the Bronsted definition of a base substance (A^-) that accepts a proton, we can write the general expression

$$\underset{(\text{Conjugate acid})_1}{A^-} + \underset{(\text{Conjugate base})_2}{H_2O} \rightleftharpoons \underset{(\text{Conjugate acid})_1}{HA} + \underset{(\text{Conjugate acid})_2}{OH^-} \tag{1.17}$$

The equilibrium constant K_{ion} for this ionization may be written in analogy with equation (1.11) as

$$K_{ion} = \frac{[HA][OH^-]}{[A^-][H_2O]} \tag{1.18}$$

Combining K_{ion} and $[H_2O]$ as previously we have in analogy with equation (1.12)

$$K_b = \frac{[HA][OH^-]}{[A^-]} \tag{1.19}$$

Equation (1.19) can be used for calculating the $[OH^-]$ of a solution of a weak base; the chemical handbooks list values for the K_b of such substances. The pOH in turn can be calculated and from this the pH may be obtained (equation (1.8)). However, there is a direct relationship between the K_b of a weak base and the K_a of its conjugate acid that is useful in obtaining directly the pH of mixtures of weak bases and their salts.

Solving equation (1.19) for $[OH^-]$, we have

$$[OH^-] = \frac{K_b [A^-]}{[HA]} \tag{1.20}$$

Similarly, solving equation (1.13) for $[H^+]$, we have

$$[H^+] = \frac{K_a [HA]}{[A^-]} \tag{1.21}$$

Then substituting for $[H^+]$ and $[OH^-]$ in the following expression, which was defined earlier (equation (1.5))

$$[H^+][OH^-] = K_w$$

$$\frac{K_a [HA]}{[A^-]} \cdot \frac{K_b [A^-]}{[HA]} = K_w \tag{1.22}$$

which simplifies to $K_a \cdot K_b = K_w$ (1.23)

Substituting the value of K_w at 25°C, we have

$$K_a \cdot K_b = 10^{-14} \tag{1.24}$$

Taking logarithms and multiplying by –1, we have

$$\log K_a + \log K_b = \log K_w$$

$$-\log K_a - \log K_b = -\log K_w \tag{1.25}$$

Then, just as pH has been defined as $-\log [H^+]$, we can define pK_a and pK_b as $-\log K_a$ and $-\log K_b$, respectively. Equation (1.25) then becomes

$$pK_a + pK_b = -\log K_w = 14 \tag{1.26}$$

1.8 THE HENDERSON–HASSELBALCH EQUATION

Henderson and Hasselbalch have rearranged the mass law as it applies to the ionization of weak acids into a useful expression known as the Henderson–Hasselbalch equation.

If we consider the ionization of a generalized weak acid HA

$$HA \rightleftharpoons H^+ + A^-$$

$$K_{ion} = K_a = \frac{[H^+][A^-]}{[HA]}$$

Rearranging the terms, we have

$$[H^+] = K_a \frac{[HA]}{[A^-]}$$

Taking logarithms, we find

$$\log[H^+] = \log\ K_a + \log\frac{[HA]}{[A^-]}$$

and multiplying −1

$$-\log[H^+] = -\log\ K_a - \log\frac{[HA]}{[A^-]}$$

If $-\log K_a$ is defined as pK_a and $\log [A^-]/[HA]$ is substituted for $-\log [HA]/[A^-]$, we obtain

$$pH = pK_a + \log\frac{[A^-]}{[HA]} \tag{1.27}$$

This form of the Henderson–Hasselbalch equation can be written in a more general expression in which we replace $[A^-]$ with the term 'conjugate base and [HA] with conjugate acid':

$$pH = pK_a + \log\frac{[\text{Conjugate base}]}{[\text{Conjugate acid}]} \tag{1.28}$$

This expression may then be applied not only to weak acids such as acetic acid, but also to the ionization of ammonium ions and those substituted amino groups found in amino acids. In this case, NH_4^+ ions or the protonated amino group RNH_3^+ are the conjugate acids which dissociate to form protons and the conjugate bases NH_3 and RNH_2, respectively:

$$NH_4^+ \rightleftharpoons NH_3 + H^+$$

$$RNH_3^+ \rightleftharpoons RNH_2 + H^+$$

Applying equation (1.28) to the protonated amine, we have

$$pH = pK_a + \log\frac{[RNH_2]}{[RNH_3^+]} \tag{1.29}$$

Biochemistry handbooks usually list the K_a (or pK_a) for the conjugate acids of substances we normally consider as bases (e.g., NH_4OH, amino acids, organic amines, etc.). If they do not, the K_b (or pK_b; see equation (1.19)) for the ionization of the weak base will certainly be listed, and

the K_a (or pK_a) must first be calculated before employing the generalized Henderson–Hasselbalch equation. Although more care must be taken to identify correctly the conjugate acid–base pairs in that expression, its usage leads directly to the pH of mixtures of weak bases and their salts.

1.9 TITRATION CURVES

The titration curve obtained when 100 ml of 0.1 N CH_3COOH is titrated with 0.1 N NaOH is shown in Figure 1.3. This curve can be obtained experimentally in the laboratory by measuring the pH of 0.1 N CH_3COOH before and after aliquots of 0.1 N NaOH. The curve may also be calculated by the Henderson–Hasselbalch equation for all the points except the first, where no NaOH has been added, and the last, where a stoichiometric amount (100 ml) of 0.1 N NaOH has been added. Clearly, the Henderson–Hasselbalch equation cannot be used to determine the pH at the limits of the titration where the ratio of salt to acid is either zero or infinite.

In considering the grosser aspects of the titration curve of acetic acid, we see visually that the change in pH per unit of alkali added is greatest at the beginning and end of the titration, whereas the smallest change in pH per unit of alkali added is obtained when the titration is half complete. In other words, an equimolar mixture of sodium acetate and acetate and acetic acid shows less

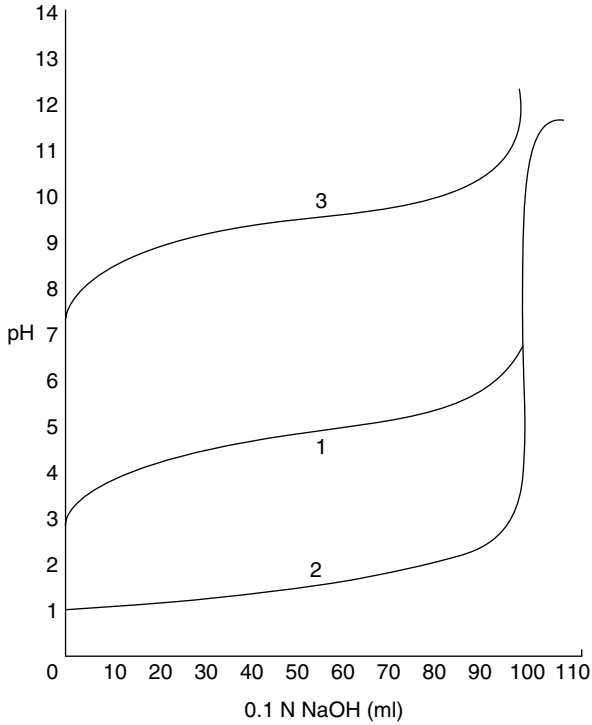


Figure 1.3 Titration curve of 100 ml of 0.1 N CH_3COOH (shown in curve No. 1), titration curve of 100 ml of 0.1 N HCl (shown in curve No. 2) and titration curve of 100 ml of 0.1 N NH_4Cl (shown in curve No. 3) with 0.1 N NaOH

change in pH initially when acid or alkali is added than a solution consisting mainly of either acetic acid or sodium acetate. We refer to the ability of a solution to resist a change in pH as its buffer action, and it can be shown that a buffer exhibits its maximum action when the titration is half complete or when the pH is equal to the pK_a. In Figure 1.3 the point of maximum buffer action is at the pH of 4.74.

Another way of representing the condition which exists when the pH of a mixture of acetic acid and sodium acetate is at the pK_a is to state that the acid at this pH is half ionized. That is, half the 'total acetate' species is present as undissociated CH_3COOH, while the other half is in the form of acetate ion, CH_3COO^-. Since at its pK_a any weak acid will be half ionized, this is one of the most useful ways of distinguishing between individual weak acids. The pK_a is also a characteristic property of each acid, because the ionization constant is a function of the inherent properties of the weak acid. The titration curve of 0.1 N HCI is also represented in Figure 1.3.

The Henderson–Hasselbalch equation is of no use in calculating the curve for HCl, since it applies only for weak electrolytes. However, the pH at any point on the HCl curve can be calculated by determining the milliequivalents of HCl remaining and correcting for the volume. Thus, when 30 ml of 0.1 N NaOH has been added, 7.0 meq of HCl will remain in a volume of 130 ml. The concentration of H^+ will therefore be 7.0/130 or 0.054 M. If the activity coefficient is neglected, the pH may be calculated from equation (1.7) as (1.27). Curve 3 in Figure 1.3 is the titration curve obtained when 100 ml of 0.1 N NH_4CI is titrated with 0.1 N NaOH. In this titration the protons contributed by NH_4^+ are neutralized by the OH^- ions provided by the NaOH

$$NH_4^+ + OH^- \rightarrow NH_3 + H_2O$$

Again, the Henderson–Hasselbalch equation is of no value when calculating the pH of the solution of NH_4Cl before any NaOH has been added. The pH of this solution may be calculated by using equation (1.19) to first obtain the $[OH^-]$. The $[H^+]$ or pH may then be calculated from equation (1.5) or equation (1.8). However, the Henderson–Hasselbalch equation can be employed to determine any point on the curve when some of the NH_4Cl has been neutralized.

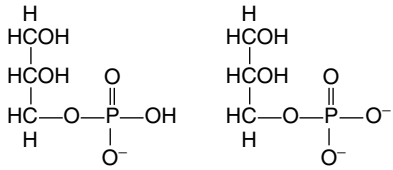
Up to this point, we have considered only the monobasic acid, acetic acid. Polybasic or polyprotic acids, commonly encountered in biochemistry, are acids capable of ionizing to yield more than one proton per molecule of an acid. In each case the extent of dissociation of the individual protons may be described by a K_{ion} or K_a. In the case of phosphoric acid (H_3PO_4) three protons may be furnished on complete ionization of a mole of this acid:

$$H_3PO_4 \rightleftharpoons H^+ + H_2PO_4^- \qquad K_{a1} = 7.5 \times 10^{-3} \qquad pK_{a1} = 2.12$$

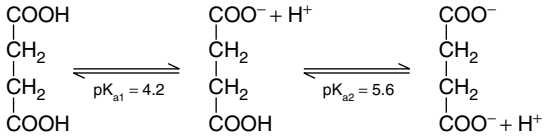
$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-} \qquad K_{a2} = 6.2 \times 10^{-8} \qquad pK_{a2} = 7.21$$

$$HPO_4^{2-} \rightleftharpoons H^+ + HPO_4^{3-} \qquad K_{a3} = 4.8 \times 10^{-13} \qquad pK_{a3} = 12.32$$

This means that at the pH of 2.12 the first ionization of H_3PO_4 is half complete; however, the pH must be 12.32 before the third and final ionization of H_3PO_4 is 50 per cent complete. At the pH of 7.0, which is frequently encountered in the cell, the second proton of phosphoric acid ($pK_{a2} = 7.21$) will be about half dissociated. At this pH both the mono- and dianions of phosphoric esters will be present in approximately equal concentration. For phosphoric acid, the two predominant ionic species will be $H_2PO_4^-$ and HPO_4^{2-}. In the case of α-glycerol phosphate the two following ions will be present in about equal concentration at pH 7.0:



Many of the common organic acids encountered in intermediary metabolism are polyprotic; for example, succinic acid ionizes according to the following scheme



At pH 7.0 in the cell, succinic acid will exist predominantly as the dianion $^{-}OOC–CH_2–CH_2–COO^{-}$. Furthermore, most of the organic acids which serve as their metabolites (palmitate, lactic, and pyruvic acids, for example) will be present as their anions (palmitate, lactate and pyruvate). This has led to the use of the names of the ions when these compounds are discussed in biochemistry. In writing chemical reactions, however, it will be the practice in this text to use the formulas for the undissociated acid.

Table 1.2 lists the pK_as for several of the organic acids commonly encountered in intermediary metabolism.

Table 1.2 The pK_a of Some Organic Acids

	pK_{a1}	pK_{a2}	pK_{a3}
Acetic acid (CH_3COOH)	4.74		
Acetoacetic acid (CH_3COCH_2COOH)	3.58		
Citric acid ($HOOCCH_2C(OH)(COOH)CH_2COOH$)	3.09	4.75	5.41
Formic acid ($HCOOH$)	3.62		
Fumaric acid ($HOOCCH{=}CHCOOH$)	3.03	4.54	
DL-Glyceric acid ($CH_2OHCHOHCOOH$)	3.55		
DL-Lactic acid ($CH_3CHOHCOOH$)	3.86		
DL-Maleic acid ($HOOCCH_2CHOHCOOH$)	3.40	5.26	
Pyruvic acid ($CH_3COCOOH$)	2.50		
Succinic acid ($HOOCCH_2CH_2COOH$)	4.18	5.56	

The meanings of gram molecular weight and gram equivalent weight (mole and equivalent, respectively) and the significance of molarity, molality and normality must be thoroughly understood. Biochemistry is a quantitative science, and the student must recognize immediately terms such as millimole and micromole. In connection with titrations it is also important to remind the student that the H^+ concentrations of 0.1 N H_2SO_4 and 0.1 N CH_3COOH are by no means similar but the 1 litre of each of these solutions contains the same amount of total titratable acid.

1.10 DETERMINATION OF pK_a

The ability to ionize is a valuable property of many biological compounds. Organic acids, amino acids, proteins, pyrimidines and phosphate esters are examples of biochemicals which are ionized to varying degrees in biological systems. Since the pH of most biological fluids is near 7, the extent of dissociation of some of these compounds may be complete there. The first ionization of H_3PO_4 will likewise be complete and the second ionization ($pK_{a2} = 7.2$) will be approximately half complete.

One of the characteristic qualitative properties of a molecule is the pK_a of any dissociable group it may possess. The experimental determination of the pK_a of dissociable groups is therefore an important procedure in describing properties of an unknown substance. The pK_a may be determined in the laboratory by measuring the titration curve experimentally with a pH meter. As known amounts of an alkali or an acid are added to a solution of the unknown, the pH is determined, and the titration curve can be plotted. From this curve the inflection point (pK_a) may be determined by following suitable procedures.

1.11 BUFFERS

With a thorough understanding of the ionization of weak electrolytes it is possible to discuss buffer solutions. Upon addition of an acid or an alkali in a solution, the change in pH is resisted and such a solution is known as a buffer solution. Most commonly, the buffer solution consists of a mixture of a weak Bronsted acid and its conjugate base; for example, mixture of acetic acid and sodium acetate of ammonium hydroxide and ammonium chloride are buffer solutions.

There are many examples of the significance of buffers in biology; the ability to prevent large changes in pH is an important property of most biological organisms. The cytoplasmic fluids which contain dissolved proteins, organic substrates and inorganic salts resist excessive changes in pH. The blood plasma is a highly effective buffer solution almost ideally designed to keep the range of the pH of blood within 0.2 pH unit of 7.2–7.3; values outside this range are not compatible with life. Further appreciation of the buffered nature of the living cell is evidenced that many of the metabolites constantly being produced and utilized in the cell are weak Bronsted acids. In addition, enzymes responsible for the catalysis of reactions in which these metabolites participate exhibit their maximum catalytic action at some definite pH.

In the laboratory, the biochemist also wishes to examine reactions in vitro under conditions where the change in pH is minimal. He or she obtains these conditions by using efficient buffers, preferably insert once, in the reactions under investigation. The buffers may include weak acids such as phosphoric, acetic, glutaric and tartaric acids or weak bases such as ammonia, pyridine and tris-(hydroxymethyl) amino methane.

When alkali (for instance, NaOH) is added to a mixture of acetic acid (CH_3COOH) and potassium acetate (CH_3COOK), the following reaction occurs

$$OH^- + CH_3COOH \rightarrow CH_3COO^- + H_2O$$

This reaction states that OH^- ion reacted with protons furnished by the dissociation of the weak acid and formed H_2O

$$CH_3COOH \rightleftharpoons CH_3COO^- + H^+ \; OH^- \rightarrow H_2O$$

Upon the addition of alkali there is further dissociation of the available CH_3COOH to furnish additional protons and thus to keep the H^+ concentration or pH unchanged.

When an acid is added to an acetate buffer the following reaction occurs

$$H^+ + CH_3COO^- \rightarrow CH_3COOH$$

The protons added (in the form of HCl, for example) combine instantly with the CH_3COO^- anion present in the buffer mixture (as potassium acetate) to form the undissociated weak acid CH_3COOH. Consequently, the resulting pH change is much less than that would occur if the conjugate base was absent.

In discussing the quantitative aspects of buffer action we should point out that two factors determine the effectiveness or capacity of a buffer solution. Clearly, the molar concentration of the buffer components is one of them. The buffer capacity is directly proportional to the concentration of the buffer components. Here we encounter the convention used in referring to the concentration of buffers. The concentration of a buffer refers to the sum of the concentration of the weak acid and its conjugate base. Thus, a 0.1 M acetate buffer could contain 0.05 mole of acetic acid and 0.05 mole of sodium acetate in 1 liter of H_2O. It could also contain 0.065 mole of acetic acid and 0.035 mole of sodium acetate in 1 liter of H_2O.

The second factor influencing the effectiveness of a buffer solution is the ratio of the concentration of the conjugate base to the concentration of the weak acid. Quantitatively it should seem evident that the most effective buffer would be one with equal concentrations of basic and acidic components, since such a mixture could furnish equal quantities of basic or acidic components to react, respectively, with acid or alkali. An inspection of the titration curve for acetic acid (Figure 1.3) similarly shows that minimum change in pH resulting from the addition of a unit of alkali (or acid) occurs at the pK_a for acetic acid. At this pH we have already seen that the ratio of CH_3COO^- to CH_3COOH is 1. On the other hand, at values of pH far removed from the pK_a (and therefore at ratios of conjugate base to acid greatly differing from unity), the change in pH for unit of acid or alkali added is much larger.

Having stated the two factors that influence the buffer capacity, we may consider the decisions involved in selecting a buffer to be effective at the desired pH value, for example pH = 5. Clearly, it would be most desirable to select a weak acid having a pK_a of 5.0. If this cannot be done, the weak acid whose pK_a is closest to 5.0 is the first choice. In addition, it is evident that we should want to use as high a concentration as is compatible with other features of the system. Too high a concentration of salt frequently inhibits the activity of enzymes or other physiological systems, however. The solubility of the buffer components may also limit the concentration which can be employed.

Table 1.3 lists the pK_a; for some buffers commonly employed in biochemistry.

1.12 PHYSIOLOGICAL BUFFERS

This would include the phosphate esters of glycolysis, the organic acids of the Krebs cycle and the free amino acids. In plants, however, certain organic acids—malic, citric and isocitric—can accumulate in the vacuoles and, in that case, play a major role in determining the pH of that part of the cell. Yeasts can also accumulate relatively large concentrations of phosphate esters during glycolysis.

In animals a complex and vital buffer system is found in the circulating blood.

Table 1.3 pK_a Values of Some Commonly Used Buffers

Compound	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
N-(2-acetamido-) iminodiacetic acid (ADA)	6.6			
Acetic acid	4.7			
Ammonium chloride	9.3			
Carbonic acid	6.1	10.3		
Citric acid	3.1	4.7	5.4	
Diethanolamine	8.9			
Ethanolamine	9.5			
Fumaric acid	3.0	4.5		
Glycine	2.3	9.6		
Histidine	1.8	6.0	9.2	
N-2-Hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES)	7.6			
Maleic acid	2.0	6.3		
2-(N-morpholino)-ethanesulphonic acid (MES)	6.2			
Phosphoric acid	2.1	7.2	12.3	
Pyrophosphoric acid	0.9	2.0	6.7	9.4
Triethanolamine	7.8			
Tris-(hydroxymethyl) aminomethane (Tris)	8.0			
N-Tris (hydroxymethyl) methyl-2-amino-ethanesulphonic acid (TES)	7.5			
Veronal (sodium diethylbarbiturate)	8.0			
Versene (ethylenediaminotetraacetic acid)	2.0	2.7	6.2	10.3

The components of this system are CO_2–HCO_3^-; NaH_2PO_4–Na_2HPO_4; the oxygenated and non-oxygenated forms of haemoglobin and the plasma proteins. Two of these components deserve further comment now. Since the pK_{a1} for H_2CO_3 is 6.1, the ratio of conjugate base to weak acid is approximately 20:1 in the normal pH range of 7.35–7.45 of blood. Consequently, one would expect that the H_2CO_3–HCO_3^- buffer system would not be very effective as a buffer, and it is therefore important to emphasize that the H_2CO_3–HCO_3^- buffer is an extremely important buffer of blood. The explanation is found in the fact that the weak acid component, H_2CO_3, is in rapid equilibrium with dissolved CO_2 in the plasma (equation (1.31)). This equilibrium is catalyzed by the enzyme carbonic anhydrase that is found in red blood cells.

$$H_2CO_3 \rightleftharpoons CO_{2diss} + H_2O \tag{1.31}$$

The dissolved CO_2 is in turn in equilibrium with CO_2 in the atmosphere and depending on the partial pressure of CO_2 in the gas phase, will either escape into the air phase (as in the lungs where CO_2 is expired) or will enter the blood (as in the peripheral tissues where CO_2 is produced by respiring cells). Thus the H_2CO_3–HCO_3^- buffer system functions, not by alteration of the ratio

of 20:1 for conjugate base (HCO_3^-) to weak acid (H_2CO_3), but instead by maintaining that ratio at 20:1 and increasing or decreasing the total amount of buffer components ($H_2CO_3 + HCO_3^-$).

The two forms of haemoglobin found in the blood (oxygenated haemoglobin, $HHbO_2$, and unoxygenated haemoglobin, HHb) constitute the other major buffer system of blood. Their buffering capacity, which is due to the imidazole group of histidine residues found in both forms, greatly exceeds that of the other plasma proteins whose buffer action is due to a number of different dissociable groups in those proteins. The haemoglobin in 1 litre of blood can buffer 27.5 meq of H^+ while the plasma proteins will neutralize only 4.24 meq of H^+. The two forms of haemoglobin differ also in the pK_as; $HHbO_2$ is the stronger acid and dissociates with a pK_{a1} of 6.2:

$$HHbO_2 \rightleftharpoons H^+ + HbO_2^-;\ pK_{a1} = 6.2$$

Therefore, in the lungs where the partial pressure of O_2 is high, $HHbO_2$ will predominate over the unoxygenated form and the blood tends to become more acidic. In the peripheral tissues where the partial pressure O_2 is relatively lower, HHb with the higher pK_{a1} of 7.7 will predominate, and the pH will tend to increase:

$$HHb \rightleftharpoons H^+ + Hb^-;\ pK_{a1} = 7.7$$

The two effects are compensated by the low concentrations of CO_2 in the lungs relative to that in the peripheral tissues, and the two effects working in tandem ensure a minimum change in pH.

EXERCISES

1. Define the Henderson–Hasselbalch equation and give the complete derivation of the same.
2. Write the concept of Bronsted acids in detail.
3. The pK_as for H_3PO_4 are $pK_{a1} = 2.1$; $pK_{a2} = 7.2$ and $pK_{a3} = 12.7$. Describe the preparation of a phosphate buffer, pH 6.7, starting with a 0.1 M solution of H_3PO_4 and 0.1 M NaOH.
4. Write short notes on:
   (a) Ionization of weak bases
   (b) Acid–base balance
   (c) Active transport
   (d) Buffers and pH
5. Write the difference between:
   (a) Acid and base
   (b) Acidosis and alkalosis
6. Write in detail about the bicarbonate buffer system.
7. Describe the preparation of 100 ml of 0.1 M phosphate buffer, pH 6.7, starting with 1 M H_3PO_4 and 1 M NaOH.
8. Write the structure of water and show its hydrogen bonding with the help of a diagram.
9. How the ionization of weak bases takes place? Explain with an example.
10. Write the dissociation of strong electrolytes taking NaCl as an example.

11. Write short notes on:
    (a) The law of mass action
    (b) Hydrogen ion concentration (pH)
10. Write the pK_a values of the following organic acids:
    (a) Acetic acid (CH_3COOH)
    (b) Acetoacetic acid (CH_3COCH_2COOH)
    (c) Formic acid (HCOOH)
    (d) Fumaric acid (HOOCCH=CHCOOH)

MULTIPLE CHOICE QUESTIONS

1. The pH of gastric juice of infants is
   (a) 2.0
   (b) 4.0
   (c) 4.5
   (d) 5.0

   Ans. d
2. The pH of blood is 7.4 when the ratio between [$NaHCO_3$] and [H_2CO_3] is
   (a) 10:1
   (b) 20:1
   (c) 25:1
   (d) 30:1

   Ans. b
3. The difference in pH between arterial and venous blood is rarely more than
   (a) 0.02
   (b) 0.03
   (c) 0.04
   (d) 0.06

   Ans. c
4. The chief buffering system in the blood is
   (a) K_2HPO_4 and KH_2PO_4
   (b) B protein and H protein
   (c) $NaHCO_3$ and H_2CO_3
   (d) B haemoglobin and H haemoglobin

   Ans. c
5. During severe muscular exercise when the blood lactic acid content rises over 100 mg per 100 ml, the pH of blood
   (a) Slightly increases
   (b) Highly increases
   (c) Slightly decreases
   (d) Markedly decreases

   Ans. d
6. The pH of urine usually is
   (a) 5.6
   (b) 6.0
   (c) 6.4
   (d) 6.8

   Ans. b
7. The osmotic pressure of a solution increases with the rise in
   (a) Temperature
   (b) Cold
   (c) Humidity
   (d) Rancidity

   Ans. a
8. For phosphoric acid the two predominant ionic species will be $H_2PO_4^-$ and HPO_4^{2-}. In the case of α-glycerol phosphate the two following ions will be present in about equal concentration at pH
   (a) 7.0
   (b) 6.5
   (c) 8.0
   (d) 9.0

   Ans. a
9. Strong electrolytes are substances that, in aqueous solution, are dissociated almost completely into charged particles known as
   (a) Ions
   (b) Cations

(c) Anions
(d) Dianions

Ans. a

10. The equilibrium constant for this dissociation reaction has been accurately measured and at 25ºC it has the value
(a) 1.6×10^{-16} mole/litre
(b) 1.8×10^{-16} mole/litre
(c) 1.7×10^{-16} mole/litre
(d) 1.8×10^{-10} mole/litre

Ans. b

11. The intracellular fluid of red cells and the red cell membrane in 0.92 per cent NaCl solution maintains a relation
(a) Hypertonic
(b) Hypotonic
(c) Isotonic
(d) None of the above

Ans. c

12. Haemolysis is caused by the dilution of RBC by
(a) Diffusion
(b) Osmosis
(c) Effusion
(d) Imbibition

Ans. b

13. The surface tension of a solution is decreased by
(a) Calcium sulphate
(b) Barium sulphate
(c) Magnesium phosphate
(d) Potassium permanganate

Ans. d

14. The surface tension of a solution is lowered by
(a) Ammonia
(b) Sodium hydroxide
(c) Potassium hydroxide
(d) Aluminium hydroxide

Ans. a

15. The surface tension of a solution is increased by
(a) Bile salts
(b) Bile acids
(c) Concentrated sulphuric acid
(d) Acetic acid

Ans. c

16. Bile salts emulsify fat using
(a) Amylase
(b) Lipase
(c) Pepsin
(d) Trypsin

Ans. b

17. The high boiling and melting points of H_2O and its high heat of vapourization are the result of an interaction between adjacent water molecules known as
(a) Hydrogen bonding
(b) Van der wall forces
(c) Covalent bonding
(d) Electrostatic force

Ans. a

18. The concentration of H_2O in aqueous solution is itself a constant, the value is
(a) 55.4 moles/litre
(b) 55.5 moles/litre
(c) 55.3 moles/litre
(d) None of these

Ans. b

19. For ammonium hydroxide (NH_4OH), the K_b is given in chemical handbooks as 1.8×10^{-5}. It is therefore important to realize that the extent of dissociation of NH_4OH is identical with that of
(a) Acetic acid ($K_a = 1.8 \times 10^{-5}$)
(b) Lactic acid ($K_a = 1.8 \times 10^{-5}$)
(c) Both (a) and (b)
(d) None of these

Ans. a

20. The water specific heat capacity require how many calories to raise the temperature of 1 g of water from 15 to 16°C?
(a) 1.0 calories
(b) 1.5 calories
(c) 2 calories
(d) None of these

Ans. a

2 Bioenergetics and Biological Oxidation

CHAPTER OBJECTIVES

2.1 Introduction to Bioenergetics

2.2 Laws of Thermodynamics or Laws of Conservation of Energy

2.3 The Concept of Free Energy

2.4 Determination of ΔG

2.5 Energy-Rich Compounds

2.6 ΔG and Oxidation–Reduction

2.7 Introduction of Electron Transport and Oxidative Phosphorylation

2.8 Components Involved in Electron Transport

2.9 The Respiratory Chain

2.10 Oxidative Phosphorylation

2.11 The Energetics of Oxidative Phosphorylation

2.12 Energy Conservation Process

2.13 Integration of Carbohydrate, Lipid and Amino Acid Metabolism

2.1 INTRODUCTION TO BIOENERGETICS

Intermediary metabolism constitutes the sum of chemical reactions which the cell's constituents undergo. In the intact cell, both synthetic (anabolic) and degradative (catabolic) processes go on simultaneously, and energy released from the degradation of some compounds may be utilized in the synthesis of other cellular components. Thus, the concept of an energy cycle has developed in biochemistry in which fuel molecules, representing a source of potential chemical energy, are degraded through known enzymatic reactions to produce a few different energy rich compounds.

Playing a key role in this energy cycle is the adenosine triphosphate–adenosine diphosphate (ATP–ADP) system. We know that ADP is able to accept a phosphate group from other energy rich compounds produced during metabolism and thereby be converted into ATP. The ATP in turn can be utilized to drive many biosynthetic reactions and in addition serve as a primary source of

energy for specific physiological activities such as movement, work, secretion, absorption and conduction. In doing so, it is generally converted back to ADP.

To appreciate the energy changes of the ATP–ADP system as well as other energy reactions in biochemistry, it is necessary to define and understand a few fundamental terms of thermodynamics, a science that relates the energy changes which occur in chemical and physical processes.

2.2 LAWS OF THERMODYNAMICS OR LAWS OF CONSERVATION OF ENERGY

First Law of Thermodynamics: According to this law energy can neither be created nor destroyed but it can change its form. It means that in an isolated system there is no change in the total energy. Since energy may change its form, in calculating the total energy, three quantities have to be known: the work done, the heat that is exchanged and the energy that is stored in the system. In these terms, the first law of thermodynamics may be expressed by the following equation

$$\Delta E = q - w$$

where ΔE is the change in internal energy of the system, q is the heat absorbed by the system and w is the work done by the system.

Second Law of Thermodynamics: This law is related to the equilibrium of the process. Two types of statements are applicable for this law.

'The complete conversion of heat into work is impossible without leaving some effects elsewhere.'

This law deals about direction of flow of energy. Systems try to reach equilibrium stage from a non-equilibrium stage. In this type of change, the systems lose their capacity to work slowly.

2.3 THE CONCEPT OF FREE ENERGY

One thermodynamic concept particularly useful is free energy (ΔG). We may speak of the free-energy content of a substance A, but this quantity cannot be measured experimentally. If A is converted to B in a chemical reaction, however,

$$\text{A} \rightleftarrows \text{B} \tag{2.1}$$

it is possible to speak of the change in free energy (ΔG). This is the maximum amount of energy made available as A is converted to B. If the free-energy content of the product B (G_B) is less than the free-energy content of the reactant A (G_A), the ΔG will be a negative quantity. That is

$$\Delta G = G_B - G_A$$
$$= \text{Negative quantity when } G_A > G_B$$

For ΔG to be negative means that the reactions occur with a decrease in free energy. Similarly, if B is converted back to A, the reaction will involve an increase in free energy, that is, ΔG will be positive. Experience has shown that reactions which occur spontaneously do so with a decrease in free energy ($-\Delta G$). On the other hand, if the ΔG for a reaction is known to be positive, that reaction will occur only if energy is supplied to the system in some manner to drive the reaction.

Reactions having a negative ΔG are termed exergonic; those that have a positive ΔG are called endergonic.

Experience has also shown that although the ΔG for a given process is negative, this fact has no relationship what so ever to the rate at which the reaction proceeds. For example, glucose can be oxidized by O_3 to CO_2 and H_2O according to equation (2.2)

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \tag{2.2}$$

The ΔG for this reaction is a very large negative quantity, approximately –686,000 cal/mole of glucose. The large Δ–G has no relationship to the rate of the reaction. Oxidation of glucose may occur in a matter of a few seconds in the presence of a catalyst in a bomb calorimeter. Reaction (2.2) also goes on in most living organisms at rates varying from minutes to several hours. Glucose can nevertheless be kept in a bottle on the shelf for years in the presence of air without undergoing oxidation.

The factor which determines the rate at which a reaction proceeds is the activation energy for that process. Chemical theory postulates that reaction (2.1) will proceed by way of an intermediate or activated complex (e.g., A*). For A to proceed to B, A must pass through the complex A*, and energy must be expended on A to convert it to A*. If little energy is required, the reaction is said to have low activation energy and the reaction will proceed readily. If the energy required is large, little perceptible conversion of A to B will occur and it will be necessary to provide sufficient energy to overcome the barrier to the reaction. The role of catalysts, including enzymes, is to lower the activation energy and allow the reaction to proceed.

The free energy change of a reaction can be related to other thermodynamic properties of A and B by the expression

$$\Delta G = \Delta H - T\Delta S \tag{2.3}$$

In this expression, ΔH is the change in heat content that occurs as reaction (2.1) proceeds at constant pressure; T is the absolute temperature at which the reaction occurs and S is the change in entropy, a term which expresses the degree of randomness or disorder in a system. The absolute heat H and entropy S contents of substances A and B are difficult to measure, but it is possible to measure the changes in these quantities as they are interconverted in reaction (2.1). The ΔH for a reaction may be measured in a calorimeter, a device for measuring quantitatively the heat produced at constant pressure. However, it follows from equation (2.3) that, as the entropy of the products increases over that of the reactants, the $T\Delta S$ term will become more positive and the ΔG will become more negative.

2.4 DETERMINATION OF ΔG

For reaction (2.1), it is possible to derive the expression

$$\Delta G = \Delta G^\circ + RT \ln [B]/[A] \tag{2.4}$$

where ΔG° is the standard change in free energy, soon to be defined; R is the universal gas constant; T is the absolute temperature and [B] and [A] are the concentrations of A and B in moles per litre. Precisely, [B] and [A] should be replaced by the activities of A and B, a_A and a_B respectively. As with pH, however, this correction is not usually made, because the activity coefficients are seldom known for the concentrations of compounds existing in the cell.

From equation (2.4) the ΔG for a reaction is a function of the concentrations of reactant and product as well as the standard free-energy change, $\Delta G°$. It is possible to evaluate $\Delta G°$ if we consider the ΔG at equilibrium. At equilibrium there is no net conversion of A to B, and hence the change in free energy ΔG is O. Similarly the ratio of [B] to [A] is the ratio at equilibrium or the equilibrium constant K_{eq}. Substituting these quantities in equation (2.4)

$$O = \Delta G° + RT \ln K_{eq}$$
$$\Delta G° = -RT \ln K_{eq} \quad (2.5)$$

When the constants are evaluated (R = 1.987 cal/mole-degree; 25°C = 298° K and ln x = 2.303 log $_{10}$x), the equation becomes (at 25°C)

$$\Delta G° = (1.987)\,(298)(2.303) \log_{10} K_{eq}$$
$$= -1363 \log_{10} K_{eq} \quad (2.6)$$

This equation relating the $\Delta G°$ to K_{eq} is an extremely useful way to determine the $\Delta G°$ for a specific reaction. [Another way discussed in Section 2.5 relates $\Delta G°$ to a difference in oxidation–reduction potential ($\Delta E°'$).] If the concentration of both reactants and products at equilibrium can be measured, the K_{eq} and in turn the $\Delta G°$ of the reaction can be calculated. Of course, if the K_{eq} is extremely large or extremely small, this method of measuring $\Delta G°$ is of little value, because the equilibrium concentration of the reactants and products, respectively, will be too small to measure. The $\Delta G°$ for each of a series of K_{eq} ranging from 0.001 to 10^3 is calculated in Table 2.1.

Table 2.1 Relation between K_{eq} and $\Delta G°$

K_{eq}	$\log_{10} K_{eq}$	$\Delta G° = -1363 \log_{10} K_{eq}$ (cal)
0.001	–3	4089
0.01	–2	2726
0.1	–1	1363
1.0	0	0
10	1	–1363
100	2	–2726
1000	3	–4089

From inspection of Table 2.1 it is clear that reactions which have a K_{eq} greater than 1 proceed with a decrease in free energy. Thus, for reaction (2.1) if the K_{eq} = 1000 (that is, if [B]/[A] is 1000), the tendency is for reaction to proceed in the direction of the formation of B. If we start with 1001 parts to A, equilibrium will be reached only when 1000 parts (or 99.9 per cent) of A have been converted to B. If reaction (2.1) has a K_{eq} of 10^{-3} (that is, if [B]/[A] = 0.001), equilibrium will be attained when only 1 part or 0.1 per cent of A has been converted to B.

It is also possible to evaluate $\Delta G°$ for the situation where both the reactants and products are present at unit concentration. When [A] = [B] = 1 M, equation (2.4) becomes

$$\Delta G = \Delta G^\circ + RT \ln 1/1$$
$$= \Delta G^\circ$$

Thus, ΔG° may be defined as the change in free energy when reactants and products are present in unit concentration, or more broadly, in their "standard state." The standard state for solutes in solution is unit molarity; for gases, 1 atm and for solvents such as water, unit activity. If water is a reactant or a product of a reaction, its concentration in the standard state is taken as unity in the expression for the ΔG° (equation (2.4)). If a gas is either formed or produced, its standard state concentration is taken as 1 atm. If a hydrogen ion is produced or utilized in a reaction, its concentration will be taken at 1 M or pH = 0.

Since in the cell few if any reactions occur at pH 0 but rather at pH 7.0, the standard free-energy change ΔG° is frequently corrected for the difference in pH. Conversely, the equilibrium of a reaction may be measured at some pH other than 0. The standard free-energy change ΔG° at any pH other than 0 is designated as ΔG°, and the pH for a given ΔG should be indicated. Of course, if a proton is neither formed nor utilized in the reaction, $\Delta G'$ will be independent of pH and ΔG° will equal $\Delta G'$.

An example will demonstrate the use of these terms. In the presence of the enzyme phosphoglucomutase, glucose-1-phosphate is converted to glucose-6-phosphate. Starting with 0.020 M glucose-1-phosphate at 25°C, it is observed that the concentration of this compound decreases to 0.001 M while the concentration of glucose-6-phosphate increases to 0.019 M. The K_{eq} of the reaction is 0.019 divided by 0.001, or 19. Therefore

$$\Delta G^\circ = -RT \ln K_{eq}$$
$$= -1363 \log_{10} K_{eq}$$
$$= -1363 \log_{10} 19$$
$$= (-1363)\,(1.28)$$
$$= -1745 \text{ cal}$$

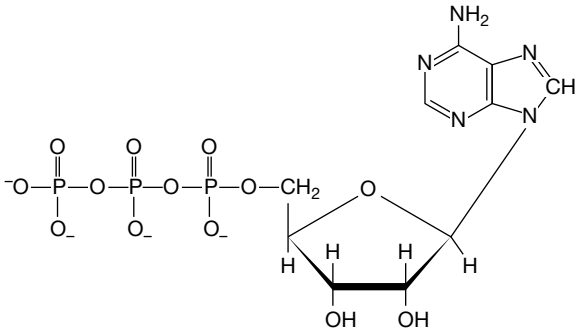
The ΔG° for this reaction will be independent of pH, since acid is neither produced nor used up in the reaction. This amount of free-energy decrease (–1745 cal) will occur when 1 mole of glucose-1-phosphate is converted to 1 mole of glucose-6-phosphate under such conditions that the concentration of each compound is maintained at 1 M, a situation quite different from the experimental situation just described for measuring the K_{eq}. Indeed, these conditions of unit molarity are difficult to maintain either in the test tube or in the cell. It should be pointed out, however, that the concentration of a particular substance (for example, glucose-6-phosphate) may frequently be maintained relatively constant at some concentration over a time interval, since it may be produced in one reaction while it is being used up in another. This condition of steady-state equilibrium undoubtedly exists in many biological systems and requires that thermodynamics be applied to the steady-state condition rather than to the equilibrium condition for which thermodynamic quantities discussed in this chapter apply only to reactions occurring in homogeneous systems, whereas much metabolism occurs in heterogeneous systems involving more than one phase. As a result, most of the values reported in the literature cannot be considered more than 10 per cent accurate. Nevertheless, the concept of the standard free-energy change has found many fruitful applications in intermediary metabolism.

2.5 ENERGY-RICH COMPOUNDS

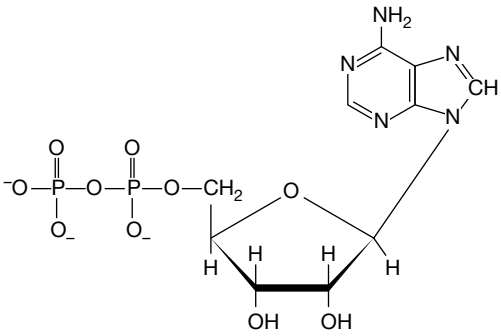
In all living forms, one compound repeatedly functions as a common reactant linking endergonic processes to others that are exergonic. This compound, ATP is one of a group of 'energy-rich' or 'high-energy' compounds whose structure will now be considered. They are called 'energy-rich' or 'high-energy' compounds because they exhibit a large decrease in free energy when they undergo hydrolytic reactions. They are in general unstable to acid, to alkali and to heat. In subsequent chapters their biosynthesis and utilization will be described in detail.

2.5.1 Pyrophosphate Compounds

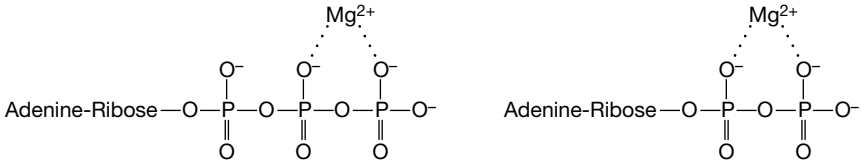
Let us now consider the structure of ATP and its partner ADP in more detail. At pH 7.0 in aqueous solution, ATP and ADP are anions bearing a net charge of –4 and –3, respectively. This results from the fact that the two dissociable protons on the interior phosphates of ATP (and the one interior phosphate of ADP) are primary hydrogens with pK_a in the range of 2–3. The terminal phosphates of ATP (and ADP) have both a primary hydrogen with pK_a of 6.5. Therefore at pH 7.0, the primary hydrogen will be completely ionized and the secondary will be about 75 per cent dissociated. In the cell, however, where a relatively high concentration of Mg^{2+} exists, both ATP and ADP will be complexed with this cation in a one-to-one ratio to form divalent and monovalent complexes, respectively.



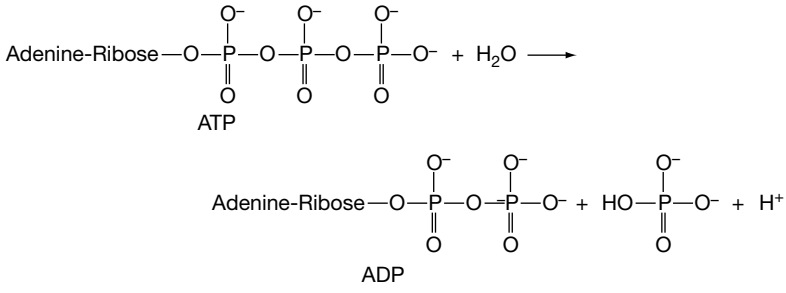
Adenosine triphosphate (ATP)



Adenosine diphosphate (ADP)

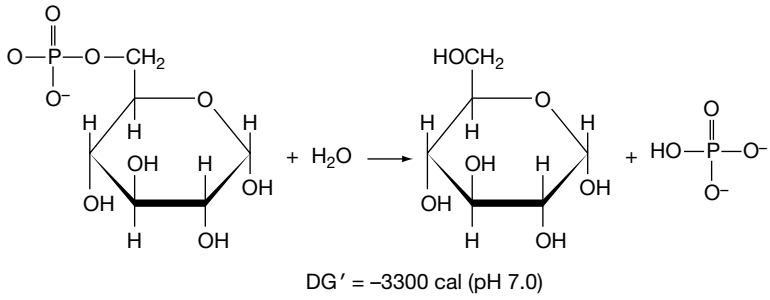


It is informative to compare the $\Delta G'$ of hydrolysis of ATP with that of other phosphate compounds. The hydrolysis of the terminal phosphate of ATP which is called orthophosphate cleavage, may be written as in reaction (2.7).



(2.7)

The $\Delta G'$ at pH 7 has been estimated to be –7300 cal/mole. This is in contrast to the hydrolysis of glucose-6-phosphate, which results in a much smaller decrease in free energy.

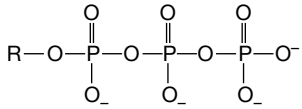


(2.8)

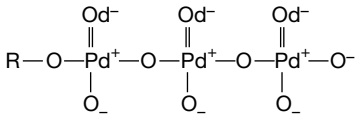
We may properly ask why this large difference in the free energy of hydrolysis exists. On examining the several types of energy-rich compounds encountered in intermediary metabolism, we note several factors which are important but not all of which apply to every energy-rich compound. Regardless of the specific factors involved, it will be seen that the large decrease in free-energy occurs during hydrolysis because the products are significantly more stable than the reactants. Important factors contributing to this stability are listed hereunder.

1. Bond strain in the reactant caused by electrostatic repulsion
2. Stabilization of the product by ionization
3. Stabilization of the products by isomerization
4. Stabilization of the products by resonance

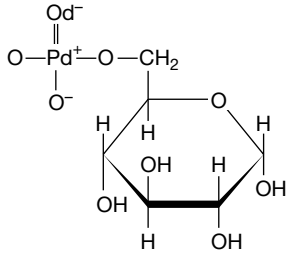
In the case of ATP, the structure of importance in determining its character as an energy-rich compound is the pyrophosphate moiety which, at pH 7.0 is fully ionized



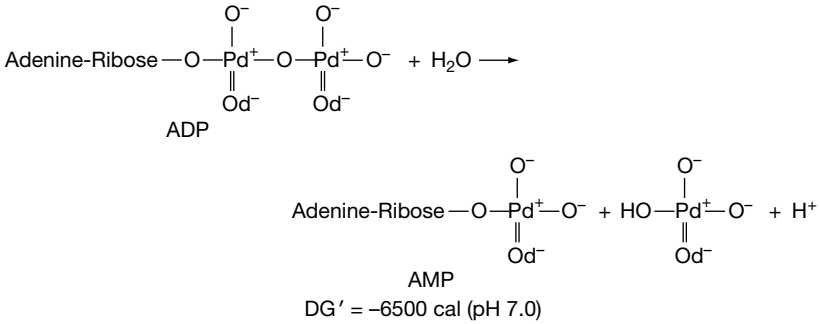
There will be a tendency for the electrons in the P = 0 bond of the phosphates to be drawn closer to the electronegative oxygen atom, thereby producing a partial negative charge (δ^-) on that atom. This is compensated by a partial positive charge (δ^+) on the phosphorus atom resulting in a polarization of the phosphorus–oxygen bonding which may be indicated as



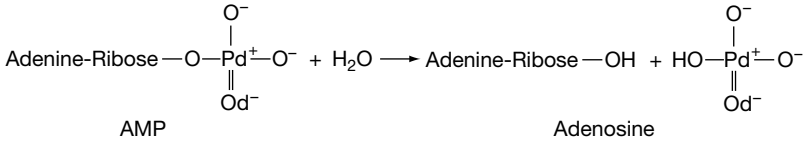
The existence of residual positive charges of this nature on adjacent phosphorus atoms in the pyrophosphate structures of ATP (and ADP) means that these molecules must contain sufficient internal energy to overcome the electrostatic repulsion between the adjacent like charges. When the pyrophosphate structure is cleaved, as on hydrolysis, this energy will be released and will contribute to the total negative ΔG of the reaction. Although the P = 0 bond in glucose-6-phosphate can also be considered to have polar character, there is no adjacent phosphorus atom with a (δ^+) charge:



The argument for instability due to charge repulsion does not exist with this compound, and the ΔG of hydrolysis will be less for this reason. Obviously, the same factor applies in the hydrolysis of ADP to AMP and inorganic phosphate where the observed ΔG on hydrolysis at pH 7 is –6500 cal/mole. On the other hand, the hydrolysis of AMP to adenosine and H_3PO_4 is less ($\Delta G = -2200$, pH7) for lack of the same reason.



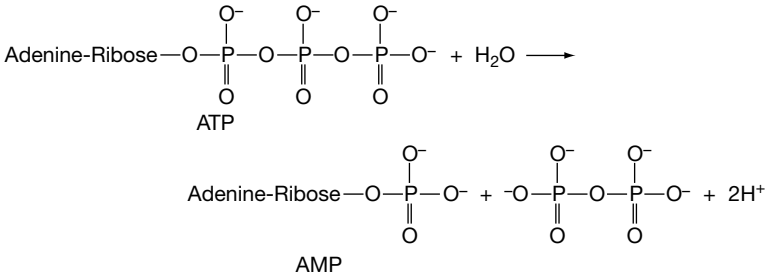
(2.9)



(2.10)

DG′ = –2200 cal (pH 7.0)

Although ATP is converted to ADP in many reactions of intermediary metabolism, there are a number of important reactions in which the interior pyrophosphate bond of ATP is cleaved to yield AMP and inorganic pyrophosphate:

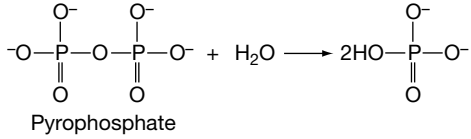


(2.11)

DG′ = –8600 cal (pH 7.0)

This type of cleavage is known as the pyrophosphate cleavage and is in contrast to the orthophosphate cleavage in which ADP is formed [reaction (2.7)].

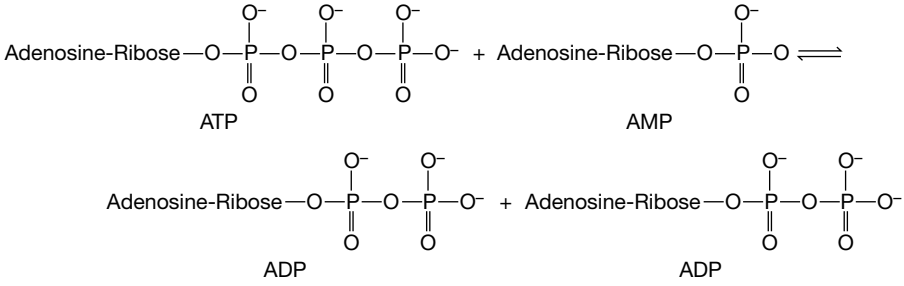
The ATP–ADP system is functional in nature because ADP, having been formed from ATP, can be rephosphorylated in energy-yielding reactions and be converted back to ATP. It is critical, therefore, that AMP and the pyrophosphate formed in the pyrophosphate cleavage be converted back to ATP. This is accomplished by two reactions catalyzed by enzymes widely distributed in nature. The first of these reactions, catalyzed by a pyrophosphatase, is the hydrolysis of pyrophosphate to yield 2 moles of inorganic phosphate:



(2.12)

DG′ = –8000 cal (pH 7.0)

The second reaction is the one in which ATP reacts to form 2 moles of ADP which in turn can be further phosphorylated in several different energy-yielding reactions to regenerate ATP

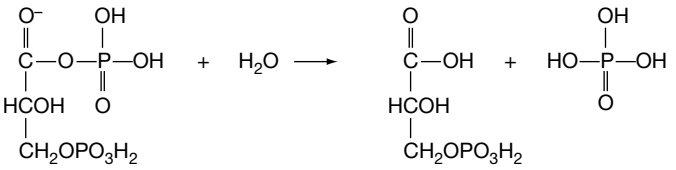


(2.13)

The ΔG for this reaction is approximately 0 because the K_{eq} is ~1.0. Examination of the means by which ADP can be converted back to ATP introduces two other energy-rich phosphate compounds, 1,3-diphosphoglyceric acid and phosphoenolopyruvic acid. Both of these are encountered during the conversion of glucose to pyruvic acid and both have standard free energies of hydrolysis more negative than that of ATP.

2.5.2 Acyl Phosphates

1,3-diphosphoglyceric acid is an example of an acylphosphate, and its standard free energy of hydrolysis is –11.8 kcal/mole



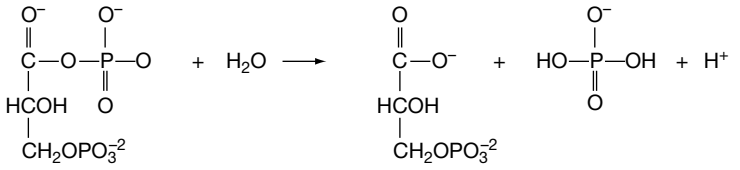
(2.14)

1,3-diphosphoglyceric acid 3-phosphoglyceric acid

DG′ = –11,800 cal (pH 7.0)

Bond strain in the acyl phosphate is a significant factor contributing to the large negative standard free energy of hydrolysis of this class of compounds. The C=O bond of the acyl phosphate group may be considered also to have considerable polar character because of the tendency for the electrons in the double bond to be drawn closer to the electronegative oxygen. Energy is required to overcome the repulsion between the partial positive charges on the carbon and phosphorus atoms, such energy being released on hydrolysis of the acyl phosphate.

The relative tendencies of reactants and products to ionize at a particular pH have an important influence on the ΔG of a reaction. This factor may also be seen in the case of 1,3-diphosphoglyceric acid. In reaction (2.14), the ionization of the reactants and products has not been indicated in the formulas. At pH7 the reaction is more accurately represented as where the primary and secondary hydrogen ions are ionised, while the tertiary hydrogen (on the inorganic phosphate) is not. The carboxylic acid group (pk = 3.7) formed on hydrolysis will also be extensively ionized. The effect of this ionization is to reduce the concentration of the actual hydrolysis product (the unionized acid) to a low level.



(2.15)

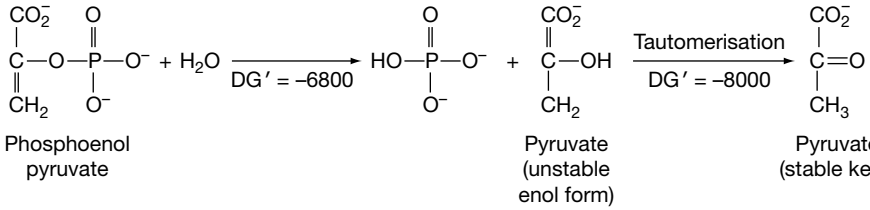
1,3-diphosphoglyceric acid 3-phosphoglyceric acid

It should be stressed that the extent to which ionization is a factor in the $\Delta G'$ of the reaction (i.e., the extent to which products are stabilized in a reaction) will be dependent on the difference in the pK_a of the newly formed ionizable group and the pH at which the reaction occurs. It may be shown that the contribution of a new group with a pK_a of 1 unit less than the pH of the medium is –1363 cal/mole. If reaction (2.15) were to occur at an acid pH (something less than 3) where

the newly formed 3-phosphoglyceric acid is not significantly ionized, the ionization factor would contribute little to the $\Delta G'$ of hydrolysis of 1,3-diphosphoglyceric acid.

2.5.3 Enolic Phosphate

The second compound encountered during the conversion of glucose to pyruvate that provides for the regeneration of ATP from ADP is phosphoenolpyruvic acid (PEP). The free-energy change on hydrolysis of this energy-rich enolic phosphate is –14,800 cal at pH 7.0



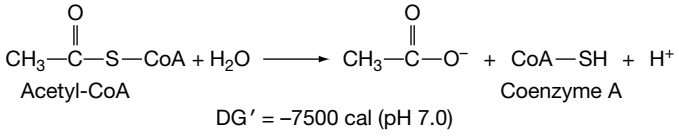
(2.16)

One can appreciate the large negative ΔG observed on hydrolysis of this compound. If one recognizes that the inherently unstable enolic form of pyruvic acid is stabilized in PEP by the phosphate ester group. On hydrolysis, the unstable enol may be thought of as being formed, but it will instantly isomerize to the much more stable keto structure. It is estimated that the tautomerization occurs with a decrease in $\Delta G'$ of about 8000 cal/mole, therefore bringing the total $\Delta G'$ to –14,800 cal/mole. This tautomerization is of major importance in making PEP one of the most 'energy-rich' phosphate compounds of biological importance.

2.5.4 Thiol Esters

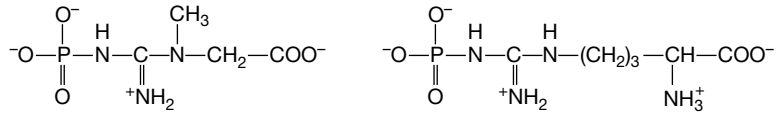
A third type of energy-rich compound that can in turn be utilized to generate ATP from ADP is the thioester, acetyl-coenzyme A.

The $\Delta G'$ of hydrolysis of this compound is approximately –7500 cal



2.5.5 Guanidinium Phosphates

A fourth type of energy-rich compound that plays an important role in energy transfer and storage is the guanidinium phosphate. This type of structure is found as phosphocreatine and phosphoarginine in muscles of vertebrates and invertebrates, respectively. These compounds are also known as phosphagens. The phosphagens are formed by the phosphorylation of creatine or arginine with ATP in the presence of an appropriate enzyme.

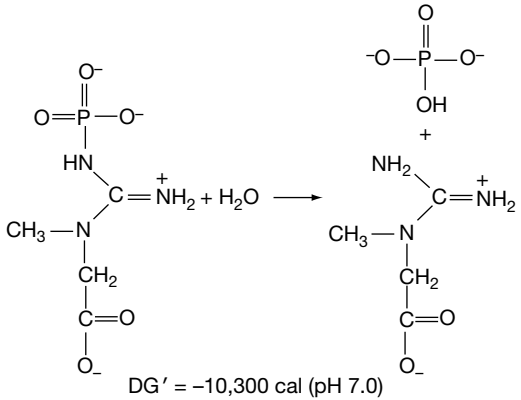


$$\text{Phosphocreatine} + \text{ADP} \underset{}{\overset{\text{Creatine kinase}}{\rightleftharpoons}} \text{Creatine} + \text{ATP} \qquad (2.17)$$

$$\Delta G' = -3000 \text{ cal (pH 7.0)}$$

Since, however, the standard free-energy change on hydrolysis of these compounds is more negative by about –3000 than that of ATP, the equilibrium actually favours ATP formation. Phosphagens carry out their physiological role by furnishing a place to store energy-rich phosphate. When the concentration of ATP is high, reaction (2.17) proceeds from right to left and phosphate is stored as energy-rich phosphocreatine. Then, when the level of ATP is depleted, reaction (2.17) proceeds from left to right, and ATP concentration is increased.

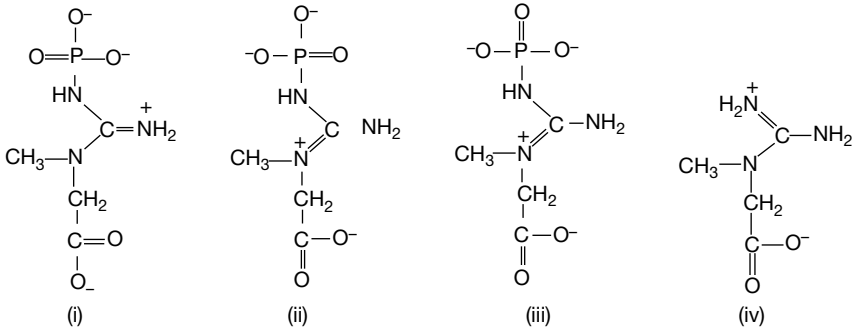
The guanidinium phosphates, represented by phosphocratine, are not inherently less stable because of bond strain as in the case of ATP and ADP. There are no obvious ionization or tautomerization processes which account for greater stability of the products over their reactants as in the case of the acyl and enolic phosphates:



(2.18)

$$\Delta G' = -10{,}300 \text{ cal (pH 7.0)}$$

Nevertheless, the hydrolysis products are significantly more stable than the guanidinium phosphate since one can write a greater number of resonance forms for the products than for the reactants. Phosphocreatine possesses 12 possible resonance forms, three of which are shown as structures I–III.



When, however, creatine lacks its phosphate group, one can write an increased number of resonance isomers which include structure IV, in which a positive charge is placed on the nitrogen atom formerly linked to the phosphate group. Since, in phosphocreatine, there is no oxygen

Table 2.2 Standard Free Energy of Hydrolysis of Some Important Metabolites

Metabolite	$\Delta G'$ at pH 7.0 (cal/mole)
Phosphoenolpyruate	–14,800
Cyclic AMP	–12,000
1,3-Diphosphoglycerate	–11,800
Phosphocreatine	–10,300
Acetyl phosphate	–10,100
S-adenosylmethionine	–10,000
Pyrophosphate	–8,000
Acetyl-CoA	–7,500
ATP to ADP and Pi	–7,300
ATP to AMP and pyrophosphate	–8,600
ADP	–6,500
UDP glucose to UDP and glucose	–8,000
Glucose-1-phosphate	–5,000
Fructose-6-phosphate	–3,800
Glucose-6-phosphate	–3,300
sn-Glycerol-3-phoshate	–2,200

atom between the P atom of the phosphate group and the ureido nitrogen, the partial positive charge on phosphorus would prevent a similar charge on an adjacent atom.

The five types of compounds discussed above may be contrasted with such compounds as glucose-6-phosphate or sn-glycerol-3-phosphate, which are phosphoric acid esters of organic alcohols and which have relatively small values for the $\Delta G'$ of hydrolysis. When all these compounds are listed in Table 2.2, one can see that there is no sharp division between 'energy-rich' and 'energy-poor' compounds, and that several compounds including ATP occupy intermediate positions in the table. For that matter, the unique ability of ATP to participate in so many different reactions involving energy transfer may be ascribed to truly intermediate position between the acyl and enolic phosphates which are generated in the breakdown of fuel molecules and the numerous acceptor molecules which are phosphorylated in the course of their metabolism.

While the discussion on pyrophosphate compounds in Section 2.4 dealt only with ATP and ADP, it should be noted that GTP, GDP, CTP, CDP, UTP, UDP as well as dATP, dGTP, dTTP and dCTP are also energy-rich compounds. Moreover, there is specificity in the biological roles that these compounds play. Thus, UTP is primarily used for the biosynthesis of polysaccharides; GTP is employed in protein synthesis and CTP is utilized in lipid synthesis.

These three together with ATP are involved in RNA synthesis, while dATP, dGTP, dCTP and dTTP are used in DNA synthesis. Although cyclic AMP (cAMP) exhibits a large decrease in free energy on hydrolysis, due to its unstable anhydride ring, it is not known to function by virtue of its 'energy-rich' nature. Rather, it is an allosteric effector and second messenger.

2.6 ΔG AND OXIDATION–REDUCTION

The G of a reaction which involves an oxidation–reduction process may be related to the difference in oxidation–reduction potentials (ΔE_o) of the reactants.

A reducing agent may be defined as a substance that tends to furnish an electron and be oxidized:

$$Fe^{2+} \xrightarrow{\text{oxidized}} Fe^{3+} + 1 \text{ electron}$$

Similarly, Fe^{3+} is an oxidizing agent because it can accept electrons and be reduced

$$Fe^{3+} + 1 \text{ electron} \longrightarrow Fe^{2+}$$

Other substances such as H^+ or organic compounds such as acetaldehyde can serve as oxidising agents and be reduced

$$H^+ + 1 \text{ electron} \longrightarrow \frac{1}{2} H_2$$

$$CH_3CHO + 2H^+ + 2 \text{ electrons} \longrightarrow CH_3CH_2OH$$

These reactions in which electrons are indicated as being consumed (or produced), but in which we have not indicated the donor (or acceptor), are called half-reactions. Clearly, the tendency or potentiality for each of these agents to accept or furnish electrons will be due to the specific properties of that compound, and hence it is necessary to have some standard for comparison. That standard is H_2, which has been arbitrarily given the reduction potential, E'_o of 0.000 V at pH 0 for the half-reaction

$$H^+ + 1e^- \longrightarrow \frac{1}{2} H_2 \tag{2.19}$$

Since a proton is consumed in reaction (2.19), the potential of this half-reaction will vary with pH, and at pH 7.0 the reduction potential E'_o of the reaction (2.19) may be calculated to be –0.420 V. With this as a standard it is possible to determine the reduction potential of any other compound capable of oxidation–reduction with reference to hydrogen. A list of such potentials, which includes several coenzymes and substrates, is given in Table 2.3. Note that these potentials are for the reactions written as reductions. When any two of the half-reactions in Table 2.3 are coupled, the one with the more positive reduction potential will go as written (i.e., as a reduction) driving the half-reaction with the less positive reduction potential backward (i.e., as an oxidation). Qualitatively one may observe that those compounds with the more positive reduction potentials (e.g., O_2 or Fe^{3+}) are good oxidizing agents, while those with the more negative reduction potentials are reducing agents (e.g., H_2 or NADH).

It is possible to derive the expression $\Delta G' = -n\mathcal{F}\Delta E'_o$ where n is the number of electrons transferred in an oxidation–reduction reaction, $\mathcal{F}$ is Faraday's constant (23,063 cal/V equiv.) and $\Delta E'_o$ is the difference in the reduction potential between the oxidizing and reducing agents. That is

$$\Delta E'_o = [E'_o \text{ of half-reaction containing oxidizing agent}] - [E'_o \text{ of half-reaction containing reducing agent}]$$

For example, consider the overall reaction resulting from coupling the two half-reactions involving acetaldehyde and NAD^+

$$\text{Acetaldehyde} + 2H^+ + 2e^- \longrightarrow \text{Ethanol} \tag{2.20}$$

$$NADH + H^+ \longrightarrow NAD^+ + 2H^+ + 2e^- \tag{2.21}$$

Table 2.3 Reduction Potentials of Some Oxidation–Reduction Half-Reactions of Biological Importance

Half-reaction (written as a reduction)	E'_o at pH 7.0 (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.82
$Fe^{3+} + 1e^- \longrightarrow Fe^{2+}$	0.77
Cytochrome a – $Fe^{3+} + 1e^- \longrightarrow$ Cytochrome a – Fe^{2+}	0.29
Cytochrome c – $Fe^{3+} + 1e \longrightarrow$ Cytochrome c – Fe^{2+}	0.25
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ Ubihydroquinone	0.10
Dehydroascorbic acid + $2H^+ + 2e^- \longrightarrow$ Ascorbic acid	0.06
Oxidized glutathione + $2H^+ + 2e^- \longrightarrow$ 2 Reduced glutathione	0.04
Fumarate + $2H^+ + 2e^- \longrightarrow$ Succinate	0.03
Cytochrome b – $Fe^{3+} + 1e^- \longrightarrow$ Cytochrome b – Fe^{2+}	–0.04
Oxalacetate + $2H^+ + 2e^- \longrightarrow$ Malate	–0.10
Yellow enzyme + $2H^+ + 2e^- \longrightarrow$ Reduced yellow enzyme	–0.12
Acetaldehyde + $2H^+ + 2e \longrightarrow$ Ethanol	–0.16
Pyruvate + $2H^+ + 2e^- \longrightarrow$ Lactate	–0.19
Riboflavin + $2H^+ + 2e^- \longrightarrow$ Riboflavin – H_2	–0.20
1,3-diphosphoglyceric acid + $2H^+ + 2e^- \longrightarrow$ Glyceraldehyde-3-phosphate + Pi	–0.29
$NAD^+ + 2H^+ + 2e^- \longrightarrow NADH + H^+$	–0.32
Acetyl-CoA + $2H^+ + 2e^- \longrightarrow$ Acetaldehyde + CoA-SH	–0.41
$H^+ + 1e^- \longrightarrow ½ H_2$	–0.42
Ferredoxin – $Fe^{3+} + 1e^- \longrightarrow$ Ferredoxin – Fe^{2+}	–0.43
Acetate + $2H^+ + 2e^- \longrightarrow$ Acetaldehyde + H_2O	–0.47

Half-reaction (2.20) will go as reduction because it has the higher reduction potential. Half-reaction (2.21) then will go on as an oxidation in the opposite direction from which it is given as shown in Table 2.3. The overall reaction is

$$\text{Acetaldehyde} + \text{NADH} + H^+ \longrightarrow NAD^+ + \text{Ethanol} \qquad (2.22)$$

The $\Delta E'_o$ for reaction (2.22) will be –0.16 – (–0.32) or 0.16 V and the $\Delta G'$ for reaction (2.22) will be

$$\Delta G' = (-2)(23{,}063)(0.16)$$
$$= -7400 \text{ cal}$$

Because this figure is a large negative quantity, the reaction is feasible thermodynamically. Whether the reaction will occur at a detectable rate is not indicated by information at hand.

In a similar manner, the $\Delta G'$ may be calculated for the oxidation of NADH by molecular O_2, a common reaction in living tissues

$$\text{NADH} + H^+ + \frac{1}{2}O_2 \longrightarrow NAD^+ + H_2O \qquad (2.23)$$

In this reaction, $n = 2$, and $\Delta E'_o = 0.82 - (-0.32)$ or 1.14 V, and

$$\begin{aligned}\Delta G' &= -n\mathcal{F}\,\Delta E'_o \\ &= (-2)(23{,}063)(1.14) \\ &= -52{,}600 \text{ cal}\end{aligned}$$

Although the $\Delta G'$ is a large negative quantity, this has no bearing on whether NADH is rapidly oxidized. As a matter of fact, NADH is stable in the presence of O_2 and will react only in the presence of appropriate enzymes.

The standard reduction potential (E_o), in analogy with the standard free-energy change ($\Delta G°$) implies some specific condition or state of the reactants in an oxidation–reduction reaction. Just as $\Delta G°$ specifies that the reactants in a hydrolytic reaction, for example, are all present in their standard state (for solutes 1 M), the term E_o specifies that the ratio of the oxidant to reductant in an oxidation–reduction reaction is unity. Therefore, just as the ΔG for a reaction in which the reactants are not present at 1 M can be related to $\Delta G°$ (equation 2.4), the E for an oxidation–reduction reaction in which the oxidized form (oxidant) and reduced form (reductant) are not present in a 1:1 ratio can be related to E_o by the Nernst equation:

$$E = E_o + 2.303\text{RT}/n\mathcal{F} \log [\text{Oxidant}]/[\text{Reductant}]$$

From this it may be calculated that the E will be 0.030 V more positive than E_o (therefore, more oxidizing) if the ratio of the oxidant to reductant is 10:1 and 0.060 V more positive if that ratio is 100:1. Since there is no reason that this ratio should be 1:1 in biological systems, it is clear that the actual reduction potential (E) can very significantly from the standard reduction potential (E_o).

2.7 INTRODUCTION OF ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

The oxidation of pryuvate and acetly CoA as it occurs in mitochondria is frequently called the aerobic phase of carbohydrate metabolism. However, the title is misleading, since both pyruvate and acetyl CoA can also be obtained from non-carbohydrate sources. In addition, the term aerobic is not strictly precise when the reactions are described as on the inside of the front cover, since the immediate oxidizing agents are nicotinamide and flavin nucleotides rather than oxygen. As in the glycolytic sequence, the amounts of nicotinamide and flavin nucleotides in the cell are limited, and the reactions cease when the supply of oxidized nucleotides is exhausted. Hence, in other for the oxidation of organic substrates to continue, the reduced nicotinamide and flavin nucleotides must be reoxidlzed.

In prokaryotic cells, the reoxidation is accomplished by enzymes located on the plasma membrane; in eukaryotes, the necessary catalysts are in the inner membrane of the mitochondrion and adjacent to the matrix where the nucleotides are reduced. In all aerobic organisms the ultimate oxidizing agent is molecular oxygen and, for the case of NADH we may write the overall reaction

$$\text{NADH} + \text{H}^+ + \frac{1}{2}\text{O}_2 \longrightarrow \text{NAD}^+ + \text{H}_2\text{O} \qquad (2.24)$$

$$\Delta G' = -52{,}500 \text{ cal (pH 7.0)}$$

The enzymes accomplishing this oxidation constitute an electron transport chain in which a series of electron carriers are alternately reduced and oxidized.

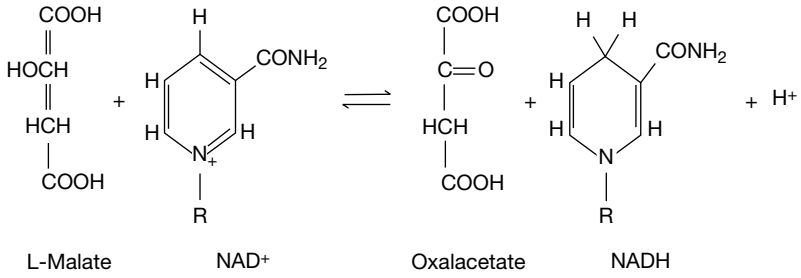
This reoxidation of NADH by O_2 is accompanied by a large decrease in free energy. The amount is sufficient to produce several moles of ATP per mole of NADH oxidized. The enzymes which catalyze the production of ATP as the NADH is oxidized are also localized in the mitochondrial inner membrane. Although the process is known as oxidative phosphorylation, it is perhaps better described as respiratory chain phosphorylation. This process will be described after the electron transport chain is discussed.

2.8 COMPONENTS INVOLVED IN ELECTRON TRANSPORT

There are five different kinds of election carriers that participate in the transport of electrons from substrates as they are oxidized in the mitochondria. A brief description of each is in order before the electron transport chain itself is described.

2.8.1 Nicotinamide Nucleotides

The general properties of these coenzymes (cosubstrates) were described in some detail in Chapter 15 (Vitamins and Minerals). Two of the oxidations in the tricarboxylic acid (TCA) cycle involve the removal of the equivalent of two hydrogen atoms from the substrates, malate and isocitrate. In two others, pyruvic dehydrogenase and α-keto-glutarate dehydrogenase, the electrons are transferred first to lipoic acid and then via a FAD-enzyme to NAD^+.

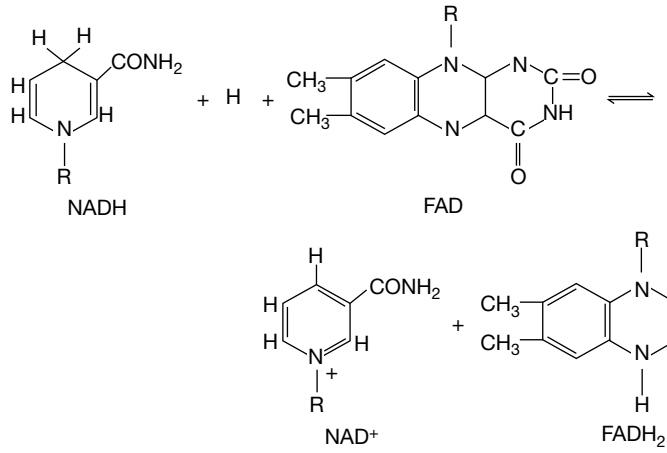


L-Malate NAD+ Oxalacetate NADH (2.25)

2.8.2 Flavoproteins

The prosthetic, groups of flavoproteins are the flavin coenzymes FAD and FMN. These cofactors, in contrast to the nicotinamide nucleotide coenzymes, are much more firmly associated to the protein moiety and in some instances (e.g., succinic dehydrogenase) are covalently bonded to that protein.

In their simplest form, the flavin cofactors accept two electrons and a proton from NADH or two electrons and two protons from an organic substrate such as succinic acid. The reactions with NADH may be represented as:



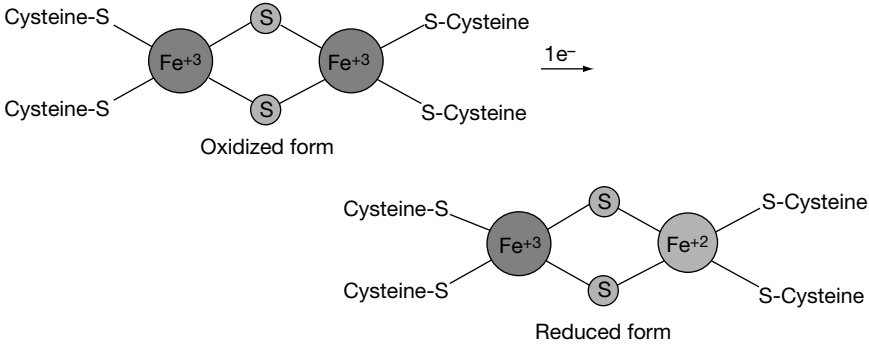
(2.26)

The flavoproteins of the mitochondrial respiratory chain are more complex in that they contain or are closely associated with non-heme iron (NHI) proteins. Thus, the NADH dehydrogenase of beef heart mitochondria contains 1 FMN and 8 Fe atoms per particle weight of 200,000. The iron is present as NHI and is associated with acid-labile sulphur atoms. Because the flavin cofactors can accept one electron at a time forming a semiquinone, the flavoproteins represent a point in the respiratory chain where electrons can be transferred one at a time rather than in pairs.

2.8.3 NHI Proteins

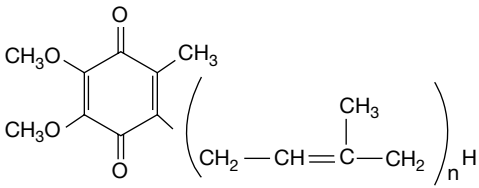
This type of protein was encountered as ferredoxin in plants, in nitrogen fixation and photosynthesis before it was recognized to function in mitochondrial electron transport. Their most characteristic chemical feature is the release of H_2S on acidification (acid labile sulphur), a treatment that also removes the iron. The iron atoms, usually two or more, are arranged in an iron-sulphide bridge that in turn is bonded to cysteine residues in the protein. All Fe–S-proteins are characterized by low E'_o values indicating a role as electron carriers.

In the oxidized state, both iron atoms in the model are in the ferric state. When reduced, one iron becomes Fe^{+2} and is detected by a characteristic electron paramagnetic resonance (EPR) signal.



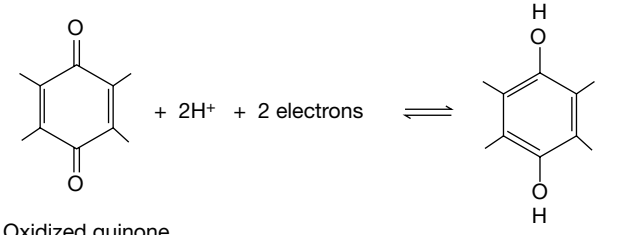
2.8.4 Quinones

Mitochondria contain a quinone called ubiqinone which has the general structure



Ubiquinone

The length of the side chain varies with the source of the mitochondria; in animal tissues, the quinone possesses 10 isoprenoid units in its side chain and is called coenzyme Q_{10} (CoQ_{10}). Because of its long aliphatic side chain ubiquinone is lipid soluble, and together with cytochrome c it is easily solubilized from the inner mitochondrial membrane. This is in contrast to all other of the enzymes in the respiratory chain. When the quinone is extracted from the mitochonodira, the transport of electrons from substrates to oxygen is inhibited; the activity is restored when the quinone is added back. Because it is easily reduced and oxidized, it serves as an additional electron carrier between the flavin coenzymes and the cytochromes.



Oxidized quinone

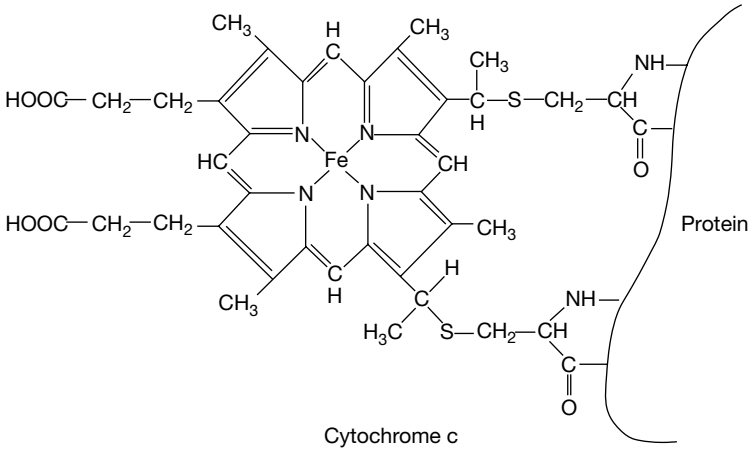
Hydroquinone
or reduced quinone

Coenzyme Q_{10} serves as an acceptor of electrons not only from NADH dehydrogenase, but also from the flavin components of succinic dehydrogenase, glycerol phosphate dehydrogenase and fatty acyl-CoA dehydrogenase (Figure 2.1).

2.8.5 The Cytochromes

These respiratory carriers were discovered in animal cells in 1886 by McMunn. He called the compounds myo- or histohematins and thought they were important for respiratory processes. His results were severely criticized and finally forgotten until these components were rediscovered by Keilin. Keilin's classical experiments in England in 1926–1927 demonstrated that these cell pigments (cytochromes) were found in almost all living tissues and implied an essential role for these substances in cellular respiration.

Keilin's research showed that in every tissue there usually are three types of cytochrome to which he assigned the letters a, b and c. The amount seemed to be proportional to the respiratory activity of the tissue, heart and other active muscles containing the largest amounts of these pigments. The research on the cytochromes was facilitated by the fact that they absorb light of



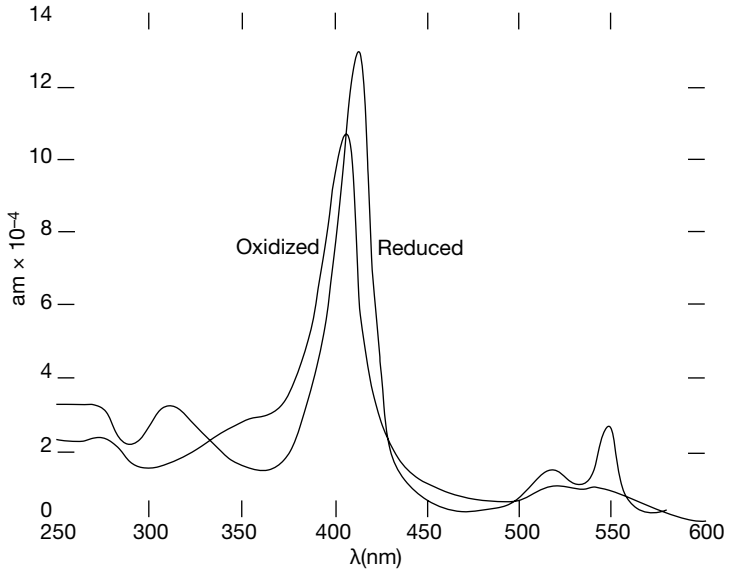


Figure 2.1 Absorption spectra of oxidised and reduced cytochrome c

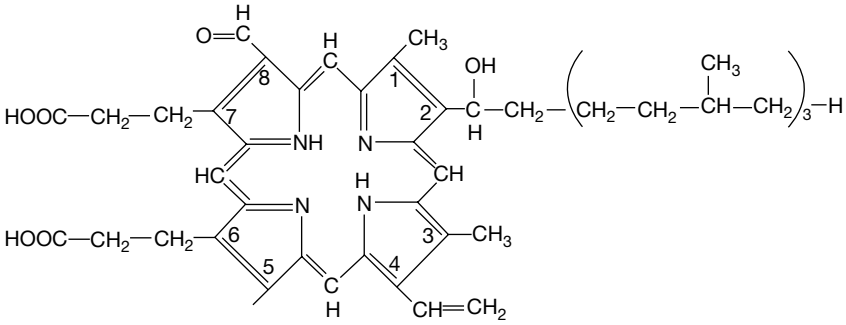
different wavelengths in a characteristic manner. The absorption spectra of oxidized and reduced cytochrome c are shown in Figure 2.1; note the positions of the maxima of the alpha, beta and gamma bands of the reduced carrier. Cytochromes b and a have their a-absorption maximum at 563 nm and 605 nm, respectively.

Only cytochrome c is readily solubilized from the mitochondrial membrane. As additional cytochromes have been detected, especially in bacteria, they have been classified according to the original cytochrome that they most closely resemble. In cells of animals, plants, yeasts and fungi,

all of which have mitochondria, the cytochromes are almost exclusively found in this organelle. In bacteria, the cytochromes are located in the plasma membrane. Mammalian mitochondria contain, in addition to cytochromes a, b and c, another cytochrome called C_1 with an a-band at 554 nm that functions in the electron transport chain. Mammalian microsomes contain cytochrome b_5 whose α-band absorbs at 557 nm.

The absorption spectra together with other properties of the cytochromes indicate that these compounds are conjugated proteins having an iron porphyrin as the prosthetic group. The structure of the prosthetic group for cytochrome c is shown hereunder.

It is a derivative of iron-protoporphyrin IX, and it is bound through thioether linkages with cysteine residues in the protein component. The iron porphyrins associated with cytochromes a and b are known to be different because of differences in their absorption spectra; this has subsequently been confirmed by chemical studies on the structure of the porphyrins. The prosthetic group of cytochrome b is known to be iron-protoporphyrin IX itself. The porphyrin of cytochrome a is porphyrin A, characterized chiefly by a long hydrophobic chain of hydrogenated isoprenoid units. In this regard, porphyrin A resembles the porphyrin of chlorophyll.



Porphyrin A

Many of the cytochromes tend to form complexes readily with HCN, CO, and H_2S; these complexes can be detected by their characteristic absorption spectra. These reagents react by virtue of their ability to occupy one or both of the two coordination positions of the Fe atom that are not occupied by the nitrogen atoms of the pyrrole rings of the porphyrin. In cytochrome c, where those two positions are occupied by other structures, complexes with HCN, CO and H_2S are not formed at neutral pH. In cytochrome a, where one position is normally occupied by O_2 as it is reduced, complexes with HCN, CO and H_2S can form. The high affinity of HCN for cytochrome a in fact accounts for the extreme toxicity of HCN to aerobic organisms.

The studies on soluble cytochrome c confirmed that cytochromes are capable of being alternately reduced and oxidized . The iron of the oxidized cytochromes is ferric iron, for it is reduced to ferrous iron by the incorporation of one electron into the valence shell of the iron atom. Indeed, it is this property that allows the cytochromes to function as carriers in the electron transport process. As indicated above, cytochromes are reduced when CoQ_{10}–H_2 is reoxidized. Since each reduced quinone can furnish two electrons for the reduction of the iron pigment, two molecules of cytochrome are required to react with one molecule of reduced quinone

$$CoQ_{10} - H_2 + 2\text{Cytochrome} - (Fe^{3+}) \longrightarrow CoQ_{10} + 2\text{Cytochrome} - (Fe^{2+}) + 2H^+ \quad (2.27)$$

The reaction is balanced by the release of two protons into the medium.

The order in which the cytochromes are reduced in the election transport chain is readily determined. If an oxidizable substrate of the TCA cycle (i.e., malate) is added to a mitochondrial suspension and the mixture is observed in a spectrophotometer under anaerobic conditions, the absorption band of reduced cytochrome b appears first, then the bands of cytochromes c_1, c and a appear in that order. Then, when O_2 is introduced to the suspension, the band attributable to cytochrome a disappears first followed by the bands from c, c_1 and b. This approach has been extended to those other carriers in the electron transport chain that have characteristic spectra in order to position them in the chain. When this is done, the position is almost directly in agreement with the reduction potential of those components starting with NADH ($E'_o = -0.32$ V) and ending with cytochrome a ($E'_o = +0.29$ V) (Table 2.3).

In discussing the cytochromes some extra attention should be paid to cytochromes a and a_3. Together they constitute cytochrome oxidase, a term used for many years to describe the last carrier or terminal oxidase in the chain of electron transport in aerobes. The reduced form of cytochrome oxidase was known to be capable of reducing O_2 to H_2O, a process requiring a total of four electrons for each mole of O_2 reduced.

The firm association of cytochrome oxidase with the inner mitochondrial membrane has made study of this iron–porphyrin system difficult. Evidence indicates that the enzyme is a complex (240,000 mol. wt.) consisting of six subunits, each containing a heme. A group and one atom of copper. The hexamer has two units which differ in their absorption spectrum from the rest; these, called cytochrome a, are not able to react directly with O_2. The other four units of the hexamer are called a_3 and the reduced form of these cytochromes in the presence of cytochrome c can react with O_2. Without implying about the mechanism of the reaction, we may write it as

$$4\text{Cytochrome } a_3 - Fe^{2+} + O_2 + 4H^+ \longrightarrow 4\text{Cytochrome } a_3 - Fe^{3+} + 2H_2O$$

Reduced cytochrome a_3, combines with carbon monoxide to form a complex that is dissociable by light. The oxidized (ferric) form of cytochrome oxidase has a very high affinity for cyanide and cannot be reduced. This accounts for the extreme toxicity of cyanide.

2.9 THE RESPIRATORY CHAIN

The carriers described above can be arranged in the order in which they are reduced in the mitochondrial membrane (Figure 2.2a). In the initial step, hydrogen atoms removed from different substrates are passed to NAD^+. In some cases, electrons enter the chain at the point where CoQ is positioned. The later carriers are the cytochromes.

The sequence of carriers shown is supported by many types of evidence with sensitive spectrophotometers. It is possible to measure the relative amounts of oxidized and reduced carriers, both in intact tissue and in mitochondrial suspensions. If an oxidizable substrate is added to the latter, NAD^+ will be observed as the more fully reduced of the carriers, whereas the cytochrome oxidase (cytochrome a + a_3) is the more fully oxidised. If the suspension is made anaerobic, NAD^+ is the first to become completely reduced, followed in turn by flavoprotein, ubiquinone and the cytochromes. If an inhibitor such as antimycin A, which inhibits the reaction between cytochrome b and cytochrome c_1 is added, at all carriers to the left of that point become fully reduced as electrons enter into the blocked chain. However, all carriers to the right become fully oxidized since they are no longer receiving electrons from the substrate pool.

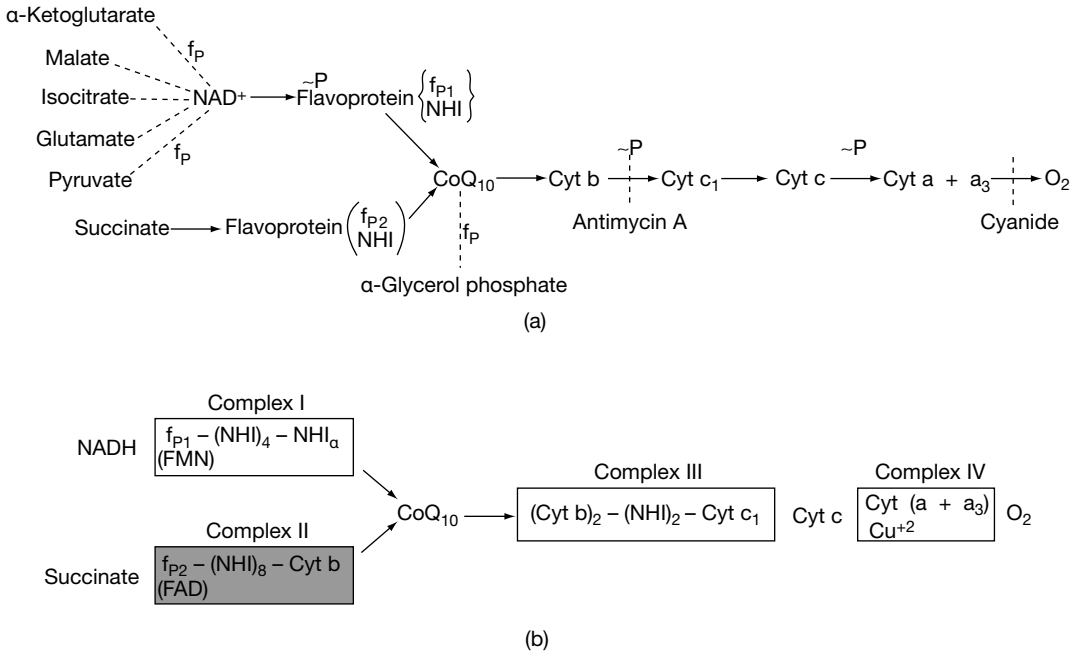


Figure 2.2 (a) The sequence of carriers and (b) The submitochondrial complexes

In recent years, evidence for the arrangement shown has been provided by the isolation of submitochondrial fragments that represent different parts of the electron transport chain. These fragments are structurally organized complexes that can carry out only part of the respiratory chain themselves but when recombined with the other complexes and coenzyme Q and cytochrome c can function as the original chain. Thus complexes I, III and IV will transfer electron from NAD+ to O_2 while complexes II, III and IV are required for the oxidation of succinate (Figure 2.2b). The position of CoQ and cytochrome c outside of any of the four complexes is intended to show the unique ability of these carriers to be extracted without disruption of the complexes. The study of these complexes has revealed how truly complicated is the arrangement of carriers in the electron transport chain.

The clarification of the sequence of carriers in the electron transport chain aided the study of the phosphorylation reactions that accompany the transport of electrons from substrate to O_2. This process of respiratory-chain phosphorylation can now be discussed.

2.10 OXIDATIVE PHOSPHORYLATION

A major objective of the degradation of carbon substrates by a living organism is the production of energy for the development and growth of that organism. In the anaerobic degradation of sugars to lactic acid, some of the energy available in the sugar molecule was conserved in the formation of the energy-rich phosphate compounds which are made available to the organism. As noted already, over 90 per cent of the energy available in glucose is released when pyruvate is oxidized to CO_2 and H_2O through the reactions of the TCA cycle. In that process, however, there was only one energy-rich compound, namely succinyl-CoA synthesized by reactions involving

the substrates of the cycle itself, in the presence of succinic thiokinase, and this thioester was utilized to convert GDP to GTP.

When the production of energy-rich compounds in organisms was investigated in more detail, two different types of phosphorylation processes were recognized. In one of the processes, phosphorylated or thioester derivatives of the substrate were produced initially and were subsequently utilized to produce ATP. Examples of these are the reactions of glycolysis in which 1.3-diphosphoglyceric acid and phosphoenol pyruvic acid are formed and react with ADP to form ATP as well as the reaction catalyzed by succinic thiokinase in the Krebs cycle. These phosphorylation processes have been referred to as substrate level phosphorylations and are to be distinguished from the phosphorylations associated with electron transports which are usually referred to as oxidative phosphorylation.

Phosphorylation occurred during the oxidation of pyruvic acid by muscle homogenates. Although the subsequent fate of the pyruvate molecule was not clear at that time, oxygen was consumed by the homogenate, and inorganic phosphate was esterified as hexose phosphates. If the reaction were inhibited by cyanide or by the removal of O_2, both the phosphorylation and the oxidation ceased. Thus, the synthesis of a sugar phosphate bond was dependent on a biological oxidation in which molecular oxygen was consumed.

Several important advances occurred which simplified the study of this important process. First, in 1948 Kennedy and Lehninger showed that isolated rat liver mitochondria catalyzed oxidative phosphorylation coupled to the oxidation of Krebs cycle intermediates. Today, it is recognized that the inner membrane of mitochondria is the locus of this type of phosphorylation enzymes. In bacteria, smaller units in the cell membrane contain the phosphorylation assemblies.

Second, it was found that the only phosphorylation reaction that could be identified in the mitochondrion was the incorporation of inorganic phosphate into ADP to form ATP.

$$ADP + H_3PO_4 \longrightarrow ATP + H_2O$$

This is clearly a reaction which requires energy; if all reactants are in the standard state, the ΔG ($\Delta G'$ by definition) would be +7300 cal/mole. Since the reactants are undoubtedly not present at the concentration of 1 M, the ΔG will be considerable larger, perhaps as much as +12,000 cal/mole.

Third, the composition of the electron transport chain of mitochondria was investigated in some detail and fourth, the oxidation of NADH itself by O_2 in the presence of mitochondria was shown to lead to the formation of ATP by the incorporation of inorganic phosphate into ADP.

If NADPH is added to a reaction mixture containing ADP, inorganic phosphate, Mg^{2+} and animal or plant mitochondria which have been properly prepared (see discussion of mitochondrial permeability that follows this text). The NADH will be oxidized to NAD^+, and one atom of O_2 will be reduced. This occurs because, as described earlier, mitochondria contain the intact electron transport chain. Simultaneously with this oxidation, inorganic phosphate will react with ADP of form ATP. Under ideal conditions, between 2 and 3 moles of ATP will be formed per atom of O_2 consumed. Since the mitochondria contain ATPase and also can catalyze side reactions which utilize ATP, it is believed that 3 moles of ATP are formed per mole of NADH oxidized or atom of oxygen consumed. This may be represented schematically as

$$NADH + H^+ + \frac{1}{2}O_2 + 3ADP + 3H_3PO_4 \longrightarrow NAD^+ + 3ATP + 4H_2O$$

This reaction is also said to have a P:O ratio of 3.0, a term used to describe the ratio of the atoms of phosphate esterified to the atoms of oxygen consumed in the oxidation. Since the oxidation

of malate and isoctrate exhibited P:O ratios of 3.0, the phosphorylations associated with these substrates were assumed to occur after the NAD^+ that serves as oxidant was reduced. A P:O ratio of 2.0 for succinate similarly indicated that one fewer phosphorylation step was involved when this compound is oxidized.

Much experimental evidence supports the conclusion that the phosphorylations occur as a pair of electrons makes its way along the electron transport chain depicted in Figure 2.2. Since only one phosphorylation occurs when reduced cytochrome c is oxidiszed by molecular oxygen (reaction catalyzed by complex IV) only one phosphorylation site is shown to the right of cytochrome c in Figure 2.2. When NADH is oxidized by cytochrome c, (complex I + III) two phosphorylations occur (two ATPs are formed per mole of NADH oxidized) and their postulated sites of formation in the chain are shown.

One of these is placed in the region between NAD^+ and CoQ because of the following observation: The P/O ratio for succinate oxidation is 2. Since the electrons from succinate share the electron transport pathway only from CoQ on to oxygen and since one phosphorylation occurs in that scheme between cytochrome c and O_2, the second phosphorylation step for succinate oxidation must occur between CoQ and cytochrome c and the third one, from NADH through to oxygen, must occur between NAD^+ and CoQ. These findings are supported by the observation that complexes I, III and IV will each catalyse phosphorylation reactions, although at reduced rates.

Further support for the localizations of the phosphorylation steps along the election transport chain is obtained from differences in E'_o values among the individual carriers.

$$\begin{array}{cccccccc} (-0.32\ V) & (-0.03\ V) & (0.1\ V) & (0.04\ V) & & (0.25\ V) & (0.29\ V) & (0.8\ V) \\ NADH \longrightarrow & \text{fp: NHI} \longrightarrow & CoQ \longrightarrow & \text{Cyt b} \longrightarrow & \text{Cyt } c_1 \longrightarrow & \text{Cyt c} \longrightarrow & a + a_3 \longrightarrow & O_2 \end{array}$$

It is obvious that three greater jumps in the E'_o values are present for NAD ⟶ fp: NHI; Cyt b ⟶ Cyt c and Cyt a ⟶ O_2. In all three cases the differences $\Delta E'_o$ warrants a ΔG approximately great enough to explain that 3ADP are phosphorylated to 3ATP during the oxidation of NADH through the respiratory chain (see also Section 2.6).

Experimental evidence based on the use of inhibitors and uncoupling agents support the positions indicated in Figure 2.2b. Uncoupling agents are compounds which uncouple the synthesis of ATP from the transport of electrons through the cytochrome system; that is, electron transport is going on but no phosphorylation of ADP takes place. In the intact mitochondria these two processes are closely associated. When they are uncoupled, the transport of electrons may actually speed up, thereby indicating that the phosphorylation of ADP has been a rate-limiting process. Further, 2, 4-dinitrophenol is one of the most effective agents for uncoupling respiratory chain phosphorylation. It does not have any effect on the substrate-level phosphorylations that occur in glycolysis. Other examples of uncouplers are salicylanilides, gramicidin and valinomycin. Oligomycin and rutamycin inhibit both electron transport and oxidative phosphorylation.

Uncoupling agents have also been utilized in studying the mechanism of oxidative phosphorylation.

2.11 THE ENERGETICS OF OXIDATIVE PHOSPHORYLATION

In this chapter, the $\Delta G'$ for the oxidation of 1 mole of NADH by molecular O_2 was calculated as approximately –52,000 cal from the reduction potentials of NAD^+/NADH and O_2/H_2O. Since the oxidation of NADH by O_2 through the cytochrome electron transport system leads to the

Table 2.4 Formation of Energy-Rich Phosphate During the Oxidation of Pyruvate by the Tricarboxylic Acid Cycle

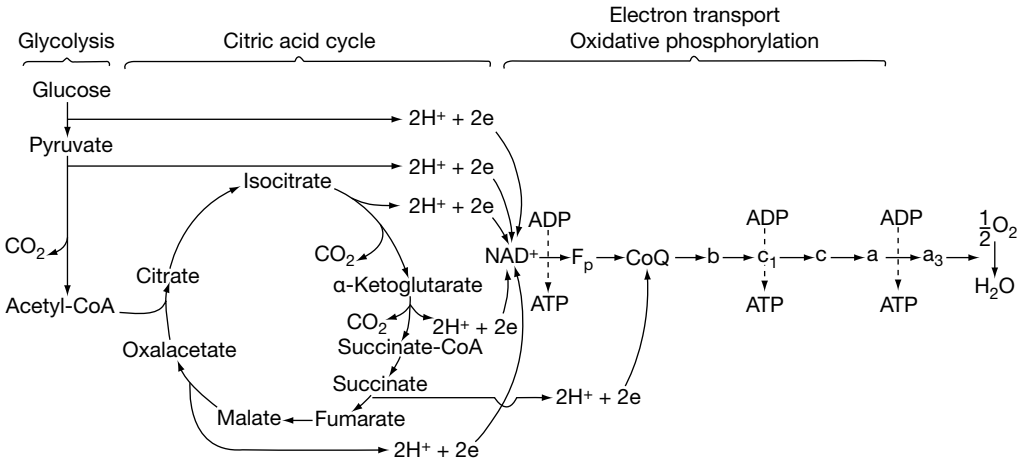
Enzyme or process	Reaction	Energy-rich phosphate produced
Pyruvic dehydrogenase	$Pyruvate + NAD^+ + CoASH \longrightarrow Acetyl\text{-}CoA + NADH + H^+ + CO_2$	0
Electron transport	$NADH + H^+ + \frac{1}{2} O_2 \longrightarrow NAD^+ + H_2O$	3
Isocitric dehydrogenase	$Isocitrate + NAD^+ \longrightarrow \alpha\text{-}Ketoglutarate + CO_2 + NADH + H^+$	0
Electron transport	$NADH + H^+ + \frac{1}{2} O_2 \longrightarrow NAD^+ + H_2O$	3
α-Ketoglutaric dehydrogenase	$\alpha\text{-}Ketoglutarate + NAD^+ + CoASH \longrightarrow Succinyl\text{-}CoA + NADH + H^+ + CO_2$	0
Electron transport	$NADH + H^+ + \frac{1}{2} O_2 \longrightarrow NAD^+ + H_2O$	3
Succinic thiokinase	$Succinyl\text{-}CoA + GDP + H_3PO_4 \longrightarrow Succinate + GTP + CoASH$	1
Succinic dehydrogenase	$Succinate + FAD \longrightarrow Fumarate + FADH_2$	0
Electron transport	$FADH_2 + \frac{1}{2} O_2 \longrightarrow FAD + H_2O$	2
Malic dehydrogenase	$Malate + NAD^+ \longrightarrow Oxalacetate + NADH + H^+$	0
Electron transport	$NADH + H^+ + \frac{1}{2} O_2 \longrightarrow NAD^+ + H_2O$	3
		Sum = 15

formation of three high-energy phosphate bonds, the efficiency of the process of energy conservation may be calculated as –21,900 (i.e., 3×-7300) divided by –52.000 or 42 per cent.

It is now possible to summarize the esterification of inorganic phosphate that accompanies the oxidation of pyruvic acid to CO_2 and H_2O by means of the TCA cycle. The oxidation steps in the process lead to the production of reduced nicotinamide and flavin coenzymes; when these are reoxidized by means of the electron transport system of the mitochondria, the process of oxidative phosphorylation leads to the production of ATP from ADP and inorganic phosphate.

Table 2.4 lists the different reactions which result in the formation of energy-rich phosphate compounds; the total number of high-energy phosphate bonds synthesized per mole of pyruvate oxidized is 15. Since the oxidation of pyruvate to CO_2 and H_2O results in a free-energy change of –273,000 cal, the efficiency of energy conservation in this process is at least –109,000 or (-7300×15) divided by –273,000 or 40 per cent.

In line with this calculation, it is possible to estimate the total number of high-energy phosphate bonds which may be synthesized when glucose is oxidized to CO_2 and H_2O aerobically as illustrated in Figure 2.3. The conversion of 1 mole of glucose to 2 moles of pyruvic acid forms two high-energy phosphates as a result of substrate-level phosphorylation in the glycolytic sequence. The further oxidation of the 2 moles of pyruvic acid in the TCA cycle forms 30 high-energy phosphates. In addition, there are four to six more high-energy phosphates to be added



Carbohydrate catabolism; electron transport; odidative phosphoylation.

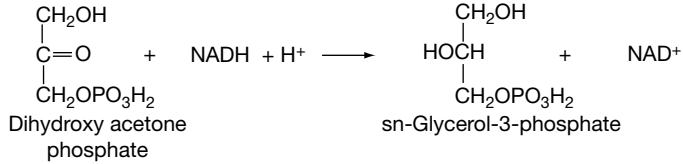
Glycolysis	2 substrate level phosphorylations – 2 ATP =		2 ATP
	2 × [2H⁺ + 2e] NAD odixative phosphorylations		= 6 ATP
Pyruvate → Acetyl-CoA	2 × [2H⁺ + 2e] NAD	–/–	= 6 ATP
Citric acid cycle	3 × 2[2H⁺ + 2e] NAD	–/–	= 18 ATP
	2 × 1[3H⁺ + 2e] FAD	–/–	= 4 ATP
	2 substrate level phosphorylations		= 2 ATP
		SUM	38 ATP

Figure 2.3 Carbohydrate catabolism; electron transport and oxidative phosphorylation

to the 32 just listed. When glucose is converted to two molecules of pyruvate in glycolysis and the latter is not reduced to lactic acid, two molecules of NADH remain in the cytoplasm to be accounted for. While the NADH might be reoxidized by other cytoplasmic dehydrogenases, in a tissue that is actively oxidizing glucose completely to CO_2 and H_2O these two molecules of NADH would be oxidized by the electron transport chain of the organism just as is the NADH produced by oxidation of Krebs cycle intermediates.

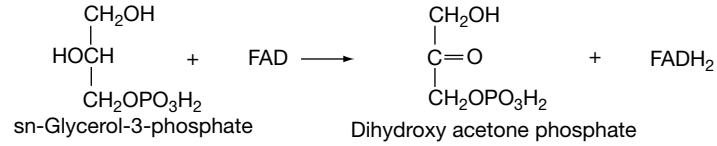
In prokaryotic organisms, there would be no particular problem as the NADH would presumably have ready access to the plasma membrane with its respiratory assemblies that contain the electron transport chain and phosphorylation enzymes. A total of 38 ATPs would therefore be formed in the complete oxidation of glucose to CO_2 and H_2O.

The inner membrane of the mitochondria of eukaryotic organisms is not permeable to NADH and a shuttle process involving glycerol-3-phosphate is employed. In this process, NADH produced in glycolysis (or in any other cytoplasmic oxidation–reduction reaction) is first reoxidized by dihydroxy acetone phosphate in the presence cytoplasmic glycerol-3-phosphate dehydrogenase.



(2.28)

The glycerol-3-phosphate formed is readily permeable to the mitochondrial membranes and enters through the inner membrane to the matrix where it is oxidized, this time by a dehydrogenase that utilizes FAD instead of NAD^+.



(2.29)

The $FADH_2$ produced by this flavoprotein then contributes electrons to the electron transport chain at the level of CoQ (see Figure 2.2) and, as with succinate, 2 moles of ATP are formed when the CoQ-H_2 is oxidized. To keep the shuttle operating, the dihydroxy acetone phosphate produced in reaction (2.29) then passes out of the mitochondria into the cytoplasm, where it can repeat the process. This shuttle operates only in the manner described, namely of the largely unidirectional movement of electrons along the electron transport chain. The transfer of reducing equivalents from the two cytoplasmic NADHs produced as 1 mole of glucose is converted to pyruvate in animal mitochondria will therefore result in the formation of four high-energy phosphates or a total of 36 ATPs for the complete oxidation of glucose to CO_2 and H_2O in eukaryotes.

As discussed previously, the $\Delta G'$ for the oxidation of glucose by O_2 to CO_2 and H_2O has been estimated from calorimetric data:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \qquad (2.30)$$

$$\Delta G' = -686{,}000 \text{ cal (pH 7.0)}$$

If there were no mechanisms for trapping any of this energy, it would be released to the environment as heat, for the entropy term is negligible. The cell can conserve a large portion of this energy, however, by coupling the energy released to the synthesis of the energy-rich ATP from ADP and H_3PO_4. If 38 moles of ATP are formed during the oxidation of glucose, this represents a total of 38×-7300 or $-277{,}000$ cal. The amount of energy that would be liberated as heat in reaction (2.30) is hence reduced by this amount, and the overall oxidation and phosphorylation may now be written as

$$C_6H_{12}O_6 + 6O_2 + 38ADP + 38H_3PO_4 \longrightarrow 6CO_2 + 38ATP + 44H_2O \qquad (2.31)$$

$$\Delta G' = -409{,}000 \text{ cal (pH 7.0)}$$

The conservation of 277,000 cal as energy-rich phosphate represents an efficiency of conservation of 277,000 divided by –686,000 or 40 per cent. The trapping of this amount of energy is a noteworthy achievement for the living cell.

2.12 ENERGY CONSERVATION PROCESS

The phosphorylation mechanism(s) associated with electron transport is/are fundamentally different from the phosphorylation step in glycolysis in that energy-rich phosphorylated forms of substrates (e.g., 1,3-diphosphoglyceric acid or phosphoenol pyruvate) have not been identified.

The difficult nature of this problem is because oxidative phosphorylation is a process intimately associated with the structure of the inner mitochondrial membrane. It is within this

membrane that the transfer of electrons and the initial phosphorylation processes occur. The membrane is also involved in transport of ions in and out of the mitochondrial matrix. As one reducing equivalent moves from NADH to O_2 during electron transport, 6 equivalents of acid ($6H^+$) are transported out of the matrix. Simultaneously, K^+ can be transported back into the matrix to maintain charge neutrality. Ca^{+2} and other divalent cations can be accumulated in the matrix during respiration in processes that are sensitive to different inhibitors. Moreover, if a concentration gradient of K^+ can be established across a mitochondrial membrane, it can be used to drive the phosphorylation of ADP.

Still another phenomenon of the inner membrane is reverse electron flow in which succinate can be used to reduce NAD^+. From the E'_o values (Table 2.3) one can readily calculate that this is a highly exergonic process and will not occur to any significant extent unless additional energy is provided. In the presence of ATP one can observe the reduction of NAD^+ by succinic acid. Presumably electrons are flowing from succinate to CoQ via complex II and then in a reverse direction through complex I to NAD^+, provided ATP is present as an additional energy source.

Studies to elucidate the mechanism of oxidative phosphorylation have therefore been of many sorts: experiments on the linking or electron transport to phosphorylation, use of inhibitors and uncoupling agents; isolation of submitochondrial complexes and other protein fractions. In the last sorts of studies obtained a protein factor called coupling factors that when added back to non-phosphorylating complex I is able to restore the activity of the complex to carry out phosphorylation. One of these factors, F_1, is an ATPase that has a molecular weight of 280,000. It has been extensively purified and contains none of the components of the electron transport chain. The name ATPase for factor F_1 is perhaps unfortunate as this enzyme functions as a special transphosphorylase which catalyzes the reaction

$$ADP + {\sim}P \longrightarrow ATP + H_2O$$

2.13 INTEGRATION OF CARBOHYDRATE, LIPID AND AMINO ACID METABOLISM

At this point it will be useful to integrate some of the information on energy production from carbohydrates and lipids that has been discussed.

Krebs and Kornberg have pointed out that many different compounds which may be classified roughly as carbohydrates, lipids or proteins can serve as sources of energy for living organisms. These authors have also emphasized that the number of reactions involved in obtaining energy from these compounds is astonishingly small, whether the organism involved is animal, higher plant or microorganism. Thus nature has practiced great economy in the processes developed for handling these compounds. These authors divide substrate degradation into three phases, as indicated in Figure 2.4.

In phase 1, polysaccharides, which serve as an energy source for many organisms, are hydrolyzed to monosaccharides, usually hexoses. Similarly, proteins can be hydrolyzed to their component amino acids, and triacylglycerols, which make up the major fraction of the lipid food sources are hydrolyzed to glycerol and fatty acids. These processes are hydrolytic, and the energy released as the reactions occur is made available to the organism as heat.

In phase 2, the monosaccharides, glycerol and fatty acids are further degraded to acetyl-CoA by processes which may result in the formation of some energy-rich phosphate compounds.

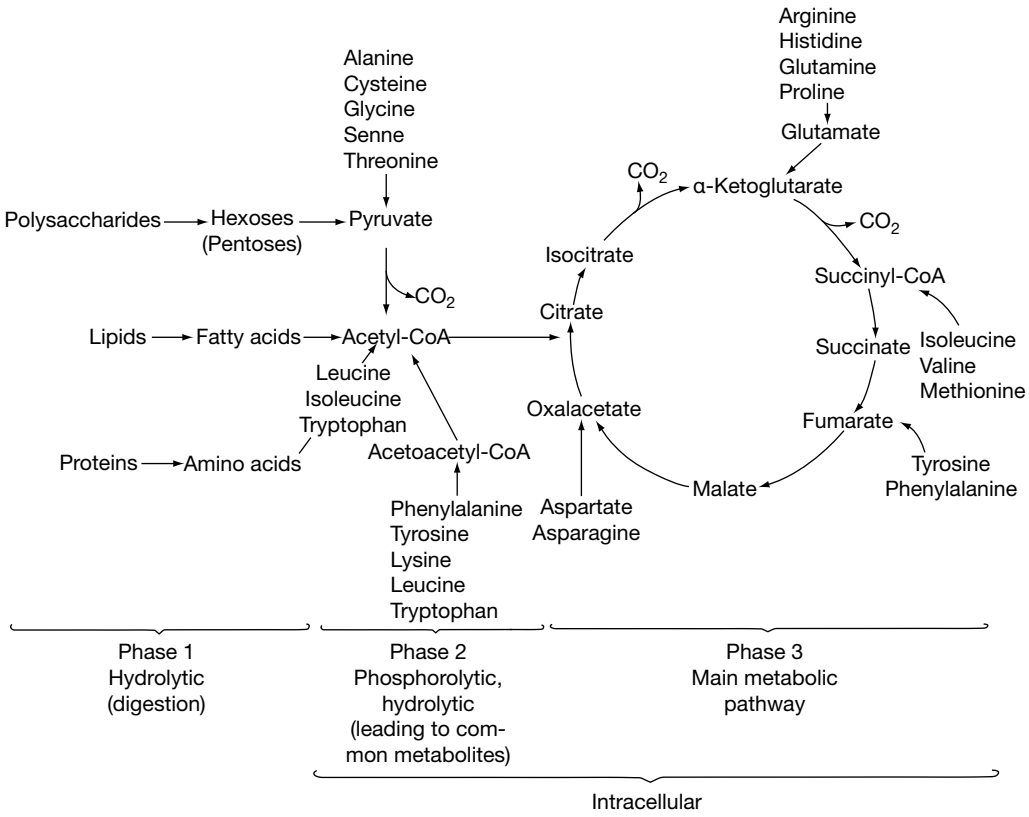


Figure 2.4 Three phases of substrate degradation

That is, in glycolysis, the hexoses are converted to pyruvate and then to acetyl-CoA by reactions involving the formation of limited number of high-energy phosphate bonds (see Chapter 9 on Carbohydrate Metabolism). Similarly in phase 2, the long-chain fatty acids are oxidized to acetyl-CoA (see Chapter 10 on Lipid Metabolism) while glycerol, obtained from hydrolysis of triacylglycerol, is converted to pyruvate and acetyl-CoA by means of the glycolytic sequence.

For the amino acids, the situation is somewhat different. In phase 2 some amino acids (alanine, serine, cysteine, etc.) are converted to pyruvate on degradation, and thus, acetyl-CoA formation is predicted if these amino acids are utilized by an organism for energy production. Other amino acids (the prolines, histidine, arginine, etc.) are converted to glutamic acid on degradation; this amino acid in turn undergoes transamination to yield α-Ketoglutarate, a member of the TCA cycle. Aspartic acid is readily transaminated to form oxalacetate, another intermediate of the cycle. The branch-chain amino acids and lysine amino acids yield acetyl-CoA or succinyl-CoA on degradation and phenylalanine and tyrosine, on oxidative degradation, produce both acetyl-CoA and fumaric acid.

Thus, the carbon skeletons of the amino acids yield either an intermediate of the TCA cycle or acetyl-CoA, that is, the same product obtained from a carbohydrate or a lipid. During the oxidation of this compound in phase 3 by means of the cycle, energy-rich ATP is produced by

oxidative phosphorylation. Specifically, 12 energy-rich bonds are produced for each mole of acetyl-CoA oxidized. Hence, hundreds of organic compounds that can conceivably serve as food for biological organisms are utilized by their conversion to acetyl-CoA or an intermediate of the TCA cycle and their subsequent oxidation by the cycle.

In considering the actual steps involved in making energy available to the organisms, the reactions of oxidative phosphorylation that occur during electron transport through the cytochrome system are quantitatively the most significant. Even here, an economy in the number of reactions is involved. As discussed in Chapter 9, the oxidation of substrates in the TCA cycle is accompanied by the reduction of either a nicotinamide or a flavin nucleotide. It is the oxidation of the reduced nucleotide by molecular oxygen in the presence of mitochondria that results in the formation of the energy-rich ATP. As pointed out, three phosphorylations occur during the transfer of a pair of electrons from NADH to O_2. We have discussed only three other reactions leading to the production of energy-rich compounds where none previously existed before. These are (a) the formation of acylphosphate in the oxidation of triose phosphate, (b) the formation of phosphoenol pyruvate and (c) the formation of thioesters. It is indeed a beautiful design which permits the energy in the myriad food stuffs to be trapped in only six different processes. Even here a single compound, ATP, is the energy-rich substance formed.

2.13.1 Interconversion of Carbohydrate, Lipid and Protein

The interconversions among the three major foodstuffs may be summarized with the help of Figure 2.5 as follows: In this figure two reactions that are effectively irreversible are indicated by heavy unidirectional arrows: (a) carbohydrates are convertible to fats through the formation of acetyl-CoA and (b) carbohydrates may also be converted to certain amino acids (alanine, aspartic and glutamic acids) provided a supply of dicarboxylic acid is available for the formation of the keto acid analogues of those amino acids. Specifically, a supply of both oxalacetate (or other C_4-dicarboxylic acid) and acetyl-CoA required in an amount stoichiometrically equivalent to the amino acid being synthesized. Several reactions exist for forming the C_4-dicarboxylic acids; the principal one is the formation of oxalacetic acid from pyruvic acid, the reaction catalyzed by pyruvic carboxylase. Another is the formation of malic acid from pyruvic acid, the reaction catalyzed by malic enzyme. (c) Fatty acids may be similarly converted to certain amino acids provided a source of dicarboxylic acid is available. (d) Fatty acids cannot be converted to carbohydrate by the reactions shown in Figure 2.5. This inability is due to the fact that the equivalent of the two carbon atoms acquired in acetyl-CoA has been lost as CO_2 prior to the production to the dicarboxylic acids. Note, however, that the glyoxylate cycle can enable an organism to form carbohydrate from fat, as it does, for instance, in some plants, some bacteria and some moulds. (e) The naturally occurring amino acids are convertible to carbohydrates and lipids. Each of the 20 amino acids may be classified as glucogenic, ketogenic or both glucogenic and ketogenic, depending on the specific metabolism of the amino acid. As an example, aspartic acid is glucogenic through formation of oxalacetic acid and its subsequent conversion to phosphoenol pyruvic acid. Similarly, glutamic acid is glucogenic by virtue of its conversion to oxaloacetic acid in the tricarboxylic acid cycle and the conversion of oxalacetate to phosphoenol pyruvic acid. The carbon skeleton of leucine is degraded to acetoacetate-CoA and acetyl-CoA. Thus, it is a ketogenic amino acid. Examples of amino acids which are both glucogenic and ketogenic are tyrosine, phenylalanine, isoleucine and lysine.

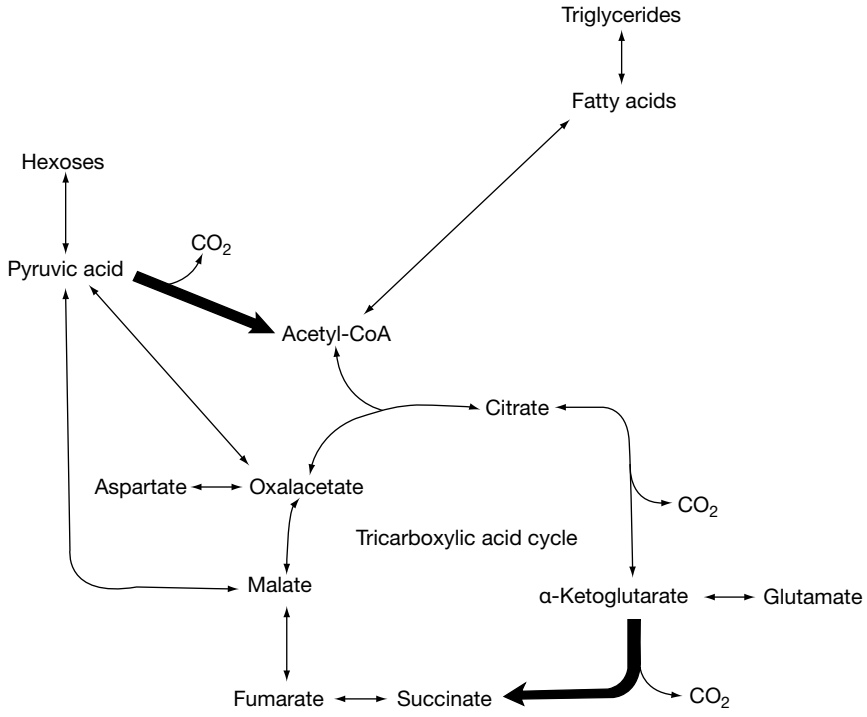


Figure 2.5 The possible interconversion between carbohydrates, lipids and certain amino acids

2.13.2 Interrelationships in Metabolic Control

The interconversions of lipids, carbohydrates and amino acids just described appear reasonable when discussed in terms of known enzymatic reactions. It is now apparent that these interrelationships exist in the area of metabolic regulation as well. While some of the following control processes have already been discussed elsewhere, they will be repeated here to emphasize the interrelation of regulation.

Consider a cell or tissue in which the energy charge value is approaching 1.0. The resulting high concentration ATP and the low level of AMP will decrease the activity of the TCA by lowering the activity of citrate synthase and isocitric dehydrogenase. An immediate decrease in ATP production by oxidative phosphorylation will occur. At the same time, citric acid can be expected to accumulate. Since this acid is known to increase the activity of acetyl-CoA carboxylases which catalyzes the first step in the conversion of acetyl-CoA to fatty acids the cell can shunt the acetyl-CoA being produced from glucose from energy production into fat storage. When ATP utilization is resumed, as it would be in fatty acid synthesis, the corresponding increase in AMP production would lower the citric acid concentration and the ability of fatty acid synthesis to compete for the acetyl-CoA.

The interrelationships possible in control can also be extended back into reactions of glycolysis. Thus, in the 'energy-saturated' cell under discussion, the low level of AMP (high level of ATP) will decrease the glycolytic degradation of glucose because of the action of these nucleotides on phosphofructokinase and fructose-1,6-diphosphate phosphates.

A secondary control can be expected due to the effect of the low levels of ADP and inorganic phosphate on the enzymes glyceraldehyde-3-phosphate dehydrogenase, phosphoglyceric kinase and pyruvic kinase. Requiring as they do either inorganic phosphate or ADP, these enzymes must compete for the available and limited amounts of ADP and inorganic phosphate, and presumably will not be operating at maximum rates. Finally, the low AMP concentration will tend to retard the action of glycogen phosphorylase because AMP is a positive effector of this enzyme. Fructose-6-phosphate and its precursor, glucose-6-phosphate , would accumulate, and the action of the latter ester, as a positive effector of UDPG-glycogen glucosyl transerase, would be to stimulate polysaccharide formation. Again, when the level of ATP is lowered (and AMP concentration is increased), the glycolytic degradation of glucose would increase and oxidation of pyruvate through the TCA cycle would provide a renewed supply of ATP.

EXERCISES

1. Write in detail about the high-energy phosphates with their relevant structures.
2. Explain the possible interconversion between carbohydrates, lipids and certain amino acids with the help of a diagram.
3. Write the formation of energy-rich phosphate during the oxidation of pyruvate by the tricarboxylic acid cycle.
4. Write in detail about the type of protein that was encountered as ferredoxin in plants, in nitrogen fixation and photosynthesis before it was recognized to function in mitochondrial electron transport. Does it belong to the non-heme iron protein group?
5. Derive the expression:

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

6. Explain diagrammatically the corelationship between, the carbohydrate catabolism; electron transport and oxidative phosphorylation.
7. Write short notes on:
   (a) The energetics of oxidative phosphorylation
   (b) Laws of thermodynamics
   (c) Pyrophosphate compounds
8. What is the difference between the pyrophosphate cleavage and orthophosphate cleavage?
9. What is the difference between acyl phosphates and enolic phosphates?
10. Write the formation of phosphagens, as they are formed by the phosphorylation of creatine or arginine with ATP in the presence of the appropriate enzyme.

MULTIPLE CHOICE QUESTIONS

1. AMP contains
   (a) High energy phosphate bond
   (b) Phosphate bond
   (c) Energy phosphate bond
   (d) Low energy phosphate bond

   Ans. d

2. The free energy is derived from respiratory chain oxidation in
   (a) Cytosol
   (b) Microsomes
   (c) Mitochondria
   (d) Lysosomes

   Ans. c

3. Cytochrome oxidase is
   (a) a_3 (b) aa_3
   (c) a (d) None

   Ans. b

4. The mitochondrial superoxide dismutase contains
   (a) Mg^{++} (b) Zn^{++}
   (c) Mn^{++} (d) Co^{++}

   Ans. c

5. The uncoupling agent of oxidative phosphorylation is
   (a) Antimycin
   (b) Dicoumarol
   (c) Barbiturates
   (d) Penicillin

   Ans. b

6. When substrates are oxidized through an NAD-linked dehydrogenase, the P:O ratio is
   (a) 1 (b) 2
   (c) 2 (d) 4

   Ans. c

7. The high-energy compound is
   (a) UDPG
   (b) ATP
   (c) ADP
   (d) Arginine phosphate

   Ans. a

8. Aerobic dehydrogenases have the prosthetic group
   (a) ATP
   (b) NAD^+
   (c) FAD^+
   (d) $NADP^+$

   Ans. c

9. The oxidation and phosphorylation in intact mitochondria is completely blocked by
   (a) Steptomycin
   (b) Gentamycin
   (c) Puromycin
   (d) Oligomycin

   Ans. d

10. NADP-linked dehydrogenases in the extramitochondrial are found to synthesize
    (a) Urea
    (b) Steroid
    (c) Ascorbic acid
    (d) Niacin

    Ans. b

11. The process by which ADP is phosphorylated by Pi to ATP in the respiratory chain is called
    (a) Oxidative phosphorylation
    (b) Phosphorylation
    (c) Non-oxidative phosphorylation
    (d) None of these

    Ans. a

12. For every proton pair passing through the Fo–Fi complex, how many ATP molecules are formed from ADP + Pi?
    (a) 1 (b) 2
    (c) 3 (d) 4

    Ans. a

13. A proton pair attacks one oxygen of Pi to form
    (a) Water (b) Hydrogen
    (c) Both (d) None

    Ans. a

14. Coenzyme Q has a structure very similar to vitamins
    (a) K and E
    (b) C and D
    (c) A and D
    (d) B_3 and B_6

    Ans. a

15. The guanidinium phosphates are also known as
    (a) Phosphagens
    (b) Phosphocreatine
    (c) Both
    (d) None of these

    Ans. a

16. The standard free energy of hydrolysis of phosphoenolpyruvate is
    (a) –14,800
    (b) 14,800
    (c) –15,800
    (d) 15,800

    Ans. a

17. A thiol ester that is an energy-rich compound that can in turn be utilized to generate ATP from ADP is
    (a) Thioester, acetyl-coenzyme A
    (b) Phosphoenolpyruvate
    (c) Both
    (d) None of these

    Ans. a

18. The standard free energy of hydrolysis of cyclic-AMP is
    (a) –12,000 (b) 12,000
    (c) –15,000 (d) 15,000

    Ans. a

19. A reducing agent may be defined as a substance that tends to furnish an electron and be
    (a) Oxidized
    (b) Reduced
    (c) Both
    (d) None of these

    Ans. a

3 Structure, Composition and Functions of Cell and Cell Membrane

CHAPTER OBJECTIVES

3.1 Introduction
3.2 Cell Walls
3.3 Plasma Membrane
3.4 Cell Organelles
3.5 Transport Processes

3.1 INTRODUCTION

In 1957, Dougherty first proposed the adjectives, prokaryotic and eukaryotic to describe cells. These terms are now in common use. By definition, the prokaryotic cell has a minimum of internal organization. It possesses no membrane-bound organelle, its genetic material is not enclosed by a nuclear membrane nor is its DNA complexed with histones. Indeed, histones are not found in this cell. Its sexual reproduction involves neither mitosis nor meiosis. Its respiratory system is closely associated with its plasma membrane. Typical prokaryotic cells include all bacteria and the blue-green algae. All other cells are of the eukaryotic type.

A eukaryotic cell has a considerable degree of internal structure with a large number of distinctive membrane-enclosed organelles. For example, the nucleus is the site for informational components collectively called chromatin. Reproduction involves both mitosis and meiosis; the respiratory site is the mitochondrion and, in plant cells, the site of the conversion of radiant energy to chemical energy is the highly structured chloroplast.

3.2 CELL WALLS

The cell walls in prokaryotic cells and in most eukaryotic cells such as algae, fungi and plants confer shape and rigidity to the cell itself. Without cell walls, cells would be spherical in shape and extremely fragile to slight osmotic changes of the external environment. This conclusion can be demonstrated by the conversion of a G(+) bacteria with its thick cell wall to a protoplast devoid of cell wall but possessing its plasma membrane and its cytosolic components. Protoplasts can be readily prepared by exposing a suspension of G(+) bacteria to the enzyme lysozyme in an osmotically stabilized medium. Lysozyme hydrolyzes the peptidoglycan components of the wall, weakening the wall and allowing the plasma membrane enclosed protoplast to escape into the isotonic environment. There, the protoplast can undergo normal replication and growth. However, if the environment is altered by the addition of water to form a hypotonic medium, immediate lysis occurs. In G(–) bacteria, lysozyme breaks the peptidoglycan skeleton of the wall forming a spheroplast which usually still has wall material attached to the cell.

One exception to this requirement for cell walls is the prokaryotic genus *Mycoplasma*, all members of which are devoid of cell walls. These organisms have adjusted to survival in the hostile osmotic environment by being parasitic, that is, living in plant and animal host cells where the osmotic environment is carefully maintained. These organisms have also included steroids in their plasma membranes. Since steroids are complex planar ring systems that allow stacking and interaction with other lipid components of the plasma membrane, considerable stability is conferred on their membranes. Similar situations occur in the animal, that is, the erythrocyte which has no rigid cell wall. It remains structurally stable in the circulating plasma but lyses instantly when transferred to water.

3.2.1 Prokaryotic Cell Walls

Cell walls of prokaryotic cells are rather complicated and markedly different from those in eukaryotic cells. Bacteria are roughly divided into Gram-positive [G(+)] and Gram-negative [G(–)] cells based on the differential staining by a crystal violet- iodine reagent. In general, Gram-positive cells have thick cell walls, up to 80 per cent of which are composed of a mesh-like macropolymer called a peptidoglycan. Variations in the structure and composition of this peptidoglycan occur in a number of bacteria.

Superimposed on the peptidoglycan are the teichoic acid polymers that consist of repeating units of either glycerol or ribitol connected by internal phosphate diesters. D-Alanine is usually attached through an ester linkage to the polyhydroxyl alcohol. The teichoic acid polymers are probably intimately involved in the cell's antigenicity and susceptibility to phage infection. They definitely confer a strong negative charge on the surface of the cell wall because of their high content of ionized phosphate groups. The teichoic acids apparently are located in the region extending from the exterior of the plasma membrane to the outer regions of the peptidoglycans. Gram-positive cell walls are also characterized by the absence of any significant lipid.

Of considerable interest is the fact that the enzyme lysozyme found in tears and saliva, in bacteria and in plants, readily hydrolyzes peptidoglycans at the β-1,4 linkage of N-acetyl muramic acid with the resulting weakening of the cell wall and subsequent rupture of the cell. Certain antibiotics such as penicillin specifically inhibit the synthesis of new cell walls in growing cells, and this leads to lysis and death of the cell. Since eukaryotic cells have entirely different cell

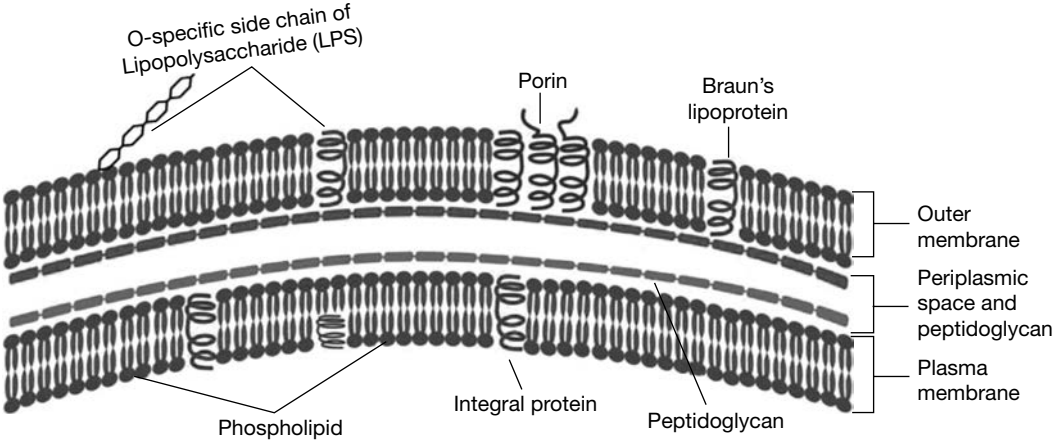


Figure 3.1 Schematic presentation of Gram-negative cell wall

walls or membranes when compared to prokaryotic cells, penicillin has no effect on animal cells. Hence, this specificity leads to its great value in the treatment of infectious diseases caused by prokaryotic cells, in particular Gram-positive organisms.

Gram-negative organisms have a somewhat more complex cell wall structure (Figure 3.1). Although little, if any, teichoic acid is found in these organisms, and although a thin strand of peptidoglycan similar in structure to those found in Gram-positive cell walls is sandwiched between the cell membrane and the outer envelope, the major component of these organisms is a giant macropolymer called a lipopolysaccharide (Figure 3.2). It is very complex and its structure is only known in some detail, especially the lipopolysaccharide of Enterobacteriaceae. A generalized structure is presented in Figure 3.3. When lipopolysaccharides are released into the blood stream of an animal, they are very toxic, causing fever, haemorrhagic shock and other tissue damage. They are, therefore, called endotoxins.

The student should not consider that the bacterial cell wall is covered with a mesh-like sheet of complex macromolecules. If this was the case, the organism would have some difficulty in obtaining metabolites for growth.

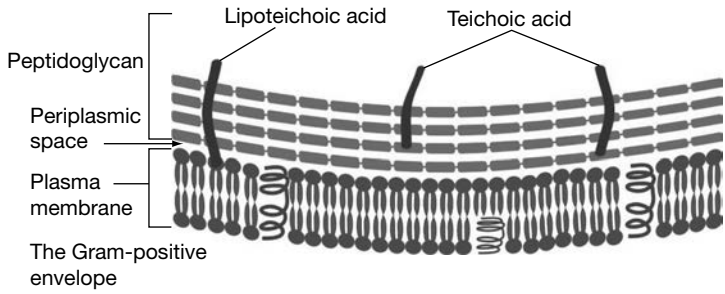


Figure 3.2 Gram-positive cell wall

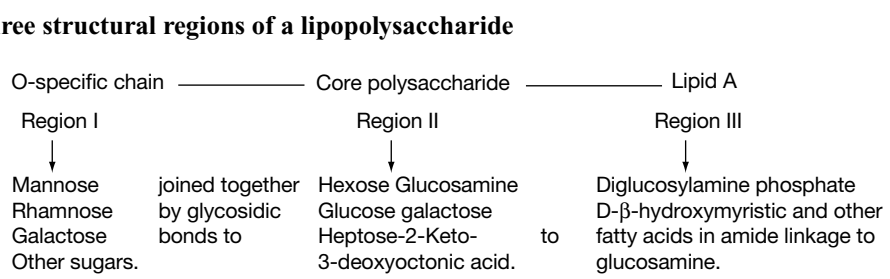


Figure 3.3 A generalized structure of a lipopolysaccharide

The cell surface is actually punctured with a large number of pores through which biochemical compounds flow, but which prevent entry of very large molecules such as proteins or nucleic acids. One could think of a bacterial cell wall as a giant molecular sieve allowing small-molecular weight compounds to pass through the plasma membrane but retaining macromolecules. At the plasma membrane, the transport mechanisms become operative.

3.2.2 Comparison of Features of Prokaryotic and Eukaryotic Cells

We now compare the important features of prokaryotic and eukaryotic cells in Table 3.1. The structure of prokaryotic and eukaryotic cells are shown in Figure 3.4.

Table 3.1 Comparison of Prokaryotic and Eukaryotic Cells

	Prokaryotes	Eukaryotes
Organisms	Bacteria, archaea	Protists, fungi, plants and animals
Cell size	~1–10 μm	~10–100 μm (sperm cells, apart from the tail, are smaller)
Complexity	Unicellular, rarely small clusters or filaments	Sometimes unicellular more often multicellular
Membrane-bound organelles	None (mesosome is infolding of cytoplasmic membrane)	Nuclei, mitochondria, chloroplasts, lysosomes, endoplasmic reticulum, Golgi complex and vacuoles
DNA	Circular (usually)	Linear molecules (chromosomes) with histone proteins
RNA-/protein synthesis	Coupled in cytoplasm	RNA synthesis inside the nucleus and protein synthesis in cytoplasm
Ribosomes	70S (50S + 30S subunit)	80S (60S + 40S subunit)
Cytoplasmatic structure	Very few structures	Highly structured by endomembranes and a cytoskeleton

(*Continued*)

Table 3.1 Continued

	Prokaryotes	Eukaryotes
Cell movement	Flagella made of flagellin	Flagella and cilia containing microtubules; lamellipodia and filopodia containing actin
Mitochondria	None	One to several thousand (though some lack mitochondria)
Chloroplasts	None	In algae and plants
Organization	Usually single cells	Single cells, colonies, higher multicellular organisms with specialized cells
Cell division	Binary fission (simple division)	Mitosis (fission or budding) and meiosis

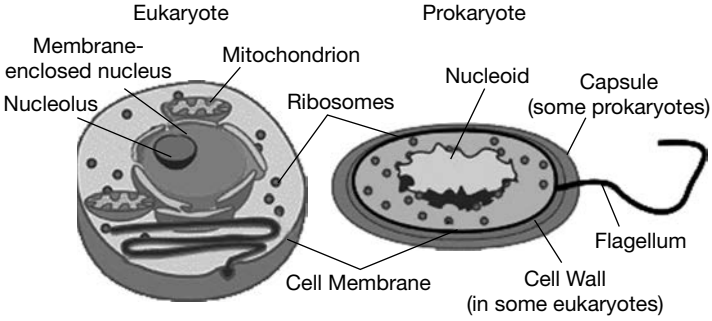


Figure 3.4 The cells of eukaryotes and prokaryotes

3.2.3 Plant Cell Walls

In mature plant cells, the cell wall is composed of three distinct parts, the intercellular substance or middle lamella, the primary wall and the secondary wall. The middle lamella is composed primarily of pectin polymers, and may also be lignified. The primary wall consists of cellulose, hemicellulose (xylans, mannans, galactans. glucans, etc.) and pectins as well as lignin. The secondary wall, which is laid down last, contains mostly cellulose, with smaller amounts of hemicellulose and lignin.

A large number of openings called pits occur in various arrays and shapes in the secondary wall. Connecting adjacent cells are thread-like structures called plasmodesmata, which penetrate through the pits and the primary wall and middle lamella to the neighbouring cell. It is thought that the endoplasmic reticulum of the cell extends through the plasmodesmata into the neighbouring cell, thereby permitting a flow of metabolites and hormones from one cell to the next.

Much work has been expended to understand the structural role of cellulose in the plant cell walls. There is considerable agreement that cellulose form microfibrils consisting of about

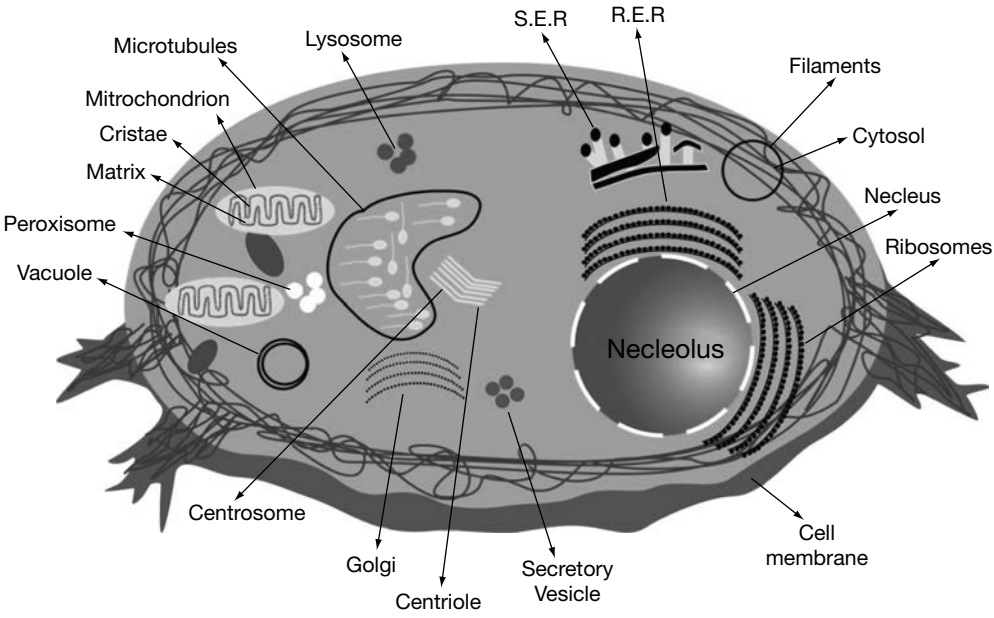


Figure 3.5 Structure of a plant cell

2000 cellulose molecules in cross section. These are arranged in orderly three-dimensional lattices around the cell particularly in the secondary cell wall, to give great strength as well as plasticity to the cell wall.

There is now reasonably good evidence that the Golgi apparatus in the cytosol participates in the formation of the middle lamella and the adjacent primary cell walls as a plant cell divides during mitosis. This organelle, rich in enzymes for phospholipid and polysaccharide synthesis, releases small vesicles which line up and fuse in a linear fashion to form first a gel-like matrix, which then develops into the middle lamella with the deposition of hemicelluloses and pectins (Figure 3.5).

It should be noted that the plant cell wall has associated with it a significant number of hydrolases including invertase, phosphatases, nucleases and peroxidases. The significance of these hydrolases in the cell wall is not clear.

3.2.4 Animal Cell Surfaces

The generalized animal cell has no rigid cell wall. However, it has a 'cell coat,' and external to the plasma membrane of most cells is a multicomponent system consisting of binding proteins, glycolipids, glycoproteins, enzymes, hormone receptor sites and antigens that confer on the cell surface the unique properties characteristic of a given cell. Thus the plasma membrane of an animal cell is not smooth in appearance but rather has a 'fuzz' over its surface (Figure 3.6).

Table 3.2 provides a comparison between the structure of animal and plant cells.

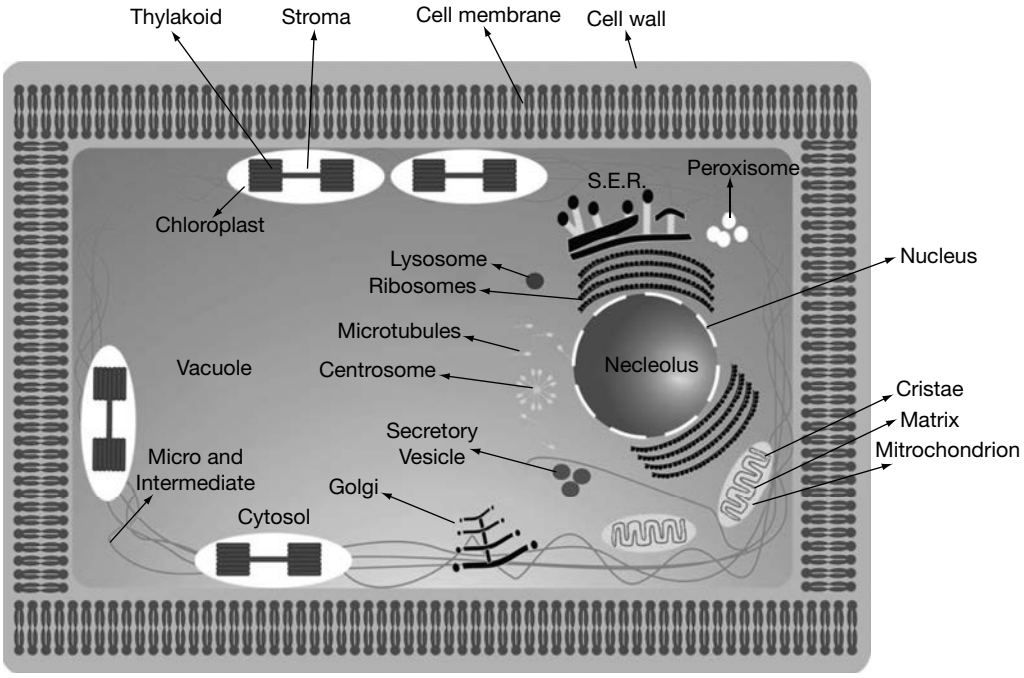


Figure 3.6 Structure of an animal cell (In this figure spelling of thylakoid need to be corrected)

Table 3.2 Comparison between the structure of animal and plant cells

Animal cell	Plant cell
• Nucleus	• Nucleus
• Nucleolus (within nucleus)	• Nucleolus (within nucleus)
• Rough endoplasmic reticulum (ER)	• Rough ER
• Smooth ER	• Smooth ER
• Ribosomes	• Ribosomes
• Cytoskeleton	• Cytoskeleton
• Golgi apparatus	• Golgi apparatus (dictyosomes)
• Cytoplasm	• Cytoplasm
• Mitochondria	• Mitochondria
• Vesicles	• Plastids and its derivatives
• Lysosomes	• Vacuole(s)
• Centrosome	• Cell wall
• Centrioles	

3.3 PLASMA MEMBRANE

The semipermeable barrier between the internal and external environment of the cell is called the plasma membrane. By means of a limiting membrane the cell organizes its internal environment for specific purposes and expends energy to maintain this environment despite changes constantly occurring externally. Since the cell may also be a component of a larger unit in multicellular organisms, intercellular coordination or interaction is necessary.

Before we begin a discussion on the structure of plasma membranes, it should be made clear that a wide array of structural models have been proposed over the past several decades. Rather than recite the pros and cons for each model, we shall assume that in most membranes the majority of the lipid, primarily as phospholipids, is in the form of a fluid bilayer and that membrane proteins and glycoproteins are both loosely bound and deeply or transversely embedded in the bilayer matrix. These assumptions, supported now by considerable experimental evidence, are embodied in **Singer's 'fluid mosaic' model** (Figure 3.7).

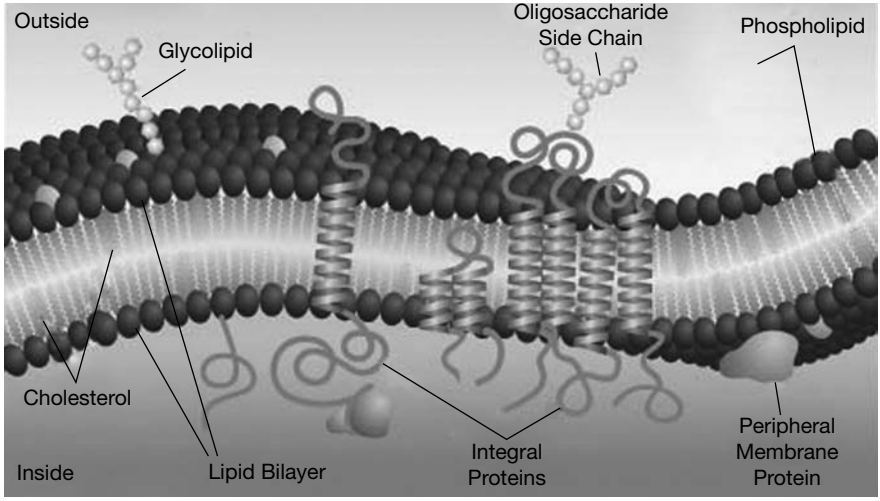


Figure 3.7 Fluid-mosaic model of biological membrane conceived by S. J. Singer and Garth Nicolson in 1972 to describe structural features of biomembrane

Chemical analyses of a large number of cell membranes have consistently revealed the presence of proteins and an array of complex polar lipids (Table 3.3). Indeed, of the total phospholipid found in the bacterial cell over 95 per cent is associated with its plasma membrane. Most, if not all, of the carbohydrate components are covalently associated with glycoproteins and glycolipids.

Table 3.3 Chemical Composition of Plasma and Organelle Membranes

Membrane	Protein %	Lipid %	Carbohydrate %	Ratio = Protein/lipid
Plasma membranes				
Mouse liver cell	44	53	3	0.85
Human erythrocyte	49	43	8	1.1

(*Continued*)

Table 3.3 Continued

Membrane	Protein %	Lipid %	Carbohydrate %	Ratio = Protein/lipid
Plasma membranes				
Amoeba	54	42	4	1.3
Gram-positive bacteria	70	20	10	3.0
Mycoplasma	59	40	1	1.6
Organelle membranes Mitochondrial outer membrane (liver)	51	47	2	1.1
Mitochondrial inner membrane (liver)	76	23	**1**	3.2
Chloroplast lamellae (spinach)	67	28	5	2.3
Nuclear membranes (rat liver)	61	36	3	1.6

3.3.1 Membrane Lipids

Membrane lipids comprise the matrix that give form and structure to membranes and in which membrane proteins are embedded (Figure 3.8). All membranes contain amphipathic lipids that include phospholipids and glycolipids (Table 3.4). These lipids are characterized by having both hydrophobic (lipophilic) and hydrophilic (lipophobic) functions.

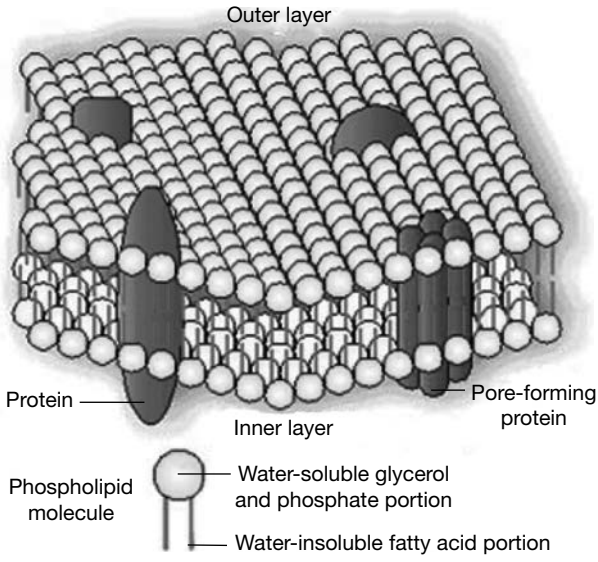


Figure 3.8 Structure of biomembrane

Although phospholipids are insoluble in water, a suspension of phospholipid aggregates can be readily rearranged into highly water soluble micelles by exposing the suspension to a short burst of sonication.

Table 3.4 Lipids of Prokaryotic and Eukaryotic Plasma and Organelle Membranes

Lipid		Lipid composition (% of total lipid)				
Membrane	**% of membrane Components**	**Phospho-glycerides**	**Glycosyl diglycerides**	**Sphingo-lipids**	**Steroid**	**Others**
Plasma membranes						
B. subtilis	18	74	16	0	0	10
Erythrocytes (human)	29	37	0	21	23	19
Liver (rat)	40	45	0	10	20	25
Organelle membranes						
Spinach chloroplast lamella	52	10	74	0	1	15
Endoplasmic reticulum	25	72	0	14	9	5
Mitochondria (liver)	26	–	–	–	–	–
Outer membrane	–	96	0	1	3	–
Inner membrane	–	97	0	2	1	–

Occurring in many forms and shapes, micelles can aggregate to form structures with particle weights from a few thousand to many thousand. They are highly stable and water soluble in their newly ordered arrangement. The hydrophobic functions of the amphipathic compounds namely the hydrocarbon chains, are arranged internally to exclude water and thus are held together by hydrophobic interaction forces. The hydrophilic functions, namely the phosphoryl base moieties, are in turn highly attracted to the aqueous environment. Artificial membranes of phospholipids are readily formed by the sonication technique as well as other techniques, and are under intensive investigation as model systems.

Membranes undergo a physical phase transition from a flexible fluid like liquid crystalline state to a solid gel structure as a function of temperature. The temperatures at which the phase transition occurs are dependent on the composition of the amphipathic lipids. Thus lipids with more unsaturated fatty acids have lower transition temperatures than those with more saturated fatty acids: longer chain lengths have higher transition temperatures than shorter chain lengths; cis-unsaturated fatty acids have lower transition temperatures than trans-unsaturated fatty acids.

What is the significance of a thermal phase transition in membranes? Obviously, homeothermic animals, by controlling their internal temperature, do not expose their membrane systems to marked changes in temperature, However, poikilothermic organisms—these include a large array of cold blooded vertebrates such as the fish as well as plants and lower organisms—are exposed to marked shifts in temperature, Undoubtedly, all membrane-bound enzymes; transport processes, receptor sites, etc. associated with membranes are surrounded by a lipid milieu: therefore, their activities would be markedly altered by the physical state of the membrane lipids, which in turn is in part a reflection of the surrounding temperature. Thus, it can be shown that lipids of liver mitochondria from homeothermic animals have a higher proportion of saturated fatty acids than those obtained from poikilothermic organisms. These results correlate well with the presence or absence of thermal phase changes in the membrane lipids of these animals, respectively.

3.3.2 Membrane Proteins

In general, two classes of proteins appear to be associated with plasma membranes. One group, called peripheral proteins, is weakly bound and can be displaced by hypotonic exposures, strong salts, mild detergents or sonication. Examples include cytochrome c, which is loosely associated with the outer face of the inner membrane of mitochondria, and α-lactalbumin, which is loosely associated with the plasma membrane of mammary gland cells. In addition, periplasmic-binding proteins are classified as peripheral proteins of the plasma membrane of bacteria. The second class of proteins, called integral proteins, is tightly bound to a lipid bilayer and may include a large number of functional proteins that participate as transport carriers, drug and hormone receptor sites, antigens and a large number of membrane-bound enzymes. For example, cytochrome b_5 is classified as an integral protein of the endoplasmic reticulum of eukaryotic cells as is the NAD-cytochrome b_5-reductase, which is tightly coupled to the hemeprotein, and cytochrome oxidase which is embedded in the inner membrane of the mitochondrion.

A final comment that can be drawn from the current picture of plasma and organelle membrane structures is that membranes are asymmetric. That is, because of the presence of two classes of proteins, the outer and inner faces of membranes may have markedly different physical, structural and biochemical properties. As we will see, for example, the inner membrane of mitochondria is markedly asymmetrical for a number of important reasons involving transport of ions, the electron transport chain and oxidative phosphorylation.

3.4 CELL ORGANELLES

3.4.1 Endoplasmic Reticulum

The network of membrane-bound channels and vesicles called the endoplasmic reticulum is missing in prokaryotic cells. However, this system is present in all eukaryotic cells.

Varying in size, shape and amount the endoplasmic reticulum divides the intracellular space into two compartments, i.e., luminal (inside the endoplasmic reticulum) and extra-luminal (rest of the cytoplasm).The extends of endoplasmic reticulum varies from cell to cell. There are two kinds of endoplasmic reticulum—the rough surfaced type known as ergastoplasm, which has ribosomes associated with it externally, and the smooth type, which lacks ribosomes (Figure 3.9). When cells are disrupted by homogenization and fractionated by differential centrifugation, the

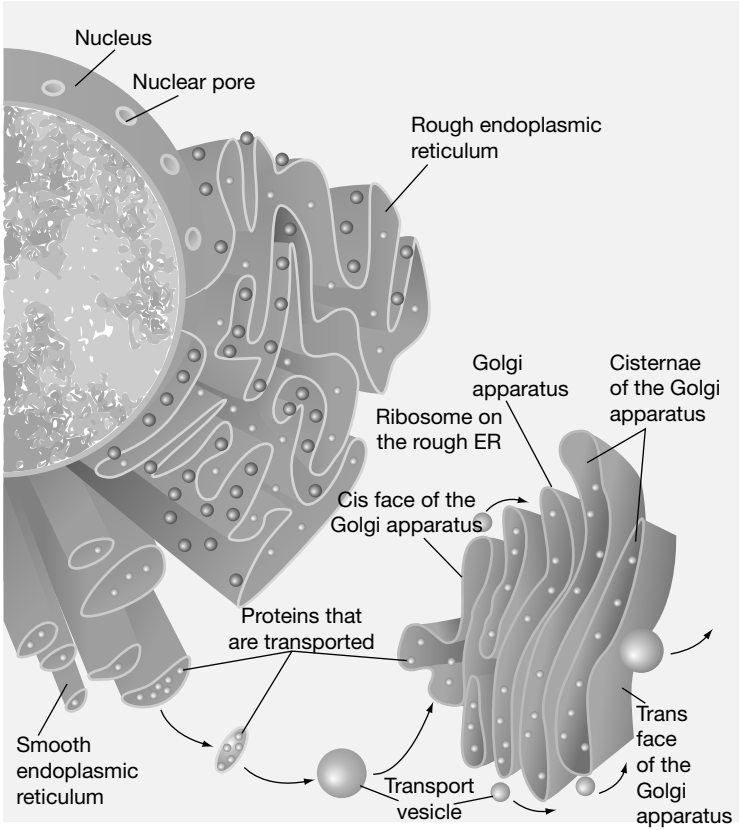


Figure 3.9 Structure of endoplasmic reticulum and Golgi apparatus

pellet, which contains whatever sediments on centrifugation at 100,000 × g for 30 min, is called the microsomal fraction (microsomes) and contains small vesicles and fragments derived from the endoplasmic reticulum. The endoplasmic reticulum of mammalian liver cells is associated with many important enzymes which are responsible for the production of sterols, triacylglycerols and phospholipids, the detoxification of drugs by modification through methylation, hydroxylation, etc. the desaturation and elongation of fatty acids and the hydrolysis of glucose-6-phosphate. As a word of caution, however, many of these activities are only associated with microsomes of liver cells, and are missing in microsomes from other eukaryotic tissues. Although cytochromes characteristic of mitochondria are absent, both cytochrome b_5, which may serve as a limited electron carrier system in the desaturation of fatty acids, and cytochromc P-450, which participates in hydroxylation reactions in animal cells, reside in the microsomes.

3.4.2 Golgi Apparatus (Dictyosomes)

Present in both animal and plant cells is a complex organization of net-like tubules or vesicles surrounded by smaller spherical vesicles. The function of this structure is not completely defined, although techniques have been devised for the isolation of this organelle. Evidence strongly suggests

that the Golgi apparatus participate in the early stage of cell wall synthesis in higher plants as well as the organization of lysosomal structure. The Golgi apparatus plays a role in the secretion of proteins and polysaccharides, and in the coupling of these two components to form glycoproteins. Intense phospholipid biosynthesis has also been observed in this organelle (Figure 3.9).

3.4.3 Ribosome

A few comments are in order here concerning the ribosomal particles. In prokaryotic cells ribosomes are grouped in clusters 10–20 nm in diameter, probably held together by mRNA to form polysomes (Figure 3.10). Because of the intense synthesis of proteins by growing bacterial cells, their cell matrix (sap) contains very many of these clusters.

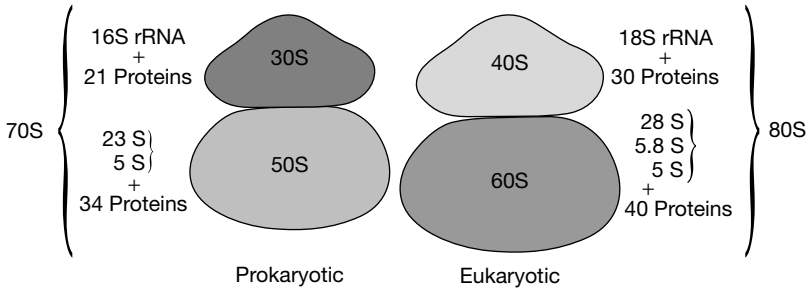


Figure 3.10 Structure of ribosomes

Table 3.5 summarizes the information concerning both prokaryotic and eukaryotic cells. In eukaryotic organisms, since ribosomes are associated closely with the endoplasmic reticulum thereby forming rough surface endoplasmic reticulum, protein synthesis occurs on the endoplasmic reticulum. Presumably, newly formed proteins are secreted into the vesicular system and then transferred to Golgi bodies to be used there in the formation of lysosomes and other microbodies.

Table 3.5 Properties of Prokaryotic and Eukaryotic Ribosomes

Components	Prokaryotic		Eukaryotic	
A. Ribosome unit				
Protein	35%		50%	
RNA	65%		50%	
Sedimentation value	70S		80S	
Molecular weight	2.5×10^6		4.5×10^6	
Ribosome subunits	30S; 50S		40S; 60S	
B. Subunit structure				
	30S	50S	40S	60S
RNA	16S	23S; 5S	18S	28S; 5S
Number of proteins	21	33	34	50

The ribosomes found in mitochondria and chloroplasts closely resemble the bacterial ribosomes in size as well as in sensitivity to protein inhibitors such as chloromycitin. We have discussed in considerable detail the functions of ribosomes in protein synthesis in Chapter 7.

3.4.4 Mitochondria (Singular: Mitochondrion)

Since the nineteenth century, microscopists have observed in all eukaryotic cells small, rod-shaped particles 2–3 μm long, which were called mitochondria. The mitochondrion was the only site for oxidative phosphorylation, the Krebs cycle and fatty acid oxidation in animal cells. Because of the importance of these systems in the total economy of the cell, much research has been expended in defining the structure and function of these bodies that are found universally in eukaryotic cells but are totally missing in prokaryotic cells.

Although prokaryotic cells have no mitochondrial bodies, their plasma membrane appears to be the sites of electron transport and oxidative phosphorylation. Thus, all the cytochrome pigments and a number of dehydrogenases associated with the tricarboxylic acid cycle, namely succinic, malic and α-ketoglutaric dehydrogenase are localized in the bacterial plasma membrane. In addition, enzymes involved in phospholipid biosynthesis and cell wall biosynthesis are also found in or on this membrane structure.

All mitochondria consist of a double membrane system. An outer membrane separated from but enveloping an inner membrane, which by invagination extends into the matrix of the organelle as cristae (Figure 3.11). Considerable evidence suggests that all the enzymes of the electron transport system, namely the flavoproteins succinic dehydrogenase, cytochromes b, c, c_1, a, and a_3, are buried in the inner membrane. In addition the inner surface of the inner membrane has projecting into the matrix, a cluster of knobs on stalks called inner membrane particles. These structures (85 Å in diameter) possess the coupling factor F_1 which has ATPase activity and a molecular weight of 280,000. It is now believed that this ATPase participates in the final step of oxidative phosphorylation by catalysing the reaction:

$$ADP + Pi \rightleftharpoons ATP + H_2O$$

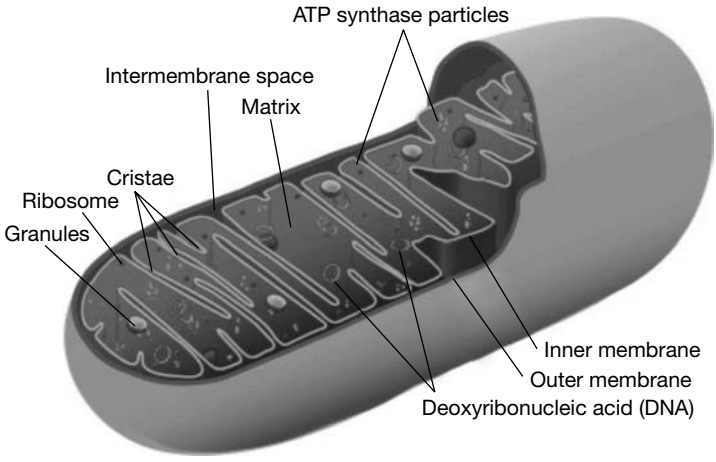


Figure 3.11 Structure of mitochondrion

The inner membrane of mitochondria possesses limited permeability, whereas the outer membrane is fully permeable to a large number of compounds with molecular weights up to 10,000. The outer membrane has a density of 1.13, and the inner membrane has a density of 1.21. The outer membrane has about three times more phospholipids than the inner membrane, and about six times more cholesterol than is found in the inner membrane; hence, it is slightly lower in density. Phosphatidyl inositol is found exclusively in the outer membrane, whereas cardiolipin occurs almost exclusively in the inner membrane. Of the total protein in a mitochondrion, 4 per cent is associated with the outer membrane, 21 per cent with the inner membrane and 67 per cent with the matrix. Ubiquinone is present only in the inner membrane.

In addition to a large number of soluble enzymes, the mitochondrial matrix contains mitochondrial DNA, a circular double-stranded molecule somewhat smaller but very similar to bacterial DNA in shape. In bacteria, mitochondria and chloroplasts the DNA is histone-free and bound to membranes. Each mitochondrion has from 2 to 6 DNA circles amounting to about 0.2–1 μg of DNA/mg mitochondrial protein. This amount of DNA can code for about 70 polypeptide chains of 17,000 mol wt. Since DNA polymerase is found in the matrix, presumably mitochondrial DNA is independently synthesized in the mitochondria. Replication appears to be semiconservative. Of further interest is the observation that 70S ribosome particles are in the mitochondrial matrix as well as tRNA, mRNA and protein-synthesizing enzymes which catalyse a limited type of protein synthesis. A DNA-dependent RNA polymerase has also been detected in the matrix. This complete complement of machinery for synthesizing protein may in some manner be concerned with the formation of a number of unknown mitochondrial proteins. Currently, very little is known about the precise function of this mitochondrial protein-synthesizing machinery. Evidence suggests that the outer membrane protein is synthesized by the cytoplasmic-nuclear protein synthesizing system, and the proteins of the matrix and the inner membrane are synthesized by both the cytoplasmic nuclear system and the mitochondrial system.

A number of biochemists have submitted the provocative speculation that both mitochondria and chloroplasts resemble very closely prokaryotic cells with respect to size, distribution of respiratory enzymes and the striking similarity of their DNA and RNA components. Perhaps, both mitochondria and chloroplasts originated from prokaryotic endosymbionts which, over a long evolutionary period, were gradually integrated into their host.

3.4.5 Chloroplast

All eukaryotic organisms with photosynthetic capabilities have chlorophyll-containing organelles called chloroplasts. Only the structure of higher plant chloroplasts will be considered here, although lower plants possess these organelles in varying size, shape and number. The size of the chloroplast is about 5–10 μm, in diameter and about 2–3 μm thick. About 50 per cent of the dry weight of the chloroplast is protein, 40 per cent lipid and the remainder water soluble small molecules. The lipid fraction consists of about 23 per cent chlorophyll (a + b), 5 per cent carotenoids, 5 per cent plastoquinone, 11 per cent phospholipid, 15 per cent digalactosyl diglyceride, 36 per cent monogalactosyl diglyceride and 5 per cent sulpholipid.

Chloroplasts have a bilayer membrane enclosing the outer envelope. Internally are found a large number of closely packed membranous structures called lamellae, which contain the chlorophyll of the organelle. In one kind of chloroplast the lamellae are arranged as closely packed discs or stacks called grana which are interconnected to each other by intergrana or stroma

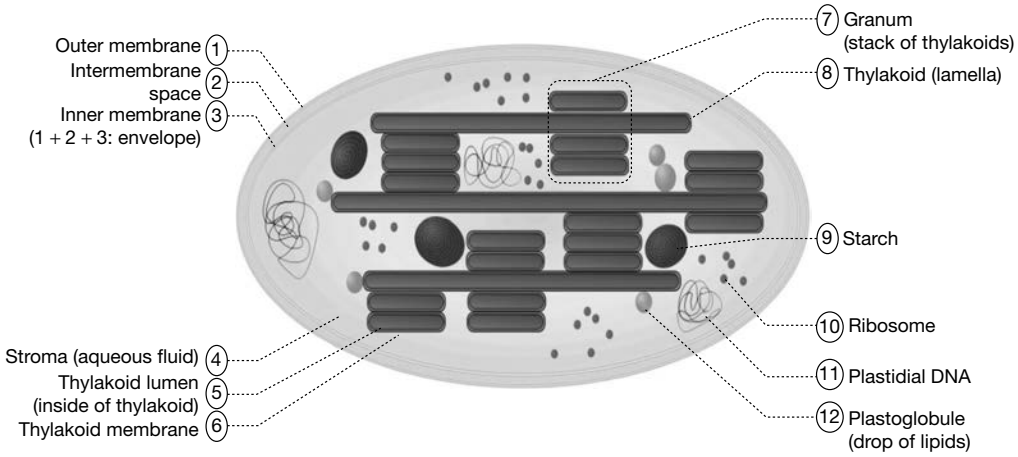


Figure 3.12 Structure of chloroplast. The thylakoids within chloroplast are suspended in the stroma. The thylakoids when stacked together form grana

lamellae. The grana stacks are the sites of oxygen evolution and photosynthetic phosphorylation. In other chloroplasts the chlorophyll-containing lamellae are not arranged in stacks but rather extend the length of the organelle (Figure 3.12). As we observe from Figure 3.12, a plant species may have both kinds of chloroplasts. The matrix embedding the lamellae is called the stroma and is the site of the carbon photosynthetic enzymes involved in CO_2 fixation, ribosomes, nucleic acid-synthesizing enzymes and fatty acid-synthesizing enzymes.

Chloroplasts are extremely fragile osmometers, since only a brief exposure to distilled water will result in a bursting of the outer envelopes, loss of stroma protein and marked changes in the appearance of the lamellar systems.

Chloroplasts contain circular chloroplast DNA. Ribosomes are of the 70S species and are very similar to those observed in mitochondria and bacteria. A DNA-dependent RNA polymerase also occurs in intact chloroplasts.

In photosynthesizing prokaryotic cells such as *Rhodospirillum rubrum*, small particles, about 60 μm, in diameter, are attached to the inner surface of the cell membrane. These particles are called chromatophores; they have no limiting membrane and they possess all the bacteriochlorophyll. They are, therefore, sites of bacterial photosynthesis. In the prokaryotic blue-green algae, no discrete chloroplasts are visible, but the photosynthetic lamellar membrane occupies most of the cell. These prokaryotic cells therefore seem to be more advanced than the chromatophore-containing bacteria but less developed than eukaryotes such as the green algae which have membrane-limiting chloroplasts.

3.4.6 Lysosomes

In 1955, the Belgian biochemist de Duve discovered and described for the first time a new organelle, the lysosome. Found in all animal cells except erythrocytes in varying numbers and types, the lysosome in general is a rather large organelle consisting of a unit membrane enclosing a matrix containing about 30–40 hydrolytic enzymes that are characterized by having an acid pH optima. Acid phosphatase is used as a marker enzyme for this organelle.

Enzyme groups in lysosomes include the following.

Ribonucleases	Cathepsins (proteinases)
Deoxyribonucleases	Acid glycosidases
Acid phosphatases	Sulphatases
Lipases	Phospholipases

Collectively, the lysosomal enzymes act on a number of biopolymers. Thus, the proteases have a wide capacity for the hydrolysis of proteins, the acid nucleases for RNA and DNA, and the acid glycosidases for polysaccharides, A family of acid phosphatases also is present. The median value for the pH optima of these enzymes is around pH 5. Thus, the lysosomal matrix must be acidic for the enzymes to be reactive. It is attractive to consider the lysosomal membrane which has a high specific activity for NADH dehydrogenase serving as a hydrogen ion pump. All the enzymes, other than the esterases and the NADH dehydrogenase, are present as soluble proteins in the matrix of the lysosome.

3.4.7 Nucleus

Although in prokaryotic cells no nucleus per se is observed, a fibrillar area can be detected on the interior side of the plasma membrane which is associated with an extremely involuted double-stranded circle of DNA. It has been estimated that in a single bacterial cell of 2 μm long, its DNA, if stretched out as a single fibre, would be over 1000 μm long, 500 times the length of its own cell body. Since histones are absent in these cells, no DNA–histone complexes exist. However, high concentrations of polyamines such as spermidine, spermine, cadaverin and putrescine have been detected in the bacterial cell, and these compounds may participate in neutralizing the negative charges on the DNA.

$\overset{+}{N}H_3 - (CH_2)_3 - NH_2 - (CH_2)_4 - \overset{+}{N}H_3$

Spermidine

$\overset{+}{N}H_3 - (CH_2)_3 - NH_2 - (CH_2)_4 - NH_2 - (CH_2)_3 - \overset{+}{N}H_3$

Spermine

In eukaryotic cells, the nucleus is a large dense body surrounded by a double membrane with numerous pores which permit passage of the products of nuclear biosynthesis into the surrounding cytoplasm. Internally, the nucleus contains chromatin or expanded chromosomes composed of DNA fibres closely associated with histones. During nuclear division, the chromosomes contract and become clearly visible in the light microscope as the DNA chains undergo their programmed changes. In addition, the nucleoplasm contains enzymes such as DNA polymerases, RNA polymerases for mRNA and tRNA synthesis; further, surprisingly, the enzymes of the glycolytic sequence, citric acid cycle and the pentose phosphate pathway have been detected in the nucleoplasm. One to three spherical structures called the nucleolus are closely associated with the inner nuclear envelope and are presumably the sites of rRNA biosynthesis, This dense suborganelle is nonmembranous and contains RNA polymerase. RNAase, NADP pyrophosphorylase, ATPase and S-adenosylmethionine-RNA methyltransferase. There is absence of DNA polymerase. Ribosomal RNAs are separately synthesized in the nucleolus and are then transported to the cytoplasm as discrete units to be assembled in the cytoplasm to form polysomes.

3.4.8 Microbodies

This term covers a number of single membrane-enclosed cytoplasmic organelles which have been found in both animal and plant cells and which contain H_2O_2-producing oxidases and catalases. The particles are approximately 0.5 μ in diameter.

In general, leaf tissues possess peroxisomes which possibly serve as sites of photorespiration in the leaf cell. This process involves the oxidation of glycolic acid (a product of photosynthetic CO_2 fixation) to CO_2 and H_2O_2. They have a single membrane and granular matrix without lamellae which houses catalase, glycolic oxidase, isozymes of malate dehydrogenase, NADP isocitrate dehydrogenase and the transaminases glyoxylate glutamate, hydroxypyruvate serine and oxaloacetate glutamate. They are approximately 1 μ in width, and they number from a few to one-seventh as many as mitochondria in the leaf cell.

In seeds rich in lipid, microbodies called glyoxysomes are the sites of the following enzymes: citrate synthetase, aconitase, the glyoxylate bypass enzymes isocitrate lyase and malate synthetase, malate dehydrogenase, fatty acyl CoA synthetase, crotonase, β-hydroxyacyl CoA dehydrogenase, thiotase, catalase, glutamate oxalacetate transaminase, glycolic oxidase and uricase.

This organelle is beautifully designed to convert fatty acids to C_4 acids which can then be further converted to sucrose, etc. Since carbohydrate-rich seeds do not draw on storage lipids for carbon skeletons or energy, this organelle is appropriately not present. Since the glyoxysome contains, in addition to the glyoxylate bypass enzymes, catalases and a H_2O_2 generating glycolic oxidase, it could be considered as a highly-specialized peroxisome.

In rat liver, the microbodies are also known as peroxisomes. Urate oxidase, D-amino acid oxidase and L-amino acid oxidase are always localized in these organelles in addition to catalase. In the liver cell, mitochondria are about 2–5 times more numerous than peroxisomes, with lysosomes being about 1.5 times less numerous than peroxisomes.

In summary, the microbodies have in common very high catalase activity and one or more H_2O_2-generating oxidases spatially separated from other important sites of metabolism. They possess neither a respiratory chain system nor an energy-conserving system such as that occurs in certain other organelles.

3.5 TRANSPORT PROCESSES

Biomembranes allows the movement of all compounds which are essential for the normal function of a cell across the membrane barrier. These compounds include a vast array of sugars, amino acids, steroids, fatty acids, anions and cations to mention a few. These compounds must enter or leave the cell in an orderly manner.

There are at least four general mechanisms by which metabolites (solutes) can pass through biomembranes.

3.5.1 Passive Diffusion

A few metabolites of low molecular weight are presumed to move or diffuse across the membrane. The rate of flow is directly proportional to the concentration gradient across the membrane. When the concentration gradient ceases to exist no further flow occurs. Active transport inhibitors such

as cyanide or azide do not affect the process. Although passive diffusion was believed to be an important transport mechanism in cells, current information suggests that this process is very limited. Water would be an example of a simple compound passing through a membrane by passive diffusion, but essentially all metabolites move through membranes by more sophisticated and hence better regulated processes.

3.5.2 Facilitated Diffusion

This type of diffusion is somewhat similar to simple diffusion in that a concentration gradient is required and the process does not involve an expenditure of energy. However, it differs in several important respects from passive diffusion. First, the membrane contains a large number of specific components called carriers which facilitate the individual transport processes, that is, speeds up the rate of diffusion much more than is predicted from simple diffusion. Second, the diffusion is mechanism-stereospecific. Third, the rate of penetration of the metabolite approaches a limiting value with increasing concentration on one side of the membrane. The rate of the process is also temperature-sensitive. The kinetics mimic simple Michaelis–Menten enzyme kinetics (Chapter 13), that is, the system can be saturated. Thus, K_m and V_{max} values are easily measured and characterize the carrier system.

The general mechanism can be explained by a specific carrier molecule present in the membrane, which forms a specific complex with the metabolite to be transported at the outer area of the membrane. The complex then, by diffusion, rotation, oscillation, or some other motion, translocate to the inward area of the membrane, where it dissociates to discharge the metabolite. However, none of these suggested mechanisms has actually been demonstrated.

Recent evidence has revealed over a 100 small molecular weight proteins localized in the outer surface of plasma membranes in Gram-negative organisms that fulfil the requirements of facilitated diffusion. Arthur Pardee, while studying the transport of sulphate in *Salmonella typhimurium*, has isolated a protein with a molecular weight of 34,000. It contains no lipid, carbohydrate, phosphorus or SH groups. One sulphate molecule is specifically bound per molecule of protein, and the binding is very strong. Binding is, however, reversible and requires no ATP. The protein exhibits no enzymatic activity. Under conditions of a low sulphate source, the organism produces about 10^4 molecules of the binding protein per bacterium. Osmotic shock treatment of cells results in considerable loss of the carrier protein, suggesting that the protein is near or on the cell membrane surface (periplasmic space). An increasing number of specific membrane transport proteins have now been isolated and crystallized. These include carrier proteins for glucose, galactose, arabinose, leucine, phenylalanine, arginine, histidine, tyrosine, phosphate, Ca^{2+}, Na^+ and K^+. They all have small molecular weights ranging from 9000 to 40,000 and occur as monomers.

3.5.3 Active Transport

This process, one of the most important properties of all plasma membranes, is very similar to facilitated diffusion with the critical exception that the metabolite or solute moves across the membrane against a concentration gradient and this requires energy input. Use of an inhibitor, such as azide or iodoacetate that markedly decreases the production of energy in the cell, greatly inhibits active transport. Neither passive diffusion nor facilitated diffusion would be affected by the use of these inhibitors.

Most models for this mechanism postulate that the outside solute combines a carrier and then the carrier–solute complex is modified in the lipophilic membrane by energy input in such a way that the carrier's affinity for the solute is lowered, the solute is released into the interior of the cell and the high affinity conformer of the carrier is regenerated and the cycle is repeated.

Recent evidence clearly indicates that the energy input required for transport involves two distinctly different systems: (a) a non–ATP utilizing respiration linked process and (b) direct utilization of ATP.

3.5.4 Respiratory Linked Active Transport

These systems are coupled to the oxidation of a suitable substrate such as D-lactate, L-malate or NADH which is catalysed by a flavin linked membrane bound dehydrogenase. By respiratory chain, electrons derived from the substrate are transferred to oxygen. The generation or hydrolysis of ATP is not involved. The role of specific binding proteins in this process is not clear at present. Under anaerobic conditions, a reducible substrate can serve in place of oxygen as a terminal electron acceptor. A large number of metabolites is now known to be transported into bacteria by this type of system. Thus in *E. coli* with D-lactate as the source of electrons, the following metabolites move into the interior of the cell: alpha galactosides, galactose, arabinose, glucuronic acid, hexose phosphates, amino acids, hydroxy, keto and dicarboxylic acids and nucleosides.

3.5.5 ATP-dependent Transport

All plasma membranes have one enzyme activity in common, namely an ATPase which is activated by Mg^{2+}, K^+ and Na^+. The overall reaction hydrolyzes ATP in a stepwise fashion involving (a) a Na^+-dependent phosphorylation of the enzyme and (b) a K^+-dependent hydrolysis of the phosphoenzyme. The enzyme–phosphate bond has been identified with an aspartyl-β-phosphate residue (Figure 3.13).

This unique membrane bound NaK ATPase has now been solubilized, has a molecular weight of about 250,000 and two subunits, one with molecular weight of 84,000 to 100,000 and the other, a glycoprotein with the molecular weight of 55,000. The larger unit is phophorylated by ATP.

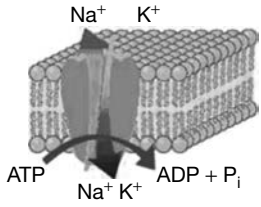


Figure 3.13 Three Na^+ bind to Na^+/K^+-ATPase. The binding and subsequent phosphorylation of the enzymes result in conformational changes and the enzymes expel 3 Na^+ while 2 K^+ bind Na^+/K^+-ATPase. This is followed by dephosphorylation of the enzymes which brings about changes in the structure of the enzyme and release of K^+ inside.

EXERCISES

1. What is the difference between a prokaryotic cell and a eukaryotic cell?
2. Write in detail the structure of mitochondria. Why mitochondria is called as power house of the cell?
3. Write short note on:
   (a) Lysosome
   (b) Golgi apparatus
   (c) Endoplasmic reticulum
4. Write the difference between plant cell and animal cell with the help of a diagram.
5. Write notes on cell wall. How the cell wall of Gram-positive bacteria differs from Gram-negative bacteria?
6. Discuss in detail the structure of a fluid membrane.
7. Write short notes on:
   (a) Membrane assembly
   (b) Disorders of membrane
   (c) Active transport
8. Write the difference between:
   (a) Osmosis and diffusion
   (b) Lysosomes and lysozymes
9. Write in brief about the transport processes.
10. Give an account of plasma lipids and plasma proteins.

MULTIPLE CHOICE QUESTIONS

1. The cell was discovered by
   (a) Robert Hooke (b) Robert Huke
   (c) Robert Monad (d) Robert Hill
   Ans. a
2. The cell theory was first developed in 1839 by
   (a) Matthias Jakob Schleiden
   (b) Matthias Jan
   (c) Jan Evangelista
   (d) Jan Monad
   Ans. a
3. Gram-positive cell wall contains
   (a) Teichoic acids (b) Tryptophan
   (c) Acetic Acid (d) Tyrosine
   Ans. a
4. Gram-negative cell wall do not contain
   (a) Teichoic acids
   (b) Lipids
   (c) Phospholipids
   (d) Lipopolysaccharides
   Ans. a
5. Ribosomes bound to rough endoplasmic reticulum synthesize
   (a) Proteins (b) Lipids
   (c) Nucleic acid (d) Carbohydrate
   Ans. a
6. The prokaryotic cells do not contain
   (a) Mitochondria (b) Circular DNA
   (c) Ribosomes (d) Flagella
   Ans. a

7. The eukaryotic cells contain the type of ribosomes
   (a) 80S (b) 70S
   (c) 60S (d) 50S
   Ans. a
8. The osmotic pressure of a solution increases with the rise in
   (a) Temperature (b) Cold
   (c) Humidity (d) Rancidity
   Ans. a
9. The osmotic pressure of a solution relating to solute molecules depends on the
   (a) Size (b) Shape
   (c) Number (d) Volume
   Ans. c
10. If a cell is immersed in a concentrated solution, it follows the phenomenon
    (a) Turgor (b) Plasmolysis
    (c) Haemolysis (d) Paralysis
    Ans. b
11. Osmosis is opposite to
    (a) Effusion (b) Transport
    (c) Confusion (d) Diffusion
    Ans. d
12. The intracellular fluid of red cells and the red cell membrane in 0.92 per cent NaCl solution maintains a relation
    (a) Hypertonic (b) Hypotonic
    (c) Isotonic (d) None of these
    Ans. c
13. Haemolysis is caused by the dilution of RBC by
    (a) Diffusion (b) Osmosis
    (c) Effusion (d) Imbibition
    Ans. b
14. By respiratory chain, electrons derived from the substrate are transferred to
    (a) Oxygen (b) Hydrogen
    (c) Both (a) and (b) (d) None of these
    Ans. a
15. This type of diffusion is somewhat similar to simple diffusion in that a concentration gradient is required and the process does not involve an expenditure of energy
    (a) Facilitated diffusion
    (b) Passive diffusion
    (c) Active diffusion
    (d) None of these
    Ans. a
16. In plant cells, the site of the conversion of radiant energy to chemical energy is the highly structured
    (a) Chloroplast (b) Mitochondria
    (c) Ribosomes (d) None of these
    Ans. a
17. The prokaryotic genus whose members are devoid of cell walls is
    (a) Bacteria
    (b) Mycoplasma
    (c) Both (a) and (b)
    (d) None of these
    Ans. b
18. Which of the following protein is loosely associated with the outer face of the inner membrane of mitochondria?
    (a) Cytochrome c
    (b) α-lactalbumin
    (c) Cytochrome b_5
    (d) None of these
    Ans. a
19. Which the following protein is loosely associated with the plasma membrane of mammary gland cells?
    (a) α-lactalbumin (b) Cytochrome c
    (c) Cytochrome b_5 (d) None of these
    Ans. a
20. Golgi apparatus is also known as
    (a) Dictyosomes (b) Polysomes
    (c) Both (a) and (b) (d) None of these
    Ans. a

4 Composition of Carbohydrates

CHAPTER OBJECTIVES

4.1 Introduction to Carbohydrate
4.2 Classification of Carbohydrates
4.3 Monosaccharides
4.4 Compound Carbohydrates
4.5 Oligosaccharides
4.6 Polysaccharides
4.7 Qualitative Tests for Identification of Carbohydrates

4.1 INTRODUCTION TO CARBOHYDRATE

There is more carbohydrate material in nature than all other organic substances combined. This is due to the fact that carbohydrates make up most of the organic structure of all plants, as well as being present to some extent in all animals. The most abundant carbohydrate is cellulose, found in the woody structures and fibres of plants.

The starches are abundant and widespread, especially in grains, tubers and roots, where they serve as reserve food material for plants and are utilized as the chief carbohydrate food of man.

Cane sugar or sucrose is present in the nectar of flowers, in fruits and in the juices of various plants. The annual production of sucrose, largely for food, is about 30,000,000 tons, approximately two-thirds from sugar cane and one-third from sugar beets. Large quantities of pentosans, so called because they are composed of simple sugars called pentoses, are found in plants, especially in seed husks, corn cobs and other fibrous structures, and in plant gums and mucilages.

The glucosides are a class of carbohydrate derivatives which are frequent constituents of plants. A number of these substances is important drugs, among which are the glucosides of *Digitalis* used in the treatment of heart disease.

The simple sugars—glucose and fructose—occur in small amounts and widely distributed in plants. Carbohydrates and their derivatives are present in all animal tissues and tissue fluids, blood and milk. To a greater or lesser extent, they are found in animal secretions and excretions.

Although a variety of carbohydrates, such as starch, dextrins, sucrose and lactose or milk sugar, are taken in the food, all the substances are eventually converted to the simple sugar, glucose, which is the primary carbohydrate utilized by the body tissues.

Glucose is the sugar of blood and other body fluids. Blood normally contains from 60 to 90 mg of glucose in each 100 ml, but in the diabetic condition more than 1000 mg may be present.

Blood glucose serves a number of purposes. The liver removes it and combines many molecules to form the polysaccharide glycogen, which normally is present in liver to the extent of a few per cent. Liver glycogen helps keep up the blood glucose level by breaking down to glucose again when absorption of sugar from the intestine tails off after a meal. Muscles and other tissues remove glucose from blood to form glycogen, which by breaking down through many and complex reactions provides energy to operate the tissue machinery. Blood glucose serves as the direct food for brain tissue, which removes it from the circulating blood as needed. Mammary glands remove glucose from the blood, convert some of it to another sugar, galactose, and then combine a molecule of galactose with a molecule of glucose to form lactose, which is milk sugar. Other sugars or sugar derivatives are formed from blood glucose and combined with proteins and other substances to make essential tissue constituents. Glucose is oxidized preferentially by all the tissues of the body to provide energy. Ordinarily, more than half of the energy of the body is provided by the oxidation of glucose. Excess glucose is readily converted to fats in the body and stored in the fat depots. A very large proportion of all the chemical changes occurring in tissues involve glucose or substances derived from it.

Chemical characteristics of the carbohydrates: The carbohydrates are all compounds of carbon, hydrogen and oxygen as are thousands of compounds that are not carbohydrates. Generally, but not always, the hydrogen and oxygen in carbohydrates are present in the proportion of two hydrogen atoms to one oxygen atom as in H_2O, from which fact the term 'carbohydrate' (carbon hydrate) was derived. Glucose has the molecular formula $C_6H_{12}O_6$ and can be written as carbon hydrate, $C_6(H_2O)_6$, which, however, is entirely erroneous from a structural standpoint. Many substances but not carbohydrates contain hydrogen and oxygen in the proportion of H_2O such as acetic and lactic acids, $C_2H_4O_2$ and $C_3H_6O_3$. Also, some carbohydrates, such as rhamnose, $C_6H_{12}O_5$, do not contain hydrogen and oxygen in the proportion of H_2O. The carbohydrates are actually or potentially (convertible into) hydroxy aldehydes or ketones, and in most cases they are polyhydroxy aldehydes or ketones. All the simple sugars contain a free sugar group in which the carbonyl group is part of an aldehyde or ketone group. All the compound sugars, which are made up of simple sugar molecules, contain the sugar group in combined form, though a number of them also possess one free sugar group.

4.2 CLASSIFICATION OF THE CARBOHYDRATES

The carbohydrates are sometimes referred to as the saccharides. The word 'saccharide' comes from the Greek word sakcharon, meaning sugar. Those carbohydrates such as glucose and fructose that cannot be hydrolysed into simpler compounds are called 'simple sugars,' or 'monosaccharides,' or 'monosaccharoses,' or at times simply 'monoses.' Certain carbohydrates are made up of two molecules of monosaccharide, such as sucrose, lactose and maltose. These carbohydrates are called 'disaccharides,' or 'disaccharoses.' Similarly, trisaccharides are composed of three and tetrasaccharides of four monosaccharide molecules. Those carbohydrates made up of many monosaccharide molecules (starches, glycogens, celluloses, etc.) are called

'polysaccharides,' or 'polysaccharoses.' All these compound carbohydrates may be readily hydrolysed into their constituent monosaccharides by heating with dilute acids or by the action of specific enzymes.

Monosaccharides containing from 2 to 10 carbon atoms have been synthesized, and many occur in natural sources. They are either hydroxy aldehydes or hydroxy ketones and are named systematically to indicate both the number of carbon atoms present and the aldehyde or ketone structure. For example, glycol aldehyde, $CH_2OH \cdot CHO$, the simplest compound to be classed as a carbohydrate, contains two carbon atoms and is referred to as a 'diose.' Since it contains an aldehyde group, it is called an 'aldose.' To indicate the presence of two carbon atoms and an aldehyde group in a single term it is called an 'aldodiose.' Similarly, glyceric aldehyde, $CH_2OH \cdot CHOH \cdot CHO$, contains three carbon atoms, is an aldehyde and is called an 'aldotriose.' Glucose, $C_6H_{12}O_6$, contains an aldehyde group and is an aldohexose. Sugars possessing a ketone group are 'ketoses.' Dihydroxyacetone, $CH_2OH \cdot CO \cdot CH_2OH$, with three carbon atoms and a ketone group, is a 'ketotriose'; and fructose, $C_6H_{12}O_6$, since it has a ketone group and carbons 18, is a 'ketohexose.'

The several ways of classifying carbohydrates vary in minor details and completeness. Since one group of carbohydrates cannot be hydrolysed into anything simpler, it is logical to refer to these substances as the simple carbohydrates, or simple sugars, or monosaccharides or monosaccharoses. However, many carbohydrates are made up of from two to a thousand or more molecules of simple sugar into which they may be split by hydrolysis. These carbohydrates may be referred to as the 'compound carbohydrates.' The following classification is based upon these considerations.

4.3 MONOSACCHARIDES

Monosaccharides, marked with an asterisk, occur in nature, whereas others are synthetic. The hexoses and pentoses are most important of the simple sugars. The monosaccharide or simple sugars are generally well-crystallized solids, soluble in water and have a more or less sweet taste. They all have the property of reducing alkaline copper solutions and giving other reactions that are characterstic of the free sugar groups, such as reactions with phenylhydrazine, hydrogen cyanide and hydroxylamine. The smallest of monosaccharides, for which $n = 3$, are dihydroxyacetone and D- and L-glyceraldehyde (Figure 4.1a). The structures of D-glucose and D-fructose are shown Figure 4.1b. The structures of D-ribose (an aldopentose) and 2-Deoxy-D-ribose (an aldopentose) are shown in Figure 4.1c.

Monosaccharides with their names and sources

Monosaccharide	
Name	**Source**
Glucose	Grape sugar, blood sugar and dextrose
Galactose	Lactose in milk
Fructose	Fruits and honey

Ribose forms the backbone structure of both RNA and DNA.

Disaccharides	
Name	**Source**
Sucrose	Glucose and fructose; table sugar, cane sugar and beet sugar
Lactose	Milk containing glucose and galactose
Maltose	Two units of glucose, found in germinating grains

Polysaccharides	
Name	**Source**
Starch	Wheat, rice, corn, oats, barley and potatoes
Cellulose	Rigid cell walls in plants consists of many glucose monosaccharide units
Glycogen	Storage form of glucose in animals

Glycogen Storage form of glucose in animals.

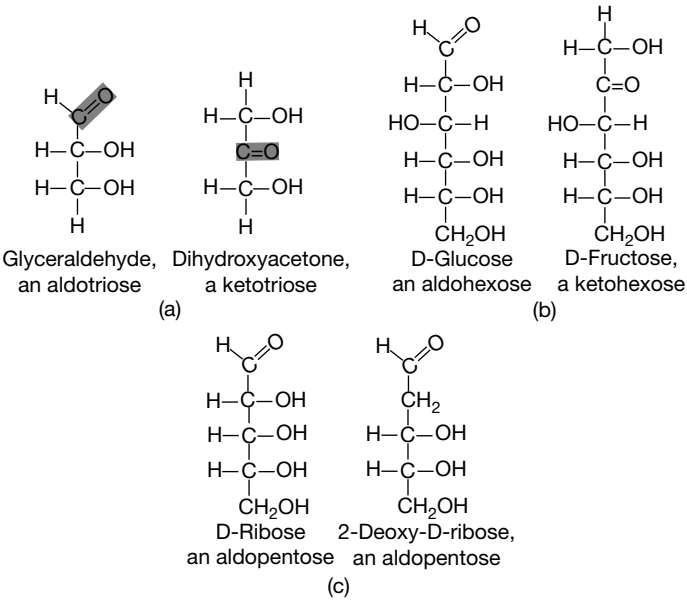


Figure 4.1 (a) Glyceraldehyde and aldotriose, Dihydroxyacetone, a ketotriose, (b) D-glucose, an aldohexose, D-fructose, a ketohexose and (c) D-ribose, an aldopentose and 2-Deoxy-D-ribose an aldopentose

The monosaccharide is called an aldose if the carbonyl group is an aldehyde. The monosaccharide is called a ketose if the carbonyl group is a ketone (Figures 4.2 and 4.3).

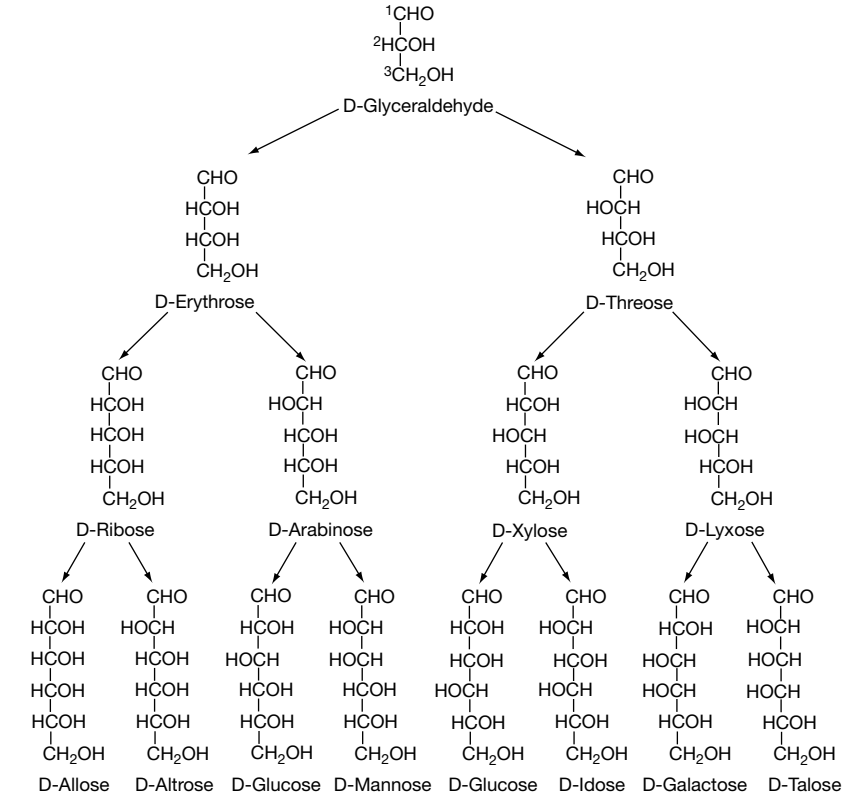


Figure 4.2 Structure of D-aldoses

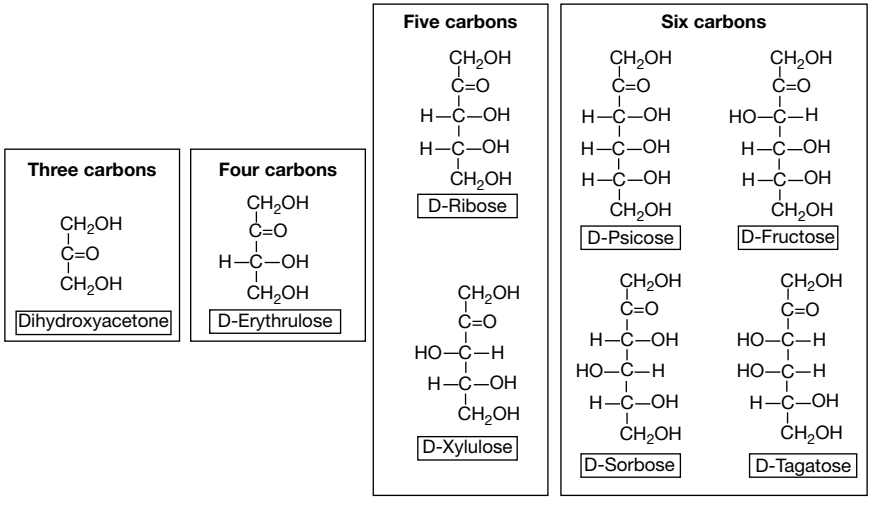


Figure 4.3 Structure of D-ketoses

4.3.1 Monosaccharides have Asymmetric Carbon Atom

A carbon atom to which four different atoms or groups of atom are attached is said to be asymmetric carbon atom. All monosaccharides except dihydroxacetone contain one or more asymmetric carbon atoms and thus occur in optically active isomeric form (Figure 4.4).

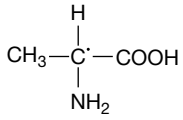


Figure 4.4 Asymmetric carbon atom

4.3.2 Enantiomer

Glyceraldehyde contains one chiral centre and therefore has two different enantiomers. An enantiomer is one of two stereoisomers that are mirror images of each other that are 'non-superimposable' (not identical), much as one's left and right hands are 'the same' but opposite.

4.3.3 D and L Forms

Sugars, amino acids, etc., related to dextro-glyceraldehydes are designated as 'D' compounds, whereas substances related to laevo-glyceraldehydes are designated 'L' compounds.

Although the optical rotations of the glyceraldehydes upon which the D- and L-configurations are based are dextro and laevo, respectively, the rotations of many members of the D-series are laevo, and the L-series are dextro. Accordingly, the prefixes 'D' and 'L' have no significance relative to the direction of optical rotation. They simply designate spatial relations of the compounds to the two forms of glyceraldehydes.

Although in general the direction of the optical rotation of substances may be indicated by the prefixes 'D' and 'L,' in the case of compounds belonging to the D- and L-series of the direction of rotation is designated by prefixes such as 'D (–),' 'D (+),' 'L (–)' and 'L (+).' Thus ordinary fructose, also called levulose because of its laevo-rotation, is D (–)-fructose, and glucose is D (+)-glucose.

The position of the OH group on the lowest asymmetric carbon atom of the sugar indicates whether the sugar is derived from D- or L-glyceric aldehyde. If this OH is on the right, the sugar is derived from D-glyceric aldehyde and is a D-sugar, and if this OH is on the left, the sugar is derived from L-glyceric aldehyde and is an L-sugar (Figure 4.5).

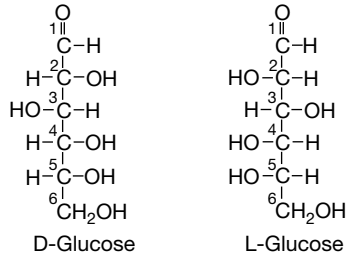


Figure 4.5 D- and L-glucose

4.3.4 Epimers

Epimers are defined as the change in the configuration of two sugars around one carbon atom only. As shown in Figure 4.6, the formation of isomers due to interchange of the OH and H on carbon atoms C-2, C-3 and C-4 of glucose is known as epimers. The mannose and galactose are the epimers of glucose formed by epimerization at C-2, C-3 and C-4. In the body, epimerization takes place by the enzyme epimerase (Figure 4.6).

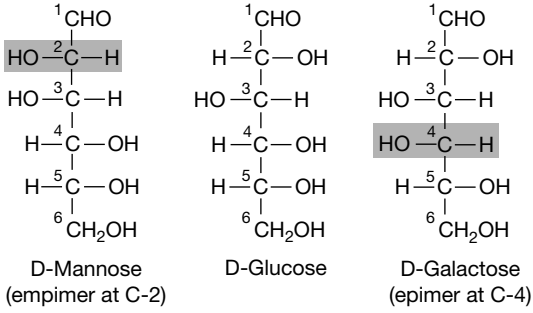


Figure 4.6 Structure of D-mannose, D-glucose and D-galactose

4.3.5 Pyranose and Furanose Rings

Haworth has suggested the six-membered ring forms of the sugars be called pyranose because pyran possesses the same ring of five carbons and an oxygen. Similarly, Haworth has suggested the five-membered ring forms of the sugars be called furanose because furan possesses the same ring of five. The pyranose and furanose ring structures of the sugar proposed by Haworth more accurately represent their actual configuration than do the older projection structures used by Fischer and other workers (Figure 4.9).

Aldehydes add alcohols to form hemiacetal and hemiacetals condense with alcohols to form acetals. In a similar manner, ketones form hemiketals and ketals (Figures 4.7a and b).

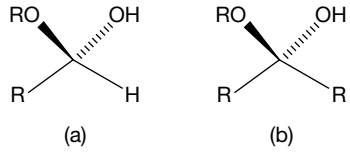


Figure 4.7 (a) Hemiacetal (b) Hemiketal

The pyranose forms of the sugars internal hemiacetals formed by combination of the aldehyde or ketone group of the sugar with the hydroxyl group on the fifth carbon from the aldehyde or ketone group. Similarly, the furanose forms of the sugars are formed by reaction between the aldehyde or ketone group with the hydroxyl group on the fourth carbon from the aldehyde or ketone group. The hemiacetal linkage is present in the aldose ring forms, and the hemiketal linkage is present in the ketose ring forms (Figure 4.8).

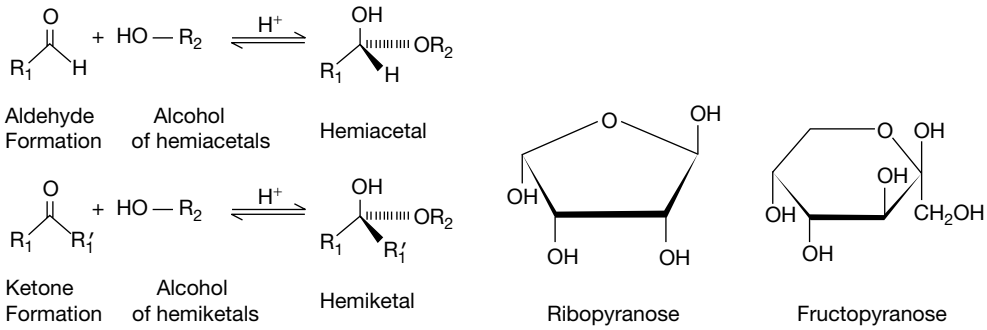


Figure 4.8 Cyclic hemiacetals and hemiketals

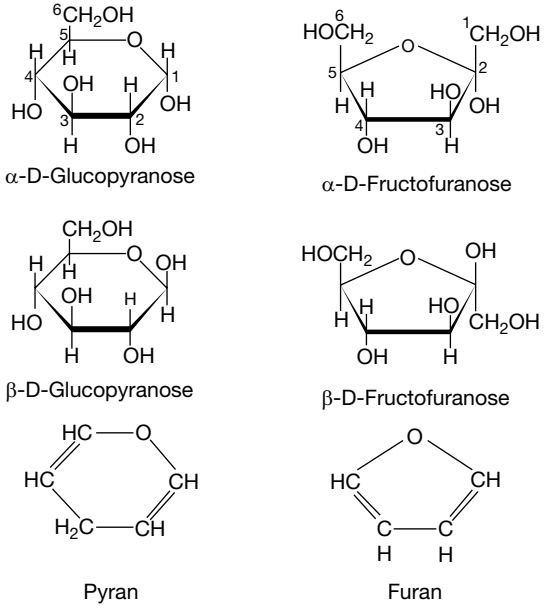


Figure 4.9 Pyran and furan rings

If the H of the reacting hydroxyl group adds to one side of the double-bonded oxygen of the aldehyde group, then α-D-glucopyranose is formed; however, if it adds to the other side of the double bond, then β-D-glucopyranose is the product. In solution the different forms exist in dynamic equilibrium, with nearly all of the sugars in the ring form.

The furanose ring forms of the sugars are produced in a manner analogous to that of the pyranoses.

In the D-series of the aldohexopyranoses, the terminal primary alcoholic group—CH_2OH—projects above the plane of the ring, while in the L-series it lies below the ring. Also, the position of the hemiacetal hydroxyl groups in the α- and β-forms are reversed in the D- and L-series. The α-D and β-L hydroxyl groups lie below the plane of the ring, while β-D and α-L hydroxyl groups lie above the plane of the ring.

4.3.6 α- and β-Anomers

The α- and β-forms of the sugars are also called anomers. The carbon atom giving rise to the α- and β-forms is called the anomeric carbon atom.

The hemiacetal (or carbonyl) carbon atom is called the anomeric carbon.

The α- and β-anomers of glucose as shown in Figure 4.10, indicate that the position of the hydroxyl group on the anomeric carbon relative to the CH_2OH group is bound to carbon 5 and regarding the position of hydroxyl group they are either on the opposite sides (α) or on the same side (β).

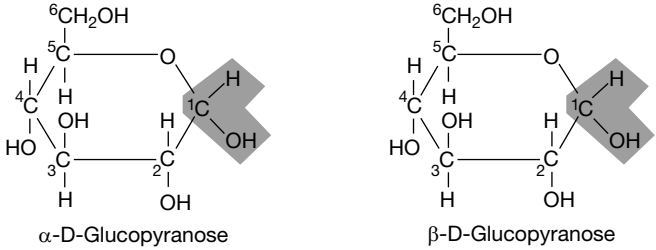


Figure 4.10 Structure of α-D-glucopyranose and β-D-glucopyranose

4.3.7 Isomerism

The topic of isomerism may be divided into **structural isomerism** and **stereoisomerism**.
Structural isomers have the same molecular formula but differ from each other by having different structures.

Stereoisomers have the same molecular formula and the same structure, but they differ in configuration, that is, in the arrangement of their atoms in space.
Structural isomers, in turn, can be of three types as discussed hereunder.

Chain isomers: in which the isomers have different arrangements of the carbon atoms. As an example, *n*-butane is a chain isomer of isobutane (Figure 4.11).

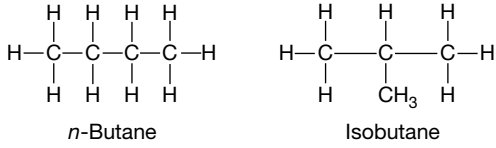


Figure 4.11 Chain isomers

Positional isomers: *n*-propyl chloride and isopropyl chloride, in which the two compounds involved have the same carbon chain but differ in the position of a substituent group, are positional isomers (Figure 4.12).

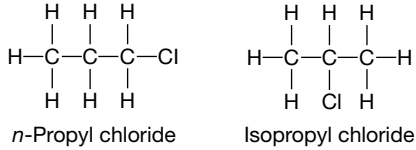


Figure 4.12 Positional isomers

Functional isomer: The third type of structural isomer is that of the functional group isomers, in which the compounds have different functional groups. Examples are *n*-propanol and methylethyl ether (Figure 4.13).

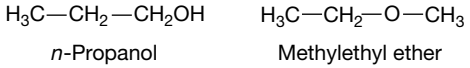


Figure 4.13 Functional isomer

Stereoisomerism can be divided further into **optical isomerism** and **geometrical** (or cis-trans) isomerism. The latter type of isomerism is illustrated by the cis-trans pair, fumaric and maleic acids.

Optical isomerism: This is the type of isomerism commonly found in carbohydrates; further, it is usually encountered when a molecule contains one or more chiral (Greek cheir = hand) or asymmetric carbon atoms.

The carbon atom has the shape of a tetrahedron in which the carbon nucleus sits in the centre of the tetrahedron and the four covalent bonds or bond axes extend out to the corners of the tetrahedron.

When four different groups are attached to those bonds, the carbon atom in the centre of the molecule is said to be a chiral centre (or a chiral carbon atom) (Figure 4.14).

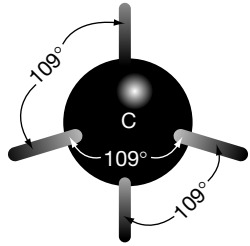


Figure 4.14 Optical isomerism

4.3.8 Projection and Perspective Formulas

In the study of carbohydrates many examples of optical isomerism are encountered and it is necessary to have a means for representing the different possible isomers. One way of representing them is to use the projection formula. The projection formula represents the four groups attached to the carbon atom as being projected onto a plane.

In the Fisher projection formula, positioning of the horizontal bonds and vertical bonds is understood to be in front of the plane of the paper and behind the plane of the paper, respectively. This understanding is more clear in the perspective formula. The dotted lines show that the bonds extending behind the plane of the page while solid wedges identify bonds standing in front of the plane of the page. The projection and perspective formulas can be used to distinguish between the compounds as shown in Figure 4.15 and its mirror image isomer. These two pairs of formula together with a simplified version of the ball-and-stick model can be used to distinguish between the compound and its mirror image isomer as shown in Figure 4.15b. These three pairs of formula constitute three different ways of writing formulas to represent the enantiomeric pairs.

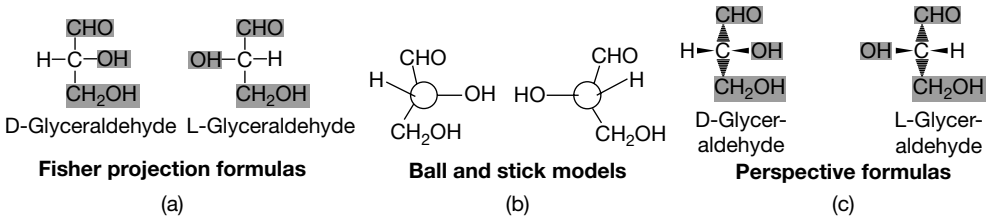


Figure 4.15 (a) Fischer projection formulas, (b) Ball and stick models and (c) Perspective formulas

4.3.9 Chemical Properties of Monosaccharides

Reducing sugars: The classification of carbohydrates may be as either reducing or non-reducing sugars. The more common reducing sugars are able to function as reducing agents because free or potentially free aldehyde and ketone groups are present in the molecule. These carbohydrates have reducing properties because they can reduce Benedict's and Fehling's solutions.

Benedict's solution is a common reagent for detecting reducing sugars; however, in this reagent Cu^{2+} is maintained in solution as its alkaline citrate complex. When the Cu^{2+} is reduced, the resulting Cu^{2+} ion is less soluble and Cu_2O precipitates out of the alkaline solution as a yellow or red solid. The reducing sugar in turn is oxidized, fragmented and polymerized in the strongly alkaline Benedict's solution.

In the aldohexoses, aldehyde group is readily oxidized (as shown by its oxidation by Cu^{2+}) to the carboxylic acid at neutral pH by mild oxidizing agents or by enzymes. The monocarboxylic acid that is formed is known as an aldonic acid (e.g., galactonic acid from galactose). The structures of several of these are shown in Figure 4.16.

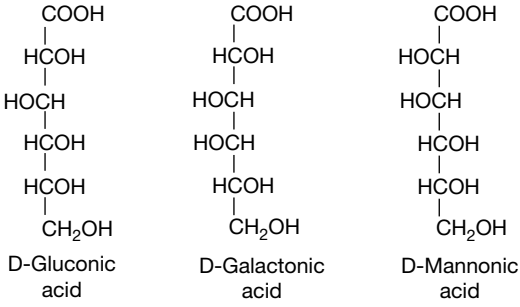


Figure 4.16 Structure of D-gluconic acid, D-galactonic acid and D-mannonic acid

Both the aldehyde and the primary alcoholic function will be oxidized in the presence of a strong oxidizing agent like HNO_3, to yield the corresponding dicarboxylic or aldaric acid (e.g., galactaric acid). One of the more important oxidation products of monosaccharides is the monocarboxylic acid obtained by the oxidation of only the primary alcoholic function, usually by specific enzymes, to yield the corresponding uronic acid (e.g., galacturonic acid, see Figure 4.17). Such acids are components of many polysaccharides.

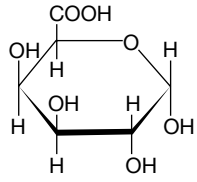


Figure 4.17 α-D-galacturonic acid

In carbohydrate analysis, much use is made of the oxidizing agent, periodic acid. This reagent will cleave carbon–carbon bonds if both carbons have hydroxyl groups or if a hydroxy and an amino group are on adjacent carbon atoms. Thus, the glycoside methyl α-D-gluco-pyranoside will react as shown below. The carbon atoms, whose bonds are severed, are converted into aldehydes (R-CHO). If there happen to be three hydroxyl groups on adjacent carbon atoms, as in this case, the central carbon atom is released as formic acid (Figure 4.18).

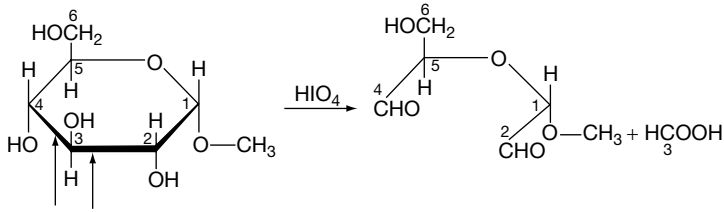


Figure 4.18 Oxidizing agent, periodic acid, will cleave carbon–carbon bonds if both carbons have hydroxyl groups

Sugar alcohols: The aldehyde and ketone functions of monosaccharides may be reduced chemically (with hydrogen or $NaBH_4$) or with enzymes to yield the corresponding sugar alcohols. Thus, D-glucose when reduced yields D-sorbitol and D-mannose products, e.g., D-mannitol. Sorbitol is found in the berries of many higher plants, especially in the Rosaceae; it is a crystalline solid at room temperature but has a low melting point. D-mannitol is found both in algae and fungi. Both compounds are soluble in H_2O and have a sweet taste (Figure 4.19).

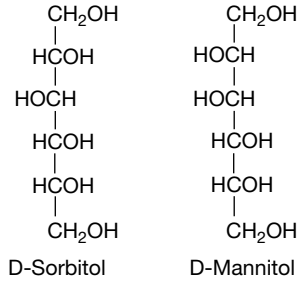


Figure 4.19 Sugar alcohols

Glycoside formation: The ability to form glycosides or acetals is one of the most important properties of monosaccharides. Consider, as an example, the formation of the methyl glycoside of glucose. When D-glucose in solution is treated with methanol and HCI, two compounds are formed (Figure 4.20).

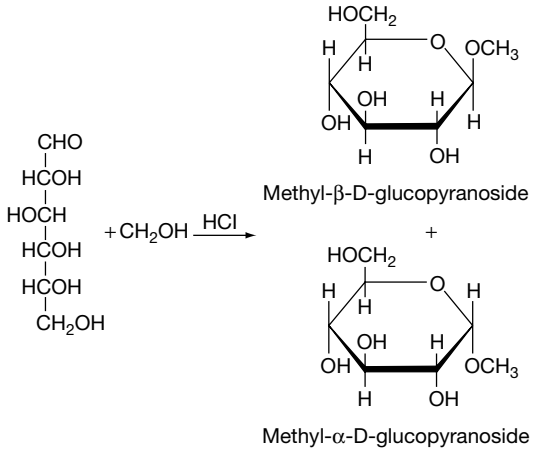


Figure 4.20 Glycoside formation

When an alcoholic hydroxyl group on a second sugar molecule reacts with the hemiacetal (or hemiketal) hydroxyl of another monosaccharide, the resulting glycoside is a disaccharide. The bond between the two sugars is called as a glycosidic bond. Polysaccharides are formed by linking together a large number of monosaccharide units with glycosidic bonds.

In the formation of methyl glycosides, the anomeric hydroxy group of sugars may be methylated with ease, much stronger methylating agents are required for the methylation of the remaining hydroxyl functions.

The remaining four hydroxyl groups of methyl-α-D-glucopyranoside can be reacted with methyl iodide or dimethyl sulphate to yield the pentamethyl derivative. Such compounds, in turn, are useful in determining the ring structure of the parent sugar as in the following example (Figure 4.21).

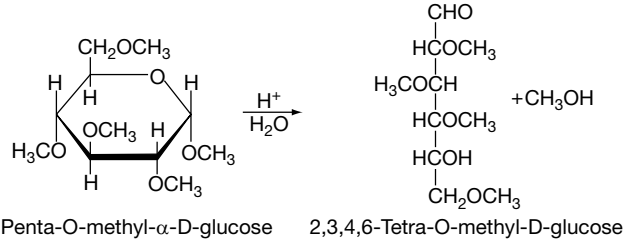


Figure 4.21 Reaction shows conversion of penta-O-methyl-α-D-glucose to 2,3,4,6-Tetra-O-methyl-D-glucose

The methyl group on the hemiacetal carbon, being a glycosidic methyl is readily hydrolysed by acid. The remaining methyl groups, being methyl ethers, are not. Therefore, treatment of the pentamethyl glucose derivative pictured here with dilute acid at 100°C will yield the 2,3,4,6-tetra-O-methyl-D-glucose (Figure 4.22). Treatment of the pentamethyl derivative in which the sugar is in a furanose ring yields 2,3,5,6-tetra-O-methyl-D-glucose instead.

Ester formation: When α-D-glucopyranose is treated with acetic anhydride, all the hydroxyl functions are acetylated to yield the penta-O-acetyl glucose pictured here. These acetyl groups, being esters, can be hydrolysed either in acid or alkali (Figure 4.22).

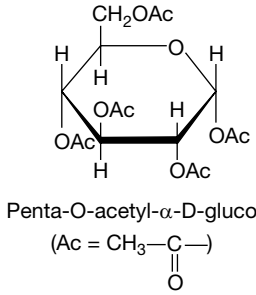


Figure 4.22 Structure of Penta-O-acetyl α-D-glucose

An important type of carbohydrate derivative encountered in intermediary metabolism is the phosphate ester. Such compounds are frequently formed by the reaction of the carbohydrate with adenosine triphosphate (ATP) in the presence of an appropriate enzyme. An example is fructose-1,6-diphosphate (Figure 4.23).

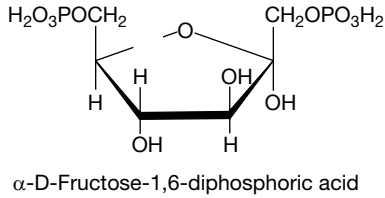


Figure 4.23 Structure of α-D-fructose-1,6-diphosphoric acid

Oxidation: Oxidation of the carbonyl (aldehyde) carbon of glucose to the carboxyl level produces gluconic acid; however, other aldoses yield other aldonic acid. Oxidation of the carbon at other end of the carbon chain-C-6 of glucose or galactose or mannose forms uronic acid like glucuronic acid, galacturonic acid or mannuronic acid. As shown in Figure 4.24, taking glucose as an example, we observe that glucose forms glucuronic acid.

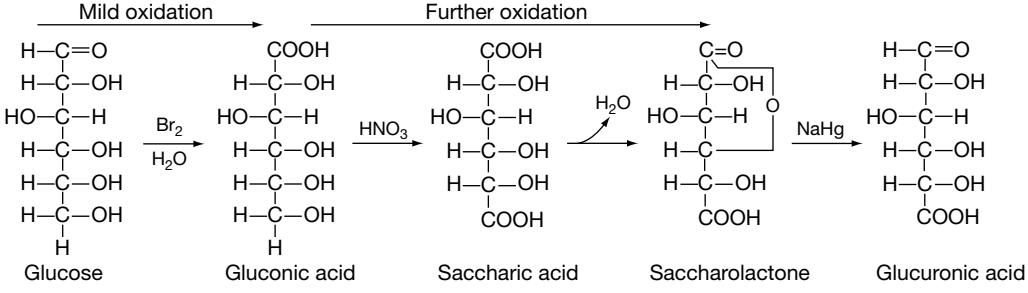


Figure 4.24 Oxidation of Glucose to Glucuronic acid

4.4 COMPOUND CARBOHYDRATES

The compound carbohydrates are derivatives of the monosaccharides in which the monosaccharides are joined together through acetal (glycoside) linkages. The molecular complexity of the compound sugars varies from those made up of two monosaccharide units to those containing hundreds or thousands of these units, such as the starches and glycogen.

The simpler compound carbohydrates containing only a few monosaccharide units are crystalline substances with a sweet taste; they form true solution in water and give the characteristic sugar reaction if a free sugar group is present in the molecule. These carbohydrates are called 'oligosaccharides' because they are composed of only a few (oligos) monosaccharide units.

The more complex compound carbohydrates such as celluloses, starches, glycogen and dextrins are composed of many monosaccharide units, and most of them do not crystallize but are amorphous solids. However, those that are soluble form colloidal solutions. Most of them are tasteless, though the dextrins are mildly sweet in taste. Some contain the free sugar group and give its characteristic reactions, though the quantity of reagent used per unit weight of carbohydrate is small owing to the high molecular weights of these carbohydrates. The complex compound carbohydrates are called 'polysaccharides' because their structures contain many (poly) monosaccharide units.

All the compound carbohydrates are optically active as a result of the optical activity of their constituent monosaccharides. In general, the specific rotations of the polysaccharides are much higher than those of monosaccharaides.

The compound carbohydrates represent an exceedingly important group of substances biochemically. Surcose, lactose, maltose, starches and dextrins form the bulk of man's carbohydrate food. Glycogen is the form in which reserve carbohydrate is stored in the liver and muscles and is the primary carbohydrate involved in supplying energy for muscle contraction. The celluloses make up the larger proportion of the woody and fibrous structures of plants and are used directly and as derivatives for many purposes. The celluloses are by far the most abundant of all organic compounds in nature.

4.5 OLIGOSACCHARIDES

The oligosaccharides are composed of the disaccharides, trisaccharides and tetrasaccharides and are so designated to indicate the number of monosaccharide units involved in their structures. Those which contain free sugar (hemiacetal) groups exist in the α- and β-forms, just as the monosaccharides do.

4.5.1 Disaccharides

The following tabulation gives the better-known disaccharides with their component monosaccharides. Those possessing a free sugar group, which consequently are reducing sugars and, give the other characteristic sugar reactions and hence are indicated as reducing sugars. Sucrose, lactose and maltose are the most important disaccharides.

	Disaccharides	Constituent
	$C_{12}H_{22}O_{11}$	Monosaccharides
1.	Reducing Sugars	
	Maltose	Glucose, glucose
	Lactose	Glucose, galactose
	Cellobiose	Glucose, glucose
	Gentiobiose	Glucose, glucose
	Melibiose	Glucose, galactose
	Turanose	Glucose, fructose
2.	Non-reducing Sugars	
	Sucrose	Glucose, fructose
	Trehalose	Glucose, glucose

Sucrose

It occurs especially in the juices of plants such as sugar beets, sugar cane, sorghum, sugar maple, pineapple and in smaller quantities in the juices of many other plants. Ripe fruits are rich in sucrose. It is by far the most abundantly distributed of all the sugars.

Hydrolysis of sucrose by a dilute acid or by the enzyme invertase or sucrose produces one molecule each of glucose and fructose with a change in optical rotation from positive to negative because D-fructose is more laevorotatory than D-glucose, which is dextrorotatory:

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6$$

Because of the inversion of the sign of rotation in the reaction, the process is referred to as 'inversion' and the mixture of glucose and fructose obtained is called 'invert sugar.' Honey contains a large proportion of invert sugar.

Sucrose is not a reducing sugar, is relatively stable toward the action of alkali and in general does not give the reactions characteristic of the sugar group (hemiacetal or hemiketal group).

The fact that sucrose has no free sugar group indicates that the linkage of glucose to fructose in the molecules involves the 1 hydroxyl of glucose and the 2 hydroxyl of fructose (the sugar group hydroxyls).

Haworth and associates prepared octamethyl sucrose and hydrolysed it. The products were 2,3,4,6-Tetramethyl glucopyranose and 1,3,4,6-Tetramethyl fructo-furanose.

Hudson followed the optical rotation of sucrose solutions hydrolysed by concentrated invertase at low temperatures. The sucrose was hydrolysed almost instantly at 0°C. The specific rotation of sucrose is +65.5°, and of stable invert sugar is –28.2° when measured at 0°C. Despite the fact that Hudson found sucrose to be very quickly converted by invertase to glucose and fructose at 0°C, the specific rotation did not quickly fall to –28.2° as expected. Instead, it dropped moderately rapidly for the first few minutes and then decreased slowly for many hours. After 25 minutes, the rotation was still +1.1°. Hudson has pointed out that only a α-β glucose-to-fructose linkage in sucrose can explain these rotary changes.

It appears that the foregoing phenomena are explainable on the basis of the following processes:

$$\text{Sucrose HOH} \xrightarrow{0^\circ C} \underset{(+\text{rotation})}{\text{D-glucopyranose}} + \underset{(+\text{rotation})}{\text{D-fructofuranose}} \longrightarrow \underset{(-\text{rotation})}{\text{D-fructopyranose}}$$

The sucrose is first split into α-D-glucopyranose and β-D-fructofuranose, both of which are dextrorotatory. However, the less stable β-D-fructofuranose then sets up an equilibrium with its more stable isomer, β-D-fructopyranose, which is strongly laevorotatory. The rate of this change increases rapidly with rising temperature.

Sucrose is hydrolysed by dilute acids more easily than any other sugar. Normal sulphuric acid hydrolyses it rapidly at 20°C, whereas lactose must be heated for a long time at 80°C to achieve the same degree of hydrolysis. Armstrong states that if the rate of acid hydrolysis of lactose is taken as 1.00, then the rate for maltose is 1.27 and for sucrose it is 1240.

Through its hydroxyl groups sucrose forms salts with bases, such as calcium sucrates. All its hydroxyl groups may be methylated or acetylated to form octa-O-methyl sucrose and octa-O-acetyl sucrose. When sucrose is heated to about 200°C, it loses water and forms a brown amorphous mass called 'caramel.'

The presence of the furanose ring in fructose component of sucrose apparently is related to the ease of hydrolysis of the glycosidic bond of sucrose. The free energy of hydrolysis of this bond is 6570 cal which represents a relatively high potential for a chemical reaction.

Sucrose is readily fermented by yeast. It is first split into glucose and fructose by invertase and the monosaccharides and then fermented by the zymase system of enzymes (Figure 4.25).

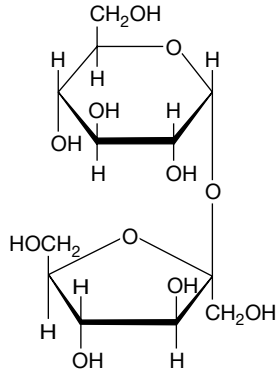


Figure 4.25 Structure of sucrose

Lactose

Lactose is formed in the mammary glands and occurs to the extent of about 5 per cent in milk (Latin lac; hence, its name). It is prepared commercially from milk whey.

Lactose is hydrolysed by acids and the specific enzyme lactase into its constituent monosaccharides, glucose and galactose.

Lactose is reducing sugar, forms osazones, a cyanhydrin and an oxime. Lactose is decomposed by alkalis. It accordingly contains a free sugar group in its structure.

When lactose is carefully oxidized with bromine, the free sugar group is oxidized to a carboxyl giving lactobionic acid. Hydrolysis of lactobionic acid forms gluconic acid and galactose, showing that the sugar group of the glucose unit in lactose is free, while that of the galactose unit is combined. Lactose is thus a glucose galactoside.

The enzyme emulsion, a β-glycosidase found in bitter almonds, selectively splits β-glycoside linkages. This enzyme hydrolyses lactose, proving that the sugar is a glucose β-galactoside.

The structure of lactose has been established by the work of Haworth and Long on methylated lactose.

It is of interest that human milk differs from cow's milk in containing, in addition to regular lactose, other oligosaccharides, such as l-fucosyl-lactose, in which the deoxy sugar l-fucose is linked to galactose at carbon 2.

Lactose generally crystallizes as the α form. It is not fermented by yeast (Figure 4.26).

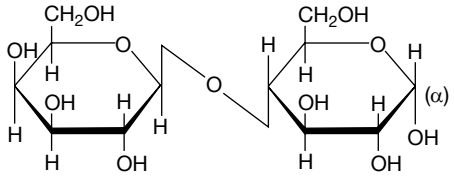


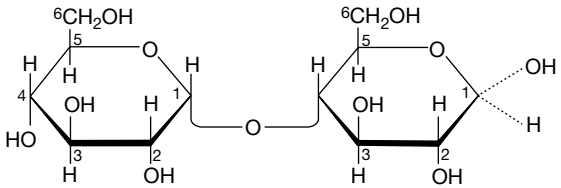
Figure 4.26 Structure of lactose

Maltose

It is composed of two glucose units and is formed when the enzyme amylase or diastase hydrolyses starch. It is a product of the action of salivary amylase (ptyalin) and pancreatic amylase (amylopsin) upon starch in the processes of digestion. Maltose is formed as an intermediate product in the acid hydrolysis of starch and consequently is an important constituent of corn syrups, which are prepared by partial hydrolysis of starch with a dilute acid. These syrups are complex mixtures of dextrins, maltose and glucose. Commercial malt sugar is a mixture of maltose and dextrins formed from starch by hydrolysis with amylase.

Maltose contains a free sugar group, since it is a reducing sugar and gives the other reactions characteristic of the sugar group. Maltase, an α-glycosidase which splits α-glycoside linkages only and which is abundant in yeast, hydrolyses maltose to give two molecules of glucose. These facts show that the linkage of glucose units in maltose involves only one sugar group, and that maltose is a glucose α-glucoside. Maltose generally crystallizes as the β-form (Figure 4.27).

Maltose is readily fermented by yeast.



α-D-glucopyranosyl-(1→4)-D-glucopyranose

Figure 4.27 Structure of maltose

Isomaltose is 6-O-α-D-glucopyranosyl-D-glucopyranose. It exists as a disaccharide unit in glycogens, amylopectins and especially certain bacterial dextrans, from which it may be obtained by partial hydrolysis. It is hydrolysed to glucose in the intestinal tract by the enzyme oligo-1,6-glucosidase.

Cellobiose

It is obtained as a product of the incomplete hydrolysis of cellulose. It is composed of two molecules of glucose into which it splits by either acids or the enzyme emulsin. Cellobiose is a reducing sugar and gives the reactions generally characteristic of the free sugar group.

Cellobiose bears the same relation to cellulose as maltose does to starch and glycogen.

4.5.2 Trisaccharides

Several oligosaccharides containing three monosaccharide units occur in nature. These are given in the following tabulation.

Trisaccharides		
1.	Reducing Sugars	
	Mannotriose	Galactose, rhamnose, glucose
	Robinose	Galactose, rhamnose, rhamnose
	Rhamninose	Galactose, rhamnose, rhamnose
2.	Non-reducing Sugars	
	Raffinose	Fructose, glucose, galactose
	Gentionose	Fructose, glucose, glucose
	Melazitose	Glucose, fructose, glucose

Raffinose: It is the most important of the trisaccharides. It occurs in sugar beets and is concentrated in sugar beet molasses. Cottonseed meal contains about 8 per cent of raffinose and is the material generally used for its preparation. Raffinose is also frequently found in the higher plants and fungi.

When hydrolysed completely, raffinose yields one molecule each of fructose, glucose and galactose. It has been shown that the fructose and glucose components are joined in a sucrose linkage and that the galactose molecule is attached to glucose. Accordingly, raffinose may yield the disaccharide melibose (galactose and glucose) and fructose, or sucrose (glucose and fructose) and galactose by appropriate hydrolysis. Examples of such hydrolysis are bottom yeasts that contain both sucrase and melibiase and consequently hydrolyse raffinose completely to glucose, fructose and galactose.

The fact that raffinose fails to give the characteristic sugar reactions means that all sugar groups of glucose, fructose and galactose are combined in its structure. Hydrolysis of raffinose by sucrose to form fructose and melibiose (glucose and galactose) shows that glucose and galactose are linked and also indicates the presence of sucrose type of linkage. Maltase is an α-glucosidase which hydrolyses α-glucoside linkages, and converts raffinose into galactose

and sucrose (fructose and glucose), showing that fructose and glucose are linked and that glucose is joined by an α-glucoside linkage. The sequence of sugar in raffinose accordingly is fructose-glucose-galactose.

Raffinose is fermented by yeast. It is hydrolysed by enzymes of the gastrointestinal bacteria of herbivorous animals and serves as food for these animals. Raffinose is not well utilized as food by man.

Gentianose: The trisaccharide gentianose occurs in gentian root and when hydrolysed it yields fructose and two molecules of glucose, and the order of linkage is fructose-glucose-glucose. The fructose and glucose are joined by a sucrose type of linkage, and the two glucose molecules are linked as in gentiobiose.

Melezitose: It is a very rare sugar. It occurs in the sap of larch, scrub pine and douglas fir. When hydrolysed completely, it yields two molecules of glucose and one molecule of fructose. Partial hydrolysis gives the disaccharide turanose and glucose. In melezitose the order of linkage is glucose-fructose-glucose. Melezitose posseses no free sugar group.

Melezitose has the structure as glucose-fructose-glucose. Melezitose possesses no free sugar group.

Robinose: Robinose is a reducing trisaccharide composed of galactose and two molecules of deoxysugar rhamnose. It is present in combination in the glucoside robinine. Robinose is reported to reduce Fehling's solution in cold Mannotriose. Mannotriose is formed by partial hydrolysis of the tetrasaccharide stachyose and is composed of glucose and two molecules of galactose linked in the order galactose-galactose-glucose. The glucose unit possesses a free sugar group.

4.5.3 Tetrasaccharides

Two tetrasaccharides are known stachyose (lupeose, β-galactan, manneotetrose), found in *Stachys tuberifera* and many other plant sources, and scorodose, found in the bulbs of onion and garlic.

4.6 POLYSACCHARIDES

The polysaccharides found in nature either serve a structural function or as an energy store for an organism. All polysaccharides upon hydrolysis yield monosaccharides. Those polysaccharides that upon hydrolysis yield only a single type of monosaccharide molecule are termed **homopolysaccharides**. **Heteropolysaccharides** on hydrolysis yield a mixture of constituent monosaccharides and derived products. D-glucose, the monomeric unit of starch, glycogen, and cellulose, is the most abundant carbohydrate building block in the biosphere.

4.6.1 Storage Polysaccharides

Starch, the storage polysaccharide of higher plants, consists of two components, amylose and amylopectin, which are present in varying amounts. The amylose component consists of D-glucose units linked in a linear fraction by α-1-4 linkages; it has a non-reducing end and a reducing end. Its molecular weight can vary from a few thousand to 150,000. Amylose gives a characteristic blue colour with iodine due to the ability of the halide to occupy a position in the interior of a helical coil of glucose units that is formed when amylose is suspended in water.

Amylopectin is a branched polysaccharide; in this molecule, shorter chains (about 30 units) of glucose units linked α-1-4 are also joined to each other by α-1-6 linkage. (from which isomaltose can be obtained). The molecular weight of potato amylopectin varies greatly and may be 500,000 or larger. Amylopectin produces a purple to red colour with iodine (Figure 4.28).

One enzyme, α-amylase, found in the digestive tract of animals (in saliva and the pancreatic juice), hydrolyses by attacking at α-1-4 linkages of amylose chain to produce maltose and glucose. β-amylase, an enzyme found in plants, attacks the non-reducing end of amylose to yield successive units of maltose.

The enzymes α- and β-amylase, attack amylopectin at α-1-4 glycosidic bond near the branching point and the α-1-6 bond in amylopectin is not hydrolysed by these enzymes. Therefore, a highly branched core—called a limit dextrin—of the original amylopectin is the product of these enzymes. A separate 'debranching' enzyme, an α-1-6 glucosidase, can hydrolyse the bond at the branch point. Therefore, the joint action of these two enzymes, α-amylase and the α-1-6 glucosidase together hydrolyse amylopectin and yield glucose and maltose.

The storage polysaccharide of animal tissues is glycogen; it is similar in structure to amylopectin in that it is a branched, homopolysaccharide composed of glucose units. It is more highly branched than amylopectin, however, having branch points about every 8–10 glucose units. Glycogen is hydrolysed by α- and β-amylases to form glucose, maltose and a limit dextrin,

A final example of a nutrient polysaccharide will suffice. This is inulin, a storage carbohydrate found in the bulbs of many plants (dahlias, Jerusalem artichokes). Inulin consists chiefly of fructofuranose units joined together by β-2-1 glycosidic linkages.

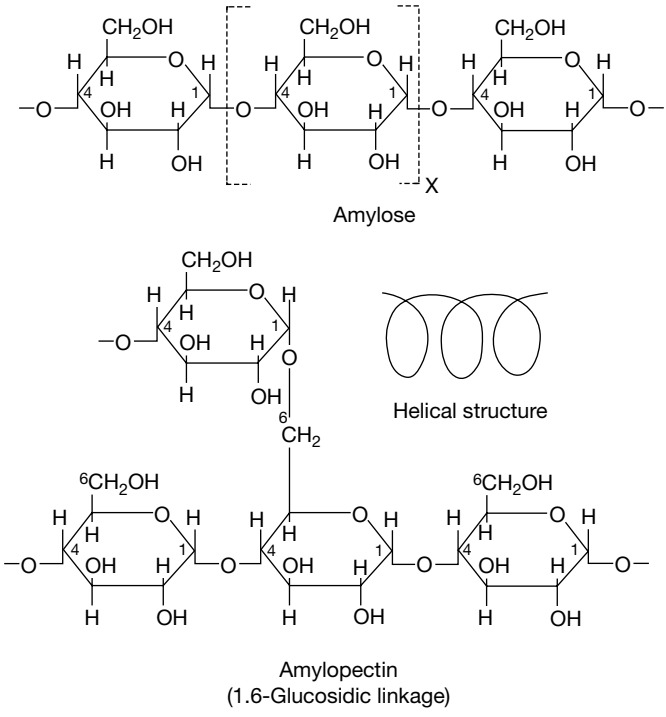
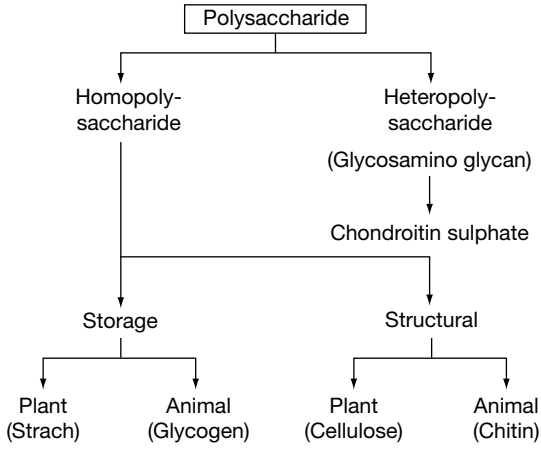


Figure 4.28 Structure of starch



4.6.2 Structural Polysaccharides

The most abundant structural polysaccharide is **cellulose**, a linear, homopolysaccharide composed of D-glucopyranose units linked β-1-4.

Cellulose is found in the cell walls of plants where it provides structure to the organism. The wood of trees is composed primarily of cellulose and another polymer called lignin.

In contrast to starch, the β-1-4 linkages of cellulose are highly resistant to acid hydrolysis; strong mineral acid is required to produce D-glucose; partial hydrolysis yields the reducing disaccharide, cellobiose. The β-1-4 linkages of cellulose are not hydrolysed by glycosidases found in the digestive tracts of humans or other higher animals. However, snails secrete a cellulase that hydrolyses the polymer; termites contain a similar enzyme. Moreover, the rumen bacteria that reside within the intestinal tract of cattle and other ruminants can hydrolyse cellulose and further metabolize the D-glucose produced (Figure 4.29).

Other examples of structural polysaccharides in plants are known. Plants contain pectins and hemicelluloses. The latter are not cellulose derivatives but rather are homopolymers of D-xylose linked β-1-4. Pectins contain arabinose, galactose and galacturonic acid. Pectic acid is a homopolymer of the methyl ester of D-galacturonic acid.

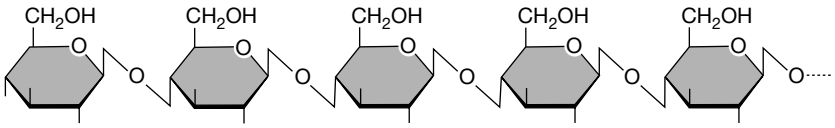


Figure 4.29 Structure of cellulose

Chitin, a homopolymer of N-acetyl-D-glucosamine, is the structural polysaccharide that constitutes the shell of crustaceans and the scales of insects (Figure 4.30).

In recent years, much effort has been expended on identifying the chemical nature of bacterial cell walls and related structures. Animal cells do not possess a well-defined cell wall but have a cell coat, visible in the electron microscope that plays an important role in the interaction with adjacent cells. These cell coats contain glycolipids, glycoproteins and mucopolysaccharides.

The mucopolysaccharides are gelatinous substances of high molecular weights (up to 5×10^6) that both lubricate and serve as a sticky cement. One common mucopolysaccharide is hyaluronic acid, a heteropolysaccharide composed of alternating units of D-glucuronic acid and N-acetyl D-glucosamine. The two different monosaccharides are linked by a β-1-3 unit to form a disaccharide which is linked (β-1-4) to the next repeating unit. Hyaluronic acid, found in the vitreous humour of the eye and in the umbilical cord, is water soluble but forms viscous solutions.

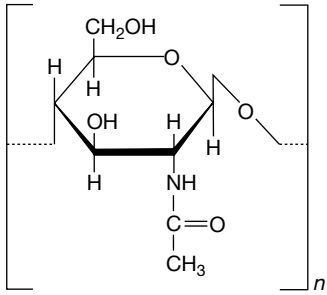


Figure 4.30 Structure of chitin

Chondroitin, similar in structure to hyaluronic acid except that the amino sugar is N-acetyl-D-galactosamine, is also a component of cell coats. Sulphate esters (at the C-4 or C-6 positions of the amino sugar) of chondroitin are major structural components of cartilage, tendons and bones.

Bacterial cell walls, which determine many of the physiological characteristics of the organism they enclose, contain complex polymers of polysaccharide linked to chains of amino acids (see Chapter 9). Since the individual chains of amino acid are not as long as in proteins, such polymers have been termed peptidoglycans rather than glycoproteins. The repeating unit of peptidoglycans is a disaccharide composed of N-acetyl-D-glucosamine (NAG) and N-acetylmuramic acid (NAMA) joined by a β-1,4-glycosidic bond. N-acetylmuramic acid consists of a N-acetyl glucosamine unit which has its C-3 hydroxyl group joined to the α-hydroxyl function of lactic acid by an ether linkage. In the peptidoglycan the carboxyl group of each lactic acid moiety is in turn, linked to a tetrapeptide (see Chapter 4) consisting of L-alanine, D-isoglutamine, L-lysine and D-alanine. While the peptidoglycan could then be represented as a linear polysaccharide chain with a tetrapeptide branching out at every second hexose amine unit, there is considerable evidence of cross-linking between adjacent, parallel polysaccharide chains. In the cross-linking (Figure 4.5), the carboxyl group of the terminal D-alanine moiety is attached to pentaglycine residue which in turn is attached to the α-amino group of lysine in the next adjacent glycan unit.

Glycogen

It is a branched chain polysaccharide composed of glucose units. Glycogen is a storage polysaccharide present in animals and stored in liver and muscles of animals. It is also formed in plants which do not have chlorophyll system, e.g., fungi and yeast. It has a branched structure with straight chain units of 12-18-α-D-glucopyranose (in a 1-4 glucosidic linkage) with branching by means of a [1–6]-glucosidic bonds. It is nonreducing, readily soluble in water and gives a red colour with iodine.

4.7 QUALITATIVE TESTS FOR IDENTIFICATION OF CARBOHYDRATES

4.7.1 Colour Reactions of Carbohydrates

A very large number of colour reactions more or less characteristic of carbohydrates is known. Most of these reactions are carried out in strongly acid solution; this hydrolyses the polysaccharides present to monosaccharides, which give the colour reactions. The basis of these colour tests involving strongly acid reagents appears to be conversion of the monosaccharide to furfural (pentose), hydroxymethylfurfural (hexose), methylfurfural (methylpentose as rhamnose), or other aldehydic compound. The aldehyde formed by action of the acid is then condensed with a phenol or other aromatic substance to give the colour. A few of the more commonly used colour reactions follow:

Molisch test: This is a very sensitive general reaction for carbohydrates. It performed by mixing a few drops of an alcoholic solution of α-napthol with the solution and stratifying the mixture over concentrated sulphuric acid. A red colour ring appears at the junction of the liquids. Amino sugars, sugar alcohols, oxylic acids do not give the test. 2-Ketoaldonic acids give green colours. This test, orcinol-hydrochloric acid test is a sensitive reaction for that of pentoses and certain uronic acids which decompose upon heating with acid to form pentoses (glucuronic, galacturonic acids, etc.). A dilute solution of orcinol in 30 per cent hydrochloric acid containing a little ferric chloride constitutes Bial's reagent. The reagent is heated to boiling, and several drops of the sugar solution are added immediately after removing from the flame. Pentoses give a green colour, as do alclohexuronic acids (glucuronic, etc.). Trioses and 5-ketoaldonic acids give a positive test. Ketoheptoses give a purple colour, and ketohexoses and methylpentoses produce orange-coloured solutions which separate dark green precipitates on standing.

Phloroglucinol-hydrochloric acid test: When pentoses are boiled with a solution of phloroglucinol in hydrochloric acid, a cherry red colour is formed. Galactose also gives this test.

Seliwanoff's test (resorcinol hydrochloric acid test): This test is given by ketohexoses. Seliwanoff's reagent is a solution of resorcinol in hydrochloric acid. When heated with the reagent, ketohexoses give a red colour.

Naphthoresorcinol test: This test is used for the detection of the common uronic acids, such as glucuronic, galacturonic and mannuronic. The sugar is dissolved in 1 N hydrochloric acid containing a small amount of napthoresorcinol and heated for 5 min in a boiling water bath. The mixture is cooled and extracted with ether. The ether solution is coloured purple, violet or pink when the test is positive.

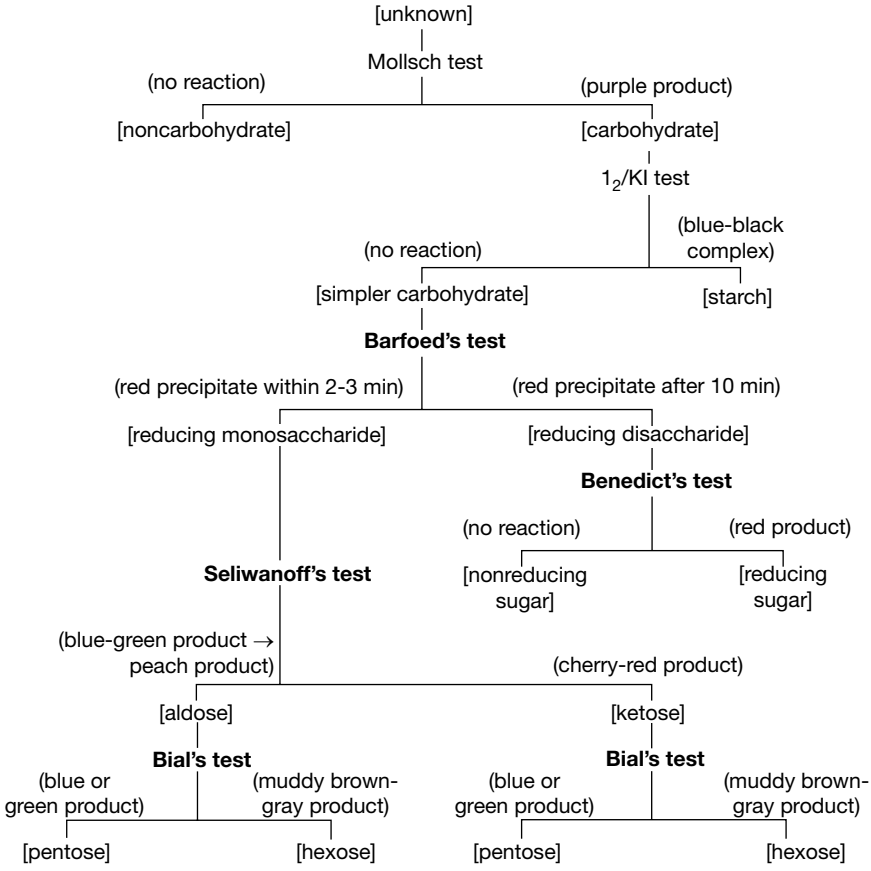
Test for deoxy sugars: When a pine splinter is placed in concentrated hydrochloric acid containing a 2-deoxy sugar, the splinter develops a green colour. Kiliani's test consists in placing the sugar in glacial acetic acid containing ferrous salt and sulphuric acid; a blue colour is produced.

Barfoed's test: This test is used in distinguishing monosaccharides from reducing disaccharides. Barfoed's reagent is a solution of cupric acetate in dilute acetic acid. When monosaccharides are boiled with this reagent, it is reduced and Cu_2O separates. The reducing disaccharides (reduce Fehling, Benedict, Shaffer-Hartman reagents) reduce Barfoed's reagent very slowly as compared with the monosaccharides. In using this test the concentrations of the sugar solutions compared should not be too different, since a more concentrated disaccharide solution will reduce faster than a dilute monosaccharide solution.

Moore's Test: This test consists in heating the carbohydrate with dilute alkali, a yellow to brown or reddish brown colour being produced if the carbohydrate contains a free sugar group (hemiacetal group).

4.7.2 Chart for Differentiating an unknown Carbohydrate

The chart for Differentiating an unknown Carbohydrate is given hereunder.



EXERCISES

1. Define carbohydrates, classify them and describe the properties of monosaccharides.
2. Write short notes on:
   (a) Disaccharides
   (b) Starch
   (c) Mucopolysaccharides

(d) Lactose
(e) Glycogen
(f) Benedict's test

3. Write an essay on blood glucose level and its regulations. Define diabetes mellitus.
4. Give the structural formula of the following:
   (a) Lactic acid
   (b) Fructose
5. Write short notes on: (a) Oligosaccharides and (b) Polysaccharides.
6. With examples write explanatory notes on optical isomerism.
7. Explain the mutarotation of carbohydrates. Describe in brief the structure and important properties of the following:
   (a) Glycogen
   (b) Starch
   (c) Cellulose
8. Name of the reducing sugars that may be found in pathological urine. Describe a method for the estimation of the concentration of reducing sugar in urine.
9. Discuss the fructose tolerance test.
10. Write notes on:
    (i) Synthesis of lactose
    (ii) Glycogen
    (iii) Benedict's test
    (iv) Glucose tolerance test
    (v) Glycosuria
    (vi) ATP

MULTIPLE CHOICE QUESTIONS

1. The heptose ketose sugar formed as a result of chemical reaction in HMP shunt is
   (a) Gulcoheptose
   (b) Galactoheptose
   (c) Sedoheptulose
   (d) Mannoheptose

   Ans. (c)

2. The general formula for a polysaccharide is
   (a) $(C_6H_{10}O_5)n$ (b) $(C_6H_{12}O_6)n$
   (c) $(C_6H_{12}O_5)n$ (d) $(C_6H_{10}O_6)n$

   Ans. (a)

3. The number of isomers of glucose is
   (a) 4 (b) 8 (c) 12 (d) 16

   Ans. (d)

4. The epimer of glucose is
   (a) Fructose
   (b) Galactose
   (c) Ribose
   (d) Deoxyribose

   Ans. (b)

5. The intermediate in hexose monophosphate shunt is
   (a) D-ribulose
   (b) D-arabinose
   (c) D-xylose
   (d) D-lyxose

   Ans. (a)

6. Iodine solution produces no colour with
   (a) Cellulose
   (b) Starch
   (c) Dextrin
   (d) Glycogen

   Ans. (a)

7. Each branch of amylopectin is at an interval of glucose units
   (a) 14–20
   (b) 24–30
   (c) 34–40
   (d) 44–50

   Ans. (b)

8. This test is used for the detection of the common uronic acids, such as glucuronic, galacturonic and mannuronic.
   (a) Naphthoresorcinol test
   (b) Moore's test
   (c) Barfoed's test
   (d) None of these

   Ans. (a)

9. This test is used in distinguishing monosaccharides from reducing disaccharides.
   (a) Naphthoresorcinol test
   (b) Moore's test
   (c) Barfoed's test
   (d) None of these

   Ans. (c)

10. This test consists in heating the carbohydrate with dilute alkali, a yellow to brown or reddish brown colour being produced if the carbohydrate contains a free sugar group (herniacetal group).
    (a) Naphthoresorcinol test
    (b) Moore's test
    (c) Barfoed's test
    (d) None of these

    Ans. (b)

11. Similar in structure to hyaluronic acid except that the amino sugar is N-acetyl-D-galactosamine, is also a component of cell coats
    (a) Chondroitin (b) Chitin
    (c) Glycogen (d) None of these

    Ans. (a)

12. A homopolymer of N-acetyl-D-glucosamine, is the structural polysaccharide that constitutes the shell of crustaceans and the scales of insects
    (a) Chondroitin (b) Chitin
    (c) Glycogen (d) None of these

    Ans. (b)

13. The mannose and galactose are the epimers of
    (a) Glucose (b) Fructose
    (c) Glycogen (d) None of these

    Ans. (a)

14. D-mannitol is found in
    (a) Algae and fungi
    (b) Berries of many higher plants
    (c) Both (a) and (b)
    (d) None of these

    Ans. (a)

15. It is fermented by yeast. It is hydrolysed by enzymes of the gastrointestinal bacteria of herbivorous animals and serves as food for animals, (a) and (b) Berries of many higher plants
    (a) Raffinose (b) Melezitose
    (c) Robinose (d) None of these

    Ans. (a)

16. Haworth has suggested the five-membered ring forms of the sugars be called
    (a) Furanose
    (b) Pyranose
    (c) Mutarotation
    (d) None of these **Ans.** (a)

17. Haworth has suggested the five-membered ring forms of the sugars be called
    (a) Furanose (b) Pyranose
    (c) Mutarotation (d) None of these

    Ans. (b)

18. The α-D and β-L hydroxyl groups lie
    (a) Below the plane of the ring
    (b) Above the plane of the ring
    (c) Both
    (d) None of these

    Ans. (a)

19. While β-D and α-L hydroxyl groups lie
    (a) Below the plane of the ring
    (b) Above the plane of the ring
    (c) Both (a) and (b)
    (d) None of these

Ans. (b)

20. *n*-butane is a chain isomer of
    (a) Isobutane
    (b) *n*-propyl chloride
    (c) *n*-propanol
    (d) None of these

Ans. (a)

5 Composition of Lipids

CHAPTER OBJECTIVES

5.1 Introduction
5.2 Classification of Lipids
5.3 Fatty Acids
5.4 Fats: Fats are Fatty Acid Esters of Glycerol
5.5 Waxes
5.6 Phospholipids or Phosphatides
5.7 Non-Phosphorylated Lipids
5.8 Steroids
5.9 Qualitative Test of Lipids

5.1 INTRODUCTION

5.1.1 Lipids as an Energy Reserve

The required energy for the human body comes through the oxidation of lipids and carbohydrates. While carbohydrates give a readily accessible source of energy, lipids function mainly as an energy store. The sum of lipids stored in the human body as a source of energy is much more as compared to glycogen. The energy produced by 1 g of lipid is 9 kcal while the energy produced by 1 g of carbohydrates and proteins is 4 kcal.

The comparative amounts of energy generated by different biochemicals are like the energy generated by free glucose in the blood is only 40 kcal energy. The reserve glycogen is stored in the liver and muscles and even after an overnight fast, generates about 600 kcal energy. Protein contains a significant energy reserve of about 25,000 kcal. Finally, the lipid stores contain 100,000 kcal of energy which can make sustain body functions without food for 30–40 days with adequate water. Lipids are stored in adipose tissues.

5.1.2 Definitions

Lipids: These are the greasy materials which may be extracted from animal and plant tissues. Lipids are extracted from tissues using hot alcohol, ether and benzene. Lipids are soluble in organic solvents such as ether and chloroform.

Fats: These are fatty acid esters of glycerol and are commonly called triglycerides.

Fatty acids: Lipids upon hydrolysis yield fatty acids.

5.2 CLASSIFICATION OF LIPIDS

Fatty acids: Generally found in ester linkage but also found free and in loose molecular complexes with protein.

Fats: Fatty acid esters of glycerol. The glycerides are the most abundant lipids in nature. Monoglycerides and diglycerides are also present in natural fats. The names neutral fat, triglyceride and fat are used synonymously.

Waxes: Fatty acid esters of alcohol, other than glycerol. The fatty acids present are generally long chain acids, or the alcohol may be aliphatic or alicyclic.

5.2.1 Phospholipids

1. Derivatives of glycerolphosphate. The most abundant are the diesters of glycerophosphoric acid in which the phosphoric acid is also ester-linked to a nitrogenous or non-nitrogenous alcohol.
2. Derivatives of sphingosine phosphate which contains a fatty acid that is ester-linked and a nitrogenous base (choline, ethanolamine) also esterified with the phosphate.

5.2.2 Non-Phosphorylated Lipids

1. **Cerebroside or glycolipid or glycosphingosides:** Derivatives of sphingosine having a fatty acid and a hexose substituent.
2. **Sulpholipid:** Derivatives of sphingosine having a fatty acid and a sulphated hexose substituent.
3. **Ganglioside:** Derivatives of sphingosine, fatty acid, hexoseamine, hexose and sialic acid substituent.
4. **Proteolipids:** Complexes of lipids and proteins.
5. **Steroids:** Derivatives of cyclopentanoperhydrophenanthrene.

5.3 FATTY ACIDS

Fatty acids: The fats upon hydrolysis yield fatty acids; fatty acids are straight chain compounds and they generally contain even number of carbon atoms.

5.3.1 Nomenclature for Fatty Acids

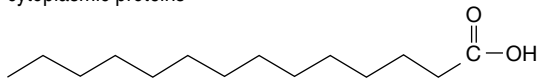
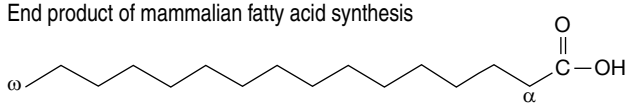
Name-carbon length: Number of double bonds (position of double bond) (See Table 5.1).

- Butyric acid-4:0
- Palmitic acid-16:0
- Oleic acid-18:1 (9)

5.3.2 Classification of Some Naturally Occurring Fatty Acids

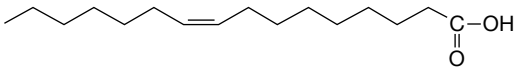
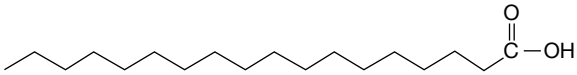
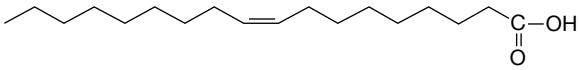
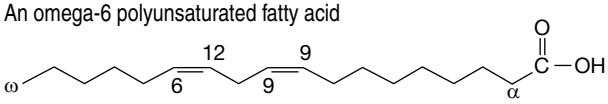
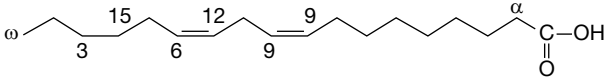
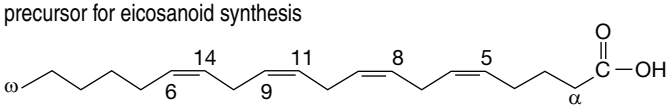
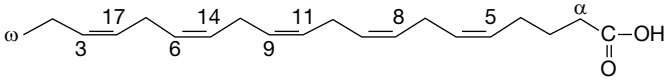
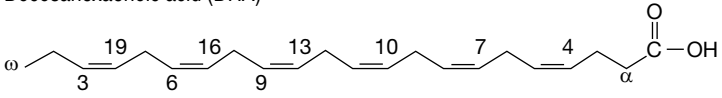
1. **Saturated fatty acids:** The saturated aliphatic acids of the general formula $C_nH_{2n}O_2$ occur in two series, that is, even- and odd-numbered series. Until recently, it was generally considered that essentially only the even carbon series is present in nature and that the few odd-carbon acids isolated are relatively unimportant. Even carbon acids from C_2 to C_{26} occur in both plant and animal fats, while acids as large as C_{34} have been found in waxes. The most common member of the even carbon saturated series is palmitic acid. However, stearic acid, the next higher homologue, occurs to some 10–30 per cent in animal depot fat. Odd carbon acids, C_3 to C_{25}, have also been isolated in animal and plant fats.
2. **Unsaturated fatty acids:** This group is characterized by having one or more double bonds or ethylenic groups. The position of unsaturation is indicated by the prefixing number in the systematic name, the carboxyl group being C-1. For example, a bond cited as 9- refers to the location of the double bond between carbon atoms 9 and 10.
3. **Branced chain fatty acids:** These fatty acids more often are found in waxes than in fats. The acids isolated from ruminant fats consist of the odd-numbered carbon acids C_{13} to C_{17}, and the odd and even series from C_{13} to C_{18}.
4. **Hydroxy and keto derivatives:** The hydroxy derivatives of the C_2, C_3 and C_4 fatty acids do not occur to any appreciable extent in lipids, although the acids glycolic, lactic and beta-hyroxybutyric are of importance in other areas of biochemistry.
5. **Cyclic acids:** Fatty acids containing cyclic structures are rare but have been identified in the lipids of *Lactobacilli* and in certain seed oils. In the *Lactobacilli*, the ring structure is a cyclopropene system. In the seed oils of the tropical family Flacourtiaceae, dextrorotatory fatty acids with a cyclopentenyl ring at the end of the fatty acids chain have been identified.
6. **Other acids:** The saturated dicarboxylic acids, the alkanedionic acids, are rarely found in natural lipids, although the lower members of the series—oxalic, malonic, succinic and glutaric acids—are well-known biochemical entities. Several higher members of the series, such as azelaic acid, are the important degradation products of the natural acids.

Table 5.1 Fatty Acids

Numerical symbol	Common name and structure
14:0	Myristic acid Often found attached to the N-terminal of plasma membrane-associated cytoplasmic proteins 
16:0	Palmitic acid End product of mammalian fatty acid synthesis 

(*Continued*)

Table 5.1 Continued

Numerical symbol	Common name and structure
16:1 $^{\Delta 9}$	Palmitoleic acid 
18:0	Stearic acid 
18:1 $^{\Delta 9}$	Oleic acid An omega-9 monounsaturated fatty acid 
18:2 $^{\Delta 9,12}$	Linoleic acid **Essential fatty acid** An omega-6 polyunsaturated fatty acid 
18:3 $^{\Delta 9,12,15}$	α-Linolenic acid (ALA) **Essential fatty acid** An omega-3 polyunsaturated fatty acid 
20:4 $^{\Delta 5,8,11,14}$	Arachidonic acid An omega-6 polyunsaturated fatty acid, precursor for eicosanoid synthesis 
20:5 $^{\Delta 5,8,11,14,17}$	Eicosapentaenoic acid (EPA) An omega-3 polyunsaturated fatty acid enriched in fish oils 
22:6 $^{\Delta 4,7,10,13,16,19}$	Docosahexaenoic acid (DHA) 

5.4 FATS: FATS ARE FATTY ACID ESTERS OF GLYCEROL

Fats—Fatty Acid Esters of Glycerol: The fats are the fatty acid esters of glycerol and are commonly called triglycerides. The fats represent the most common and widespread class of lipids in nature, being especially abundant in nuts, seeds and the fat depots of animals. The oils such as olive, cottonseed and soya bean are for the most part fats that are liquid at normal temperatures. The fats serve three major physiological functions in the animal body: they are reserve food material; they serve as insulation against the loss of body heat and they are important as padding material to support and project internal organs.

Chemical composition of the fats: Natural fats are generally complex mixtures of triglycerides and reflect the physical and chemical properties of the component fatty acids. The simple glycerides are those in which the three glycerol hydroxyls are esterified with identical fatty acids. The mixed glycerides contain two or three different fatty acids in ester combination. The following structures represent sample triglycerides and illustrate the method of naming the compounds, the three carbons of glycerol being designated α, β, α′ or 1, 2, 3, respectively (Figure 5.1).

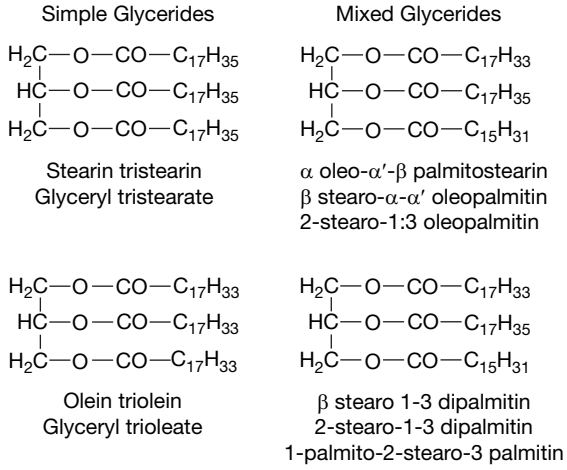


Figure 5.1 Structure of simple and mixed glycerides

5.4.1 Physical Properties of Fats

The glycerides of lower fatty acids, such as butyric, are somewhat soluble in water, whereas those of the higher acids are insoluble All glycerides are soluble in ether, chloroform and benzene. They are only slightly soluble in cold methyl and ethyl alcohol and acetone, but much more soluble in these solvents when hot. Boiling ethyl alcohol is one of the best solvents for extraction of fats as well as all other lipids from tissues. The specific gravity of fats is generally lower than that of water.

The melting point of a fat depends upon its component glycerides. The melting points of the glycerides are generally higher than those of the fatty acids present and vary with the acids. Glycerides of the higher saturated fatty acids have the highest melting points and those of unsaturated acids have the lowest. Pure glycerides have no colour, odour or taste.

Chemical properties and characterization of fats: The chemical properties of the fats are the properties of their component glycerides and these in turn depend in particular upon the character of the fatty acids present.

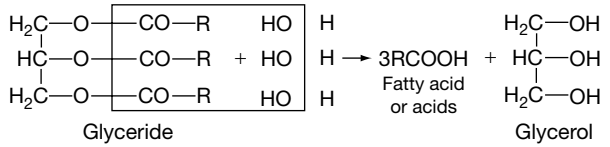


Figure 5.2 Hydrolysis of glycerides of fat

Hydrolysis: The hydrolysis of the glycerides of a fat may be readily accomplished by heating with water at high temperatures and pressured in an autoclave, preferably with addition of catalysts such as acids. When boiled at ordinary pressure, the action is very slow (Figure 5.2).

Saponification: The glycerides of the fats may be readily decomposed into glycerol and salts of the constituent fatty acids (soaps) by boiling with strong bases such as sodium or potassium hydroxide. Since fats are insoluble in water, the process is facilitated by the addition of alcohol which dissolves the fat (Figure 5.3).

Saponification number: The saponification number is defined as the milligrams of KOH required to saponify 1 g of fat. Since fats are mixtures of glycerides and the glycerides in turn contain various chain length fatty acids, the saponification number is an index of the average molecular size of the fatty acids present.

Reactions of fats due to unsaturation: The double bonds of unsaturated acids in glycerides undergo the reactions characteristic of these bonds in the free acids.

Hydrogenation: The unsaturated glycerides of fats may be hydrogenated by treatment with hydrogen in the presence of nickel catalyst (Figure 5.4).

Halogenation: Chlorine, bromine, iodine, iodine chloride and iodine bromide may be added to the double bonds of the unsaturated glycerides in fats. One atom of halogen adds to each side of the double bond producing a saturated halogenated glyceride.

Iodine number: Since the quantity of halogen absorbed by the glycerides of a fat can be measured accurately, it is possible to calculate the relative unsaturation of fats. This is done by determining the so-called iodine number, which is defined as the per cent of iodine absorbed by the fat, or the grams of iodine absorbed by 100 g of fat.

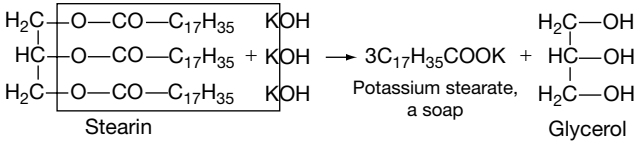


Figure 5.3 Saponification of glycerides of fats

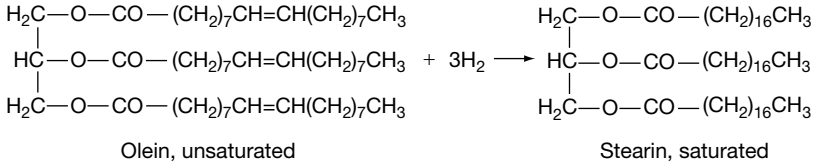


Figure 5.4 Hydrogenation of unsaturated glycerides of fats

Acetyl number: The 'acetyl number' is defined as the milligrams of KOH required to combine with the acetic acid liberated by the saponification of 1 g of acetylated fat.

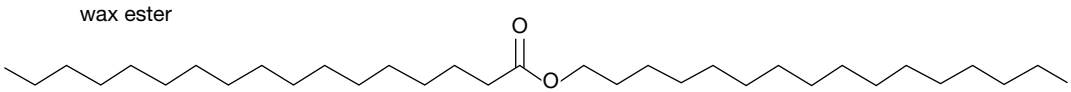
Oxidation of unsaturated glycerides of fats: Oxidation of unsaturated bonds in the glycerides of fats proceeds as previously outlined for the fatty acids. Ozone and oxygen may add at the double bonds to form ozonides and peroxides respectively, which may then decompose into other substances.

Rancidity of fats: The unpleasant odour and taste developed by most natural fats upon aging is referred to as 'rancidity.' Rancidity may be due to hydrolysis of component glycerides of a fat into free fatty acids and glycerol or monoglycerides and diglycerides. This is often hastened by the presence of lipolytic enzymes (fat-splitting enzymes or lipases) in the fats, which in the presence of moisture and at warm temperature bring about hydrolysis. Rancidity may also be caused by various oxidative processes. For example, oxidation at the double bonds of unsaturated glycerides may form peroxides which then decompose to form aldehydes of objectionable odour and taste. This process is greatly increased by exposure to light.

Acid number: This represents, by definition, the milligrams of KOH required to neutralize the free acids present in 1 g of fat and is of value in determining rancidity due to free fatty acids.

5.5 WAXES

Waxes are widespread in nature as the secretions of certain insects and as protective coatings of the skins and furs of animals and the leaves and fruit of plants. Certain animal oils, such as that of the sperm whale, are composed largely of waxes rather than of fats.



Waxes commonly are defined chemically as the esters of higher fatty acids and of higher monohydroxy alcohols. Dihydroalcohols may occasionally be present (Figure 5.5).

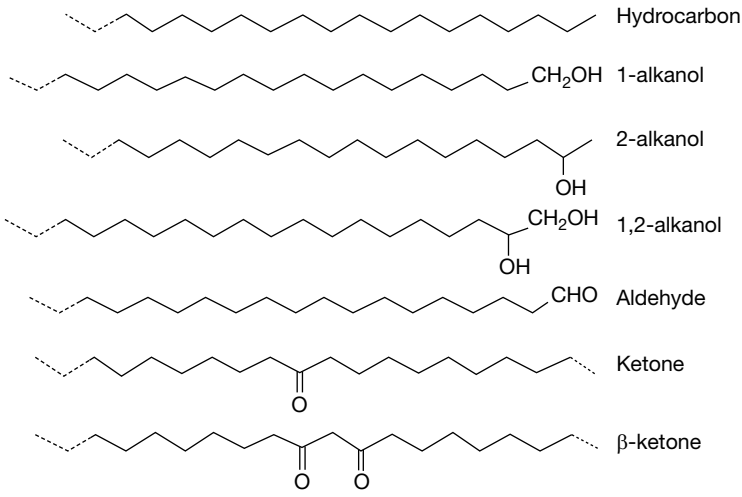
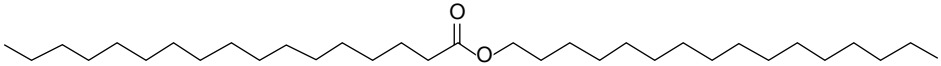


Figure 5.5 Structure of waxes

Lanolin or wool fat: This material forms a protective coating over the wool fibres and is a wax rather than a fat. It is a very complex mixture and is characterized among waxes by containing both free and esterified cholesterol ($C_{27}H_{45}OH$), as well as the sterols lanosterol ($C_{30}H_{49}OH$) and agnosterol ($C_{30}H_{47}OH$). Lanolin is used as a medium for making ointments and cosmetics.

Waxes are used chiefly in the manufacture of polishes (carnauba wax), lubricants (sperm oil), candles (spermacetic) and ointments (lanolin).

Beeswax: The insect wax is a complex mixture of esters, some free fatty acids, alcohol and hydrocarbons. The beeswax is the ester of myricyl alcohol ($C_{30}H_{61}OH$) and palmitic acid $C_{15}H_{31}COOC_{30}H_{61}$ (myricyl palmitate). Even carbon fatty acids from C_{24} to C_{34} and primary monohydroxy alcohols from C_{24} to C_{34} are present as esters or in the free condition. Odd carbon paraffin hydrocarbons from C_{25} to C_{31} are also present.



5.6 PHOSPHOLIPIDS OR PHOSPHATIDES

These are the heterogeneous group of compounds that are classed together partially on the basis of solubility and partially on the basis of the ester phosphorous present in the compounds. Phosphatides are found in every cellular organism studied and are likely a constituent of every living cell. In some cellular structures, e.g., membrane units, the phosphatides may be the chief cellular lipid components.

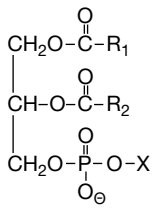


Figure 5.6 Basic composition of a phospholipid. X can be a number of different substituents

5.6.1 Basic Structure of Phospholipids

The glycerophosphatides are the most common of the phospholipids. These lipids are derived from phosphatidic acid in which a primary hydroxyl group of glycerol is esterified with phosphoric acid. The two other hydroxyl groups of the glycerol are esterified to fatty acids, or, in some cases, one hydroxal forms an ether linkage with a long unsaturated aliphatic chain. Since the middle hydroxal is asymmetric, it is necessary to indicate the optical isomer involved (Figure 5.6).

5.6.2 Classification of the Phosphatides

These compounds can be separated into a variety of subgroups depending upon the structural components present in the parent molecule. The following classification is an example of this grouping.

(i) Glycerophosphatides or phosphoglycerides
  (a) Phosphatidyl cholines (lecithin)
  (b) Phosphatidyl ethanolamine (cephalin)
  (c) Other substituents attached to the phosphatidyl groups
    - Nitrogenous: serine, threonine, methylethanolamine and hydroxyproline
    - Carbohydrate-like: inositol, phosphoinositol, glycerol and polyglycerol
  (d) Plasmalogen

(ii) Phosphosphoingosides or sphingomyelin

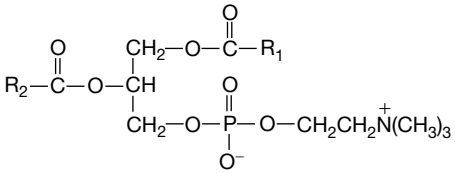


Figure 5.7 Structure of phosphatidylcholine (PC) or lecithin

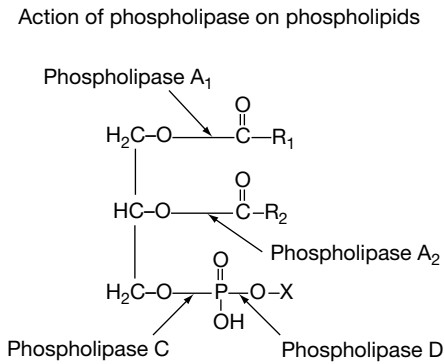


Figure 5.8 Sites of action of the phospholipases A_1, A_2, C and D

5.6.2.1 Glycerophosphatides or Phosphoglycerides

These are the most common of the phospholipids. These lipids are derived from a phosphatidic acid in which a primary hydroxyl group of glycerol is esterified phosphoric acid. The two other hydroxyl groups of glycerol is esterified to fatty acids, or one hydroxyl group forms a ether linkage with long unsaturated aliphatic chain.

(a) **Phosphatidylcholine (PC):** It is also known as **lecithin**. Lecithin contains glycerol, fatty acids, phosphoric acid and choline (Figure 5.7).

Action of phospholipase:

(i) Phospholipase A_1 attacks the ester bond in position 1 of phospholipid.

(ii) Phospholipase A_2 attacks β-position and form lysolecithin and one molecule of fatty acid.

(iii) Phospholipase C hydrolyses phosphate ester bond and produces α, β-diacyl glycerol and phosphoryl choline.

(iv) Phospholipase D splits off choline and phosphatidic acid is formed (Figure 5.8).

(b) **Phosphatidylethanolamine:** It is also known as **cephalin.** Cephalin contains glycerol and fatty acids, phosphoric acid and ethanolamine (Figure 5.9). Cephalins are structurally identical with the lecithins, with the exception that the base ethanolamine instead of choline is present.

(c) **Other substituents attached to the phosphatidyl groups**.

Phosphatidylserine (PS): It contains glycerol and fatty acids, phosphoric acid and serine. It is positioned completely on the inner monolayer surface of the plasma membrane and in other cellular membranes also. For the activation of protein kinase C, phosphatidylserine is a necessary cofactor. Phosphatidylserine is implicated in many biological processes, like blood coagulation and apoptosis (Figure 5.10).

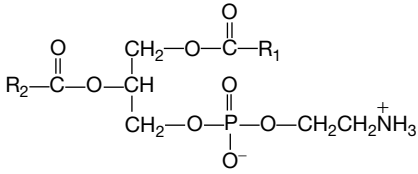


Figure 5.9 Structure of phosphatidylethanolamine (PE) also known as cephalin

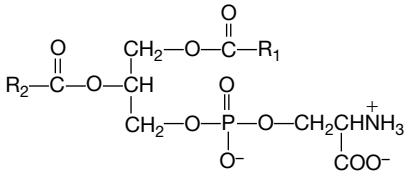


Figure 5.10 Structure of phosphatidylserine (PS)

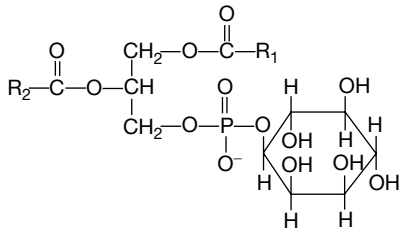


Figure 5.11 Structure of phosphatidylinositol

Phosphatidylinositol (PI): It contains glycerol and fatty acids, phosphoric acid and inositol. It acts as a second messenger in Ca^{++}-dependent hormone action. **Phosphatidylinositol**, containing the optically inactive form of inositol, *myo*-insitol, is a common constituent of animal, plant and microbial lipids (Figure 5.11).

Phosphatidylglycerol (PG): It is a trace component of most tissues, but it is frequently the major constituent of some bacterial membranes. It has significant role as a lung surfactant, and in plant chloroplasts it has an important role in photosynthesis. It is the precursor of cardiolipin (Figure 5.12).

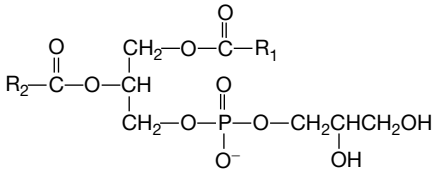


Figure 5.12 Structure of phosphatidylglycerol

Cardiolipin or (diphosphatidylglycerol, DPG): It is formed from phosphatidyl glycerol. Chemically, it is diphosphatidyl glycerol. It is found in inner membrane of mitochondria and bacterial cell wall. Heart muscle is a rich source of cardiolipin (Figure 5.13).

(d) **Plasmalogens:** Plasmalogens make up an appreciable amount of total phosphatides in brain and muscles. These compounds yield upon hydrolysis one molecule each of long chain aliphatic aldehyde, fatty acid, glycerol phosphate and nitrogenous base. The structure of an ethanolamine **plasmalogen** is shown in Figure 5.14.

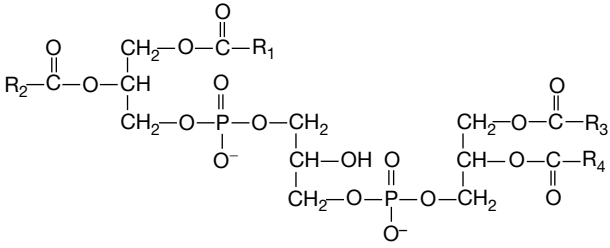


Figure 5.13 Structure of cardiolipin

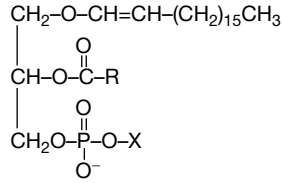


Figure 5.14 Structure of plasmalogen

5.6.2.2 Basic Structure of Phosphosphingosides or Sphingomyelins

Sphingomyelins upon hydrolysis yield the unsaturated nitrogen-containing alcohol sphingosine, phosphate, fatty acid and a nitrogenous base. Choline is the main nitrogenous base, but ethanolamine has been identified as a constituent (Figure 5.15).

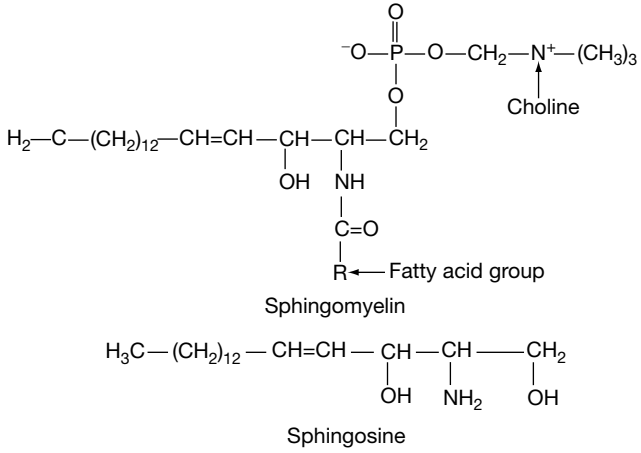


Figure 5.15 Structure of sphingosine

The sphingosine molecules in which a fatty acyl group is substituted in the amino group is called a 'ceramide.' When choline group is removed from a sphingosine, a ceramide phosphate is found (Figure 5.16).

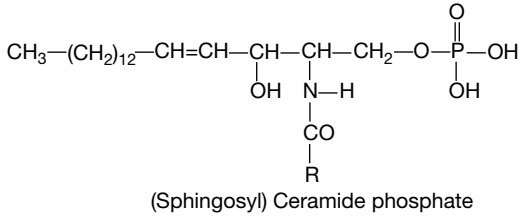


Figure 5.16 Structure of sphingosyl ceramide phosphate

Sphingomyelins are present in large amount in brain and nerve tissues and in smaller quantities in other tissues and blood. Large accumulations of sphingomyelins in the brain, liver and spleen of persons lead to Niemann-Pick disease.

5.7 NON-PHOSPHORYLATED LIPIDS

In addition to fats and phosphorylated lipids, there are many other complex, non-phosphorylated lipids found in tissues.

5.7.1 Non-Phosphorylated Lipids

1. **Cerebroside or glycolipid or glycosphingosides:** Derivatives of sphingosine having a fatty acid and a hexose substituent.

2. **Sulpholipid:** Derivatives of sphingosine having a fatty acid and a sulphated hexose substituent.
3. **Ganglioside:** Derivatives of sphingosine, fatty acid, hexoseamine, hexose and sialic acid substituents.
4. **Proteolipids:** Complexes of lipids and proteins.
5. **Steroids:** Derivatives of cyclopentanoperhydrophenanthrene.
   (a) Cholesterol
   (b) Mycosterol
   (c) Phytosterols
   (d) Bile acids

1. **Cerebrosides:** Contain a single moiety, principally galactose. Cerebrosides when hydrolysed yield sphingosine, a fatty acid and the sugar galactose. Sphingoglycosides or cerebrosides occur in large amount in the white matter of brain and in the myeline sheaths of nerves (Figure 5.17).

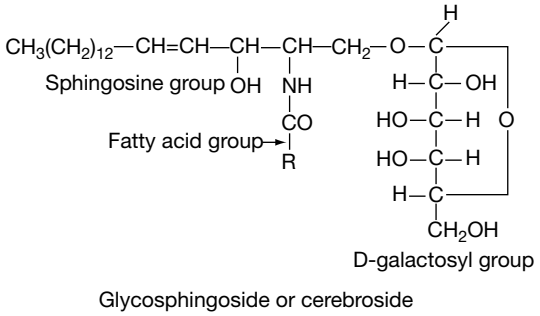


Figure 5.17 Structure of cerebroside

2. **Sulpholipids:** Lipid material has been known to be present in tissues, and found in liver, kidney, testicle, salivary gland, brain and tumours, being most abundant in the white matter of brain (Figure 5.18).

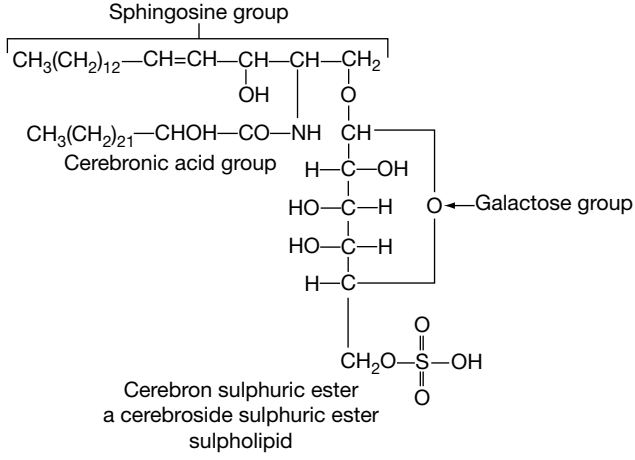


Figure 5.18 Structure of sulpholipid

3. **Globosides:** Contain 2 or more sugars. Its structure is similar to gangliosides and only sialic acid is absent.
4. **Gangliosides:** It is similar to globosides except that it also contains sialic acid. It is isolated from beef brain, nerve cells, spleen and red blood cell stroma. Gangliosides upon hydrolysis yield fatty acids, hexosamine, sphingosine, galactose, glucose and neuraminic acids (sialic acid). A large amount of accumulation of gangliosides in brain is observed in cases of Tay-Sachs disease and Niemann-Pick disease (Figure 5.19).

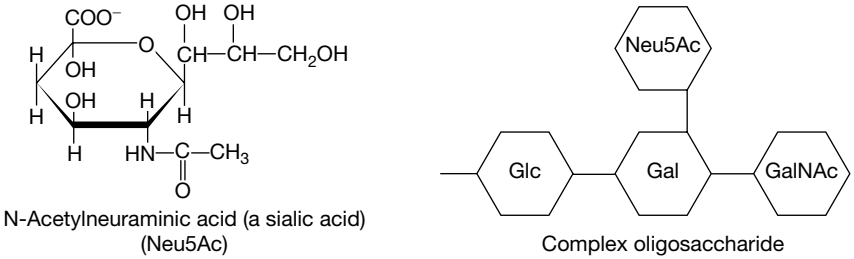


Figure 5.19 Structure of gangliosides

5. **Proteolipids:** Proteolipids are the complexes of protein and lipids having solubility properties of lipids class of being soluble in chloroform–methanol mixtures and insoluble in water. The protein part of proteolipids contains small amounts of aspartic, glutamic acids, arginine, lysine and histidine and large amount of 'neutral' amino acids. The lipids of proteolipids contain phosphatides with phosphatidyl serine. It is found in brain and nerve tissue (Figure 5.20).

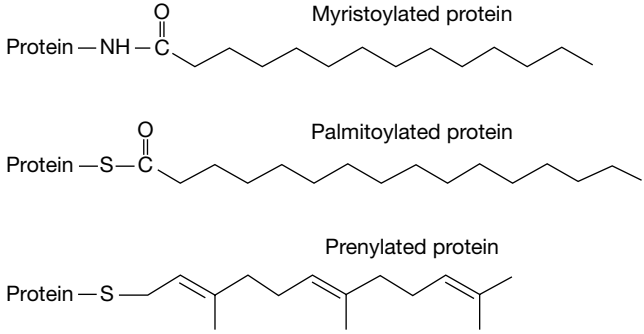


Figure 5.20 Structure of proteolipid

5.8 STEROIDS

A large number of compounds found in nature belong to the class of compounds known as steroids. These have the parent nucleus of perhydrocyclopentanophenanthrene, which consists of three six-membered rings (A, B and C) and a five-membered ring (D). These rings are joined as shown with a total of 17 carbon atoms (Figure 5.21).

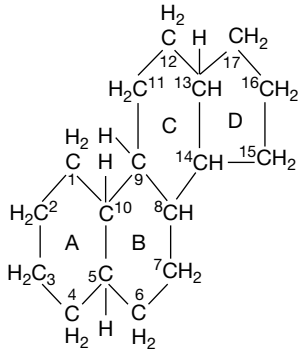
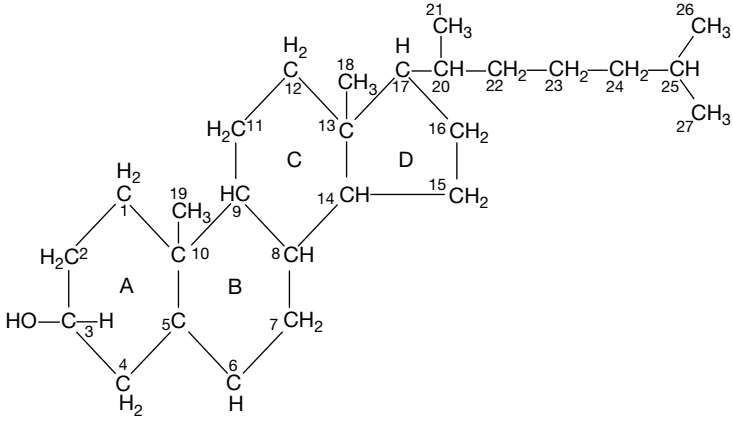


Figure 5.21 Perhydrocyclopentanophenanthrene

5.8.1 Animal Sterols

(a) **Cholesterol:** In the animal kingdom, the most abundant steroid is sterol cholesterol. Cholesterol has been found in all animal tissues but not in plant tissue. It is usually accompanied by dihydrocholesterol and 7-dehydrocholesterol (Figure 5.22).



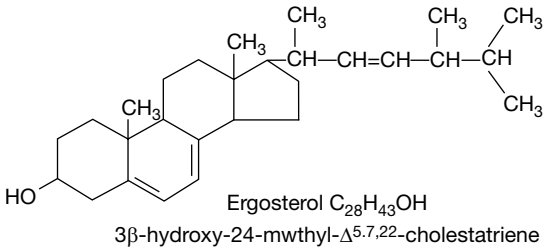
Cholesterol C_{27}-$H_{45}OH$, 3β-hydroxy-Δ^5-cholestene, or Δ^5-cholestene-3β-ol

Figure 5.22 Structure of cholesterol

The structure of cholesterol is shown in Figure 5.22, considering the number of asymmetric carbon present in the preceding structure. Cholesterol is named, in reference to the saturated parent structure cholestane, as 3β-hydroxy-Δ^5-cholestene or Δ^5-cholestene-3 β-ol.

(b) **Sterols of yeast and fungi: Mycosterols**

Ergosterol: This sterol is the principal sterol of fungi and yeast and is classed as a mycosterol. Its name is derived from the fact that it was first discovered in ergot bodies which form on rye and other cereal plants infected with ergot fungi. It is produced commercially in large quantities from certain strains of yeast. Ergosterol has the formula as shown in Figure 5.23.



Ergosterol $C_{28}H_{43}OH$

3β-hydroxy-24-mwthyl-$\Delta^{5,7,22}$-cholestatriene

Figure 5.23 Structure of ergosterol

(c) **Sterols of higher plants, phytosterol**

Stigmasterol: This sterol occurs especially in calabar and soya bean oils. It possess a ring system of cholestane type. The formula of stigmasterol is identical to that of cholesterol with the exception of the side chain attached to C-17, stigmasterol having an ethyl group on C-24 and a double bond between C-22 and C-23 of the side chain.

(d) **Bile acids:** The bile acids—cholic, deoxycholic and lithocholic—are found in the bile of higher animals, where they are largely conjugated with glycine and taurine to form compounds such as glycocholic and taurocholic acids (Figure 5.24).

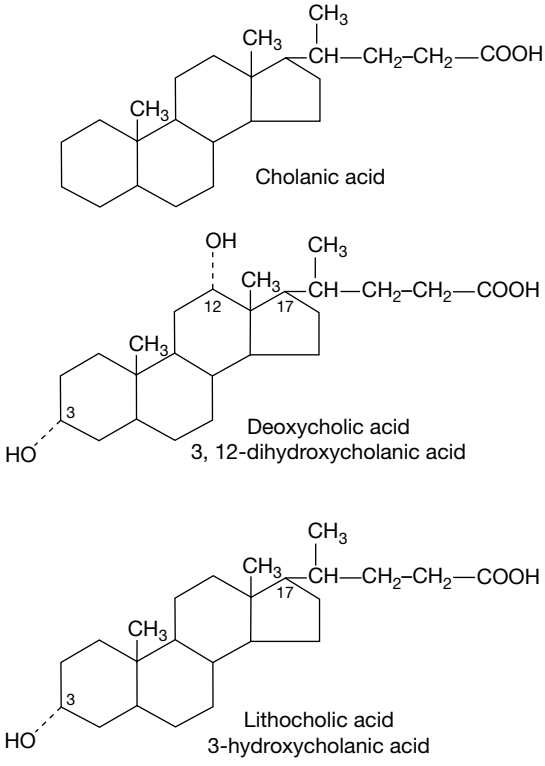


Figure 5.24 Structure of bile acids

The bile acids are conjugated with glycine and taurine, the side chain carboxyl groups, as shown hereunder (Figure 5.25).

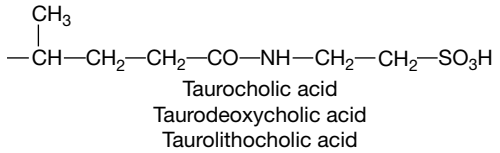


Figure 5.25 Structure of taurocholic and taurolithocholic acids

The salts of the bile acids lower surface tension and are good emulsifying agents. They play an important role in the absorption of lipids from the intestine.

5.9 QUALITATIVE TEST FOR LIPIDS

(a) **Greese spot test:** A drop of oil is placed over a piece of ordinary paper. A translucent spot is visible. This indicates the presence of fat.

(b) **Emulsification test:** To begin with, 2 ml water is taken in one test tube and 2 ml of diluted bile salt solution in another test tube. Add 3 drops of the given oil to each test tube and shake vigorously. Note the stability of the emulsification formed.

(c) **Saponification test:** Take 10 drops of coconut oil in a test tube and add glycerol 2 ml and 40 per cent NaOH (20 drops). Gently boil for about 3 min until complete saponification occurs. If oil globules are visible, boiling must be continued. Divide the solution into 3 parts to carry the following experiments in test tubes 1 to 3.

To test tube No. 1, add saturated solution of NaCI. See to it that the soap is separated out and floats to the surface (salting out process).

To test tube No. 2, add a few drops of conc. HCI. An oily layer of the fatty acids rises to the surface.

To test tube No. 3, add a few drops of $CaCl_2$ solution. The insoluble calcium soap is precipitated.

Unsaturation test: Add 10 drops of Huble's iodine reagent to 10 ml of chloroform. The chloroform assumes a pink colour due to the free iodine. The solution is divided equally into three test tubes as 1, 2 and 3 and three types of oil are added.

Add the oil No. 1 to the test tube (a) drop by drop shaking the tube vigorously after each addition till the pink colour of the solution just disappears. The number of oil drops required is noted. The experiment is repeated by taking oil 2 and 3 and adding to test tubes (b) and (c), respectively. The more the number of drops required to discharge the pink colour, the less is the unsaturation.

5.9.1 Colour Reaction to Detect Sterols

Liebermann-Burchard reaction: A chloroform solution of a sterol when treated with acetic anhydride and sulphuric acid gives a green colour. This reaction is the basis of a colorimetric estimation of blood cholesterol.

Salkowski's test: A red-to-purple colour appears when a chloroform solution of the sterol is treated with an equal volume of concentrated sulphuric acid.

EXERCISES

1. What are lipids? How are they classified? Discuss the characteristics and functions of a class of lipids having nitrogen base and phosphoric acid as constituents.
2. Give the classification and characteristics of lipids. Discuss briefly the chemistry and importance of phospholipids.
3. Write short notes on:
   (a) Essential fatty acids
   (b) Sterol
   (c) Phospholipids
   (d) Saponification number
   (e) Hydrogenated fats
   (f) Lecithin
   (g) lodine number
   (h) Lipoproteins
   (i) Unsaturated fats
   (j) Saponification
   (k) Rancidity
   (l) Cholesterol
4. How can the lipids be classified? Enumerate some neutral fats present in our body.
5. Name with test and reactions to show the presence of characteristic functional groups occurring in glycerol.
6. With structures, give examples of two unsaturated fatty acids essential for our body.
7. Give an account of the metabolism of fat with particular reference to β-oxidation.
8. What is lipogenesis? Describe the cytoplasmic system of fatty acid synthesis.
9. Describe the oxidation of fatty acids in our body.
10. How are fatty acids synthesized and degraded in our body?
11. Describe the fate of lipids in our body. What are essential fatty acids?
12. Describe the complete oxidation of oleic acid in our body. State how many molecules of ATP are formed during the process.
13. Describe briefly the chemistry of cholesterol and its physiological importance. What is the normal blood cholesterol level?
14. Describe metabolism of cholesterol with reference to atherosclerosis. Discuss the role of lipoproteins in it.
15. What are ketone bodies? Describe how ketosis is produced in the body.
16. What is ketosis? How are the ketone bodies produced in the body? How does body try to combat ketosis?
17. Discuss the following:
   (a) β-oxidation of fatty acids
   (b) Transport of lipid
18. Write in detail about the biosynthesis of cholesterol.

19. Define lipids and write in detail about the classification of lipids and their physiological function.
20. Write short notes on:
    (i) Essential fatty acids
    (ii) Cholesterol
    (iii) Lipoproteins
    (iv) Phospholipids
    (v) Phosphorylation
    (vi) Ketosis
    (vii) Ketone bodies
    (viii) Ketogenesis
    (ix) Unsaturated fatty acids

MULTIPLE CHOICE QUESTIONS

1. Fats are solids at
   (a) 10°C (b) 20°C
   (c) 30°C (d) 40°C
   Ans. b
2. Esters of fatty acids with higher alcohols other than glycerol are said to be
   (a) Waxes (b) Fats
   (c) Both (a) and (b) (d) None of these
   Ans. a
3. The combination of an amino-alcohol, fatty acid and sialic acid form
   (a) Phospholipids (b) Sulpholipids
   (c) Glycolipids (d) Aminolipids
   Ans. c
4. Hydrolysis of a fat by an alkali is called
   (a) Saponification number
   (b) Saponification
   (c) Both (a) and (b)
   (d) None of these
   Ans. b
5. The rate of fatty acid oxidation is increased by
   (a) Phospholipids (b) Glycolipids
   (c) Amino lipids (d) All of these
   Ans. a
6. Cardiolipin found in mitochondria is formed from
   (a) Lipositol
   (b) Phosphatidyl ethanolamine
   (c) Phosphatidyl glycerol
   (d) None of these
   Ans. c
7. Lecithin contains a nitrogenous base named as
   (a) Ethanolamine (b) Choline
   (c) Inositol (d) All of these
   Ans. b
8. Phosphatidyl inositol is found in
   (a) Cabbages (b) Soya beans
   (c) Cauliflowers (d) Apples
   Ans. b
9. The alkyl radical in plasmalogen is an _____ alcohol
   (a) Saturated (b) Unsaturated
   (c) Both (a) and (b) (d) None of these
   Ans. b
10. The concentrations of sphingomyelins are increased in
    (a) Gaucher's disease
    (b) Fabry's disease
    (c) Febrile disease
    (d) Niemann-Pick disease
    Ans. d

11. Sphingomyelins contain a complex amino alcohol named as
    (a) Serine (b) Lysolecithin
    (c) Sphingosine (d) Glycol
    Ans. c
12. Glycolipids contain an amino alcohol
    (a) Sphingosine
    (b) Iso-sphingosine
    (c) Both (a) and (b)
    (d) None of these
    Ans. c
13. Cerebrosides may also be classified as
    (a) Sphingolipids (b) Sulpholipids
    (c) Aminolipids (d) Glycolipids
    Ans. a
14. Gangliosides are the glycolipids occurring in
    (a) Liver (b) Brain
    (c) Kidney (d) Muscle
    Ans. b
15. The protein moiety of lipoprotein is known as
    (a) Apoprotein
    (b) Pre-protein
    (c) Post-protein
    (d) Pseudoprotein
    Ans. a
16. The iodine number of essential fatty acids of vegetable oils is
    (a) High (b) Very high
    (c) Very low (d) Low
    Ans. d

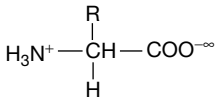
6 Composition of Amino Acids

CHAPTER OBJECTIVES

6.1 Introduction to Amino Acids
6.2 General Structure and Properties of Amino Acids
6.3 Classification of Amino Acids
6.4 Isomerism
6.5 Ionic Properties of Amino Acids
6.6 Peptides
6.7 General Reactions of Amino Acids
6.8 N-Terminal Analysis
6.9 Reactions of Specific Amino Acids
6.10 Separation of Amino Acid Mixtures

6.1 INTRODUCTION TO AMINO ACIDS

Amino acids are organic molecules of low relative molecular mass which contain at least one carboxyl group (COOH) and one amino group (NH_2) group and are essential constitution of both plant and animal tissues. The variations which occur between the different amino acids lie in the nature of their R groups (side chains).



6.1.1 Essential Amino Acids

The essential amino acids are those which cannot be synthesized in the human body and must be supplied through the diet. The essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

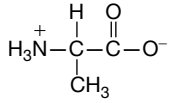
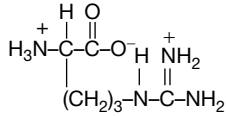
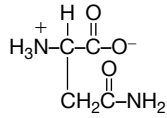
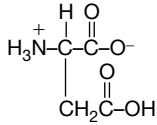
6.1.2 Non-essential Amino Acids

These amino acids are those which can be synthesized in the human body and can be made available to the body by synthesis. The non-essential amino acids are asparagine, aspartate, alanine, cysteine, glutamate, glutamine, glycine, proline, tyrosine and serine.

6.2 GENERAL STRUCTURE AND PROPERTIES OF AMINO ACIDS

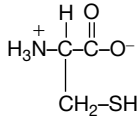
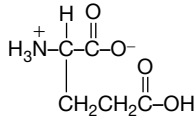
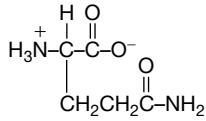
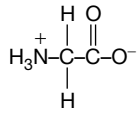
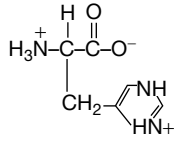
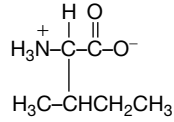
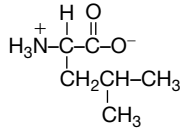
There are approximately 20 amino acids found in proteins, all of which are alpha-amino acids with the exception of two alpha-amino acids proline and hydroxyproline which for the purpose of this discussion will be considered with the amino acids because of their similarity. The alpha-amino acids are so called because the amino group is attached to the alpha-carbon of the chain which is, by convention, the carbon atom adjacent to the carboxyl group. Succeeding carbon atoms are designated β, γ, σ and ε. Hence, in α-amino acids both the amino group and carboxyl group are attached to the same carbon atom (Table 6.1).

Table 6.1 Structure of Amino Acids

R = Any number of carbons in a hydrocarbon chain				
Amino acid name	**Three-letter abbreviation**	**One-letter abbrevlation**	**Structure of amino acid R group**	**Comments**
Alanine	ala	A		Neutral, nonpolar
Arginine	arg	R		Basic, polar
Asparagine	asn	N		Neutral, polar
Aspartic acid	asp	D		Acidic, polar

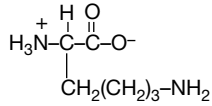
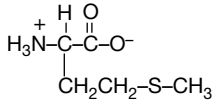
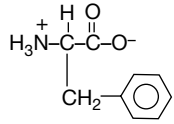
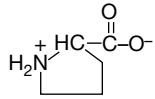
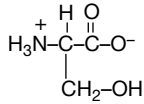
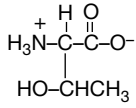
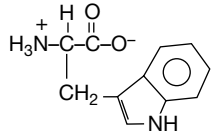
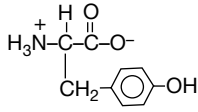
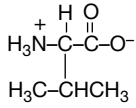
(*Continued*)

Table 6.1 Continued

R = Any number of carbons in a hydrocarbon chain				
Amino acid name	**Three-letter abbreviation**	**One-letter abbrevlation**	**Structure of amino acid R group**	**Comments**
Cysteine	cys	C		Neutral, slightly polar
Glutamic acid	glu	E		Acidic, polar
Glutamine	gln	Q		Neutral, polar
Glycine	gly	G		Neutral, nonpolar
Histidine	his	H		Basic, polar
Isoleucine	ile	I		Neutral, nonpolar
Leucine	leu	L		Neutral, nonpolar

(*Continued*)

Table 6.1 Continued

R = Any number of carbons in a hydrocarbon chain				
Amino acid name	**Three-letter abbreviation**	**One-letter abbrevlation**	**Structure of amino acid R group**	**Comments**
Lysine	lys	K		Basic, polar
Methionine	met	M		Neutral, nonpolar
Phenyl-alanine	phe	F		Neutral, nonpolar
Proline	pro	P		Neutral, nonpolar
Serine	ser	S		Neutral, polar
Threonine	thr	T		Neutral, polar
Tryptophan	trp	W		Neutral, slightly polar
Tyrosine	tyr	Y		Neutral, polar
Valine	Val	V		Neutral, nonpolar

6.3 CLASSIFICATION OF AMINO ACIDS

It is convenient to group together amino acids with similar groups which show common chemical or physical characteristics. The classification may be based on the chemical nature of the R group and such a system makes it easier to remember the general properties of each amino acid. However, a classification system based on the polarity of the R group may also be useful and this, together with the structural formula and abbreviated forms of the alpha-amino acids is given in Tables 6.2 and 6.3.

Table 6.2 Classification of Amino Acids as per Timberlake

Classification	Amino acids
Nonpolar	Glycine, alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine and tryptophan
Polar	Serine, threonine, asparagine, glutamine, cysteine, tyrosine
	aspartic acid and glutamic acid
Basic (polar)	Lysine, arginine and histidine

Table 6.3 Classification of Amino Acids as per Koolman

Classification	Amino acid
Aliphatic (do not contain N, O, S in side chain)	Glycine, alanine, valine, leucine, isoleucine
Sulphur-containing	Cysteine, methionine
Aromatic (benzene ring in side chain)	Phenylalanine, tyrosine, tryptophan
Neutral (hydroxyl or amide groups in side chain)	Serine, threonine, asparagine, glutamine
Acidic (carboxylate groups in side chain)	Aspartic acid, glutamic acid
Basic (amino group in the side chain)	Lysine, arginine
Imino acid (special case)	Proline

6.4 ISOMERISM

The α-carbon of all amino acids, with the exception of glycine, has four different substituent groups and is therefore an asymmetric carbon atom. Such an atom can exist in two different spatial arrangements which are mirror images of each other. These structural forms of molecules are known as stereoisomers and the common notation of D- and L-forms is used, a nomenclature which refers to their absolute spatial configuration when compared with that of glyceraldehyde (Figure 6.1).

All amino acids except glycine exist in these two different isomeric forms but only the L-isomers of the α-amino acids are found in proteins although many D-amino acids do occur naturally, for example, in certain bacterial cell walls and polypeptide antibiotics. It is difficult to differentiate between the D- and the L-isomers by chemical methods and when it is necessary to resolve a racemic mixture, an isomer-specific enzyme provides a convenient way to degrade the unwanted isomer, leaving the other isomer intact. Similarly, in a particular sample, one isomer may be determined in

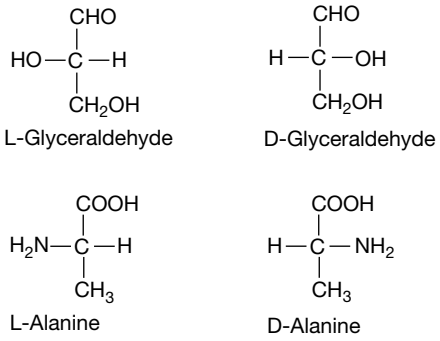


Figure 6.1 The stereo chemical relationship between amino acids and glyceraldehyde. The designation of D- or L to an amino acid refers to its absolute configuration relative to the structure of D- or L-Glyceraldehyde, respectively. The D- and L-forms of a particular compound are called enantiomers

the presence of the other using an enzyme with a specificity for the isomer under investigation. The other isomer present will not act as a substrate for the enzyme and no enzymic activity will be demonstrated. Enzyme L-amino acid oxidase, for example, is an enzyme which only shows activity with L-amino acids but will not react with the D-amino acids.

Although the D- and L-notation is still commonly used in amino acid and carbohydrate terminology, modern nomenclature employs a system which permits the configuration of an asymmetric atom to be specified. This is called the R-S convention or the sequence rule and involves assigning a priority (a > b > c > d) to the four different substituent atoms attached to the asymmetric atom on the basis of the atomic number of each. The sequence of substituent groups relative to the axis between the asymmetric carbon atom and the substituent group with the lowest priority (d) is used to designate the atom as either R (rectus, right) or S (sinister, left) (Figure 6.2).

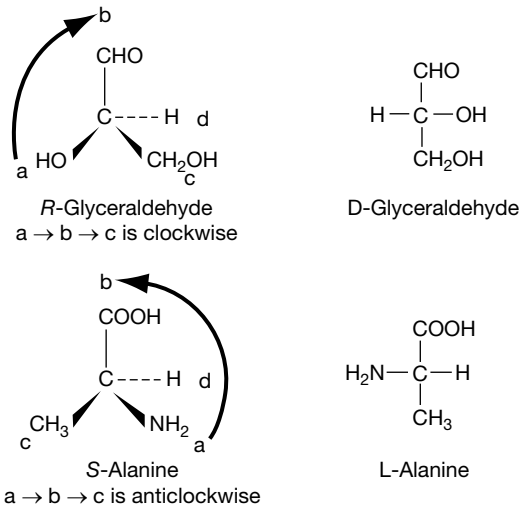


Figure 6.2 Systematic naming of an amino acid using the R-S convention. The compound is named by designating the configuration of the asymmetric carbon atom as either R or S. For amino acids containing more than one asymmetric centre, e.g., threonine and isoleucine, the configuration about each asymmetric atom is specified

6.5 IONIC PROPERTIES OF AMINO ACIDS

Amino acids contain both acidic (COOH) and basic (NH_2) groups and as a result can act both as weak acids and weak bases and are therefore called ampholytes. Their behaviour is termed amphiprotic because they can either accept or donate a proton, a reaction which can be represented by the following equation:

$$\text{R. } NH_3^+ \cdot COOH \rightleftharpoons \text{R. } NH_2 \cdot COOH \rightleftharpoons \text{R. } NH_2 \cdot COO^- + H^+$$

Even this representation is not completely true because it implies that an amino acid exists in an uncharged form (RNH_2COOH), whereas the molecule in this state carries one negative and one positive charge and as a result shows no net charge. This is known as the dipolar form or zwitterion of the amino acid (Figure 6.3).

In solution the dissociation of each ionizable group in the molecule may be represented as follows:

$$COOH \rightleftharpoons COO^- + H^+$$

$$NH_3^+ \rightleftharpoons NH_2 + H^+$$

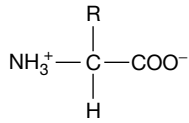


Figure 6.3 Dipolar or zwitterionic form of an amino acid. Amino acids exist in a charged form in aqueous solution, the carboxyl group being dissociated and the amino group associated. Some amino acids also have an extra ionizable group present in their side chain (R group). The ionization of each group is pH dependent and for each amino acid there is a pH at which the charges are equal and opposite and the molecule bears no net charge. This is called the isoionic pH (pI)

The dissociated and undissociated forms of each group exist in equilibrium with each other and the position of the equilibrium (or the tendency of each of the groups to dissociate) may be expressed in terms of the equilibrium (dissociation constant) K, often termed as K_a because it refers to the dissociation of the groups which liberate protons, i.e., acids. The actual value of K_a is often very small and is conventionally expressed as the negative logarithm of the value, a term often known as pK_a value:

$$pK_a = -\log K_a$$

The function results in a numerical value which is less cumbersome to use and is comparable to expressing the hydrogen ion concentration of a solution, the pH value:

$$pH = -\log [H^+]$$

The concentration of hydrogen ions liberated by the dissociation of an acid is related to the dissociation constant for that acid and this relationship can be expressed by the Henderson–Hasselbalch equation (see Chapter 1).

$$pH = pK_a + \log \frac{[salt]}{[acid]}$$

where the square brackets indicate the molar concentration of the named substance.

An examination of this equation reveals the fact when the concentrations of a salt and an undissociated acid are equal, then the pH of the solution is numerically equal to the pK_a for that acid. The lower the value of pK_a for an acid, the greater is the ability of an acid to dissociate yielding hydrogen ions, a characteristics known as the strength of the acid. Amino acids with two ionizable groups, an alpha-carboxyl and an alpha-amino group, will be characterized by a pK_a value and the actual value will give an indication of the strength of the acidic or basic group concerned.

The ionization of an amino acid is most easily demonstrated in an a titration curve which can be prepared by titrating a solution of the amino acid in the fully protonated form solution of sodium hydroxide and plotting the amount of the alkali added against resulting pH of the solution (Figure 6.4). The titration curve for a simple amino acid will show two regions where the addition of alkali results in only a small change in the pH value of the mixture. The buffering action of an amino acid is most significant over these pH ranges.

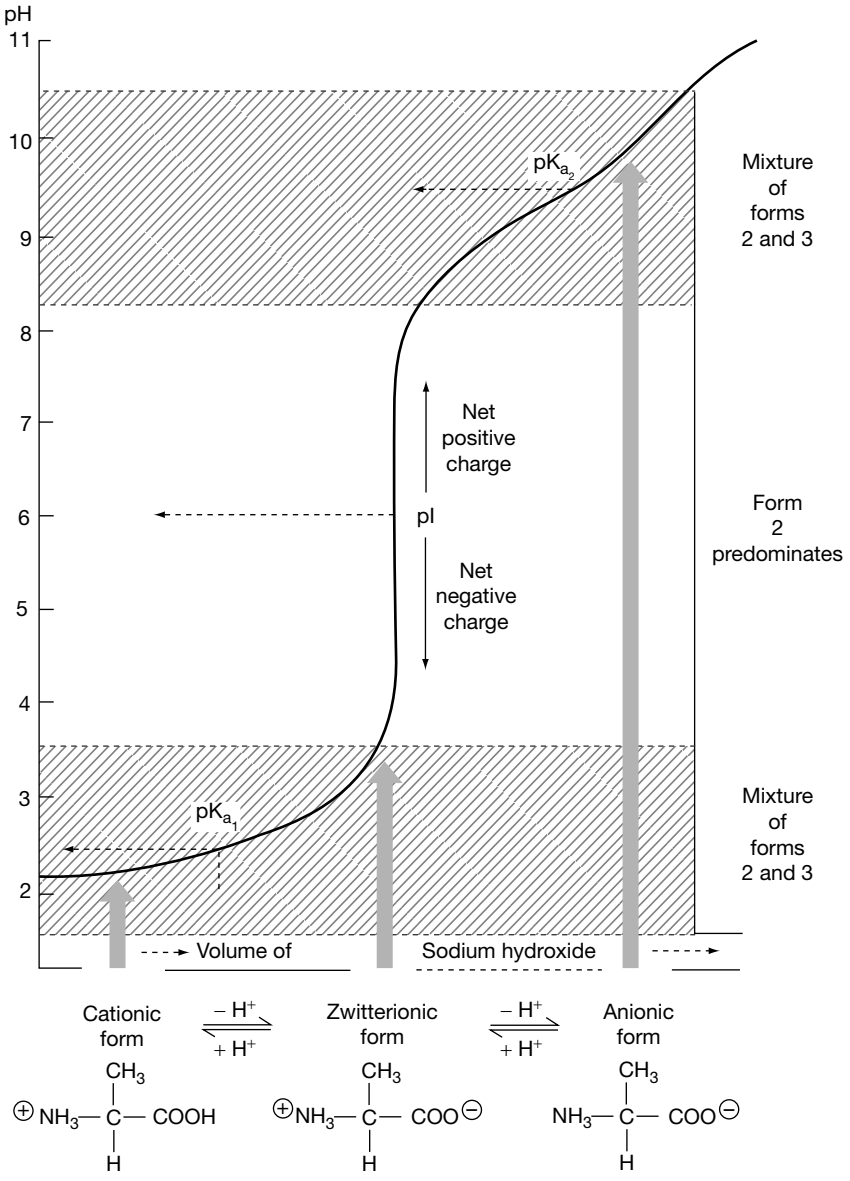


Figure 6.4 Titration curve of alanine, a solution of alanine (0.1 mol l^{-1}) in the fully protonated form at pH 2.0 is titrated with 0.1 mol l^{-1} sodium hydroxide. The volumes of sodium hydroxide added are recorded and plotted against the resulting pH values to give a titration curve which is typical of an amino acid with only two ionizable groups (one carboxyl and one amino). The two shaded areas show the pH range over which the addition of alkali results in only a very small change in pH and where the amino acid exhibits its most significant buffering action. At a pH equivalent to pK_{a1} there are equal amounts of forms 1 and 2 while at a pH equivalent to pK_{a2} forms 2 and 3 are in equal concentrations. The pI value for alanine in 6.0 and is the mean of pK_{a1} (2.4) and pK_{a2} (9.6). At a pH below its pI value, an amino acid will carry a net positive charge but it will carry a net negative charge at pH values greater than its pI

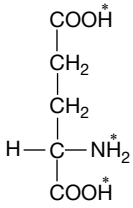
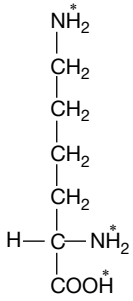
The first end point in such a titration is due to the carboxyl group and pK_a value for this is called pK_{a1} while the second pK_a value is for the amino group and is called pK_{a2}. In practice, each acid and its salt will act as a buffer over a pH range of approximately one unit on either side of its amino acid value. For the amino acid alanine, where pK_{a1} is 2.4 and pK_{a2} is 9.6, the most effective buffering action occurs over the pH ranges 2.4 ± 1 and 9.6 ± 1.

In addition to the alpha-amino and alpha-carboxyl groups, those amino amino acids with an extra ionizable group will also have a pK_a value. Glutamic acid is an example of an amino acid with an extra acidic group (COOH) on the gamma carbon and lysine is an example of an amino acid with an extra amino group on the ε-carbon atom. As a result, they each have three ionizable groups and three pK_a values can be demonstrated (Table 6.4), the pK_{a3} value being for the extra group. Other functional groups present in an amino acid may also be ionizable and will have characteristic pK_a values (Table 6.5). Such amino acids result in complex titration curves.

The overall charge carried by an amino acid depends upon the pH of the solution and the pK_a values of the ionizable groups present. If the pH is greater than the pK_a value for a group, a proton will be lost and the molecule will carry a negative charge (Figure 6.4) but if the pH is less than the pK_a value a positive charge will predominate. The fact that at different pH values different amino acids will be present in different ionic forms and will carry different net charges is utilized in many analytical methods, e.g., electrophoresis and ion-exchange chromatography.

The isoionic point of a molecule is the pH at which the number of negative charges due to proton loss equals the number of positive charges due to proton gain and the zwitterionic form

Table 6.4 Glutamic acid is an example of an amino acid with an extra acidic group (COOH) on the gamma carbon and lysine is an example of an amino acid with an extra amino group on the ε-carbon atom. As a result, they each have three ionizable groups and three pK_a

Amino acid	Formula	pK_a values
Glutamic acid (extra COOH group)		4.1 9.5 2.1
Lysine (extra NH_2 group)		10.8 9.2 2.2

The asterisk (*) shows where proton gain or loss can occur.

Table 6.5 pK_a Values for some Amino Acids

Amino acid	Extra ionizable group	α-Carboixyl pK_{a1}	α-Amino pK_{a2}	Extra group pK_{a3}
Arginine	Guanidinium	1.8	8.9	12.5
Cysteine	Sulphydryl	1.7	10.8	8.3
Tyrosine	Phenolic	2.0	9.1	10.1
Histidine	Imidazole	1.8	9.2	6.0

predominates. The isoelectric point (pI) is the pH of the solution at which the molecules show no migration in an electric field and can be determined experimentally by electrophoresis and for amino acids it is equal to the isoionic point. The isoionic point of an amino acid with one carboxylic and one amino group is the mean of the two pK_a values (Figure 6.4). However, when three ionizable groups are present, the effect of an extra acid group will be to reduce the ionic character of the other acid group and hence the pI value will not be the mean of the three separate pK_a values but will more closely approximate to the mean of the closest pK_a values.

6.6 PEPTIDES

When two amino acids are linked together by the condensation of the alpha-amino group from one amino acid and the alpha-carboxyl group from another to form a peptide bond, the resulting compound is called a dipeptide (Figure 6.5). The ionic character of the constituent amino acids will be modified due to the loss of either an amino or a carboxyl group and the properties of the dipeptide will depend not only on the terminal amino and carboxyl groups but also on any ionizable R groups. For peptides containing increasing number of amino acids, the significance of the two terminal groups (COOH and NH_2) becomes less important and the ionic nature of the R groups becomes more important. Molecules containing many amino acids linked in such a manner are known as polypeptides and are generally only classed as proteins when they are composed of more than 50 amino acids and their relative molecular mass exceeds 5000.

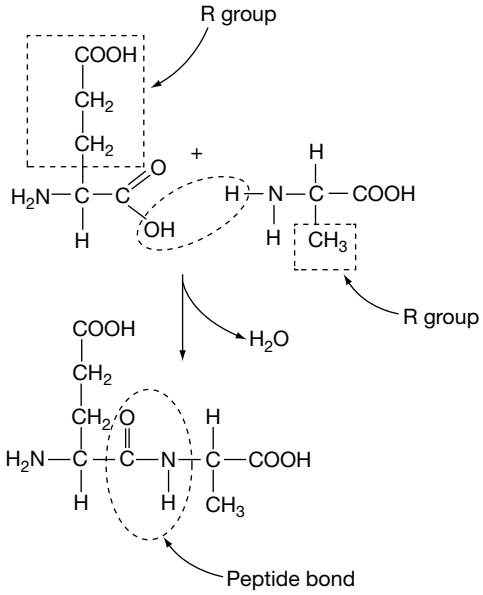


Figure 6.5 Structure of a dipeptide. The peptide bond joins glutamic acid and alanine by condensation of the alpha-carboxyl group of glutamic acid and the alpha-amino group of alanine. The resulting dipeptide is called glutamylalanine, which can be abbreviated to NH_2-Glu-Ala-COOH or Glu-Ala

The peptide bonds in proteins are between the alpha-amino and the alpha-carboxyl groups but peptides do occur naturally where the peptide linkage involves a carboxyl or amino group which is attached to a carbon atom other than the alpha-carbon. The amino acid glutamic acid contains two carboxyl groups attached to the alpha- and gamma-carbon atoms and either may be involved in peptide linkages. A dipeptide formed between the gamma-carboxyl group of glutamic acid and the amino group of alanine is called gamma-glutamylalanine (Figure 6.6). Linkages may also be formed between the ε-amino group of lysine and other amino acids. Peptides are also found whose constituents are amino acids other than the alpha-amino acids; carnosine, for instance, is a dipeptide found in muscle and consists of beta-alanine and histidine.

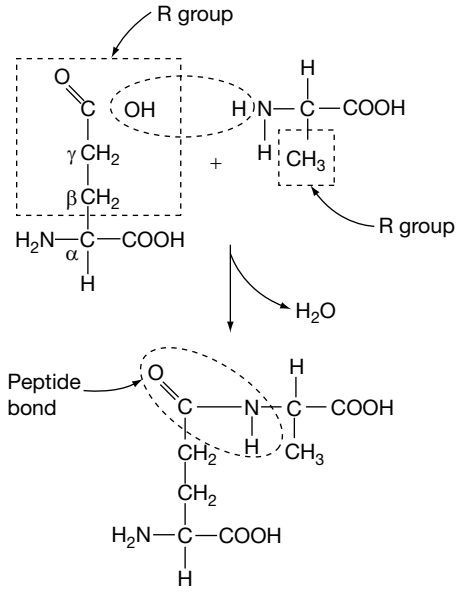


Figure 6.6 Structure of γ-glutamyalanine. The dipeptide consists of glutamic acid linked by a peptide bond which involves the carboxyl group attached to the γ-carbon atom and the α-amino group of alanine

The only residual evidence of the amino acids which make up polypeptides is their R group and the individual components are now termed amino acid residues. It is conventional when representing peptides to show the terminal amino acid residue with the free amino

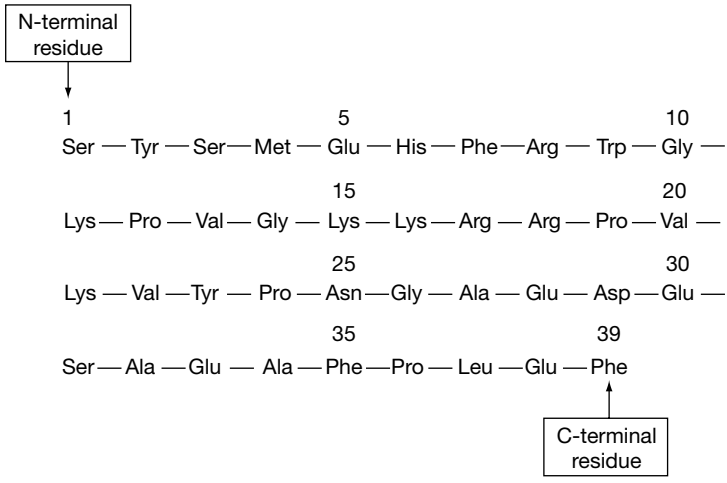


Figure 6.7 Amino acid sequence of human adrenocorticotrophin (ACTH). The amino acid residues in this polypeptide hormone are linked by peptide bonds and each residue is given a number starting with the N-terminal residue (number 1) to the C-terminal residue (number 39)

group to the left of any diagram and to designate it Residue 1 (the N-terminal residue) and the one with the free carboxyl group (the C-terminal residue) on the right of any diagram (Figure 6.7).

Many naturally occurring hormones and antibiotics are polypeptides and investigation into both the amino acid constituents and their sequence in the polypeptide chain are important areas of research. These investigations may reveal information regarding the biologically active part of the molecule, a fact which may then be used in the commercial production of a synthetic peptide containing only that small part of the original polypeptide but showing a physiological activity comparable with the whole molecule. This is true for several hormones, a good example being the anterior pituitary hormone, adrenocorticotrophic hormone (ACTH) which is naturally composed of 39 amino acid residues but a synthetic peptide containing only residues 1 to 23 of the original hormone shows comparable physiological activity (Figure 6.7).

6.7 GENERAL REACTIONS OF AMINO ACIDS

There are several compounds which will react with amino acids to give coloured or fluorescent products and as a result can be used in qualitative or quantitative methods. Fluorimetric methods are gaining in popularity and offer some important advantages over absorption spectrophotometry for amino acid analysis.

6.7.1 Ninhydrin

Ninhydrin (triketohydrindene hydrate) reacts with an amino acid when heated under acidic conditions (pH 3 to 4) to produce ammonia, carbon dioxide and a blue-purple complex. This reactions forms the basis of many widely used and methods (Figure 6.8). The most precise technique is that of Van Slyke which uses the gasometric reaction in which 1 mole of carbon dioxide is liberated from each mole of amino acid, exception being the dicarboxylic amino acids which produce 2 moles of carbon dioxide and the alpha-amino acid, proline and hyroxy-proline, which do not react gasometrically. Gasometric method is regarded as reference method. However, as it is rarely used the more common colorimetric method is used.

The ninhydrin colour reaction is very useful in qualitative work and is widely used in the visualization of amino acid bands after electrophoretic or chromatographic separations of mixtures.

The overall reactions of amino acids with ninhydrin are listed hereunder.

1. Oxidative decarboxylation of the amino acid and the production of reduced ninhydrin ammonia and carbon dioxide;
2. Reduced ninhydrin reacts with more ninhydrin and the liberated ammonia and
3. A blue-coloured complex is formed.

6.7.2 o-Phthalaldehyde

Primary amino acids will react with o-phthalaldehyde in the presence of the strongly reducing 2-mercaptoethanol (pH 9 to 11) to yield a fluorescent product (emission maximum, 455 nm; excitation maximum, 340 nm). Peptides are less reactive than alpha-amino acids and secondary amines do not react at all. As a result, proline and hydroxyproline must first be treated with a suitable oxidizing agent such as chloramine T (sodium N-chloro-p-toluene-sulphonaminde) or

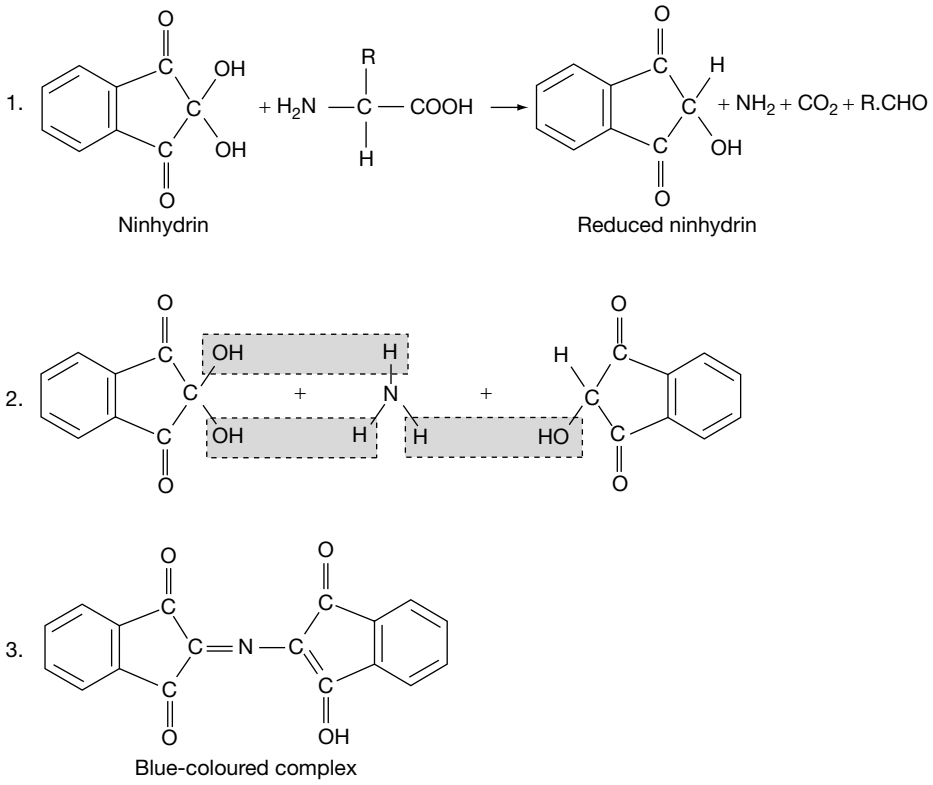


Figure 6.8 The ninhydrin reaction

sodium hypochlorite, to convert them into compounds which will react. Similarly, cystine and cysteine should also be first oxidized to cysteic acid. The fluorescent yield of individual amino acids varies and fluorescence values must be determined for accurate quantitative work in the same manner as the colour values for ninhydrin.

The aqueous reagent is stable at room temperature and the reaction proceeds quickly without requiring heat. The method is approximately 10 times more sensitive than the ninhydrin method and is particularly useful when the quantitation of many amino acids is being carried out using amino acid analysers.

6.7.3 Fluorescamine

All primary amines react with fluorescamine under alkaline conditions (pH 9 to 11) to form a fluorescent product (Figure 6.9) (excitation maximum, 390 nm; emission maximum, 475 nm). The fluorescence is unstable in aqueous solution and the reagent must be prepared in acetone. The secondary amines, proline and hydroxyproline, do not react unless they are first converted to primary amines which can be done using N-chorosuccinimide. Although the reagent is of interest because of its fast reaction rate with amino acids at room temperature, it does not offer any greater sensitivity than the ninhydrin reaction.

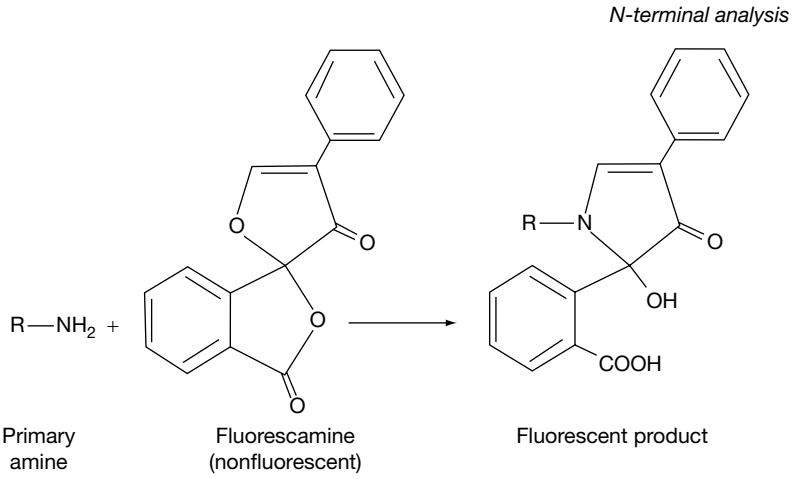


Figure 6.9 The reaction of fluorescamine with a primary amino group. The reaction of an amino acid containing a primary amino group with a solution of flourescamine in acetone at pH 9.0 results in the conversion of the non-fluorescent fluorescamine to a fluorescent product

6.8 N-TERMINAL ANALYSIS

A yellow dinitrophenyl (DNP) derivative is formed upon reaction of FDNB (1-fluoro 2,4 dinitrobenzene) with the free amino group of an amino acid or a peptide (Figure 6.10). This reaction is useful in qualitative methods of analysis and not for quantitation of mixtures of amino acids because of the variation in the the molar absorption coefficients of the DNP derivatives of different amino acids. After the separation by paper or thin layer chromatography, the yellow DNP derivatives of free amino acids in a sample are seen and R_f values are compared with the known amino acid for identification. The amino acid composition of di- and tripeptides may also be determined using the FDNB reaction if the peptide bonds joining the amino acid residue are first broken to yield the free amino acids.

The reaction at pH 9.5 between 1-fluoro, 2,4 dinitrobenzene (FDNB) and amino acids or peptides results in the formation of yellow-coloured dinitrophenyl derivatives

The FDNB reaction is used for the analysis of the N-terminal amino acid of peptides, this reaction is widely used. A yellow dinitrophenyl (DNP) derivative is formed after the reaction of FDNB reagent with the amino group of N-terminal amino acid. A yellow dinitrophenyl (DNP) derivative is detached from the peptide by acid or enzymic hydrolysis of the peptide bond. This reaction is identified by Dr. F. Sanger and therefore the reagent is named after his name often called as Sanger's reagent. For the first time Dr. F. Sanger used this reaction for the identification of primary structure of insulin.

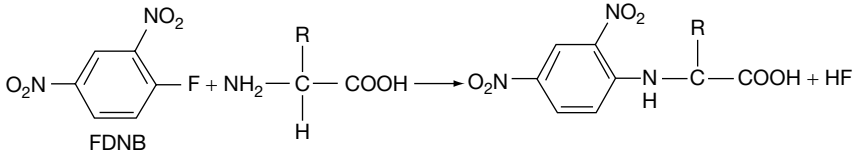


Figure 6.10 The reaction of FDNB with compounds containing a free amino group

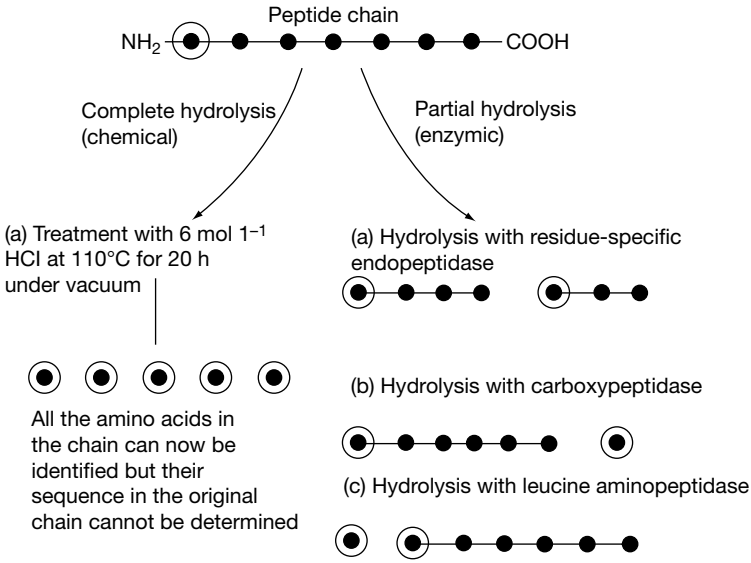


Figure 6.11 Schematic representation of the use of hydrolytic procedures in the determination of the amino acid sequence of a peptide. The presence of a free amino group @, with which an N-terminal reagent can react, enables that amino acid to be identified after complete hydrolysis of the peptide. The peptide fragments released after partial hydrolysis of the intact peptide with a particular hydrolytic enzyme can be subjected to further hydrolysis with different enzymes

The elucidation of the sequence of amino acids in a polypeptide chain is a complex process and only a brief outline of such a scheme is included (Figure 6.11). The polypeptide chain is split into smaller peptides using peptidases which are residue specific and will only hydrolyse peptide bonds where those residues occur. Pancreatic trypsin, for example, will only hydrolyse a peptide bond involving the carbonyl group of either arginine or lysine. The peptide fragments are then isolated and subjected to N-terminal analysis. If the native polypeptide is degraded by another proteolytic enzyme, different peptide fragments will result which may be subsequently analysed in a similar manner. Chemical hydrolysis of the complete polypeptide and the isolated peptide fragments will yield all the free amino acid components which may then be identified. By the use of several reagents and hydrolytic procedures, the structure of the original polypeptide may be deduced.

The free amino group of amino acid reacts with **dansyl chloride** (dimethylaminonaphthalene-5- sulphonyl chloride) to form fluorescent derivatives (Figure 6.12). This method can also be used in combination with chromatographic procedures for amino acid identification in a similar manner to the FDNB reagent but shows an approximately 100-fold increase in sensitivity.

6.9 REACTIONS OF SPECIFIC AMINO ACIDS

It is often difficult to quantitate one particular amino acid in the presence of others because of chemical similarities. Interference from substances other than amino acids is also a problem in many reputedly specific methods. Ultraviolet spectroscopy is of little value in the detection of aromatic amino acids because they have similar absorbance maxima and considerably different molar absorption coefficients.

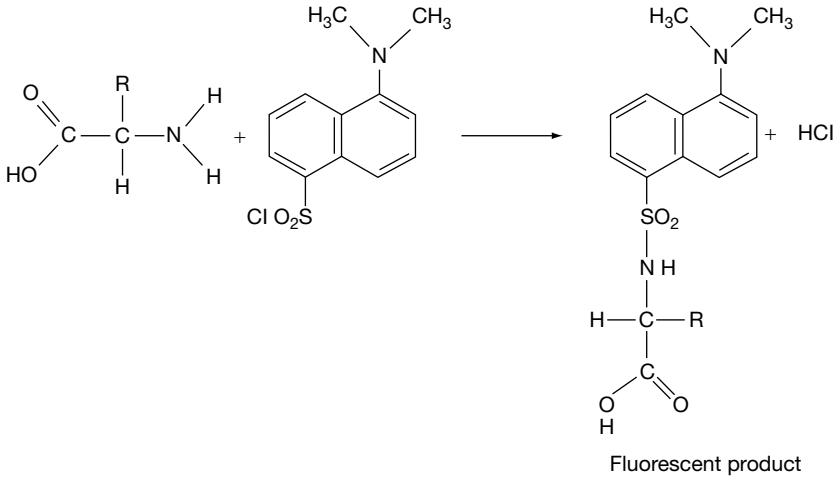


Figure 6.12 The reaction of dansyl chloride with compounds containing a free amino group. At an alkaline pH, the reaction results in the formation of fluorescent derivatives of free amino acids and the N-terminal amino acid residue of peptides

6.9.1 Colorimetric Methods

There are several colour reagents which are of little quantitative value without the prior removal or interfering substances, although in some cases it may be possible to increase the specificity of the reagent for the determination of a particular amino acid by modifying its composition or altering the reaction conditions. Pauly's reagent (diazotized sulphanilic acid reagent) for instance, reacts with histidine and tyrosine to give a red-coloured product but other phenolic compounds also give this reaction. Erhlich's reagent (p-amino benzaldehyde in HCI) gives a purple-red product with tryptophan and other indoles and a yellow-coloured product with aromatic amines and ureides, of which urea is the most widely distributed in biological fluids. They are, however, useful qualitatively, especially as locating reagents after the separation of amino acids by electrophoresis or chromatography and their use in multiple-dip sequences aids identification.

The fluorimetric methods often offer improved specificity and sensitivity over colorimetric procedures and the quantitative assays for the aromatic amino acids tyrosine and phenylalanine illustrate these points.

Tyrosine

1-Nitroso-2-naphthol reacts with tyrosine in the presence of sodium nitrite to form an unstable red compound which is converted, by heating with nitric acid, to a stable yellow fluorescent product. After removal of the excess unreacted nitroso-naphthol, the fluorescense is measured at 570 nm with excitation at 460 nm. A modification of this method is suitable for use in continuous flow automated analysers.

Phenylalanine

Phenylalanine reacts with ninhydrin in the presence of a dipeptide (usually glycyl-L-leucine or L-leucyl-L-alanine) to form a fluorescent product. The fluorescence is enhanced and stabilized

by the addition of an alkaline copper reagent to adjust the pH to 5.8 and the resulting fluorescence is measured at 515 nm after excitation at 365.

6.9.2 Microbiological Methods

Microbiological assays have been widely used for the quantitation of amino acids because they were, until recently, the most reliable, sensitive and specific tests available. They are applicable to any type of biological material although the presence of activators or inhibitors sometimes causes problems. Such methods lend themselves to the analysis of large batches of samples and are inexpensive but they are time consuming and not suitable for all amino acids. They have now been largely superseded by ion-exchange chromatography for the quantitation of specific amino acids and will be discussed only briefly.

Certain micro-organisms require amino acids for growth and without them they cannot replicate. Many strains of such micro-organisms have been produced which show dependence on a particular amino acid. Hence attempts to culture such a micro-organism in the presence of only a small amount of that amino acid will result in a limited degree of growth. This can be assessed using turbidimetry or by measuring the increase in lactic acid production by either microtitration or pH change.

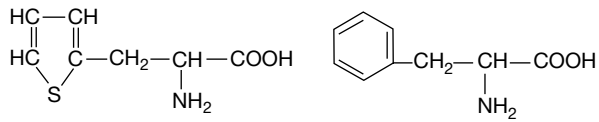
A modification of the microbiological assay which utilizes diffusion in gels has been successfully introduced into clinical biochemistry laboratories for the mass screening of blood samples for raised phenylalanine levels. It is often called the 'Guthrie test' after its originators, Guthrie and Susi, and is the most extensively used microbiological assay for the measurement of an amino acid. It is a bacterial inhibition assay and is based on the ability of phenylalanine to counteract the effects of a competitive metabolic antagonist β-2-theienylalanine on the growth of a special strain of *Bacillus subtilis* which requires phenylalanine as a growth factor (Figure 6.13).

The assay is performed on a layer of agar in which is incorporated a mixture of the suspension of *B. subtilis* spores, the minimum amount of growth nutrients and a fixed amount of the metabolic antagonist β-2-thienylalanine.

Blood soaked filter paper discs of identical diameter (~4 mm) are placed on the surface of the agar together with a range of phenylalanine standards, also in the form of blood discs, and the agar plates are incubated overnight at 37°C. Bacterial growth will only occur when the concentration of phenylalanine in the blood discs is sufficient to overcome the effects of the metabolic antagonist resulting in zones of growth around each disc. The following day the diameter of bacterial growth around each disc is measured and is related to phenylalanine concentration.

6.9.3 Enzymic Methods

There are several enzymes which in theory, may be used for quantitation but because they react with more than one amino acid cannot be used to measure an individual amino acid in a mixture.



β-2-Thienylalanine Phenylalanine

Figure 6.13 The Guthrie test. The similarity in structure between phenylalanine and its metabolic antagonist β-2-thienylalanine provides the basis for a microbiological assay for phenylalanine

The specificity of an enzyme for a particular isomer may be used for measurement of D-amino acids in the presence of the L-isomers, or vice versa and the amino acid oxidases are useful in this respect. They catalyse the oxidative deamination of amino acids.

$$\text{Amino acid} + O_2 \xrightarrow{\text{Amino acid oxidase}} \text{Oxoacid} + H_2O_2 + NH_3$$

D-amino acid oxidase extracted from sheep kidney possesses low selectivity and at pH 8 to 9 will oxidize many D-amino acids, whereas the L-amino acid oxidase from snake (*Crotalus adamanteus*) venom at pH 8 to 9 catalyses the oxidation of many L-amino acids. However, as these enzymes show different reactivity towards different amino acids, the results for a sample which contains several D- and L-amino acids may be difficult to interpret. The use of these enzymes is therefore only recommended for the measurement of one isomer of an isolated amino acid. They may also be used to remove an unwanted isomer from a sample containing both to allow subsequent measurement of the other.

One approach to the measurement of amino acids using an amino acid oxidase is to measure the amount of ammonia formed during the reaction either using in ion selective electrode or by linking it to the oxidation of NADH by the enzyme glutamate dehydrogenase. In an alternative method, the amount of hydrogen peroxide formed is measured either using an ion-selective electrode or by the oxidation of a suitable chromogen using a peroxidase. These procedures are common to other assays employing oxidases (e.g., glucose oxidase).

L-amino acid oxidase has been used to measure L-phenylalanin and involves the addition of a sodium arsenate–borate buffer which promotes the conversion of the oxidation product, phenylpyruvic acid, to its enol form which then forms a borate complex having an absorption maximum at 308 nm. Tyrosine and tryptophan react similarly but their enol–borate complexes have different absorption maxima at 330 nm and 350 nm, respectively. Thus, by taking absorbance readings at these wavelengths the specificity of the assay is improved. The assay for L-alanine may also be made almost completely specific by converting the L-pyruvate formed in the oxidation reaction to L-lactate by the addition of lactate dehydrogenase and monitoring the oxidation of NADH at 340 nm.

A group of enzymes which may be employed in the measurement of L-amino acids are the L-amino acid decarboxylases of bacterial origin many of which are substrate specific. They catalyse reactions of the type:

$$RCHNH_2COOH \longrightarrow RCH_2NH_2 + CO_2$$

These assays have not been widely used in the past but with the advent of a carbon dioxide-selective electrode, their popularity may increase.

6.10 SEPARATION OF AMINO ACID MIXTURES

The identification and quantitation of the individual amino acids in a mixture is often required in metabolic studies and investigations of protein structure. The use of paper chromatography or electrophoresis may be adequate to indicate the relative amounts and number of different amino acids in a sample but the use of gas–liquid chromatography or an amino acid analyser is essential for quantitative analysis.

6.10.1 Paper and Thin Layer Chromatography

Paper chromatography has been used successfully for many years and is still a useful tool despite the fact that thin layer techniques, especially with the advent of commercially prepared plastic or foil-backed plates, offer advantages of speed, resolution and easier handling. Larger volumes of a sample can be applied to paper permitting the subsequent elution of a particular amino acid for further purification and analysis, a feature which is of particular importance in the identification of an unknown sample constituent.

Prior to chromatography, it may be necessary to remove interfering substances such as protein, carbohydrates and salts and this may be done using an ino-exchange resin. A small column containing a cation-exchange resin, e.g., Zeo Karb 225, is prepared in the acid form by treating it with hydrochloric acid (2 mol^{-1}). After the resin column has been washed with water, the acidified sample is applied and the interfering substance washed through with water and discarded. The amino acids remain on the resin and may be subsequently eluted by adding a small volume of ammonia solution (2 mol l^{-1}) to the column and washing through with distilled water. The alkaline eluent is collected and reduced in volume using a rotary evaporator. Other techniques such as solvent extraction, dialysis or protein precipitation may also be used to separate the amino acids from the other components of the sample.

The identification of an amino acid is achieved by comparison of R_f with those of reference solution and the use of at least three different solvent systems is recommended before its identity can be established with any degree of certainty.

EXERCISES

1. Define isomerism. Explain the stereo chemical relationship between amino acids and glyceraldehydes.
2. Write the ionic properties of amino acids.
3. Please draw the titration curve of alanine, a solution of alanine (0.1 mol l^{-1}) in the fully protonated form at pH 2.0 is titrated with 0.1 mol l^{-1} sodium hydroxide.
4. Write the overall reaction of amino acids with ninhydrin with a suitable diagram.
5. The FDNB reaction is used for the analysis of the N-terminal amino acids of peptides. Explain.
6. Write in detail the microbiological assays used for the quantitation of amino acids.
   What are the sources and main functions of amino acids in our body?
7. Name the essential amino acids and describe the specific metabolic role of any two of them.
8. Write notes on:
   (a) Essential amino acids
   (b) Acidic amino acids
   (c) Methionine
   (d) Tryptophan
   (e) Aminotransferase

MULTIPLE CHOICE QUESTIONS

1. Out of 200 different amino acids found in nature, the number of amino acids present in protein is
   (a) 20 (b) 25
   (c) 30 (d) 35
   Ans. a
2. The amino acid containing hydroxyl group is
   (a) Alanine (b) Isoleucine
   (c) Arginine (d) Threonine
   Ans. d
3. The amino acid which is used in the synthesis of many hormones is
   (a) Valine (b) Phenylalanine
   (c) Alanine (d) Histidine
   Ans. b
4. Insulin degradation or disulphide bond formation is effected by
   (a) Pyruvate dehydrogenase
   (b) Xylitol reductase
   (c) Glutathione reductase
   (d) Xanthine oxidase
   Ans. c
5. The example of globulins is
   (a) Leucosin (b) Tuberin
   (c) Oryzenin (d) Legunelin
   Ans. b
6. The amino acids abstracted from the liver are not utilized for repair or special synthesis but are broken down to
   (a) Keto acids
   (b) Sulphur dioxide
   (c) Water
   (d) Ammonia
   Ans. d
7. Amino acids provide the nitrogen for the synthesis of
   (a) The bases of the phospholipids
   (b) Uric acid
   (c) Glycolipids
   (d) Chondroitin sulphates
   Ans. a
8. Phenylalanine reacts with ninhydrin in the presence of a dipeptide to form a fluorescent compound whose fluorescence is measured at
   (a) 515 nm (b) 415 nm
   (c) 315 nm (d) 615 nm
   Ans. a
9. Tyrosine and tryptophan react similarly but their enol–borate complexes have different absorption maxima at
   (a) 330 nm and 350 nm, respectively
   (b) 230 nm and 340 nm, respectively
   (c) 430 nm and 550 nm, respectively
   (d) 230 nm and 330 nm, respectively
   Ans. a
10. All primary amines react with fluorescamine under alkaline conditions (pH 9 to 11) to form a
    (a) Fluorescent product
    (b) Non-Fluorescent product
    (c) Both (a) and (b)
    (d) None of these
    Ans. a
11. When two amino acids are linked together by the condensation of the alpha-amino group from one amino acid and the alpha-carboxyl group from another to form a peptide bond, the resulting compound is called a
    (a) dipeptide (b) peptide
    (c) tripeptide (d) none of these
    Ans. a
12. All amino acids exist in two different isomeric forms
    (a) Except glycine
    (b) Except lycine
    (c) Except alanine
    (d) None of these
    Ans. a
13. A dipeptide formed between the gamma-carboxyl group of glutamic acid and the amino group of alanine is called

(a) gamma-glutamylalanine
(b) alpha-glutamylalanine
(c) beta-glutamylalanine
(d) None of these

Ans. a

14. The alpha-amino acids are so called because the amino group is attached to the
(a) alpha-carbon (b) beta-carbon
(c) gamma-carbon (d) none of these

Ans. a

15. There are approximately 20 amino acids found in proteins, all of which are alpha-amino acids with the exception of two
(a) Proline and hydroxyproline
(b) Glycine
(c) Lysine
(d) None of these

Ans. a

16. The isoionic point of a molecule is the pH at which the number of negative charges due to proton loss equal the number of
(a) Positive charges (b) No charge
(c) Both (a) and (b) (d) None of these

Ans. a

17. The secondary amines, proline and hydroxyproline, do not react unless they are first converted to primary amines which can be done using
(a) N-chorosuccinimide
(b) N-chorohexamide
(c) Chloroform
(d) None of these

Ans. a

18. The ACTH is naturally composed of how many amino acid residues.
(a) 39 amino acid residues
(b) 49 amino acid residues
(c) 59 amino acid residues
(d) None of these

Ans. a

19. Carnosine, for instance, is a dipeptide found in muscle and consists of beta-alanine and
(a) Histidine (b) Tyrosine
(c) Tryptophan (d) Histamine

Ans. a

20. Glutamic acid is an example of an amino acid with an extra acidic group (COOH) on the
(a) Gamma-carbon (b) Alpha-carbon
(c) Beta-carbon (d) None of these

Ans. a

21. Lysine is an example of an amino acid with an extra amino group on the
(a) ε-carbon atom
(b) Gamma-carbon
(c) Beta-carbon
(d) None of these

Ans. a

22. Both glutamic acid and lysine have three ionizable groups and
(a) Two pK_a (b) Three pK_a
(c) One pK_a (d) None of these

Ans. b

7 Composition of Proteins

CHAPTER OBJECTIVES

7.1 Introduction to Proteins
7.2 Importance and Structure of Proteins
7.3 General Methods of Quantitation
7.4 Separation Techniques of Proteins
7.5 Mechanism of Protein Synthesis
7.6 Control of Protein Synthesis
7.7 Control at Translational Level
7.8 Glycosylation of Proteins
7.9 Denaturation of Proteins
7.10 Nitrogenous Constituent of Urine

7.1 INTRODUCTION TO PROTEINS

The word protein describes only one type of polymer involving mainly alpha- amino acids and yet it includes many thousands of different molecules. It is possible, for instance, to measure the total protein content of a sample despite the fact that relatively simple preparative techniques may be capable of demonstrating the presence of different proteins. It may be necessary on occasions to measure any one of these proteins and in such a situation a measure of the total protein content would be completely inappropriate. Methods for the quantitation of proteins may either be suitable for all proteins or be designed to measure individual proteins. Such specific methods may depend on either a preparative stage in the analysis or the use of a specific characteristic of the protein in question.

The quantitation of a protein which has a specific biological function, a hormone for instances may not give a true indication of its biological activity due to the inactivation of some of the protein. For proteins which have definite biological function the choice is between chemical quantitation and bioassays. For this reason the measurement of the catalytic activity of an enzyme is more frequently used than is the chemical determination of the protein content.

7.2 IMPORTANCE AND STRUCTURE OF PROTEINS

7.2.1 Primary Structure of Proteins

The sequence of amino acids in a polypeptide chain is known as the primary structure. This feature is genetically determined and is responsible not only for the final shape of the protein but also for its physical characteristics and, ultimately, for its biological function. Proteins are made up of about 22 amino acids which are linked by the peptide bond, an amide linkage involving the amino group of one amino acid and the carboxyl group of another (Figure 7.1).

The formation of a peptide bond results in the loss of the amino and the carboxyl groups of each amino acid, the remaining portions of the molecules (the residues) being the major components in the structure of the protein. However, the amino acids at each end of a polypeptide chain retain either an amino group (the N-terminal residue) or a carboxyl group (the C-terminal residue). By convention, the N-terminal residue is always designated as the number one residue in a numerical sequencing of the amino acids in the protein.

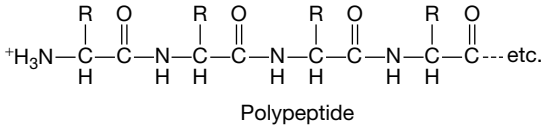


Figure 7.1 Structure of polypeptide

7.2.2 Secondary Structure of Proteins

The three-dimensional (3D) shape which a polypeptide chain or a portion of a chain assumes is known as the secondary **structure.** In its simplest form, it is the fully extended polypeptide chain. However, it often assumes a helical structure, which is stabilized by intrachain hydrogen bonds formed between the amide nitrogen of one peptide bond and the oxygen of a carbonyl group of another (Figure 7.2). Many proteins show not only these structural features but also intermediary forms. The presence of the amino acids proline and hydroxyproline induces bends in the chain due to the variant of the resulting peptide bond. Glycine, which effectively has no side chain (H), permits greater flexibility at the peptide bond than do other amino acid residues. Interchain bonding can also occur between parallel extended chains to produce pleated sheet structures in which the hydrogen bonds are formed between hydrogen and oxygen atoms in different chains. Depending upon the arrangement of the chains, these are known as either parallel pleated sheets or anti-parallel pleated sheets (Figure 7.3).

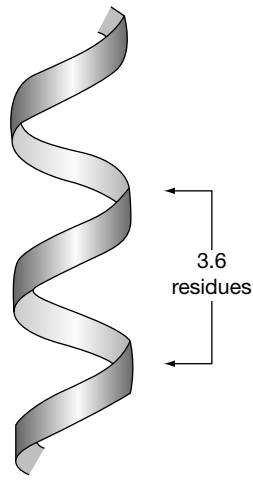


Figure 7.2 Structure of alpha helix

7.2.3 Tertiary Structure

The **tertiary structure** of a protein is defined as its overall shape and at this level of organization the role of the protein becomes significant. Those proteins, which have overall

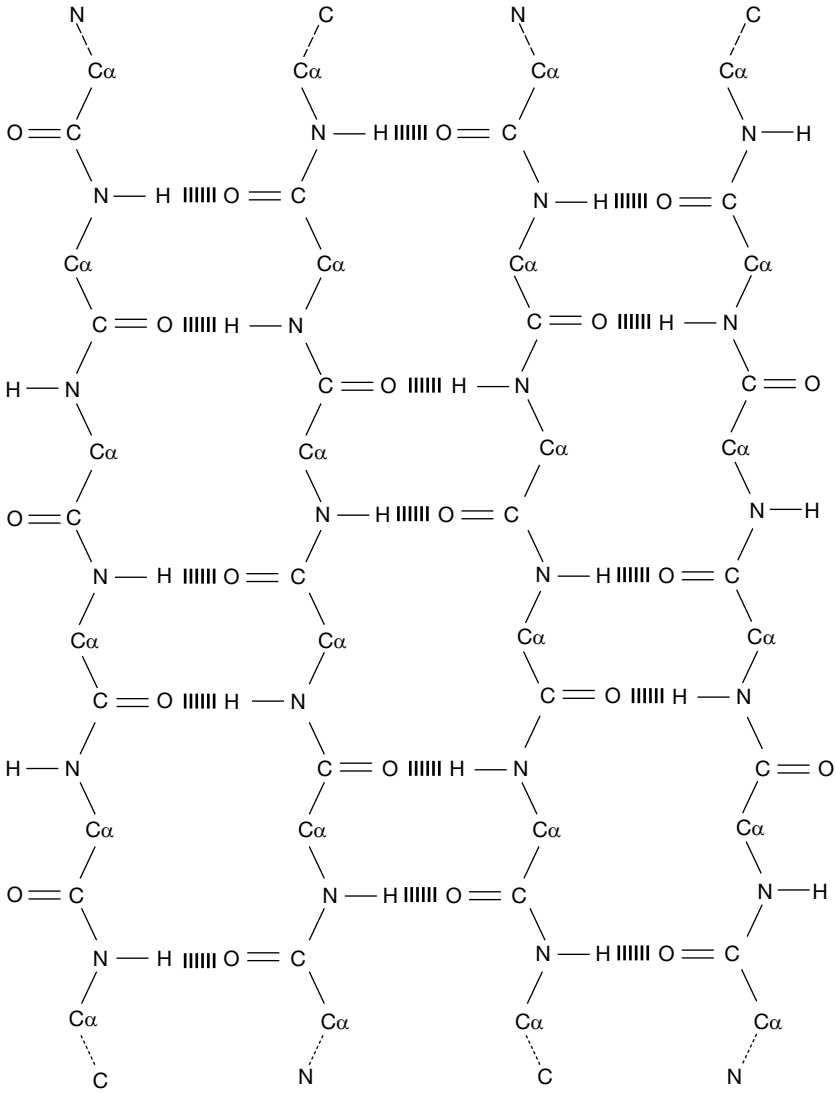


Figure 7.3 Beta-Sheet structure of proteins

spherical or globular structure—due to the internal folding of the chain—are known as globular proteins (Figure 7.4). They are semi-soluble in water forming colloidal solutions and in the solid form often exhibit a crystalline structure. They are usually the functional proteins of the cell, enzymes and immunoglobulins being examples. The fibrous proteins are the structural proteins and are linear polypeptide chains which are associated with each other to form strands or sheets. In general, those fibrous proteins which are helical in structure tend to be the elastic proteins, e.g., keratin, while those proteins with a pleated sheet structure tend to be nonelastic, e.g., silk.

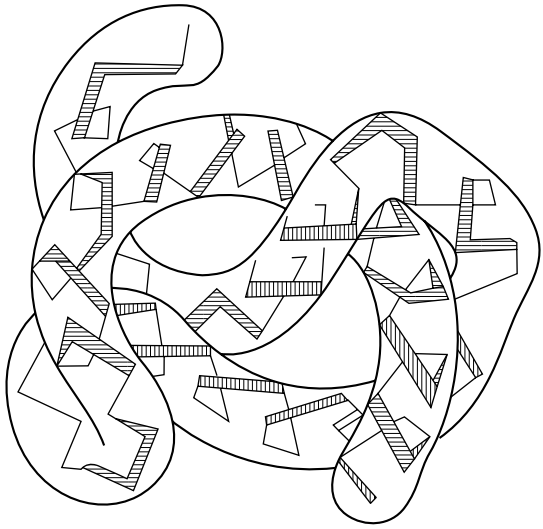


Figure 7.4 Globular proteins. The folding of a polypeptide chain in a globular form is stabilized by hydrophobic interactions and some covalent bonding, particularly the disulphide bond between cysteine residues. The polypeptide chain shows some sections which are regular and helical in nature and other sections, particularly at bends and folds, where the conformation of the chain is distorted

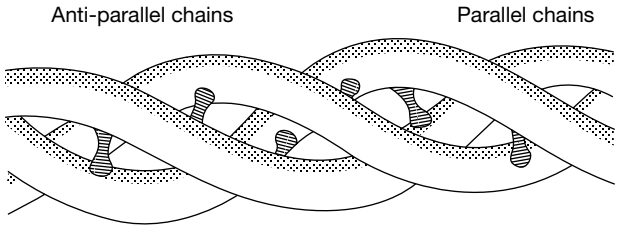


Figure 7.5 Collagen. The collagens are major constituents of cartilage and other connective tissue and contain large amounts of the amino acids proline and hydroxyproline. The basic structure involves three polypeptide chains with a considerable degree of interchain hydrogen bonding

However, helical proteins become nonelastic if there is a high degree of bonding between the individual helices, collagen being a good example (Figure 7.5).

The nature of the amino acid residues is of prime importance in the development and maintenance of protein structure. Polypeptide chains composed of simple aliphatic amino acids tend to form helices more readily than do those involving many different amino acids. Sections of a polypeptide chain which are mainly non-polar and hydrophobic tend to be buried in the interior of the molecule away from the interface with water, whereas the polar amino acid residues usually lie on the exterior of a globular protein. The folded polypeptide chain is further stabilized by the presence of disulphide bonds which are produced by the oxidation of two cysteine residues. Such covalent bonds are extremely important in maintaining protein structure, both internally in the globular proteins and externally in the bonding between adjacent chains in the fibrous proteins.

The residues of those amino acids which are classed as either acidic or basic are capable of accepting or donating a proton and will at any given pH carry a charge of characteristic sign and intensity. The presence of all these ionizable groups results in proteins showing not only acidic and basic features but also the characteristics of an electrolyte. Such substances are known as ampholytes. At low pH values the ionization of the basic groups will be dominant and the protein will carry a net positive charge while at high pH values the ionization of the anionic groups will be most evident. At a pH peculiar to the molecule the anionic nature will exactly balance the cationic nature and the protein will carry no net charge. This is known as the isoionic pH of the protein.

At the isoionic pH the repulsive effect of like-charges, which is the major stabilizing force of colloidal suspensions, is lacking and the protein will show minimal solubility and may precipitate. Whether or not precipitation occurs will depend mainly upon the degree of hydration, a phenomenon in which molecules of water are bound to the polar exterior of the protein. The effective charge carried by a colloid is known as the zeta potential and is affected by not only the ionic nature of the amino acid residues but also external factors. In the presence of salts, adsorption of ions occurs resulting in a reduction in the charge carried by the colloid. At some point the concentration of salts will be such that the zeta potential is reduced to zero and the protein will again tend to precipitate, a process known as 'salting out.' This effect is enhanced by competition for the water molecules by these high concentrations of salts.

7.2.4 Quaternary Structure

Many globular proteins have a further level of organization known as the quaternary structure, which describes the association of protein units to produce an aggregate protein with a definite functional property. The bonds involved are usually noncovalent and mainly hydrophobic between non-polar regions on the surfaces of the molecules concerned. Haemoglobin, for instance, is composed of four polypeptide chains, normally in two identical pairs forming the haemoglobin tetramer which is more effective in oxygen transport than is the monomer. In addition to the four protein chains, haemoglobin also incorporates an iron porphyrin which facilitates the binding of oxygen. Proteins, such as haemoglobin, which involve non-protein components are known as conjugated proteins.

7.3 GENERAL METHODS OF QUANTITATION

The nature of the sample and the presence of any interfering substances are major considerations in the selection of a suitable method. Fluid samples are the most convenient to handle but some methods are appropriate for the analysis of solid material. The presence of interfering substances may necessitate an initial purification of the protein components. This may be achieved by precipitating the soluble proteins and after washing, quantitating, by using a suitable method. The use of heat or strong acids results in irreversible precipitation and a method such as the Kjeldahl will usually be necessary. However, the precipitate produced by salts, alcohol, etc. can be redissolved in alkali and a method such as the biuret reaction could subsequently be used. Alternatively, the substances causing the interference which are often small molecules such as amino acids and salts may be removed by dialysis against a large volume of a suitable buffer. The sample may either be contained in a sealed bag made from suitable Visking tubing or one of the

commercial hollow fibre units may be used in which a long length of narrow bore dialysis tubing gives a very large surface area for rapid dialysis to take place.

7.3.1 Spectroscopic Methods

The presence of aromatic amino acid residues results in proteins showing an absorption maximum at 280 nm and comparison of the absorbance value of a test solution with that of a standard solution provides a sensitive method of quantitation. This cannot be considered to be an absolute method of quantitation because variations in the amino acid content mean that values for absorption coefficients vary from one protein to another. Absorption in the region of 220 nm due to the peptide bond has been used in the quantitation of proteins but many other compounds also absorb at this wavelength and as a result such methods suffer from a considerable degree of interference.

7.3.2 Chemical Methods

Kjeldahl Method

The nitrogen content of a compound is determined by Kjeldahl method, and hence may be used to determine the protein content of a sample so that the proportion of nitrogen in the protein is known. Protein determinations are complicated by the presence of nitrogen from non-protein sources. The simplest way of eliminating this source of error is to precipitate the proteins using a suitable method and hence determine the nitrogen content of the precipitate.

Biuret Method

The name given to this method is in some ways unfortunate and misleading. The method was developed following the observation that biuret reacts with an alkaline solution of copper sulphate to give a purple-coloured complex. Protein and some amines react in a similar manner to biuret. In the absence of a more suitable but equally simple title, the name biuret was retained although its chemical relevance to the quantitation of proteins is only vague. It was useful, however, in elucidating the nature of the copper complex formed. The cupric ions form a coordination complex with the four nucleophilic—NH groups which in the reaction with proteins are provided by the peptide bonds linking the amino acids. The complex shows absorption maxima at 330 nm and 545 nm. The absorbance is usually measured at 545 nm and although the sensitivity at 330 nm is greater measurements are more prone to interference.

Lowry Method

This method is used for the finding of phenolic groups by the reagent known as the Folin and Ciocalteu reagent, and was used in the quantitation of proteins. The tyrosine residues are detected by this reagent due to their phenolic nature. This sensitivity of Lowry's method was improved considerably by the incorporation of cupric ions. A copper–protein complex produced using a dilute version of the biuret reagent causes the reduction of the phosphotungstic and phosphomolybdic acids, the main constituents of the Folin and Ciocalteu reagent, to tungsten blue and molybdenum blue. The precise composition of these compounds is not known but they show broad absorption peaks in the red portion of the visible spectrum (600–800 nm). Approximately 75 per cent of the reduction which occurs is due to the copper–protein complex while tyrosine (and to a lesser

extent, tryptophan) residues are responsible for the remainder. Folin and Ciocalteu's reagent is of complex composition and is prepared by the reflux heating of sodium tungstate and sodium molybdate with orthophosphoric acid. Other ingredients have been used in order to improve the stability of the reagent which is normally pale yellow and of limited shelf life. Because of the complexity of preparation it is advisable to purchase the reagent.

The method is more sensitive than the biuret method and has an analytical range from 10 μg to 1.0 mg of protein. Using the method outlined hereunder, this is equivalent to sample concentrations of between 20 mg l^{-1} and 2.0 g l^{-1}. The relationship between absorbance and protein concentration deviates from a straight line and a calibration curve is hence necessary. The method is also subject to interference from simple ions, such as potassium and magnesium, as well as by various organic compounds, such as Tris buffer and ethylenediaminetetraacetic acid (EDTA). Phenolic compounds present in the sample will also react and this may be of particular significance in the analysis of plant extracts.

Dye-Binding Methods

Observations that the presence of protein affects the colour change of some indicators used in acid–base titrations led to the development of methods for the quantitation of proteins based on these altered absorption characteristics of such dyes. As the presence of protein alters the colour produced by these indicators when measuring pH, so in the quantitation of proteins using dye-binding methods, the control of pH is vital.

Methyl orange buffered at pH 3.5 binds to albumin with greater affinity than to other proteins and the resulting complex shows reduced absorbance at 550 nm. The method is unsuitable as a general method for protein determination because of considerable variation in the binding of the dye with different proteins.

Coomassie brilliant blue has been used extensively as a general quantitative method for proteins and when complexed with protein shows a shift in its absorption maximum from 464 nm to 595 nm and the increase in absorbance at 595 nm can he used as a measure of the protein concentration. The maximum absorbance is developed very rapidly (2 to 5 min) and is stable for at least an hour.

Bromcresol purple has been suggested as showing improved specificity for albumin and less variation in colour intensity compared with bromcresol green. The dye–albumin complex shows an absorption maximum at 603 nm and the use of a reagent buffered at pH 5.2 appreciably reduces the tendency of the complex to precipitate.

7.3.3 Physical Methods

The case with which proteins can be precipitated has resulted in a wide range of methods based on this phenomenon being described for their quantitation. The use of a specified precipitating agent and the comparison of the resulting turbidity with that produced with a known concentration of protein have commonly been used quantitatively. Organic acids such as trichloracetic acid, picric acid and salicylsulphonic acid are widely used and the resulting turbidity measured either visually or photometrically. Antibodies may be used as precipitating agents resulting in methods which are very specific while the use of nephelometers to measure the resulting turbidity significantly improves the sensitivity.

The fact that the specific gravity of protein solutions increases with the protein content has been used in a simple quantitative method. A series of copper sulphate solutions of varying specific gravity are prepared and a drop of protein solution allowed to fall into each solution. The cupric ions cause a skin of precipitated protein to form around the drop maintaining its integrity but not significantly altering the specific gravity. Usually the drop will either fall or float depending upon its specific gravity compared with the solution but when these are the same the drop will remain suspended. A calibration curve or nomogram can he prepared, using protein solutions of known concentration, and may be used to determine the protein content of the samples. The method is not very accurate and results will be affected by the presence of other solutes as well as proteins but it provides a very simple and convenient method for samples of similar composition.

7.4 SEPARATION TECHNIQUES OF PROTEINS

Most of the methods described earlier do not differentiate between different proteins but it may be necessary on occasions to determine the amount of one particular protein in the presence of others. Although proteins are composed of amino acids the problems involved in the separation of individual proteins are considerably increased compared with the separation of amino acids and the large relative molecular mass means that some of the simpler separation techniques such as paper chromatography are inappropriate. The fact that the tertiary and quaternary structure of a protein can be seriously and often permanently altered by even fairly mild conditions presents an additional problem.

7.4.1 Precipitation

High concentrations of a variety of salts including sulphates, sulphites and phosphates may be used to precipitate proteins. Although reproducible separations can be effected, each fraction still consists of a mixture of proteins. Despite these limitations, salt fractionation techniques are still very useful but are grossly lacking in selectivity.

Salt fractionation techniques are used to prepare protein fractions precipitated by successively increased concentrations of the salt. Sufficient salt is added to the sample to give the lowest selected concentration and the resulting precipitate removed, usually by centrifugation or filtration. More salt is then added to the sample to increase its concentration to the next selected level and the protein which precipitates is also removed in a similar manner. The process can be repeated at increasing salt concentrations and a series of precipitates obtained which can be redissolved in a suitable buffer. The salt can subsequently be removed from the protein preparation by either dialysis or gel permeation chromatography.

Various alcohols may also be used and the classical Cohn fractions of serum proteins are separated using specific concentrations of ethanol under carefully controlled conditions of temperature and pH. Excessive denaturation of the protein is avoided by the use of low temperatures and the technique is commonly used in the commercial preparation of various protein fractions.

7.4.2 Electrophoresis

This technique has a major application in the separation of proteins. Separation is achieved mainly on the basis of the charge carried by the protein and although careful selection of pH will facilitate separation the resulting zones are still often mixtures of protein.

Quantitative Aspects of Electrophoresis

Zonal electrophoresis uses a solid supporting medium on which the sample is applied as a streak. The zones or bands of protein which develop during electrophoresis can be precipitated in the pores of the supporting medium by trichloracetic acid and stained using a suitable dye (Ponceau S, nigrosin, etc.). The amount of dye bound is often directly correlated to the amount of protein but this quantitative relationship is open to criticism for reasons discussed under the section on dye-binding methods. However, it provides a convenient semi-quantitative method with the various fractions usually being expressed as a percentage of the total rather than in absolute amounts. If the total protein content of the original sample is determined using one of the general methods described earlier, then it is possible to calculate the amount of protein in each fraction.

The amount of dye bound by each fraction can be determined by cutting out the stained bands and eluting the dye into a fixed volume of a suitable solvent. The absorbance of each solution is measured and the sum of the absorbance values assumed to be proportional to the total amount of protein. Hence, the amount of protein in each fraction can be calculated as a percentage of the total.

An alternative and more sensitive method of quantitation is to scan the stained electrophoretic strip using a densitometer which is a modified photometer in which the electrophoretogram replaces the usual glass cuvette.

A very narrow light path must be used in order to ensure that closely adjacent bands are resolved. Usually it is necessary to make the supporting membrane translucent in order to measure the amount of light transmitted by the strip. This can be done by either impregnating the strip with art oil with a high refractive index or for some types of cellulose acetate material using an ethanol–acetic acid–ethylene glycol, reagent which causes the collapse of the porous structure of the membrane rendering it transparent.

Some densitometers are designed to measure reflected light rather than transmitted light and so enable the use of opaque strips. This simplifies the technique but does introduce a further factor in the relationship between absorbance and the amount of protein present in the sample. This relationship is still valid for reflectance measurements provided that the amount of protein involved is very small and hence such instruments are usually designed for microanalytical techniques.

7.4.3 SDS Electrophoresis

Proteins can be dissociated into their constituent polypeptide chains by the detergent, sodium dodecyl sulphate (SDS) after the reduction of any disulphide bonds. The SDS binds to the polypeptide chain producing a rod-shaped complex, the length of which is dependent upon the relative molecular mass of the protein. The large number of these strongly anionic detergent molecules bound by the protein (approximately equal to half the number of ammo acid residues) effectively masks the native charge of the protein and at a neutral pH, results in a relatively constant charge-to-mass ratio for all proteins. As a result, the electrophoretic mobility of all protein–SDS complexes is approximately equal but the molecular sieving effect of polyacrylamide gel results in a relative mobility which is inversely related to the size of the complex. Under certain conditions, this inverse relationship can be demonstrated by a linear plot of the relative mobility of the protein against the logarithm of its relative molecular mass (Table 7.1).

Table 7.1 Determination of the Relative Molecular Mass of a Protein by SDS Electrophoresis

Number	Protein	RMM	Log_{10}RMM
1	Cytochrome (muscle)	11,700	4.068
2	Myoglobin (equine skeletal muscle)	17,200	4.236
3	γ-Globulin (L-chain)	23,500	4.371
4	Carbonic anhydrase (bovine)	29,000	4.462
5	Ovalbumin	43,000	4.634
6	Albumin (human)	68,000	4.832
7	Transferrin (human)	77,000	4.886

Prior to electrophoresis the sample is diluted in a buffer containing SDS (10–25 g l^{-1}) and β-mercaptoethanol (10–50 ml l^{-1}) which reduces any disulphide bonds stabilizing the protein. It is then heated at 100°C for 2 to 5 min in order to denature the protein and expose the total length of the polypeptide chain to the detergent. After cooling, electrophoresis is performed on polyacrylamide gel and the bands subsequently visualized using an appropriate dye.

7.4.4 Chromatographic Methods

Various chromatographic techniques may be applied to the study of protein mixtures. Column techniques have the advantage that the resulting fractions are amenable to quantitation using the general method described earlier. Gel permeation chromatography is frequently used to separate protein mixtures but it is necessary to have some prior knowledge regarding the proteins present in order to select the most suitable gel. Ion-exchange chromatography using the substituted cellulose ion-exchangers, diethylaminoethyl cellulose (DEAE) and carboxymethyl cellulose (CM), is frequently used but as with gel permeation chromatography the major applications are in the preparative aspects of protein analysis.

7.5 MECHANISM OF PROTEIN SYNTHESIS

Protein synthesis takes place in the cytoplasm on ribosomes. The sequence of amino acids in the protein is controlled by DNA through m-RNA. The relation between DNA and protein synthesis is summarized in hereunder.

$$\text{DNA} \longrightarrow \text{RNA} \longrightarrow \text{Proteins}$$

The arrows indicate the direction of the transfer of information from the genetic material.

The activation of amino acids and the role of t-RNA. The amino acids to be incorporated into protein are activated by forming their adenylates with ATP. In the synthesis of adenylates the carboxylic group of amino acids is attached by high energy bonding to adenylic acid (AMP) and this reaction is catalysed by the enzyme, aminoacyl synthetase.

$$\text{AA} + \text{ATP} \xrightarrow[\text{synthetase}]{\text{Aminoacyl}} \text{AA} \sim \text{AMP} + \text{P–P}$$

Normally the synthetase is complexed into the AA–AMP and this complex collide with the appropriate r-RNA; the enzyme is released and at the same time amino acid is transferred to 2′ or 3′ OH group of ribose moiety of terminal adenylic acid residue of r-RNA, by esterification of amino acid carboxyl group.

$$\text{AA–AMP} + \text{t-RNA} \xrightarrow[\text{synthetase}]{\text{Aminoacyl}} \text{AA–t-RNA} + \text{AMP}$$

AMP and P–P thus released can be used in the formation of ATP again,

$$\text{AMP} + \text{P–P} \longrightarrow \text{ATP}$$

There are at least 20 different synthetases recognizing 20 amino acids. In several cases, however, more than one species or RNA has been found to be specific for the same amino acid. The synthetase has at least two active sites: one for recognizing the amino acid and the other for t-RNA. The maximum frequency at which a wrong amino acid is recognized by the enzyme and is inserted into the protein is about 1 in 1000. Some amino acid analogues, however, are readily charged and subsequently incorporated into proteins. Generally, charging of the amino acid is more accurate than the activation. For example, isoleucine t-RNA synthetase, converts valine as well as isoleucine to the corresponding aminoacyl adenylate but only isoleucine is transferred to the t-RNA.

7.5.1 Transcription of RNA on DNA

The genetic information of DNA is transferred to protein by m-RNA molecules. To begin with, RNA molecules are synthesized on DNA templates with the help of the enzyme RNA polymerase. Along each gene, only one strand of DNA works as a template for the m-RNA. However, RNA chains synthesized are single stranded linear molecules. They are synthesized in the 5′ to 3′ direction and hence the 5′ terminal nucleotide always contains a P—P—P group. Apparently, all normal RNA chains start with either adenine or guanine. Messenger RNA after synthesis diffuses from the nucleus to the cytoplasm and attaches to the ribosomes.

7.5.2 Role of Ribosomes

Protein synthesis never occurs in cytoplasmic fluid but only on the surface of ribosomes. Ribosomes are small cellular particles made up of r-RNA and proteins. There are about 15,000 ribosomes in a rapidly growing *Escherichia coli* cell. The size of ribosomes and other particles is expressed as S (Svedberg unit). A high Svedberg indicates that particle sediments rapidly because of its bigger size. Ribosomes isolated from *E. coli* are of the 70S type, but those isolated from cytoplasm of higher organism have a size equivalent to 80S. All ribosomes are constructed of two subunits, the larger subunit is about twice the size of the smaller unit. Each ribosome has two holes, the peptidyl (P) and the amino acyl (A). Both of these holes are made partly of smaller unit and partly of larger subunit. Most of the proteins in the ribosomes have a structural role. Some proteins, however, serve in the formation of polypeptide chain also. Role of r-RNA, however, is not known. Three types of r-RNA are found in which the smaller subunit contains a smaller r-RNA while the bigger subunit contains a bigger and a very small (5S) r-RNA.

During protein synthesis, one m-RNA may be translated at several ribosomes simultaneously and the ribosomes appear to be joined together. Such clusters of ribosomes are called polyribosomes or polysomes.

7.5.3 Synthesis of Polypeptide Chains

Synthesis of a polypeptide chain is initiated when an initiator t-RNA corresponding to an initiator codon joins with ribosomes. However, several initiation factors, ribosomal subunits and GTP molecules are required. The initiator t-RNA in bacteria, mitochondria and chloroplasts appears to be formyl methionine t-RNA. The formylation of the amino group of methionine occurs after it has attached to t-RNA. The formyl donor is formyl tetra hydrofolate and the enzyme catalysing this transfer is methionine t-RNA transformylase.

The absence of a free amino group in formyl methionine allows the chain to proceed in only one direction of a polypeptide. The formyl or formyl methionine may be removed and hence many bacterial proteins have methionine, alanine or threonine as N-terminal amino acids. In the cytoplasmic ribosomes of eukaryotic cells it appears that the initiator amino acid is methionine which is usually removed from the completed protein by the enzyme amino peptidase.

The initiator codons are AUG and GUG for formyl methionine t-RNA and AUG for methionine t-RNA.

At least three ribosomal proteins F_1, F_2 and F_3 are necessary for the initiation of protein synthesis. Further, F_3 (M. Wt. 30,000) is required for the binding of 30S ribosomal unit to m-RNA. The second protein F_1 (M. Wt. 8000) attaches to F-met-t-RNA and helps it bind to 30S m-RNA initiation complex. It is released when 50S unit joins the complex. How the third protein F_2 (M. Wt. 75,000) exactly operates remains unclear though it is known to bind GTP and also to be needed in the formation of the 30S initiation complex. This factor is also called translocase.

The requirement of GTP in protein synthesis has been demonstrated experimentally. In invitro systems, protein synthesis does not occur in the absence of GTP. In fact, GTP functions in two sequential reactions—(i) in the binding of F-met-t-RNA to the ribosomes and (ii) in the movement of the initiation complex from A to P site of the ribosome.

The series of reactions during the initiation of protein synthesis may be summarized as given hereunder.

1. A complex is formed between the smaller 30S subunit F-met-t-RNA and m-RNA containing initiator codon (complex I). Formation of this complex is catalysed by factors F_3 and F_1 and also requires a GTP molecule.
2. 50S subunit attaches to the complex I and complex II is formed which is supposed to be attached to the A site of the ribosome.
3. F-met-t-RNA is translocated to site P (complex II); however, GTP is hydrolysed to facilitate this translocation. Hydrolysis of GTP is activated by factor F_2. One GTP molecule is hydrolysed for every translocation.
4. New t-RNA charged with amino acid enters the A site and peptidyl bond formation takes place between F-met and the new amino acid. The enzyme involved in this process is peptidyl transferase which is present in the 50S subunit.
5. Three elongation factors (EF Tu, EF Ts and EF G) (EF refers to elongation factor while Tu/Ts refers to temperature unstable/stable) help in the elongation of the polypeptide chain.
6. Discharged t-RNA is released from P site and a dipeptide is formed.

During protein synthesis, the growing peptide chain is esterified to the terminal adenosine residue of t-RNA molecule. When the growing chain is completed this polypeptide t-RNA

linkage is cleaved releasing the polypeptide from t-RNA and from ribosomal complex. For release of polypeptide chain, the termination codons (UAA, UAG and UGA) and termination factors (RF) (RF1, RF2 and RF3) are required. The enzyme peptidyl transferase is itself responsible for the release of polypeptide. During termination process, the enzyme catalyses the transfer of nascent polypeptide to water instead of a t-RNA. The inhibition of peptidyl transferase by antibiotics, such as chloramphenicol and puromycin, also causes the release of polypeptide.

Release of t-RNA from ribosome is catalysed by another factor, transfer releasing (TR) factor. This release does not require GTP.

Upon completion of protein synthesis, the ribosomes dissociate into monomeric units and the subunits. RNAs released are probably degraded and the nucleotides are reused in their synthesis (Figure 7.6). Polypeptide chains synthesized are folded and refolded to assume a definite physical configuration to perform the role assigned to them. Sometimes, the proteins assume their 3D structure while they are on polysomes.

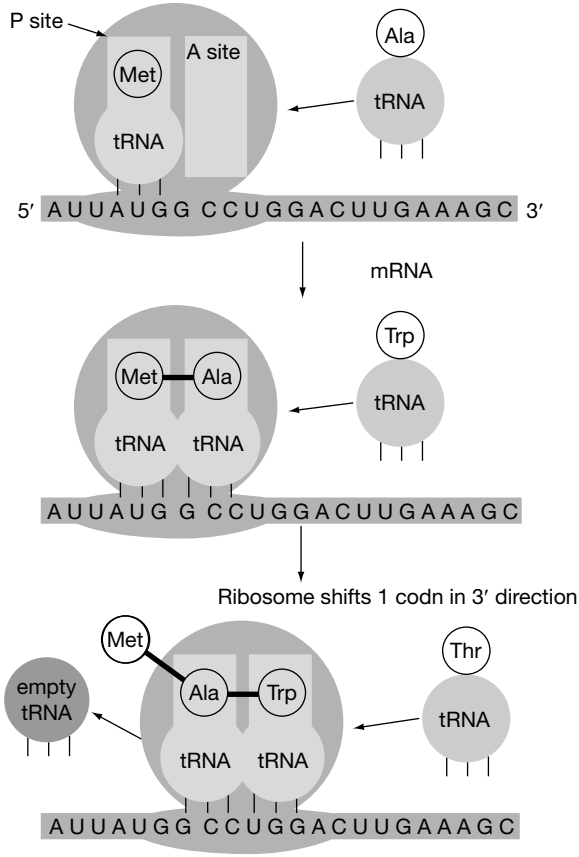


Figure 7.6 The t-RNA amino acid complex moves to the ribosomes, the site of protein synthesis

7.6 CONTROL OF PROTEIN SYNTHESIS

Rate of protein synthesis in a cell is controlled by the genetic make-up and the external chemical environment. To accommodate various known facts about the genetic control of the protein synthesis in bacteria F. Jacob and J. Monod (1961) proposed that genes existed in groups or clusters. One cluster consisted of one regulatory gene, an operator gene and one or more structural genes. The structural genes under the control of a single operator gene are called operons.

The synthesis of enzymes, β-galactosidase, β-galactoside permease and galactoside acetylase in *E. coli* were studied and their regulatory mechanism was consistent with the model.

The regulatory genes code for some special group of molecules called repressors. These repressor molecules are proteins and are present in very small amounts in the cell. Some bacterial cells do not have functional regulatory genes and hence they produce proteins independent of the need. These mutants are called constitutive mutants and these proteins produced in fixed amounts independent of need are called constitutive proteins.

Repressors inhibit protein synthesis by binding to the operators. An operator gene is about 10 to 12 nucleotide long and the repressor molecule binds with it probably by the formation of hydrogen bonds. The repressors exist in either active or inactive form. When a repressor is combined with an inducer molecule, repressor is inactivated and then does not bind to the operator. For example, when combined with β-galactoside (inducer) the β-galactosidase repressor cannot bind to its specific operator. Binding of a co-repressor, on the other hand, activates the repressor molecule. For example, several amino acids which inhibit the induction of nitrate reductase may bind with the specific repressor to activate it and stop the synthesis of the enzyme. Binding between a repressor and a compressor or an inducer is weak and involves hydrogen bonds, salt linkages or Van der Waal's forces. Thus, a repressor is rapidly able to adjust the physiological need of the cell.

Binding of repressor to the operator prevents the transcription of adjacent structural gene (genes) and hence the synthesis of protein directed by them.

The control of β-galactosidase synthesis by operator gene is negative in character because activity of the repressor molecule prevents protein synthesis. Other operators are under positive control. Arabinose operon is one such molecule studied in *E. coil*. Arabinose is converted into L-ribulose, L-ribulose-5-P and D-xylulose 5-P by the successive action of the enzymes isomerase, kinase and epimerase.

$$\text{Arabinose} \xrightarrow{\text{isomerase}} \text{L-ribulose} \xrightarrow{\text{kinase}} \text{L-ribulose-5-P} \xrightarrow{\text{epimerase}} \text{D-xylulose-5-P}$$

In the absence of arabinose all of these enzymes are present only in small amounts but the addition of arabinose increases the amount of all these three enzymes. The induction is controlled by a fourth gene 'C' which probably has a positive role. A arabinose–C-product complex is necessary for the expression of arabinose operon.

7.7 CONTROL AT TRANSLATIONAL LEVEL

The polypeptide chains start assuming 3D shape while still attached to the polyribosomes. It has been proposed that the altered configuration of the nascent polypeptide may inhibit the further growth of chain by any one of the following three processes:

1. By rather preventing the incoming aa-t-RNA
2. By preventing the translocation of t-RNA or
3. By preventing the peptide bond formation.

First evidence for the translational control of protein synthesis came from the study of haemoglobin synthesis. As mentioned earlier, a haemoglobin molecule is made up of a heme moiety and two α- and two β-polypeptide chains. Two polypeptide chains are the products of two different m-RNAs but their synthesis was found to be interdependent. If the structure of one chain was altered the translation of the m-RNA for the other chain was affected.

Control of protein synthesis in T_2-infected *E. coli* cells at translational level was studied by Sueoka and Sueoka (1970). There are 5 t-RNAs for leucine corresponding to CUG codon of the m-RNA. Messenger RNA synthesized after T_2 infection carries little or no CUG codon that may code for leucine. Thus, after the infection of T_2, leucine t-RNAs are degraded. Regarding the mechanism of degradation it is proposed that phage T_2 infection induces a specific ribonuclease which nicks leu-t-RNA-I at the site of translation of *E. coli* m-RNA on ribosomes which in turn leads to the cessation of protein synthesis. This process eliminates half of the leu t-RNA-I molecule in the cell.

7.8 GLYCOSYLATION OF PROTEINS

A protein can be modified by attachment of sugar residue to the protein by an enzymatic process. This process is known as glycosylation and the modification of the protein can take place along with its synthesis (co-translation) or after the protein synthesis (post-translational modification). A large number of membrane proteins which are usually transmembrane proteins are glycosylated. The carbohydrates which are usually added include glucose, galactose, mannose, xylose, fructose, N-acetylgalactosamine, N-acetylgucosamine and N-acetylnemuramic acid.

7.9 DENATURATION OF PROTEINS

In a protein molecule, the spatial arrangement of polypeptide chains is changed from a native form to a disordered arrangement in certain conditions. This has been termed as denaturation of protein by Kauzmann in 1959. Thus, during denaturation, only the primary structure of a protein is retained. Both physical and chemical properties of denatured proteins are different than native proteins and they lose most of their biological activities. During denaturation, soluble globular proteins are changed into insoluble fibrous proteins. Commercial fibres are obtained from globular proteins by treating them with various denaturing agents.

There are several chemicals which can denature the proteins. Mineral acids and alkalis, acetone, alcohols, salts such as ammonium sulphate, urea and tungustic, picric and trichloroacetic acids are common denaturing chemicals. Hydrostatic pressure, ultraviolet and ionizing radiations also cause denaturation. These agents cleave the hydrogen and/or salt linkages and thus the polypeptide chain is unfolded.

7.10 NITROGENOUS CONSTITUENT OF URINE

7.10.1 Composition of Urine

The composition of urine is determined by the quantities of substances that must be removed from the body by the kidneys in order to maintain the composition of blood and other body fluids within physiological limits.

Besides water, major amount of waste products in urine arises from the metabolism of protein derived from food and tissues. Generally, the gross amount of substance excreted in urine is proportional to the protein eaten. The major waste product from protein is urea. During starvation the qualitative composition of urine remains essentially unchanged, but the quantitative relations of the constituents are markedly altered. A few substances, such as creatinine, are formed largely as the result of endogenous metabolism (tissue metabolism) and show relatively less variation with diet as compared to other substances, such as urea, that normally are mainly the result of exogenous metabolism (metabolism of food).

The composition of urine in pathological states generally differs from the normal in the relative amounts of constituents as indicated hereunder.

Parameters	Normal State	Pathological States
Glucose	About 0.1 g (per day)	150 g (diabetic state) (per day)
Ketone	bodies Traces	50 g (severe diabetic state)
Protein	Less than 100 mg daily	Many grams daily (nephritic patient)

7.10.2 Urea

Urea is the principal end product of protein (amino acid) metabolism in human beings, mammals in general and in certain lower forms. In the humans, urea usually represents 80 to 90 per cent of the total urinary nitrogen. In general, the proportion of nitrogen as urea increases as the total urinary nitrogen increases and vice versa. On very low protein diets, urea may represent 60 per cent or less of the total nitrogen. Therefore, the percentage of urea changes with the change in the amount of protein in diet.

The quantity of urea excreted, in general, is proportional to the total protein metabolism, irrespective of whether this protein represents food protein or the tissues' protein (tissues undergoing catabolism). The excretion of urea is decreased in certain diseases as shown hereunder.

Diseases	Conc. of urea in urine
In acute yellow atrophy and cirrhosis (liver diseases)	Capacity to form urea is decreased (less urea is excreted in urine)
In severe acidosis	Amount of urea excreted is reduced (due to diversion of amino nitrogen to ammonia formation)
In nephritis	Amount of urea excreted is reduced (ability of the kidneys to excrete urea is severely impaired) This may cause greatly increased concentrations of urea in the blood (uremia) and other body fluids.

7.10.3 Ammonia

Ammonia, normally, is the second most important nitrogenous substance of urine quantitatively. Ordinarily, 2.5 to 4.5 per cent of the total urinary nitrogen is composed of ammonium salts. On the average, this represents about 0.7 g per day.

Since both urinary ammonia and urea are derived from the amino groups of the amino acids, for a given quantity of nitrogen excreted an increase in the amount of the one leads to a decrease in the amount of the other. Ammonia is formed in the kidney to be substituted for Na^+ and K^+ so that they may be conserved in the plasma. Accordingly, the quantity of urinary ammonia per day varies from practically 0 in alkalosis condition to 8 to 10 g in case of severe diabetic acidosis condition.

Urinary ammonia may be markedly decreased in case of severe nephritis (in which the capacity of the kidney to form ammonia has been impaired). This reduces the capacity of the kidneys to conserve base and contributes to the development of acidosis condition.

The quantity of ammonia in the urine may be enormously increased through hydrolysis of urea by bacteria in the bladder (cystitis) or other parts of the urinary tract. This bacterial production of ammonia from urea in normal urine may take place if the samples are without preservative (alkaline fermentation).

7.10.4 Uric Acid and Other Purines

Uric acid is the chief end product of purine metabolism in man, the higher apes and the Dalmatian dog. The quantity of uric acid in human urine is generally from 0.5 to 1.0 g per day, though the amount is subject to wide variation. On low-purine diet the uric acid excretion may fall to 0.1 g per day, while on a high-purine diet the excretion may rise to 2 g per day.

7.10.5 Creatinine and Creatine

The quantity of creatinine excreted in urine by the normal adult generally is from 1.2 to 1.7 g per day and depends partly upon the amount of creatinine in the diet, since this is excreted unchanged in the urine. The quantity excreted on a creatinine-free diet is practically constant for a given individual over long periods and is independent of the total nitrogen output. Creatinine coefficient is defined as the milligram of creatinine, or creatine plus creatinine (when creatine is present), excreted per kilogram of body weight per day. The creatinine coefficient averages 20 to 26 mg in normal men and 14 to 22 mg in women. The creatinine coefficient varies in proportion to muscular development; however, there is no significant change in creatinine coefficient due to variations in diet. Prolonged illness and old age, both of which are associated with lose of muscular tone and muscular activity, may cause marked decreases in the creatinine coefficient.

Adult females show much greater variation in creatine excretion, although in the great majority of cases it amounts to two or two-and-a-half times as much as for adult males. In about one-fifth of the females, the creatine excretion is similar to that of males.

The excretion of creatine is greatly increased in infants and children, during starvation, and particularly in patients with certain muscular dystrophies.

7.10.6 Amino Acids

The total amino acid excretion in urine per 24 h normally amounts to about 1.5 g, of which more than half is in peptide or other combinations from which the free amino acids are liberated by acid hydrolysis.

The quantity of urinary amino acids may be increased in diseases associated with excessive tissue destruction such as severe diabetes, typhoid fever and acute yellow atrophy of the liver.

7.10.7 Hippuric Acid

Hippuric acid is a peptide of benzoic acid and glycine, benzoylglycine. The name is coined as hippuric acid because it was first found in the urine of horses.

The amount of hippuric acid excreted per day in the urine averages about 0.7 g and ranges from 0.1 to 1.0 g. In man and the rabbit, synthesis of hippuric acid takes place largely in the liver but in the dog hippuric acid formation occurs in the kidneys. The capacity of an individual to synthesize hippuric acid can be tested by a clinical test of liver function by giving a test dose of sodium benzoate.

7.10.8 Allantoin

Human urine generally contains 10 to 25 mg of allantoin per 24-h specimen.

7.10.9 Proteins

Normally, human urine contains exceedingly little protein, i.e., 20–80 mg per day, which is composed of a small quantity of insoluble mucoid or nucleoprotein, albumin and enzymes. However, in various kidney diseases large quantities of protein may be excreted in the urine, as much as 20 g per day in cases of nephrosis.

The kinds of protein that may be found in urine under various pathological conditions include serum albumin and globulin, nucleoprotein, fibrin, myoglobin, haemoglobin and related substances, proteases and peptones and Bence Jones protein. Of these proteins, serum albumin and globulin are most frequently present in urine, with albumin predominating, apparently because of its smaller molecular size and more ready passage through the glomerular membrane. Protein in the urine is usually referred to as ‘albuminuria.’

7.10.10 Ketone Bodies

The ketone bodies found in urine are derived chiefly from the metabolism of fatty acids and consist of acetone, acetoacetic acid and β-hydroxybutyric acid.

Acetoacetic acid is the primary ketone body from which β-hydroxybutyric acid is formed by reduction and acetone by decarboxylation. The liver is the site of formation of the ketone acids.

A normal person on a mixed diet generally excretes less than 100 mg of ketone bodies per 24 h. Typical ketonuria results whenever the rate of production of ketone bodies in the liver exceeds the capacity of the body tissues to oxidize them.

This occurs on very high fat diets, during starvation when a large amount of body fat is metabolized, in diabetes mellitus, in both normal and toxemic pregnancies, during ether anaesthesia and often in conditions of alkalosis. In severe diabetic acidosis, 75 g or more of ketone bodies may be excreted in 24 h.

EXERCISES

1. Write the mechanism of protein synthesis and the role of ribosomes in protein synthesis.
2. Write the nitrogenous constituent of urine with detail description of the quantity of creatinine excreted in urine by the normal adult.
3. The structural genes under the control of single operator gene are called operons. Comment on the statement and explain with a suitable example.
4. Proteins can be dissociated into their constituent polypeptide chains by the detergent, sodium dodecyl sulphate (SDS). Explain the SDS electrophoresis.
5. What is salting in and salting out? Write in brief about the estimation of protein by Lowry's method.
6. What is the primary structure of proteins? Discuss the biochemical functions of proteins.
7. Discuss in brief the classification and biological importance of proteins.
8. Write short notes on:
   (a) Denaturation
   (b) Basic proteins
   (c) Isoelectric point
   (d) Glycoproteins
9. What is the difference between the beta-pleated sheet and alpha-helix?
10. What are the characteristics of globular and fibrous proteins? Explain.
11. How protein is determined by biuret method?
12. Write short notes on:
    (a) Kjeldahl method
    (b) Lowry's method
    (c) Collagen
13. Write the series of reactions that take place during the initiation of protein synthesis.

MULTIPLE CHOICE QUESTIONS

1. Each turn of α-helix contains the numbers of amino acids
   (a) 2.8 (b) 3.2
   (c) 3.4 (d) 3.6
   Ans. d
2. The distance travelled per turn of α-helix in nm is
   (a) 0.34
   (b) 0.44
   (c) 0.54
   (d) 0.64
   Ans. c
3. α-helix is disrupted by certain amino acids like
   (a) Proline (b) Arginine
   (c) Histidine (d) Lysine
   Ans. a
4. The effective charge carried by a colloid is known as the
   (a) zeta potential
   (b) electrostatic potential
   (c) both (a) and (b)
   (d) none of these
   Ans. a

5. Ribosomes are small cellular particles made up of r-RNA and
   (a) Proteins
   (b) Carbohydrates
   (c) Nucleic acids
   (d) Lipids

   Ans. a
6. Ordinarily, 2.5 to 4.5 per cent of the total urinary nitrogen is composed of
   (a) Ammonium salts
   (b) Sodium salt
   (c) Calcium salts
   (d) None of these

   Ans. a
7. In various kidney diseases, large quantities of protein may be excreted in the urine, however, as much as 20 g per day in cases of
   (a) Nephrosis (b) Nephritis
   (c) Both (a) and (b) (d) None of these

   Ans. a
8. A protein can be modified by attachment of sugar residue to the protein by an enzymatic process. This process is known as
   (a) Translation (b) Glycosylation
   (c) Transcription (d) None of these

   Ans. b
9. A haemoglobin molecule is made up of a heme moiety and two α- and two β-polypeptide chains. Two polypeptide chains are the products of two different
   (a) r-RNA (b) t-RNA
   (c) m-RNA (d) None of these

   Ans. c
10. The control of β-galactosidase synthesis by operator gene is negative in character because activity of the repressor molecule prevents
    (a) Protein synthesis
    (b) Lipid synthesis
    (c) Both (a) and (b)
    (d) None of these

    Ans. a
11. The formyl donor is formyl tetra hydrofolate and the enzyme catalysing this transfer is
    (a) Methionine t-RNA transformylase
    (b) Dehydrogenase
    (c) Transferase
    (d) None of these

    Ans. a
12. Methyl orange buffered at pH 3.5 binds to albumin with greater affinity than to other proteins and the resulting complex shows reduced absorbance at
    (a) 540 nm
    (b) 550 nm
    (c) 560 nm
    (d) None of these

    Ans. b
13. Coomassie brilliant blue has been used extensively in a general quantitative method for
    (a) Proteins
    (b) Carbohydrates
    (c) Lipids
    (d) None of these

    Ans. a
14. This method is used for the finding of phenolic groups by the reagent known as the Folin and Ciocalteu reagent and was used in the quantitation of proteins.
    (a) Biuret method
    (b) Lowry method
    (c) Kjeldahl method
    (d) None of these

    Ans. b
15. The three-dimensional shape which a polypeptide chain or a portion of a chain assumes is known as the
    (a) Secondary structure
    (b) Primary structure
    (c) Tertiary structure
    (d) None of these

    Ans. a

16. Bromcresol purple has been suggested as showing improved specificity for albumin and less variation in colour intensity compared with bromcresol green. The dye–albumin complex shows an absorption maximum at
    (a) 603 nm
    (b) 503 nm
    (c) 703 nm
    (d) None of these

    Ans. a

17. The synthetase has at least two active sites, one for recognizing the amino acid and the other for
    (a) t-RNA (b) m-RNA
    (c) r-RNA (d) None of these

    Ans. a

18. The genetic information of DNA is transferred to protein by
    (a) m-RNA molecules
    (b) r-RNA molecules
    (c) t-RNA molecules
    (d) None of these

    Ans. a

8 Composition of Nucleic Acids and DNA Structure and its Replication

CHAPTER OBJECTIVES

8.1 Introduction of Nucleic Acids
8.2 The Structure of Nucleic Acids
8.3 Structure of RNA
8.4 Types of RNA
8.5 Structure of DNA
8.6 Different Forms of DNA
8.7 Chemistry of the Nucleic Acids
8.8 Nucleoproteins
8.9 Viruses
8.10 Nucleotides of Biological Importance
8.11 Characteristics of DNA Replication

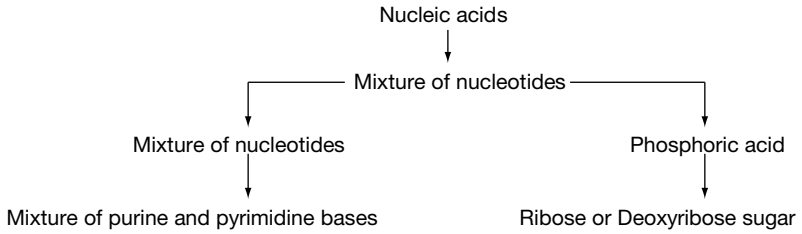
8.1 INTRODUCTION OF NUCLEIC ACIDS

Nucleic acids like proteins occur in all living cells. They derive their name because of their primary occurrence in the nucleus and acidic nature. Deoxyribonucleic acid (DNA), one of the two nucleic acids, is a major component of chromosomes. Small amount of DNA are also found associated with chloroplasts and mitochondria. Certain animal viruses and many bacteriophages are DNA–protein particles. Ribonucleic acid (RNA), the other nucleic acid is found mostly in the cytoplasm and in the plant viruses. Very little of DNA and RNA occur in free forms. Nuclear DNA is associated with arginine- and lysine-rich proteins.

8.1.1 Chemical Composition of Nucleic Acids

Elements taking part in the constitution of nucleic acids are C, H, O, N and P. In almost all nucleic acids, there are approximately 15 to 16 per cent nitrogen and 9 to 12 per cent phosphorous. Nucleic acids are made up of monomeric units of nucleotides which are analogous to the amino acids of proteins. However, the number of nucleotides taking part in the synthesis of

nucleic acids is fewer. Nucleotides are not stable units and can be further hydrolysed to purine and pyrimidine bases, ribose or deoxyribose sugars and phosphoric acids. After partial hydrolysis, nucleosides and phosphoric acid are obtained. Thus, stepwise degradation of nucleic acids will yield different compounds in the manner shown hereunder.



8.2 THE STRUCTURE OF NUCLEIC ACIDS

8.2.1 Nucleic Acid

Nucleic acids are of two types, DNA and RNA. Their basic structures consist of chains of alternating phosphoric acid and sugar residues. In RNA, the sugar is D-ribofuranose; however, in DNA, as its name implies, the sugar is 2-deoxy-D-ribofuranose. In deoxyribonucleic acid, de means without oxygen at carbon No. 2 position in the D-ribofuranose sugar, whereas RNA has oxygen at carbon No. 2 position (Figure 8.1).

8.2.2 Purines and Pyrimidines

Both RNA and DNA contain the purine bases which are adenine and guanine. The general structure of a purine and the specific structure of adenine and guanine are given here, as is the numbering of the atoms in the purine. Several unusual bases have been found in the transfer RNAs. These include hypoxanthine, 1-methyl hypoxanthine, N^2-dimethyl guanine, 1-methyl guanine; N^6-(Δ^2-isopentenyl) adenine and threonylcarbamoyl adenine. Both RNA and DNA also contain the pyrimidine and cytosine, but the two kinds of nucleic acids differ in the fourth nitrogenous base, i.e., RNA contains uracil, whereas DNA contains thymine (Figure 8.2).

8.2.3 Bases, Nucleosides and Nucleotides

Bases

Bases are of two types, pyrimidine and purine. Pyrimidine bases are derivatives of the pyrimidine ring. In DNA the pyrimidine bases are cytosine and thymine, in RNA instead of thymine uracil is present. Purine bases are derivatives of the purine ring. Both in DNA and RNA, the purine bases are adenine and guanine.

Nucleosides

The base and sugar combination is known as a nucleoside. The nucleosides are compounds in which purines and pyrimidines are linked to D-ribofuranose or 2-deoxy-D-ribofuranose in a N-β-glycosidic bond, which is the configuration in the polymeric nucleic acids. The point of

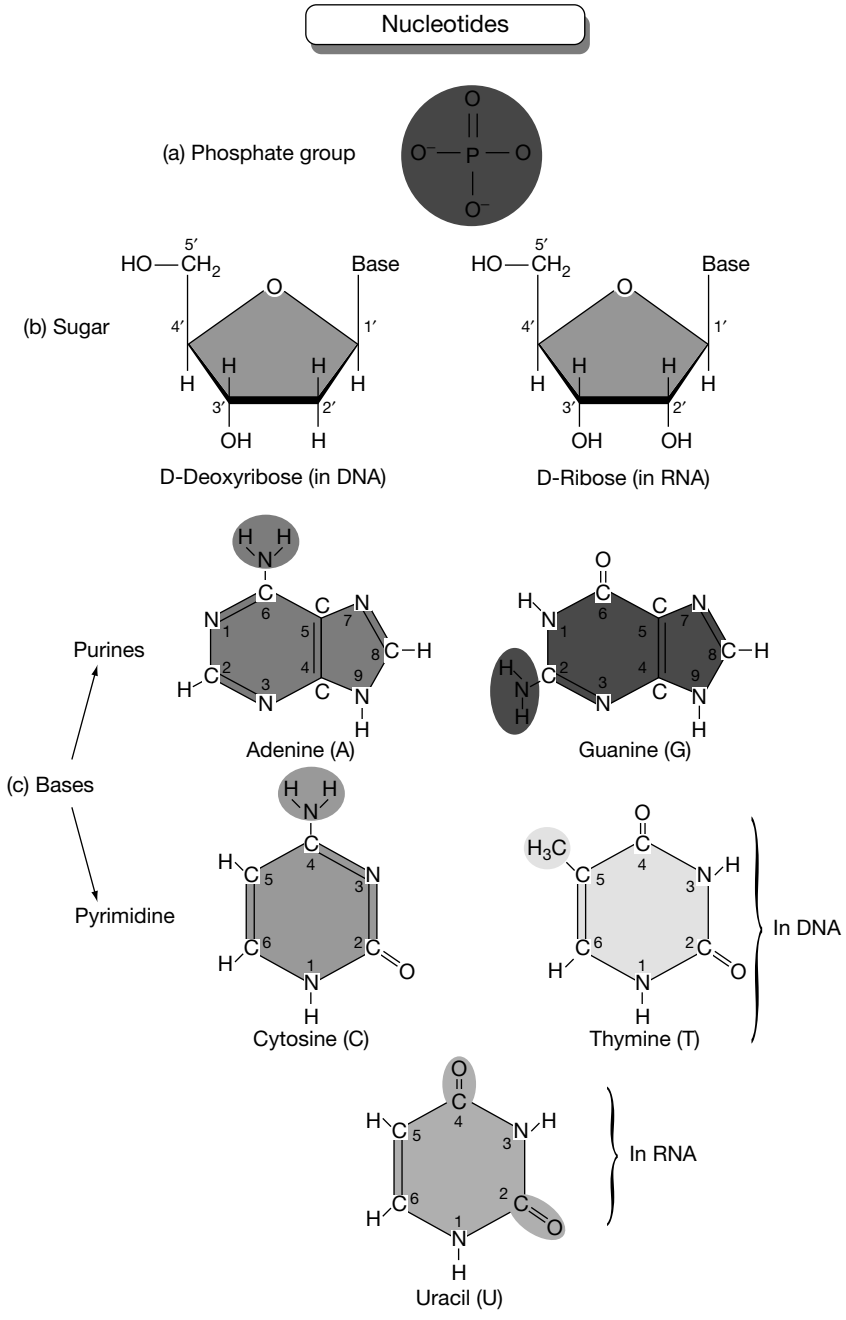


Figure 8.1 Structure of components of nucleotides in DNA and RNA. (a) Phosphate, (b) Structure of D-Deoxyribose and D-ribose sugar and (c) Structure of bases, adenine, guanine, cytosine, thymine and uracil

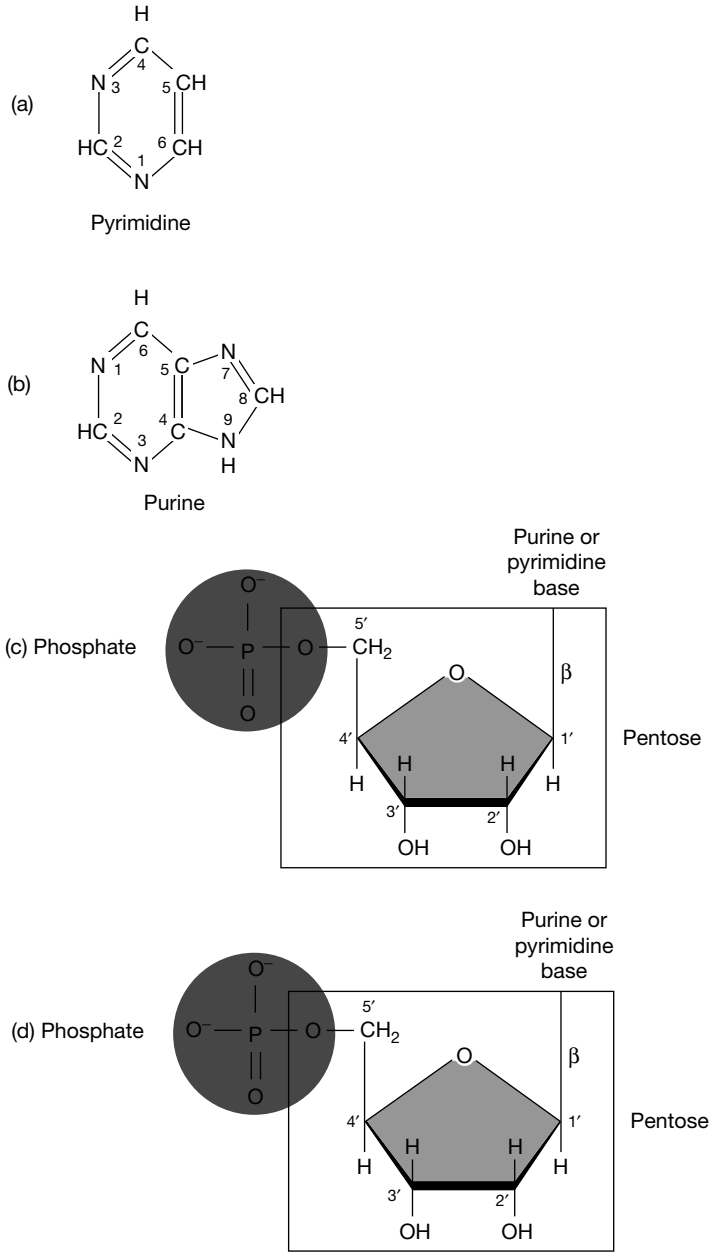


Figure 8.2 Structure of nucleotides. (a) Pyrimidine ring, a six-membered ring, with two nitrogen atoms and three double bonds. (b) Purine ring, which contains a six-membered pyrimidine ring fused to five-membered imidazole ring. It contains four nitrogen atoms, two in the pyrimidine ring and two in the imidazole ring. (c) General structure showing the numbering for the pentose ring. This is a ribonucleotide. (d) Deoxyribonucleotide

Table 8.1 Names of Nucleosides

Base	Nucleoside	Nucleotide	Nucleic acid	Symbol
Purines	Adenosine	Adenylate	RNA	A
Adenine	Deoxyadenosine	Deoxyadenylate	DNA	dA
Guanine	Guanosine	Guanylate	RNA	G
	Deoxyguanosine	Deoxyguanylate	DNA	dG
Pyrimidines	Cytidine	Cytidylate	RNA	C
Cytosine	Deoxycytidine	Deoxycytidylate	DNA	dC
Thymine	Thymidine or Deoxythymidine	Thymidylate or Deoxythymidylate	DNA	T dT
Uracil	Uridine	Uridylate	RNA	U

attachment of the base to the sugar is the hemiacetal hydroxyl on the C-1′ carbon atom of the sugar. In the purines, it is the N-9 nitrogen atom which participates in the N-glycosyl bond. In the pyrimidines, the N-1 nitrogen atom is the point of attachment. Table 8.1 lists the trivial names of the purine and pyrimidine nucleosides which are related to the bases that occur in RNA and DNA.

Nucleotides

Nucleotides are phosphoric acid esters of the nucleosides. The ribose portion of the ribonucleoside has three positions (the 2′, 3′ and 5′ hydroxyl group) where the phosphate could be esterified, whereas the 2-deoxyribonucleoside has only the 3′ and 5’ positions available (Figures 8.3 and 8.4). All these can be formed on partial hydrolysis of nucleic acids by various methods.

One of the most important naturally occurring nucleotides is adenosine-5′-monophosphate (also called 5′-adenylic acid). The compound AMP, together with two of its derivatives, ADP and ATP, plays an important role in the conservation and utilization of energy released during cellular metabolism.

A cyclic 3′, 5′-phosphate of adenosine occurs and has important regulatory properties. Mild acid hydrolysis of DNA has yielded 3′, 5′-diphosphate derivatives of deoxythymidine and deoxycytidine.

8.2.4 Nucleoside 5′-Diphosphates and 5′-Triphosphates

Corresponding derivatives of guanosine, cytidine and uridine as well as deoxyadenosine, deoxyguanosine, deoxycytidine and deoxythymidine exist and play important roles in cellular metabolism (Table 8.2). For example, the nucleoside 5′-triphosphates serve as the precursors for the synthesis of RNA and DNA. Derivatives of certain nucleoside 5′-diphosphates act as coenzymes to supply sugar residues in certain reactions; however, other derivatives of adenosine-5′-diphosphate function in oxidation–reduction reactions. Thus, uridine-5′-diphosphate linked to glucose

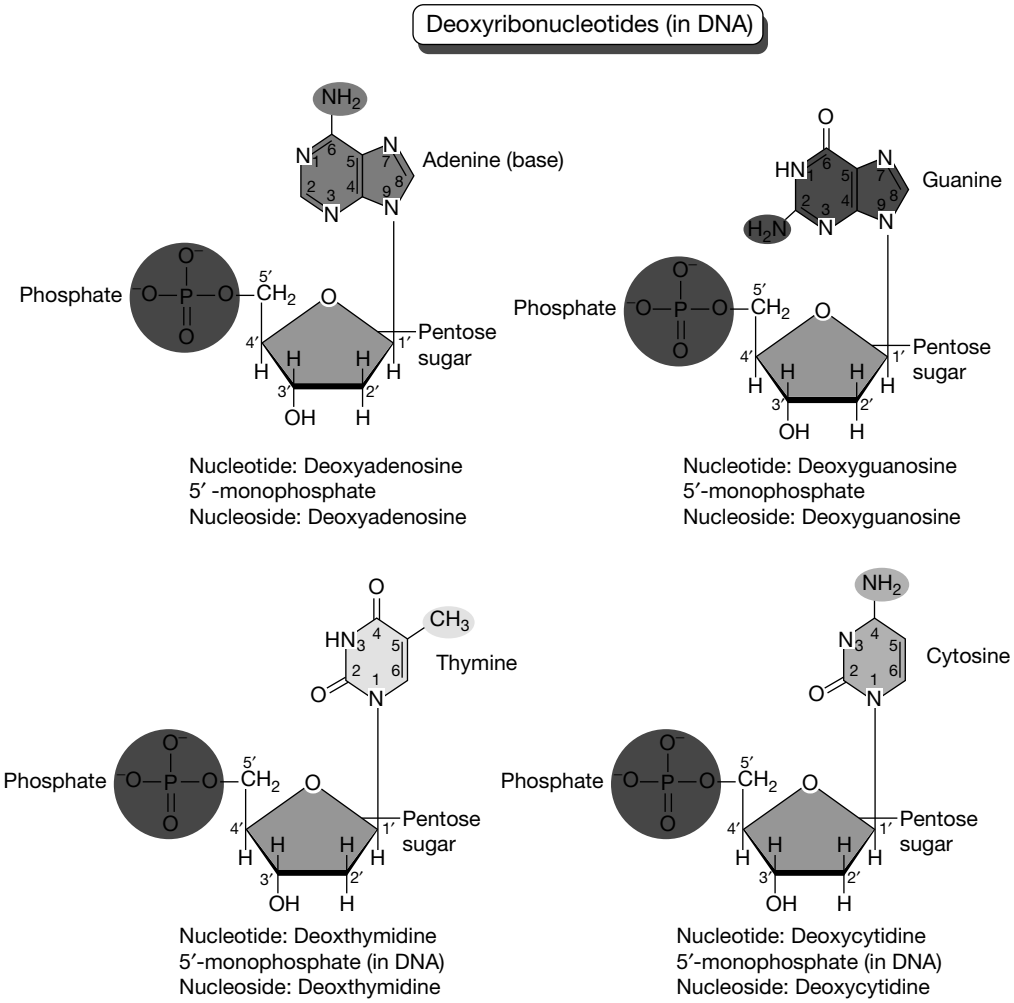


Figure 8.3 The structure of a nucleotide. The structure of deoxyguanosine depicts the base, sugar and phosphate moieties. The phosphate group is positioned at 5′ carbon, whereas the hydroxyl group is present on 3′ carbon

serves as a glucose donor. Adenosine-5′-diphosphate linked to nicotinamide forms the extremely important oxidation–reduction coenzyme, nicotinamide adenine dinucleotide, NAD^+.

8.2.5 Polynucleotides

The polynucleotides are the condensation of two or more nucleotides, each successive nucleotide being united to its immediately preceding one by forming a diester with phosphate. Only one of the hydroxyl groups of ribose is esterified in nucleotide formation leaving two more hydroxyl groups in case of ribose and one more hydroxyl groups in case of deoxyribose free for

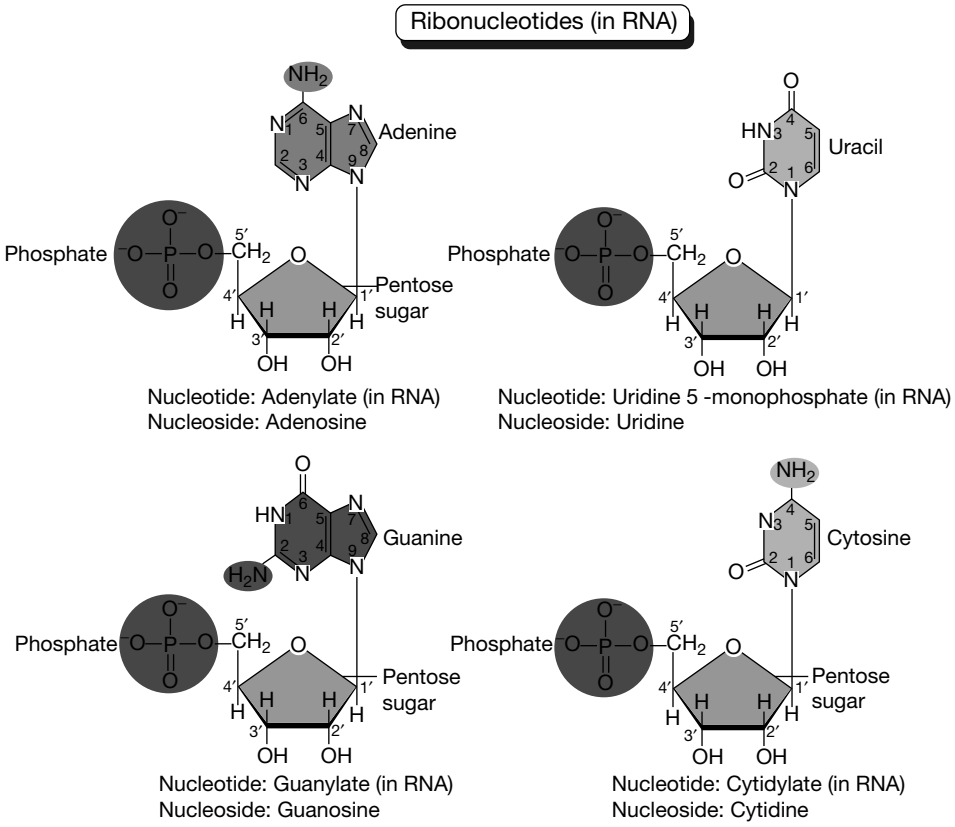


Figure 8.4 The structure of ribonucleotide (in RNA) has an extra hydroxyl group on the 2′ carbon of ribose. The phosphate group and the hydroxyl group are positioned on 5′ and 3′ carbon, respectively

Table 8.2 The Common Ribonucleotides and 2′-Deoxyribionucleotides

RNA	DNA
Adenosine-5′-monophosphate	Deoxyadenosine-5′-monophosphate
(adenylic acid; AMP)	(deoxyadenylic acid; dAMP)
Guanosine-5′-monophosphate	Deoxyguanosine-5′-monophosphate
(guanylic acid; GMP)	(deoxyguanylic acid; dGMP)
Cytidine-5′-monophosphate	Deoxycytidine-5′-monophosphate
(cytidylic acid; CMP)	(deoxycytidylic acid; dCMP)
Uridine-5′-monophosphate	Deoxythymidine-5′-monophosphate
(uridylic acid; UMP)	(deoxythymidylic acid; dTMP)
Each of the 5′-monophosphate exists as the 5′-diphosphate and 5′-triphosphate. Thus, as an example, there occurs GMP, GDP, GTP, dGMP, dGDP and dGTP.	

esterification with the phosphate of the neighbouring nucleoside. Figure 8.5 illustrates the 3-to-5 phosphate diester linkages of DNA.

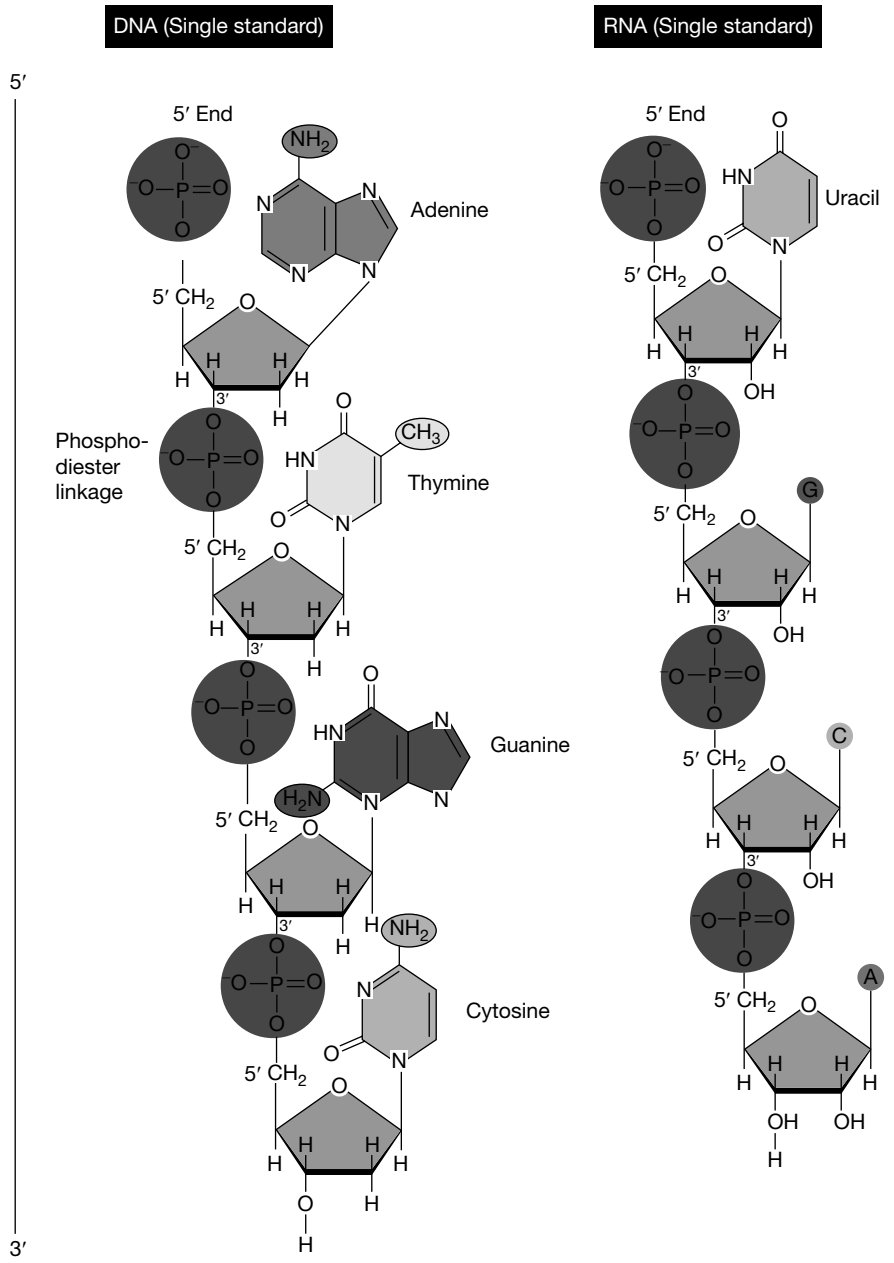


Figure 8.5 DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) are polymers of nucleotides linked in a chain between the hydroxyl at 3′ carbon of one nucleotide and the phosphate at 5′ carbon of another nucleotide through a phosphodiester bond

8.3 STRUCTURE OF RNA

The second nucleic acid component of the cell, RNA, occurs in multiple forms, each serving as extremely important informational links between DNA, the master carrier of information, and proteins. The smallest of these polymers is called transfer RNA (tRNA) and has a molecular weight of about 25,000. Transfer RNA consists of about 60 different molecular species. The tRNAs have several functions, the most important of which is to act as specific carriers of activated amino acids to specific sites on the protein-synthesizing templates. The tRNAs comprise about 10–15 per cent of the total RNA of the cell. A second group of RNAs include the ribosomal RNAs (rRNAs). These nucleic acids are always associated with a large number of proteins in a highly ordered complex called the ribosome. They make up about 75–80 per cent of the total RNA of a cell. The third important group of RNAs are the messenger RNAs (mRNAs) which comprise about 5–10 per cent of the total RNA of a cell. In bacterial cells, mRNAs are highly unstable in the sense that they are constantly being degraded and resynthesized. In eukaryotic cells, the turnover rate is much lower. These nucleic acids with a base composition corresponding very closely to that of DNA are intimately involved in the transcription and translation of information programmed by DNA for the synthesis of proteins.

RNAs are usually single stranded, but secondary structures are found in several RNA molecules due to formation of intramolecular loops between the complementary base pairing (Figure 8.6). A simple example of this is shown in Figure 8.7, and much more extensive and complex examples are known. Base pairing in RNA follows exactly the same principles as with DNA: the two regions involved in duplex formation are antiparallel to one another and the base pairs that form are A-U and G-C.

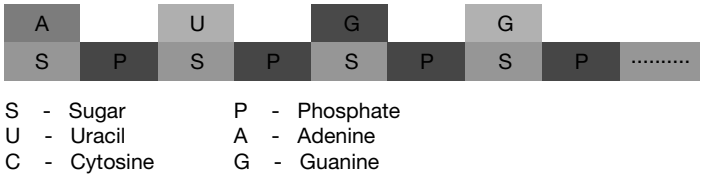


Figure 8.6 The nucleotide bases of the RNA molecule

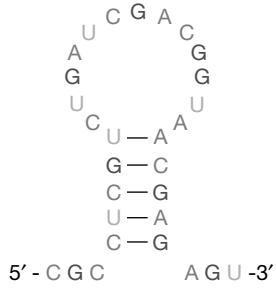


Figure 8.7 Secondary structure of RNA molecules in which intramolecular loops are formed by complementary base pairing

8.4 TYPES OF RNA

8.4.1 Transfer RNA

Transfer RNA (tRNA) plays a key role in protein synthesis. Each molecule carries an amino acid to the ribosome and decodes the genetic information in the messenger RNA in terms of the proper amino acids to be placed in the correct sequence.

Since the hydrodynamic properties of all the tRNAs are very similar, it follows that molecular weights of all are in the vicinity of 25,000 with a corresponding 4.3S value. Additional evidence suggests that 60–70 per cent of tRNA exists as a helical structure. This and other evidence point to postulated 'cloverleaf' structure in all tRNAs with the anticodon (i.e., the nucleotide triplet necessary for the positioning of the specific RNA in the messenger RNA template during protein biosynthesis) located in the central petal of the cloverleaf (Figure 8.8). By employing high resolution X-ray analysis of tRNA crystals, a number of workers have proposed a tertiary structure of tRNA that is probably closer to reality than the postulated cloverleaf structure.

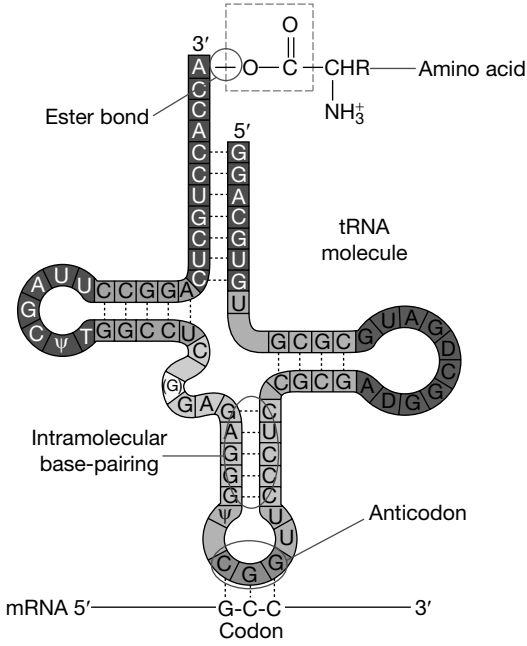


Figure 8.8 Structure of tRNA

8.4.2 Ribosomal RNA

Several species of RNA occur in prokaryotic and eukaryotic ribosomes. Ribosomal RNA (rRNA) has a helical structure. However, rRNA does not occur as a double-stranded polymer. Furthermore, since rRNA does not have the extremely rigid and stable double helical structure of DNA, it may exist in several conformations. Thus, in the absence of electrolytes, or at high temperatures, a single-stranded conformation may occur. At low ionic strengths, a compact rod

with regularly arranged helical regions can exist and at high ionic strengths, a compact coil will occur. Moreover, the concentration of Mg^{2+} ion plays an important role in the macromolecular structures of RNA, presumably since the Mg^{2+} ions form coordination bonds with the phosphate groups of the nucleic acid. At a low Mg^{2+} concentration, dissociation of RNA complexes occurs, whereas at high Mg^{2+} concentration, association of complexes is favoured.

8.4.3 Messenger RNA

Because of the metabolic instability and heterogeneity of this species of RNA, careful characterization has only recently become possible. Messenger RNA appears to be principally single stranded and complementarity with the base sequences of DNA has been demonstrated through the formation of artificial DNA–RNA double stranded hybrid molecules.

8.5 STRUCTURE OF DNA

In prokaryotic cells, DNA normally occurs as a highly twisted, double-stranded circle, in part associated with the inner side of the plasma membrane but free of protein complexes. In contrast, over 98 per cent of the total DNA in a typically differentiated eukaryotic cell is found in the nucleus as a highly twisted, double-stranded polymer bound to basic proteins called histones, the complex is known as chromatin. Much smaller amounts of DNA are always found in the matrix of eukaryotic mitochondria and in chloroplasts as small, double-stranded circles free of protein complexes.

The observation by Chargaff that the ratio of adenine to thymine and that of cytosine to guanine is very close to 1 was of basic importance in working out the structure of DNA. It was then shown that the adenine and thymine nucleotides can be so paired structurally that a maximum number of two hydrogen bonds can be drawn between these bases, whereas cytosine and guanine can be arranged spatially to permit the formation of three hydrogen bonds. In double-stranded DNA, the adenine binds to thymine or thymine to adenine and guanine always binds to cytosine or cytosine to guanine. This bonding is specific and forms a double-stranded system as shown in Figure 8.9.

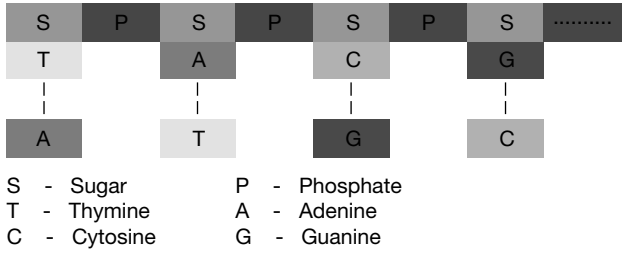


Figure 8.9 The nucleotide bases of the DNA molecule form complementary pairs

A breakthrough in the investigation of DNA structure came when Wilkins, in England, observed that DNA from different sources had remarkably similar X-ray diffraction patterns. This suggested a uniform molecular pattern of all DNA. The data also suggested that DNA consisted of two or more polynucleotide chains arranged in a helical structure with evidence based on (a) the available X-ray data, (b) the data of Chargaff and others on base pairing and equivalence

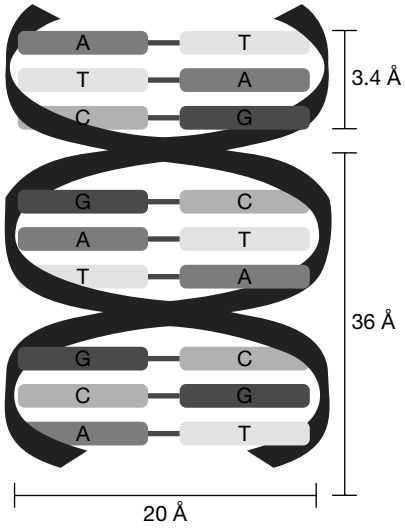


Figure 8.10 Watson and Crick model of double-stranded structure of DNA

and (c) titration data which suggested that the long nucleotide chains were held together through hydrogen bonding between base residues.

Watson and Crick constructed their model of DNA in 1953 (Figure 8.10). In the Watson and Crick model of DNA, two polynucleotide chains are wound into a right-handed double helix. The chains consist of deoxyribotide phosphates joined together by phosphate diesters with the bases projecting perpendicularly from the chain into the central axis. For each adenine projecting toward the central axis, one thymine must project toward adenine from the second parallel chain and be held by hydrogen bonding to adenine. Cytosine or guanine do not fit in this area and are rejected. Similarly, the specificity of hydrogen bonding between cytosine and guanine dictates their association only with each other. Thus, we have a spatial structure of two chains coiled around a common axis and held together by the specific bonding of adenine with thymine, and cytosine with guanine. Note, however, that the chains are not identical, but because of base pairing, are precise complements of each other. Also, the chains do not run in the same direction with respect to their internucleotide linkages but rather are antiparallel. That is, if two adjacent deoxyribosides, T and C, in the same chain are linked 5′-3′, the complementary deoxyribosides A and G in the other chain will be linked 3′-5′ (Figure 8.11).

The planes of the adjacent pairs are 3.4 Å apart. Each turn of the helix has a length of 34 Å and includes 10 base pairs. The structure differs in aqueous solution from the structure that in fibres, i.e., instead of 10 base pairs having 10.5 base per helical turn, and each turn of the helix has a length of 36 Å. The double helical structure also forms alternating major and minor grooves along its axis. The proteins of the nucleoprotein interact with the DNA molecules in these grooves.

The covalent backbone of DNA and RNA is subject to slow, nonenzymatic hydrolysis of the phosphodiester bonds. In vitro, RNA is hydrolyzed rapidly under alkaline condition to 2′, 3′-cyclicmonophosphate derivative, then further hydrolyzed to yield a mixture of 2′ and 3′-nucleoside monophosphate. Under alkaline condition, in vitro RNA is hydrolysed rapidly

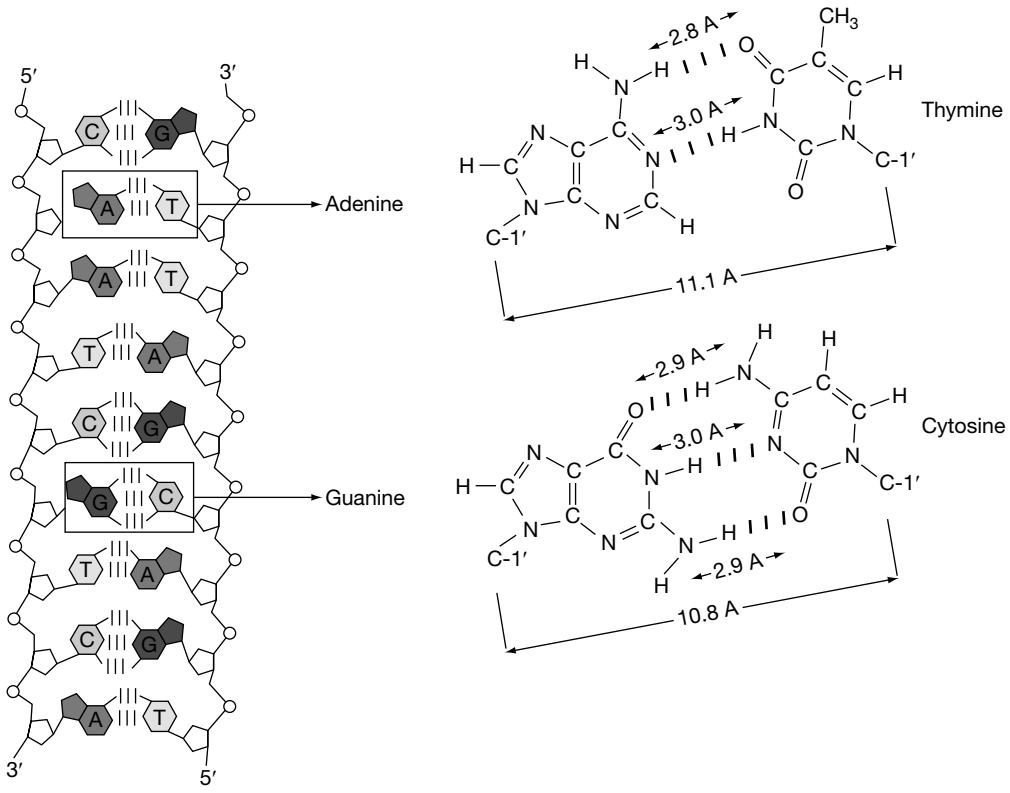


Figure 8.11 Watson and Crick hydrogen bonding pattern in the base pairs. The two chains do not run in the same direction with respect to their internucleotide linkages but rather are antiparallel, one strand is aligned 5′ to 3′, while the other strand is aligned 3′ to 5′. The bases are represented as A, T, G and C here and elsewhere

to 2′, 3′-cyclicmonophosphate derivative, then further hydrolysed to yield a mixture of 2′ and 3′-nucleoside monophosphate. Under alkaline conditions DNA is not subjected to hydrolysis because the 2′-hydroxyl group is absent in DNA, but in RNA 2′-hydroxyl group is present which is directly involved in the process.

The ability of DNA to form a double helix is of prime importance in considering its function in the cell. The double helical structure immediately suggests a mechanism for the accurate replication of genetic information. Because of the complementarity of the helical structure, each strand serves as a template to specify the base sequence of a newly synthesized complementary strand. As a result, in the synthesis of two daughter molecules of DNA, each will be precisely identical to that of the parent DNA.

Numerous unusual DNA structures involve three or even four DNA strands. The N-7, O^6, and N^6 of purines, the atoms that participate in hydrogen bonding of triplex DNA, are often referred to as Hoogsteen positions, and the non-Watson-Crick pairing is called Hoogsteen pairing. In 1963, Karst Hoogsteen first recognized the potential of Hoogsteen pairing which lead

to the formation of triplex DNAs. In triplex DNAs there could be Hoogsteen pairing between two pyrimidine strands and one purine or between two purine strands and one pyrimidine strand.

The pairing of four DNA strands is called as tetraplex (quadruplex), but tetraplex can occur in guanosine rich DNA sequence. The DNA sequences which have high content of guanosine residues and form tetraplex are known as guanosine tetraplex or G tetraplex.

In polypyrimidine or polypurine tracts, mainly unusual DNA structures are found; these are known as H-DNA.

8.6 DIFFERENT FORMS OF DNA

The different forms of DNA are shown in Table 8.3.

Table 8.3 Parameters of Major DNA Helices

Parameters	A Form	B Form	Z Form
Direction of helical rotation	Right	Right	Left
Residues per turn of helix	11	10	12 base pairs
Rotation of helix per residue (in degrees)	33	36	–30
Base tilt relative to helix axis (in degrees)	20	6	7
Major groove	Narrow and deep	Wide and deep	Flat
Minor groove	Wide and shallow	Narrow and deep	Narrow and deep
Orientation of N-glycosidic bond	Anti	Anti	Anti for pyrimidines but syn for purines

8.7 CHEMISTRY OF THE NUCLEIC ACIDS

8.7.1 Isolation of Nucleic Acids

In the presence of concentrated phenol and a detergent, cell homogenate will form two liquid phases. Proteins are denatured and become insoluble in the aqueous phase, while the nucleic acids remain soluble in that phase. The aqueous phase can be readily separated from the phenol-rich phase in which some proteins have been dissolved. Addition of ethanol to the aqueous phase precipitates out the nucleic acids and many polysaccharides while the residual phenol remains in solution. The mixture of DNA and RNA can then be further treated either with a ribonuclease to degrade RNA into soluble fragments but leave the DNA intact, or alternately, the mixture can be treated with deoxyribonuclease to split the DNA, leaving RNA undegraded. After digestion of one of the nucleic acids, aqueous phenol can again be added to denature and remove any remaining protein, and the intact nucleic acid is then precipitated with ethanol. Since native DNA consists of an extremely long helical coil, addition of ethanol to a DNA solution results in the formation of long, fibrous precipitates which can be readily removed by winding the fibrous material around a stirring rod. The mass can then be dried with appropriate solvents such as acetone, and the dry DNA can be removed from the glass rod. Further purification of DNA can be carried out by chromatography, which will yield two fractions, one containing single-stranded and the other double-stranded DNA.

When the procedure is used to isolate RNA, one obtains a heterogeneous mixture of tRNA, mRNA, rRNA and degraded RNA. Either column chromatography of this mixture or gradient centrifugation in sucrose solution will usually yield three fractions, the 4S (tRNA), and the 16S and 23S peaks from *E. coli* RNA which are derived in turn from 30S and 50S ribosomes or the 18-22S and 28-34S peaks from mammalian RNA.

8.7.2 Thermal Properties of DNA

Hypochromism

The bases of DNA have characteristic absorption maxima and molar absorbencies in the region of 260 mμ. On the basis of analytical determination of nucleotide composition, one can predict the absorbency of a DNA assuming that the bases do not interact in any way that could affect their optical properties. However, at neutral pH and room temperatures, the extinction coefficient per mole of nucleotide in solutions of DNA is only 40 per cent of the value predicted. This decrease in molar extinction coefficient is called the 'hypochromism' of DNA. Apparently, the effect arises from the interaction of the electrons in paired bases stacked in parallel array. However, neither hydrogen binding nor helical structure is necessary for the effect, which can be observed with synthetic polynucleotides. Interaction between pi electrons of the bases at the distance of 3.36 Å (in the helical stack) is a possible explanation.

When a solution of DNA is heated, little change in absorbency at 260 mμ is noted until a 'melting temperature' is reached. At this temperature, breakdown in the helical structure of DNA commences and absorbency rises sharply. The temperature at which the process is half completed—as estimated by half the total change in optical density—is called Tm or midpoint melting temperature, and usually the range of temperature in which this change occurs is very narrow. The concentration of reagents in the DNA solution may exert a strong effect upon this temperature; for example, 6 M urea lowers the Tm.

Salt concentration has an effect upon the absorbency of DNA solutions. In the case of NaCl, dilution below a critical level (0.001 M) causes an increase in absorbency due to disruption of helical structure. The change can be reversed to some extent by increasing the salt concentration; however, at very high concentrations (from 2 to 8 M) increases in absorbency also occur, but this effect is completely reversible. Similar effects are produced by pH changes due to disruption of base pair hydrogen bonding. The kinds of change produced by OH^- and H^+ on pairs of hydrogen-bonded bases.

The melting point (Tm) of DNA solutions shows a remarkable correlation with the guanine and cytosine base pairs of the DNA sample. The relation of guanine–cytosine content to Tm for DNA from various sources, as determined by changes in absorbency at 260 mμ.

8.8 NUCLEOPROTEINS

In eukaryotic cells, lysine- and arginine-rich proteins called histones are bound to chromosomal DNA to form nucleoproteins. These basic proteins are held by ionic bonds with the internucleotides phosphate residues of the nucleic acids, thereby probably functioning as chromosomal structural proteins to stabilize chromosomal structure.

Five major classes of histones have been associated in roughly equimolar amounts with DNA, obtained from a variety of eukaryotic cells. Each class of histones is defined by a characteristic

lysine/arginine ratio as indicated in Table 8.4. Of considerable interest is the fact that the complete sequence for histone lV obtained from pea seeds and calf thymus show that of the 102 amino acids residues from both sources, only two are genetically different, with the remaining 100 residues being identical in their sequence. These results strongly suggest that histone V is the most genetically stable of all proteins so far observed comparatively and implies that any marked change in the structure of this histone could not be tolerated but had to be conserved during the evolutionary process. There is good evidence that histones bind on or are closely packed in the major groves of the alpha-helix of the DNA molecule.

In prokaryotes, histones are entirely absent. However, the anionic charges of each internucleotide phosphate in DNA may be neutralized by the binding of cations such as Mg^{++} or polyamines such as cadverine, putrecine and spermine.

Table 8.4 Classification of Histones

Class	Predominant amino acids	Lysine/Arginine	Molecular weight	Lysine	Arginine
I	Lysine-rich	22	~20,000	28	1.4
IIb1	Slightly lysine-rich	2.5	~15,000	16	6
IIb2	Slightly lysine-rich	2.5	~14,000	16	6
III	Arginine-rich	0.8	~15,000	10	14
IV	Arginine-rich	0.7	~12,000	10	14

8.9 VIRUSES

W. M. Stanley in 1935 isolated the tobacco mosaic virus, and Bawden and Prie showed that it is a ribonucleoprotein. Both DNA- and RNA-containing viruses occur in nature. The viruses containing chicken pox, mumps, rabies, etc. are DNA viruses. Influenza, poliomyelitis and encephalitis are caused by RNA viruses. Viruses which infect bacteria are called 'bacteriophages.' They can be either DNA or RNA viruses.

Some unusual bases like 5-methyl cytosine, 5-hydroxymethyl cytosine, 6-methyl aminopurine, 2-methyl adenine, etc. occur in some DNA and RNA molecules, particularly associated with viruses, bacteriophages and some bacteria. The nucleic acid is located at the centre of the 'core' of the virus. The protein moiety surrounds the core forming structures called 'capsomeres.' The entire structure may be further enclosed in an envelope.

8.10 NUCLEOTIDES OF BIOLOGICAL IMPORTANCE

Other than the nucleotides which form nucleic acids, several others are present in nature or synthesized in the laboratory, which have biologic importance.

8.10.1 Adenosine Derivatives

cAMP is a powerful second messenger and is involved in signal transduction process. It helps in passing signals from the cell surface to internal proteins, for example, cAMP-dependent protein kinase, PKA, and a number of proteins gets phosphorylated by PKA thus affecting their activity

either positively or negatively. Ion channels are regulated by AMP via direct interaction with the channel proteins, for example, in the activation of odorant receptors by odorant molecules. cAMP is formed, in response to activation of receptor coupled adenylate cyclase. These receptors can be of any type, e.g., hormone receptors.

8.10.2 Guanosine Derivatives

cyclic GMP (cGMP) is implicated as a second messenger molecule and is found in cells. It was noticed in many cases that the role cGMP is to alienate the effects of cAMP (that means to counter balance). Formation of cGMP occurs in response to receptor-mediated signals like those for activation of adenylate cyclase. Nevertheless, here it is the guanylate cyclase that is coupled to the receptor.

Photoreception is the most important cGMP coupled signal transduction cascade. The absorption of a photon of light, rhodopsin or other opsins gets activated which activates transducin which sequentially activates a cGMP specific phosphodiesterase enzyme which leads to hydrolysis of cGMP to GMP. This reduces the effective concentration of cGMP bound to gated ion channels consequential in their closure and a simultaneous hyperpolarization of the cell.

8.10.3 Hypoxanthine Derivatives

Hypoxanthine derivatives: Inosine mono-, di, and triphosphate (IMP, IDP, and ITP) and cyclic GMP.

8.10.4 Uracil Derivatives

Uracil mono-, di- and triphosphate (UMP, UDP and UTP), uridinediphosphoglucose and uridinediphosphoglucuronic acid (UDP-glucose and UDP-glucuronic acid).

8.10.5 Cytosine Derivatives

Cytosine mono-, di- and triphosphate (CMP, CDP and CTP).

8.10.6 Synthetic Nucleotide Analogues

Chemically synthesized many nucleotide analogues are used for their beneficial potential, can be utilized to inhibit specific enzymatic activities. Many of nucleotide analogues are used as anti-tumour agents, for illustration, because they interfere with the synthesis of DNA and thus kill tumour cells. The nucleotide analogues which are commonly used in chemotherapy are 6-thioguanine, 5-fluorouracil, 6-mercaptopurine and 5-iodo-2′-deoxyuridine. These nucleotide analogues disrupt the normal DNA replication process by disrupting the correct Watson–Crick base pairing. Several nucleotide analogues are used to interfere with the replication of human immunodeficiency virus (HIV), for instance azidothymidine and dideoxyinosine.

Several purine analogues are used to treat gout, like the drug allopurine which is a xanthine oxidase inhibitor. This drug is a structural analogue of hypoxanthine and xanthine and has a higher affinity for xanthine oxidase. Allopurinol inhibits xanthine oxidase, the enzyme that catalyses the conversion of purines to uric acid. Furthermore, after organ transplantation, several nucleotide analogues are given so as to suppress the immune system.

8.11 CHARACTERISTICS OF DNA REPLICATION

During the cell division a cell divides into two, and each daughter cell receives the exact copy of the parental DNA. Therefore, DNA is replicated earlier to the cell division. The process of DNA replication is semiconservative. This means, at the end of replication when two molecules of DNA are synthesized, each has one strand of parental origin and another newly synthesized. In a simple model of DNA replication, double-stranded DNA is denatured and each strand is copied separately.

The replication of DNA is always initiated from a specific region in the DNA which is called as oriC (means the origin of replication). Once initiated, it can proceed in one direction (unidirectional) or in both directions (bidirectional). If a circular DNA replicates bidirectionally, the replication would finish in half the time than that required for unidirectional synthesis. The replication in bacteria is fast, its speed of replication being around 1000 nucleotides per second. Therefore, once initiated bidirectionally from the origin, it would take around 40 min to replicate 4×10^6 nucleotides long circular DNA.

The replication of DNA in eukaryotic cells is confined to the part of cell cycle. The period between mitosis (interphase) can be divided into phases. The phases are G1, S, G2 and M. G1 and G2 are the preparatory phases for a cell to enter S and M phase, respectively. The act of DNA replication is restricted to a synthetic phase, respectively. The act of DNA replication to a synthetic phase (S phase) that lasts for around 7 to 8 h then follows mitotic phase leading to cell division. The speed of replication in eukaryotes is much slower, around 50 nucleotides per second. With this slow speed, replication of entire DNA molecule would take several hours and would not finish in S phase. However, for most of the cells, S phase lasts for 7 to 8 hours during which all the chromosomes in the nucleus must replicate. To overcome this problem, cells have adopted the strategy of initiating replication at several positions along the DNA so that the replication of entire DNA would finish in a short time.

The replicon is defined as the DNA molecule in which a single act of replication can take place and is under the control of single origin. Each replicon contains an origin of replication, and a terminator for replication. In prokaryotes, the bacterial chromosome is a single replicon bearing a single origin of replication and a single terminator, whereas eukaryotic DNA has multiple replicons. Both in eukaryotes and prokaryotes, DNA replication is basically similar. The major difference is that eukaryotic DNA is replicated as a linear molecule, and not as a bare DNA but as chromatin, in which the DNA is complexed with proteins called histones. We would first discuss the DNA replication in prokaryotes.

8.11.1 DNA Replication in Prokaryotes

When two parental strands of DNA unwind, they get separated from each other, and each strand used as templates for synthesis of new strands. This is semiconservative mode of replication, following the rule of usual base pairing, i.e., A pairs with T and G with C (Figure 8.12). However, the two strands of DNA have opposite polarity. Does it mean that the replication machinery is able to make DNA in both 3′-5′ and 5′-3′ directions?

No, because DNA replication proceeds only in the 5′-3′ direction. That is, it inserts 5′ most nucleotide first and extends the chain towards the 3′ end by adding the nucleotides to the 3′ end of a growing chain. This means when replication begins at one point on DNA, both strands cannot

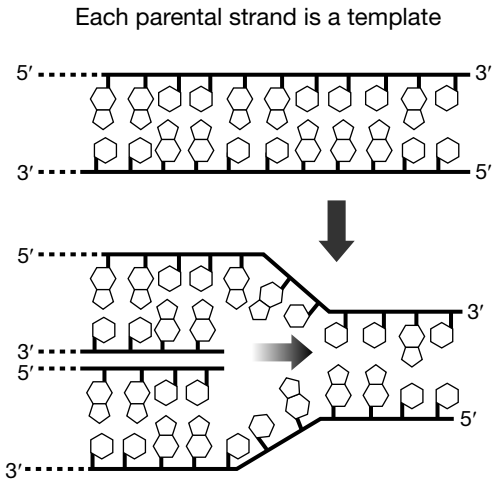


Figure 8.12 Two parental strands unwind, get separated from each other, and are used as templates for synthesis of new strands

be replicated continuously. It is not possible to replicate both strands of DNA simultaneously, as two strands with opposite polarity would have to separate totally from each other and then replicate in the 5′ to 3′ direction initiating the event at two far ends. However, cells cannot afford to allow two strands to completely separate from each other. To overcome this problem, one of the strands is made continuously in the 5′-3′ direction, while another strand is made discontinuously in the same direction (Figures 8.13 and 8.14).

In leading strand the DNA synthesis proceeds continuously, whereas in lagging strand (the other strand of DNA) DNA synthesis takes place discontinuously. The discontinuity of DNA synthesis is because the direction of DNA synthesis is opposite to the direction in which replication fork moves. Therefore, as the fork opens up and exposes a region of DNA to replicate, the lagging strand grows away from the fork (Figure 8.14). Therefore, to replicate this newly opened region, the only way out is to restart DNA synthesis at the fork behind the piece of DNA that has already been made. This starting and restarting of DNA synthesis occurs over and over again. The short pieces of DNA thus created would have to be joined together to produce the continuous strand. These short fragments make a discontinuous strand. These short fragments made discontinuously are called as 'Okazaki fragments.' The average length of Okazaki fragments is about 1200 nucleotides.

For the DNA replication process, number of proteins are involved. The role of the initiator proteins is to recognize the origin region from where the replication begins, proteins to synthesize new DNA molecule, proteins to open up the DNA helix to be copied, proteins to join short matured Okazaki fragments and proteins to help relieve helical winding and tangling problems. DNA replication can be divided into three steps as discussed hereunder.

1. Initiation,
2. Elongation and
3. Termination.

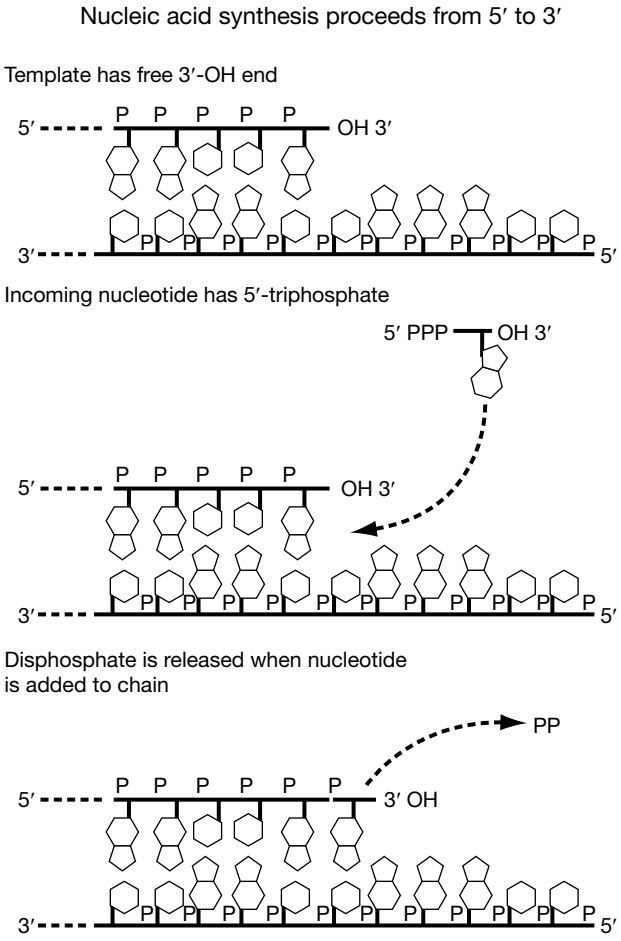


Figure 8.13 Nucleic acid synthesis proceeds from 5′-3′ direction

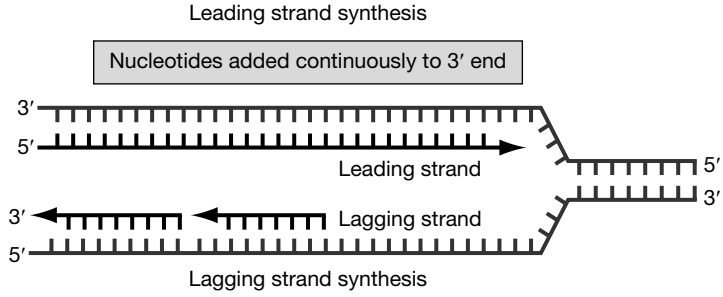


Figure 8.14 The leading strand is synthesized continuously while the lagging strand is synthesized discontinuously

Initiation of DNA Replication in Prokaryotes

The DNA replication initiation begins with identification of the origin region in the DNA, which is known as OriC, and synthesis of primer necessary to begin with polymerization of a polynucleotide chain. The origin region called OriC consists of 245 nucleotides and contains four 9 nucleotides long consensus sequence TTATCCACA and three 13 nucleotide long consensus sequence GATCTNTTNTTTT at the left end of OriC. DnaA protein recognizes and binds to the 9 nucleotide long sequence (dna box). A huge complex of DnaA binds to the OriC along with HU protein and induces bending in the DNA as well as destabilization of adjacent 13 mer repeats and causes local DNA melting. Now the next protein, DnaB, binds to the melted region, followed up with the binding of DnaC to DnaB protein and helps deliver it to be DNA. DnaB is a helicase, which can unwind the DNA in the direction 5′-3′. At this stage, the pre-priming complex is ready. Finally DnaG, a primase binds to the pre-priming complex and converts it to the primosome, which can synthesize the primer to initiate DNA replication (Figure 8.15). DnaG is a RNA polymerase that synthesizes small 8–10 nucleotides long RNA at the priming site. Even

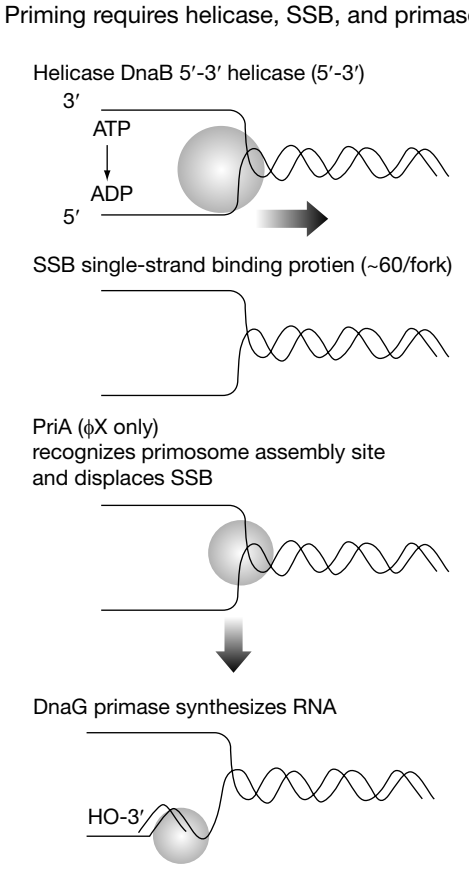


Figure 8.15 Initiation requires several enzymes like helicase, SSB and primase

in eukaryotes, several proteins are required for identifying the origin region (referred to as ORC, origin recognition complex) and initiating the process.

Elongation of DNA Replication in Prokaryotes

The elongation of DNA replication starts the moment a primer is in place. This includes leading strand synthesis and lagging stand synthesis. DNA polymerases are the enzymes involved in DNA synthesis. All DNA polymerases require a template, a primer, deoxynucleotide triphosphates, magnesium and ATP for the catalytic reaction. Polymerization reaction involves adding a nucleotide to the growing 3′ OH end in a template-dependent fashion. A nucleotide triphosphate, is the precursor for DNA synthesis, which loses the two terminal phosphate groups as pyrophosphate in the reaction and the polynucleotide chain grows by one nucleotide in the direction 5′-3′. Polymerase then scrutinizes the base pair that it has added and in case it is wrong, excises it out with its proofreading activity.

In *E. coli*, three DNA polymerases occurs namely DNA polymerase I, II and III. Of these, DNA polymerase III is a major polymerase in DNA replication. However, DNA polymerase I also has a role to play in DNA synthesis. DNA polymerase III is a complex enzyme of several subunits and has two catalytic activities, namely 5′-3′ polymerase and 3′-5′ exonuclease activity, and it is assembled at the site of replication (Figure 8.16). It does not exist as a holoenzyme in the cell. The core enzyme consists of subunits α, ε and θ, wherein 5′-3′ polymerase activity is in α-subunit, and 3′-5′ exonuclease is with the ε-subunit, and both of these are held together by the θ-subunit. The core by itself is a poor polymerase. It puts together about 10 nucleotides and then falls off the template. Again, it has to reassociate with the template and start synthesis. However, in reality, the replication fork moves with the speed of 1000 nucleotides per second. Another subunit called β-ring a and both of these are held together by hly processive enzyme, allowing it to remain engaged with the template while polymerizing at least 50,000 nucleotides before it stops. The β-subunit is called β-ring because it forms a dimer that is ring shaped. This ring fits around the DNA template and interacts with the α-subunit of the core to tether the polymerase and the template together. Thus, the enzyme can stay on template for a long time. β-subunit is therefore called as the 'clamp of polymerase.' The clamp needs help from another subunit called the γ-complex to load on the DNA template. This complex is named as 'clamp loader' and consists of many subunits such as γ, δ, δ', X and Ψ. This clamp loader has both loading and unloading activities for β-clamp (Figure 8.16).

At replication, the same DNA polymerase coordinates fork synthesis of both leading and lagging strands (Figure 8.17). The enzyme at the replication fork consists of two core polymerases linked through a dimer of τ-subunit to a clamp loader. One of the core enzymes would be busy synthesizing leading strand and another one would be involved in the synthesis of lagging strand. Polymerase III core, making the lagging strand, does not really dissociate completely from the template. It remains tethered to it by its association with the core that is making the leading strand through the τ-subunit. So, it releases its grip on the template strand without straying away from the DNA. This helps it find the next primer and reassociate with the template within a fraction of a second instead of several seconds that would be required if it completely left the DNA.

The dissociation and reassociation of polymerase core with the template is dependent on the clamp. The clamp interacts both with the core and the clamp loader. It must associate with the

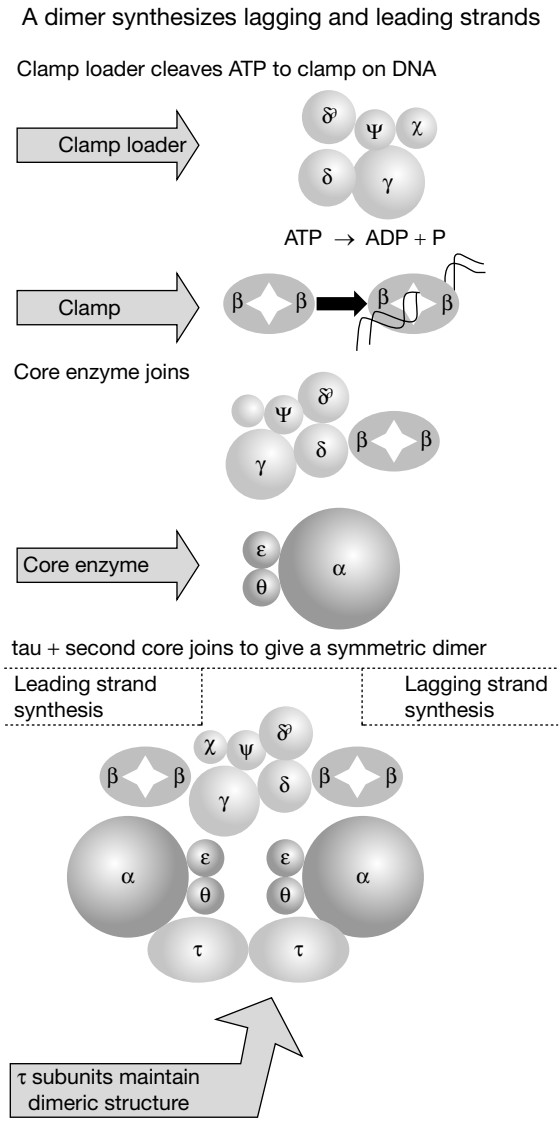


Figure 8.16 Synthesis of lagging and leading strands by DNA polymerase holoenzyme

core during synthesis of DNA to keep the polymerase on the template. Then, it must dissociate from the template so that it can move to a new site on DNA where it can interact with another core to make a new Okazaki fragment. This movement requires interaction of clamp with loader. The processive synthesis of an Okazaki fragment takes place when the loaded clamp loses it affinity for clamp loader and get associated with core polymerase. Once the fragment is completed, the clamp loses its affinity for core polymerase and associates with the complex which unloads it

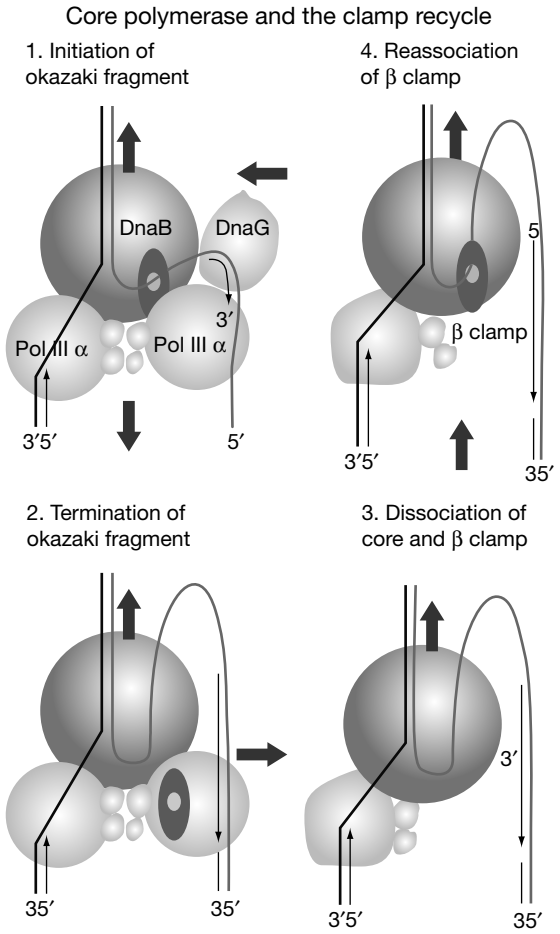


Figure 8.17 The dissociation of core polymerase and β-clamp upon completion of Okazaki fragments and reassociate at start of Okazaki fragments

from the DNA and can be loaded again by clamp loader to the next primer and repeat the process. Thus, once initiated, one of the template strands is synthesized continuously and another one discontinuously with several Okazaki fragments of about 1200 nucleotides long (Figure 8.18).

8.11.2 Maturation of Okazaki Fragments

The maturation of Okazaki fragments in the continuous DNA strand, completes in two steps as listed hereunder.

1. A small RNA primer at the 5′ end of each Okazaki fragment needs to be removed and replaced by DNA. DNA polymerase I uses nick translation to replace RNA primer with DNA.
2. All of these Okazaki fragments need to be joined to each other. DNA ligase seals the nick.

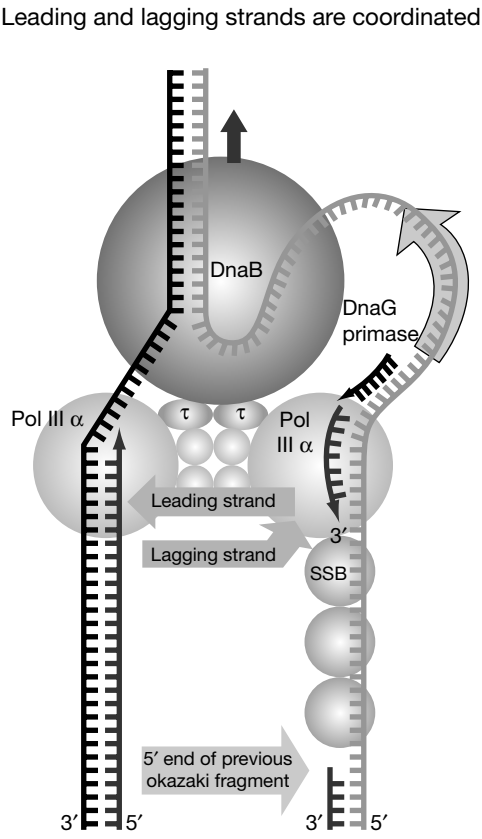


Figure 8.18 Each catalytic core of polymerase III synthesizes a daughter strand. DnaB is responsible for forward movement at the replication fork

DNA polymerase I and DNA ligase do these jobs, respectively. DNA polymerase I has three catalytic activities, namely 5′-3′ polymerase, 3′-5′ exonuclease and 5′-3′ exonuclease. Each Okazaki fragment synthesis ends when DNA polymerase approaches the RNA primer attached to the 5′ end of the earlier fragment. DNA polymerase I with 5′-3′ exonuclease removes ribonucleotides, simultaneously extending the 3′ end of Okazaki fragment. Finally, the nick is sealed by ligase by making a phosphodiester bond between 3′-OH and 5′-PO_4 ends (Figure 8.19).

8.11.3 DNA Replication in Eukaryotes

The DNA replication in eukaryotes is different from prokaryotes only in terms of that in eukaryotic cells the DNA molecules are significantly larger than those in prokaryotes and are structured into nucleoprotein complex. The essential features of replication are the same in eukaryotes and prokaryotes and many of the protein complexes are structurally and functionally conserved. Eukaryotic DNA has several origins of replication spaced 30,000 to 3,00,000 base pairs apart and replication proceeds bidirectionally from these origins. Initiation of pairs apart and replication requires a multisubunit protein, the ORC complex controls the origin and interacts with many

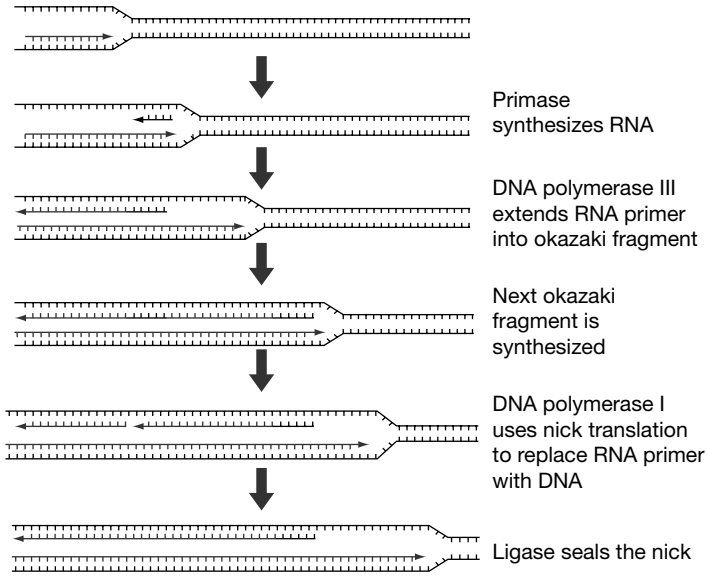


Figure 8.19 Okazaki fragments synthesis requires enzymatic action

other proteins. Two other proteins, CdC6 (Cell Division Cycle 6) and CDT1 (Cdc 10 Dependent Transcript 1), bind to ORC and load complex of another protein called minichromosome maintenance (MCM) proteins. CdC6 and CDT1 are functionally similar to DnaC, which loads DnaB at the origin of replication in bacteria. MCM proteins are ring-shaped replicative helicases similar to DnaB helicase of *E. coli* and unwind the DNA at the origin of replication (Figure 8.20).

Eukaryotic cells have several DNA polymerases: DNA polymerase α, β, γ, δ, ε, η, ζ, etc. Of these, DNA polymerase α, δ and sometimes ε are involved in nuclear DNA replication. In mitochondrial DNA replication, DNA polymerase γ is involved and the remaining (β, η and ζ) DNA polymerases are primarily involved in different DNA repair processes. DNA polymerase α is a multisubunit enzyme. One of the subunits has primase activity and the other subunits contain polymerase activity. However, this polymerase has no proofreading activity, making it unsuitable for high fidelity replication. This polymerase is basically involved in the synthesis of short primers containing RNA and DNA called initiator DNA (i-DNA), once on leading strand and many times on the lagging strand for Okazaki fragments. DNA polymerase d then extends these primers. This enzyme is associated and stimulated by a protein called proliferating cell nuclear antigen (PCNA) whose three-dimensional structure is very similar to that of the β-subunit of *E. coli* DNA polymerase III. Both leading and lagging strand synthesis is carried out by DNA polymerase δ, and has 3′-5′ proofreading activity. Sometimes, DNA polymerase ε can replace DNA polymerase δ. In eukaryotic cell at the replication fork, the replication is co-ordinated by DNA polymerase α and two molecules of DNA polymerase δ. During the same time, when DNA is being synthesized, lot of histone proteins are made by the cell, as both the newly synthesized DNA and the parental DNA, must form nucleoprotein complex on completion of replication.

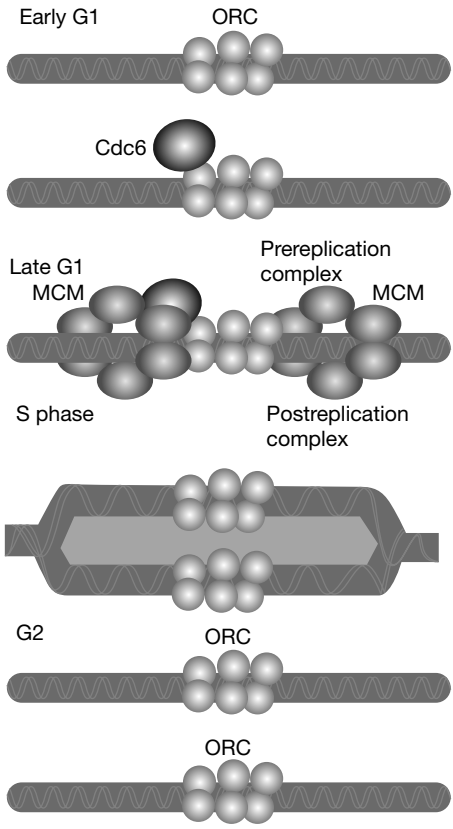


Figure 8.20 Proteins at the origin control susceptibility to initiation in eukaryotes.
ORC is a protein complex that remains associated with yeast origin throughout the cycle. Cdc6 protein is unstable and synthesized only in G1.
Cdc6 binds to ORC and allows MCM proteins to bind. When initiation of DNA replication starts, Cdc6 and MCM proteins are displaced. The degradation of Cdc6 prevents reinitiation. Some MCM proteins are in the nucleus throughout the cycle, but other proteins may enter only after mitosis.

Separate class of proteins called 'chromatin assembly proteins' carry out the assembly of DNA into nucleoprotein complex called 'chromatin.'

8.11.4 Termination of Replication

The 'Ter sequences' present on the DNA are responsible for terminating activity of the process of DNA replication. The Ter sequences are 22 base pair long that bind specific proteins called terminator utilization substances (TUS) protein. Ter sequences are six in all and out of six, three Ter sequences work as terminator for the fork moving in a clockwise direction from OriC, and the remaining three work as terminator for the fork moving in anti-clockwise direction.

Thus, these Ter sequences are orientation-specific with respect to replication fork. Tus protein is a contra-helicase. The replication fork is led by helicase, continuously unwinding the DNA in the 5′-3′ direction. If the helicase is removed, replication fork cannot proceed. Tus protein being anti-helicase knocks off the helicase, thus terminating the progression of replication fork. Upon completion of circular DNA replication, two daughter duplexes remain entangled, and these need to be separated so that they can be passed on to the two daughter cells. The two daughter duplexes formed during replication remain entwined as two interlocked rings called 'catenates.' These are unlinked or decatenated by the enzyme called topoisomerase IV.

Eukaryotic chromosomes are linear not circular and have multiple replicons; so, replication forks from neighbouring replicons approach one another just as the two replication forks of a bacterial chromosome approach each other near the termination point. Eukaryotic chromosome also forms catenates that must be disentangled, and this is done by eukaryotic topoisomerase II.

At the end of the eukaryotic DNA replication, eukaryotes face the difficulty of filling the gaps left during DNA synthesis, when the first cannot be filled, as DNA cannot be extended in the direction 3′-5′. If this is left unfilled, then the DNA would shorten with every round of replication. A special enzyme called 'telomerase' solves this problem. In eukaryotes, chromosomes have special structures known as telomeres at their ends. One strand of these is composed of tandem repeats of short GC-rich regions whose sequence varies from one species to another. G-rich strand is made by an enzyme called telomere synthesis. C-rich telomere strand is then synthesized by RNA primed DNA synthesis. This ensures that chromosome ends are rebuilt and do not suffer shortening with each round of replication.

8.11.5 Regulation of Replication

The DNA replication is strongly regulated in a cell. The DNA replication takes place only once during a cell division and the replication is initiated at the origin of replication, the regulation ensures that the origin is not reactivated before the cell divides. This is achieved by many ways. OriC contains 11 copies of GATC sequence which is a target for methylation at the N^6 position of adenine by a specific enzyme called Dam methylase. Before replication, this sequence is methylated on the adenine on each strand. Replication inserts normal base during replication which needs to be methylated later. Thus, replication generates hemimethylated DNA, wherein the adenine on the parental strand is methylated but that on the daughter strand is nonmethylated. Hemimethylated origins cannot initiate again until the Dam methylase has converted it into fully methylated origins. At the origin the GATC sites remain hemimethylated for 13 min after replication, whereas other GATC sites begin to get methylated within 1.5 min. Another region that behaves like origin is the promoter of DnaA gene. This also shows delay in remethylation and remains repressed in hemimethylated state. This does not let transcription proceed from this promoter that causes a reduction in the level of DnaA protein. DnaA is the first protein required to bind to the origin before replication can initiate, and unavailability of it would not activate the origin. The delay in remethylation of these regions is because they remain sequestered by binding of the protein called SeqA. This protein binds to hemimethylated DNA very strongly and prevents it from being methylated.

Hemimethylated DNA do not allow the binding of DnaA to the origin because it remains attached to the membrane and thus occupies the binding site of DnaA, whereas fully methylated

DNA cannot be attached to the membrane. Thus, several mechanisms are involved in regulating the replication. These include physical sequestration of the origin, delay in remethylation, inhibition of DnaA binding and repression of transcription from DnaA reducing the amount of DnaA protein.

Eukaryotic cells have several origins, and it must be ensured that each origin is activated only once and the entire DNA is replicated only once during S phase. During the cell division, the cell divides, and it goes through four phases of cell cycle, namely G1, G2, S and M. At the end of G1, the cell enters S phase where DNA replicates, then the cell enters G2 phase and finally divides in M phase after which it again enters G1. The protein, which regulates the replication and restricts it only to S phase, is called as 'licensing factor.' It is present in the nucleus prior to replication. The licensing factor in the nucleus is inactivated after replication, and another round of replication is not possible until further factor s provided. Factor in the cytoplasm can only gain access to the nuclear material at the subsequent mitosis when the nuclear envelope breaks down. Thus, by removing the necessary components after replication, it prevents more than one cycle of replication from occurring. It also provides a feedback loop that makes the initiation of replication dependent on passing through the cell division.

The entrant proteins for being a licensing factor are Cdc6 and at the origin allow MCM proteins to bind to origin, only after which the replication can initiate. When origin of replication enters S phase, it is in pre-replication complex containing ORC, Cdc6 and MCM proteins. When initiation occurs, Cdc6 and MCM are displaced, thereby leaving origin with only ORC complex. Cdc6 is rapidly degraded on displacement and is not available for loading of MCM proteins, necessary for activation of origins. This ensures that each origin is used only once during a single S phase.

EXERCISES

1. Give a brief account of nucleotides.
2. Differentiate between nucleosides and nucleotides.
3. Draw the structure of purine and pyrimidine rings along with the structure of their bases.
4. What is the difference between DNA and RNA? Explain with the help of a diagram.
5. How the maturation of Okazaki fragments takes place in DNA replication? How the DNA replication is regulated?
6. How the initiation and elongation of DNA replication takes place? Explain with suitable diagrams.
7. What are histones? Write about the nucleotides of biological importance.
8. What is hypochromism? How the DNA is isolated?
9. Write the structure of DNA. What are different forms of DNA?
10. Write the structure of RNA and tRNA with the help of a diagram.

MULTIPLE CHOICE QUESTIONS

1. The best role of purine and pyrimidine nucleotides is to serve as the monomeric precursors of
   (a) RNA
   (b) DNA
   (c) Both of the above
   (d) None of the above
   Ans. c
2. The purine nucleotides act as the components of
   (a) FAD^+
   (b) NAD^+
   (c) $NADP^+$
   (d) All of these
   Ans. d
3. The chemical name of thymine is
   (a) 2-oxy-4-aminopyrimidine
   (b) 2, 4-dioxy-5-methylpyrimidine
   (c) 2, 4-dioxypyrimidine
   (d) None of these
   Ans. b
4. The chemical name 2-amino-6-oxypurine is said to be
   (a) Adenine (b) Xanthine
   (c) Guanine (d) Hypoxanthine
   Ans. c
5. The most abundant intracellular free nucleotide is
   (a) ATP (b) FAD^+
   (c) NAD^+ (d) $NADP^+$
   Ans. a
6. The epimerization of galactose to glucose and vice versa takes place by
   (a) UTP (b) CTP
   (c) GTP (d) ATP
   Ans. a
7. The biosynthesis of phosphoglycerides in animal tissue requires
   (a) ATP (b) CTP
   (c) GTP (d) TPP
   Ans. b
8. The chemical name 4-hydroxypyrazole pyrimidine is used for
   (a) Thioguanine
   (b) Mercaptopurine
   (c) Azathioprine
   (d) Allopurinol
   Ans. d
9. Guanosine nucleotide is held by the cytosine nucleotide by the number of hydrogen bonds
   (a) 1 (b) 2
   (c) 3 (d) 4
   Ans. c
10. DNA is denatured by
    (a) Heat (b) Acid
    (c) Alkali (d) All of these
    Ans. d
11. Each transfer RNA molecule contains the number of nucleotides
    (a) 70 (b) 75
    (c) 80 (d) 85
    Ans. b
12. The carbon atoms at positions 4 and 5 and the N atom at position 7 of purine base are supplied from
    (a) Valine (b) Alanine
    (c) Glycine (d) Serine
    Ans. c
13. The polynucleotide is the condensation of two or more nucleotides, each successive nucleotide being united to its immediately preceding one by forming a diester with
    (a) Phosphate (b) Sodium
    (c) Hydrogen (d) None of these
    Ans. a
14. When origin of replication enters S phase, it is in pre-replication complex containing ORC, Cdc6 and
    (a) MCM proteins (b) MDM protein
    (c) Both (a) and (b) (d) None of these
    Ans. a

15. Eukaryotic chromosome also forms catenates that must be disentangled, and this is done by eukaryotic
    (a) Topoisomerase II
    (b) Topoisomerase I
    (c) Both (a) and (b)
    (d) None of these

    Ans. a

16. In almost all nucleic acids, there are approximately 15 to 16 per cent nitrogen and
    (a) 2 to 6 per cent phosphorous
    (b) 9 to 12 per cent phosphorous
    (c) 15 to 20 per cent phosphorous
    (d) None of these

    Ans. b

17. Adenosine-5′-diphosphate linked to nicotinamide forms the extremely important oxidation–reduction coenzyme
    (a) NAD^+ (b) FAD^+
    (c) Both (a) and (b) (d) None of these

    Ans. a

18. RNAs are usually single stranded but secondary structures are found in several RNA molecules due to
    (a) Intramolecular loops' formation between the complementary base pairing
    (b) Intermolecular loops' formation between the complementary base pairing
    (c) Intramolecular loops' formation between the uncomplementary base pairing
    (d) Intermolecular loops' formation between the uncomplementary base pairing

    Ans. a

19. A 'cloverleaf' structure in all tRNAs with the anticodon, i.e., the nucleotide triplet that is necessary for the positioning of the specific RNA in the messenger RNA template is observed during
    (a) Protein biosynthesis
    (b) Transcription
    (c) Reverse transcription
    (d) None of these

    Ans. a

20. The observation by Chargaff that the ratio of adenine to thymine and that of cytosine to guanine is very close to
    (a) 1 (b) 2
    (c) 3 (d) None of these

    Ans. a

9 Metabolism of Carbohydrates

CHAPTER OBJECTIVES

9.1 Introduction to Carbohydrate Metabolism
9.2 Glycolysis–Anarobic Pathway or Embden-Meyerhof Pathway
9.3 Gluconeogenesis
9.4 Cori Cycle
9.5 Utilization of Other Carbohydrates
9.6 Biosynthesis of Some Carbohydrates
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9.12 Regulation of Blood Glucose Levels
9.13 Carbohydrate Metabolic Disorder and Diseases
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9.1 INTRODUCTION TO CARBOHYDRATE METABOLISM

Carbohydrate metabolism is essentially the metabolism of glucose in the animal body. The characteristics sugar of blood and of tissue fluids is glucose. The digestion of food carbohydrates, such as starch, sucrose and lactose, produces the monosccharides glucose, fructose and galactose, which pass into the blood stream. Fructose and galactose then are converted to glucose in the liver. The glucose of circulating blood and tissue fluids are drawn up by all the cells of the body and used for the production of energy. Normally, carbohydrate metabolism supplies more than half of the body energy requirement. In fact the brain depends largely upon carbohydrate metabolism as a source of energy and stops working properly when glucose levels in the blood are below the normal range.

The glycogen of liver, muscles and other tissues is formed primarily from glucose and serves as a source of reserve energy. Normally, much of the reserve fat in the body depots is formed from glucose. The mammary glands synthesize lactose from blood glucose. Tissue glycolipids and mucopolysaccharides are formed from glucose.

The metabolism of many of the proteins and amino acids proceeds by the glucose pathway, and some of the products of glucose metabolism is intimately related to the metabolism of both lipids and proteins.

9.2 GLYCOLYSIS–ANAEROBIC PATHWAY OR EMBDEN-MEYERHOF PATHWAY

The sequence of reactions by which glucose is degraded anaerobically is called the glycolytic sequence. Strictly speaking, this refers to the production of two molecules of lactic acid from one molecule of glucose. Monosaccharaides other than glucose can be broken down by glycolysis provided they can be converted into an intermediate in that sequence. Energy is released in the form of ATP as the monosaccharaides are degraded and several important metabolites are produced for use elsewhere in intermediary metabolism. Those cells and tissues that actually convert pyruvic acid to lactic acid as a major end product are much more limited. Notable examples are the skeletal muscles of animals, lactic acid bacteria and some plant tissues. Skeletal muscles with its poor oxygen supply and relatively few mitochondria, but high concentration of glycolytic enzymes are ideally designed for carrying out glycolysis; however, heart muscle, well supplied with oxygen and mitochondria, will convert only small quantities of pyruvic acid to lactic acid. Most tissues that have adequate supply of oxygen utilize the pyruvic acid directly by oxidizing it via acetyl-CoA in the aerobic phase of carbohydrate metabolism (Figures 9.1a and 9.1b).

In glycolysis there is only one oxidative step (dehydrogenation) in the conversion of glucose to pyruvic acid in which the coenzyme NAD^+ is reduced to $NADH^+ H^+$. If oxygen is available, the reduced coenzyme is reoxidized to NAD^+ through the respiratory chain. The pyruvic acid itself is converted to acetate and is utilized in synthetic reactions or is oxidized in the citric acid cycle.

Under anaerobic conditions, the reduced $NADH^+ H^+$ is converted to the oxidized form of NAD^+ by passing on the hydrogen to end product, pyruvic acid, which is reduced to lactic acid. Hence, the formation of lactic acid is only a side reaction in glycolysis to regenerate the coenzyme for further use in glycolysis. Otherwise, the steps in glycolysis remain the same whether oxygen is available or not.

Alcoholic fermentation: In alcoholic fermentation, two moles of CO_2 and ethanol are produced from one mole of glucose. This process, which occurs principally in yeast and in other organisms, is identical to glycolysis except for two reactions at the end of the glycolytic sequence. Both these processes proceed without the participation of molecular oxygen, even though oxidation has occurred in both the processes.

9.2.1 Reactions of Glycolysis Pathway

In glycolysis pathway, the breakdown of 6-carbon glucose into two molecules of the 3-carbon pyruvate takes place in 10 steps. The glycolysis pathway consists of two phases, (a) preparatory phase and (b) payoff phase.

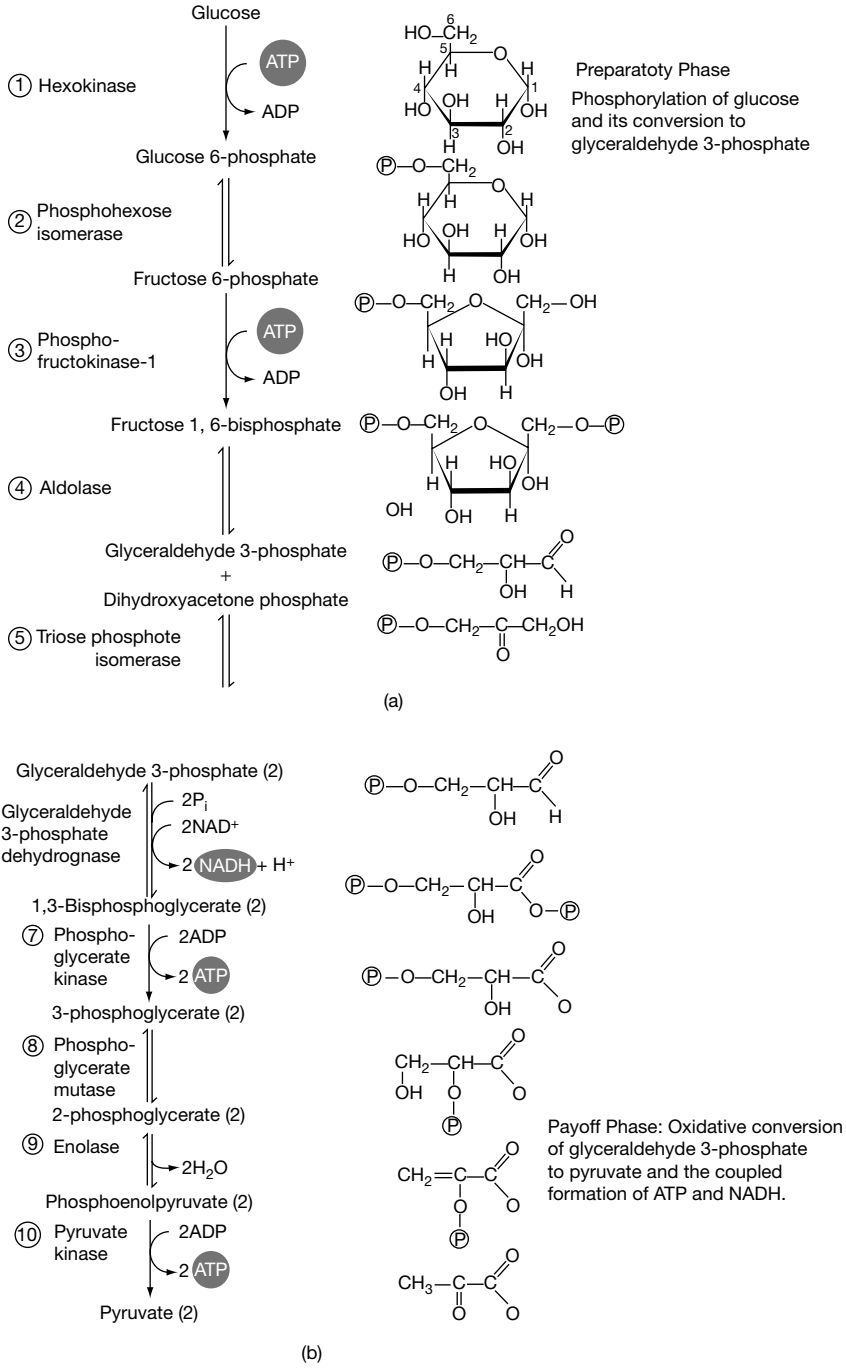
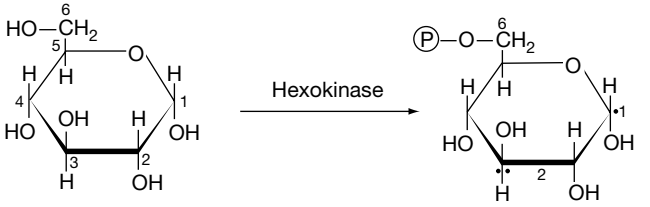


Figure 9.1 (a) and (b) Glycolysis

9.2.2 Preparatory Phase of Glycolysis

This first five steps of glycolysis takes place in preparatory phase. In this phase, phosphorylation of glucose and its conversion to glyceraldehyde-3-phosphate takes place. In this phase, two molecules of ATP are invested before cleavage of glucose into 3-carbon pieces that is glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.

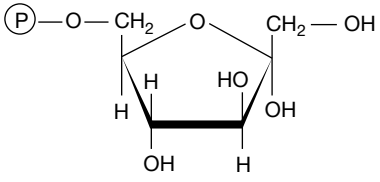
Step 1. First priming reaction: D-Glucose is first phosphorylated at the hydroxyl group on C-6. This reaction is catalysed by the enzyme hexokinase. This first reaction is endothermic and uses ATP as a source of energy. ATP is eventually hydrolysed to ADP. In this reaction, there is net loss of one ATP molecule.



Glucose **Glucose-6-phosphate**

This reaction is endothermic, ATP is used and hydrolysed to ADP. One ATP molecule is lost in this reaction.

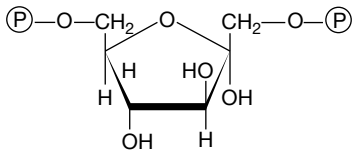
Step 2. Isomerization: The change in structure is an isomerization, the glucose-6-phosphate is changed into an isomer, fructose-6-phosphate. This reaction is catalysed by the enzyme phosphohexose isomerase. This reaction is freely reversible under normal cell conditions.



Fructose-6-phosphate

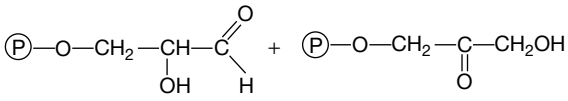
Step 3. Second priming reaction: This reaction is nearly the same to step 1. In this step, fructose-6-phosphate is phosphorylated, but in this reaction phosphate is added to C-1. This reaction is catalysed by phosphofructokinase. This is again an endothermic reaction and thus ATP is the source of energy and gets hydrolysed to ADP. Another ATP energy expenditure is given in two ways as discussed hereunder.

The glycolytic process is now irreversible, and the molecule gets destabilized by the supplied energy. This is because phosphofructokinase 1 (PFK-1) catalysed the reaction and is coupled to ATP hydrolysis. This makes the reaction a key regulatory point. This is also the rate-limiting step. This is an energetically favourable step; it is irreversible, and for the reverse conversion of the reaction, different pathways must be used during gluconeogenesis.



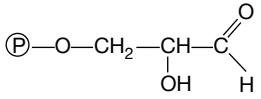
Fructose 1,6-bisphosphate

Step 4. Split molecule in half: In this step, fructose 1,6-bisphosphate is split into two 3-carbon compounds, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. This reaction is catalysed by the enzyme aldolase.



Glyceraldehyde-3-phosphate and dihydroxyacetone phosphate

Step 5. Lysis: In this step, dihydroxyacetone phosphate is isomerized to second molecules of glyceraldehyde-3-phosphate. This reaction is catalysed by the enzyme triose phosphate isomerase (TPI). This is the last reaction of the preparatory phase of glycolysis.

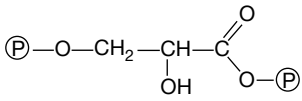


Glyceraldehyde-3-phosphate (2)

Step 6. Payoff phase of glycolysis: The second half of the glycolysis is the payoff phase. Payoff phase means a net gain of ATP and NADH, and whatever the energy lost in the form of ATP in preparatory phase is recovered in this phase. The next five steps are the part of payoff phase. The payoff phase deals with the oxidative conversion of glyceraldehyde-3-phosphate to pyruvate and the coupled formation of ATP and NADH. In glycolysis pathway from one glucose molecule, 2 NADH molecules and 4 ATP molecules are produced during payoff phase, which leads to a net gain of 2 NADH and 2 ATP molecules.

Step 7. Oxidation and phosphorylation: Each molecule of glyceraldehyde-3-phosphate is oxidized and phosphorylated by inorganic phosphate (not by ATP) to form 1, 3-Bisphosphoglycerate. This reaction is catalysed by the enzyme glyceraldehyde-3-phosphate dehydrogenase.

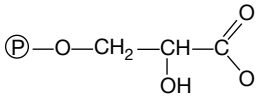
This reaction is first an oxidation involving the coenzyme NAD^+. The aldehyde is oxidized to an acid as an intermediate through the conversion of NAD^+ to $NADH^+$ H^+. Then, an inorganic phosphate is added in a phosphate ester synthesis.



1, 3-Bisphosphoglycerate (2)

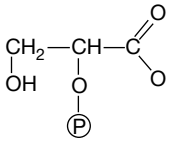
Step 8. First ATP forming reaction (substrate-level phosphorylation): In this step energy is released to molecule of 1, 3 bisphosphoglycerate are converted to 3 phosphoglycerate. This reaction

is catalysed by the enzyme phosphoglycerate kinase. In this step, two molecules of ATP are formed. In this step, enzymatic transfer of a phosphate group takes place, phosphate group is transferred from 1,3-bisphosphoglycerate to ADP by the enzyme phosphoglycerate kinase, forming ATP and 3-phosphoglycerate.



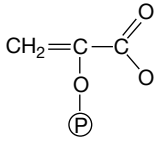
3-Phosphoglycerate

Step 9. In this step, 3-phosphoglycerate is converted to 2-phosphoglycerate. This reaction is catalysed by the enzyme phosphoglycerate mutase.



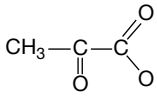
2-Phosphoglycerate (2)

Step 10. In this reaction, 2-phosphoglycerate acid is converted to two molecules of phosphoenol-pyruvate. This reaction is catalysed by the enzyme enolase with the release of $2H_2O$ molecules. In this reaction, which is the dehydration of an alcohol, the –OH on C-3 and the –H on C-2 are removed to make a water molecule. At the same time, a double bond forms between C-2 and C-3. This change makes the compound somewhat unstable, but provides energy for the final step of glycolysis.



Phosphoenolpyruvate (2)

Step 11. Second ATP forming reaction (substrate-level phosphorylation): In this step, two molecules of phosphoenolpyruvate is converted to two molecules of 3-carbon molecule pyruvate (pyruvic acid). This reaction is catalysed by the enzyme pyruvic kinase. In this step, two ATP molecules are formed.



Pyruvate (2)

Much of this energy is conserved by the coupled phosphorylation of four molecules of ADP to ATP. The net yield is two ATP molecules per molecule of glucose used, because two molecules of ATP were invested in the preparatory phase. Energy is also conserved in the payoff phase in the formation of two molecules of NADH per molecule of glucose.

In the sequential reactions of glycolysis, three types of chemical transformations are particularly noteworthy: (1) degradation of the carbon skeleton of glucose to yield pyruvate, (2) phosphorylation of ADP to ATP by high-energy phosphate compounds formed during glycolysis and (3) transfer of a hydride ion to NAD^+ forming NADH.

The overall process of glycolysis is given hereunder.

$$\text{Glucose} + 2NAD^+ + 2ADP + 2P_i \rightarrow 2\text{pyruvate} + 2NADH + 2H^+ + 2ATP + 2H_2O$$

9.2.3 Fate of Pyruvate after Glycolysis

The pyruvate formed by glycolysis is further metabolized via one of the three catabolic routes as discussed hereunder.

1. The first route: Pyruvate is oxidized, with loss of its carboxyl group as CO_2 to yield acetyl-coenzyme A; the acetyl group is then oxidized completely to CO_2 by the citric acid cycle.
2. The second route: Pyruvate is reduced to lactate via lactic acid fermentation.
3. The third route: Pyruvate catabolism leads to ethanol.

9.2.4 Regulation of Glycolysis

Glycolysis is regulated by hexokinase, phosphofructokinase and pyruvate kinase.

Hexokinase Enzyme

Pancreas, in combination with liver, is a vital part of homeostasis in regulation of blood glucose levels in animals. For the conversion to glycogen, extra glucose-6-phosphate (G6P) in liver cells, may be converted to G1P or converted by glycolysis to acetyl-CoA and then citrate, alternatively. Surplus citrate is exported to the cytosol, then in cytosol ATP citrate lyase will regenerate acetyl-CoA and OAA. Both hexokinase and glucokinase enzymes are present in liver, the latter catalyses the phosphorylation of glucose to G6P, this conversion takes place when glucose levels in blood are high. During hypoglycaemia, the liver-specific enzyme glucose-6-phosphatase leads to the conversion of the glycogen back to glucose-6-phosphate and then to glucose. During fasting, liver cells play an important role through reverse reaction to maintain blood sugar levels.

Export of excess citrate to cytosol where citrate regenrates acetyl-CoA and oxaloaceticacid (OAA) by the enzyme ATP citrate lyase. The acetyl-CoA which is regenerated here is used for the synthesis of fatty acid and cholesterol.

Phosphofructokinase Enzyme

In glycolysis, the enzyme phosphofructokinase is an important control point, which converts fructose-6-phosphate to fructose-1,6-bisphosphate and it is one of the irreversible step in glycolytic pathway.

The powerful activator of phosphofructokinase (PFK-1) is fructose 2, 6-bisphosphate (F2, 6BP), which is synthesized when the second phosphofructokinase (PFK2) enzyme phosphorylates F6P. When blood sugar levels are low, then the hormone glucagon increases cAMP, and PFK2 is phosphorylated by protein kinase A. The enzyme PFK2, gets inactivated upon phosphorylation,

and another domain on this protein gets activated as fructose 2,6-bisphosphatase, which converts F2, 6BP back to F6P. In liver, both these hormones glucagon and epinephrine are responsible for high levels of cAMP. Low levels of liver fructose 2,6-bisphosphate is the result of decreased activity of phosphofructokinase and increased activity of fructose 1,6-bisphosphatase, so that gluconeogenesis ('glycolysis in reverse') is favoured.

Pyruvate Kinase Enzyme

The last step of glycolysis is catalysed by the enzyme pyruvate kinase, and leads to the formation of pyruvate and ATP. This regulation of the enzyme pyruvate kinase is as follows:

- The enzymatic activity of pyruvate kinase is enhanced by its own substrate PEP and fructose 1,6-bisphosphate, an intermediate in glycolysis. If substrate is more, the glycolysis operation is faster.
- ATP is a negative allosteric inhibitor and alanine is a negative allosteric modulator of pyruvate kinase. This accounts for parallel regulation with PFK 1.
- Whether citrate plays a role in negative allosteric inhibition of the enzyme pyruvate kinase is not known but it is believed that acetyl-CoA does act as negative allosteric inhibitor.

9.2.5 Glycerophosphate Shuttle

The $NADH^+ + H^+$ produced in the glyceceraldehyde-3-phosphate dehydrogenase reaction in cytosol is not permeable to mitochondria. In order to enter the mitochondria, it follows a pathway known as 'glycerophosphate shuttle.' The dihydroxy acetone phosphate formed in the aldolase reaction is reduced to α-glycerophosphate by glycerophosphate dehydrogenase. The α-glycerophosphate is permeable to mitochondria, where reverse reaction takes place using the mitochondrial enzymes and flavoprotein as a coenzyme.

9.2.6 Malate–Aspartate Shuttle

Malate–Aspartate shuttle is similar to glycerophosphate shuttle. The $NADH^+ + H^+$ produced in the mitochondria is not permeable to cytosol. This is circumvented by reducing oxaloacetate to malate in the mitochondria. The malate is permeable to cytosol and again part with its hydrogen to NAD^+ to form $NADH + H^+$. The oxaloacetate cannot re-enter the mitochondria, but can enter after transamination and formation of aspartate.

9.3 GLUCONEOGENESIS

Another example of reversal is the regeneration of glucose termed as gluconeogenesis from lactic acid produced by higher animals during exercise. Skeletal muscles with its poorer oxygen supply but enriched supply of glycolytic enzymes, utilize glycolysis to meet its short term ATP needs. In doing so, muscles produce relatively large amount of lactic acid that is secreted into the blood and transported to the liver.

Gluconeogenesis can also occur from compounds other than lactic acid. Pyruvic acid and oxaloacetate, an intermediate of TCA cycle, can be converted to glucose. This occurs in animals during starvation or other wasting conditions.

9.3.1 Pathway of Gluconeogenesis

Gluconeogenesis is a pathway consisting of 11 enzyme-catalysed reactions. Gluconeogenesis pathway can begin either in the mitochondria or in cytoplasm, depends on which substrate is being used. Many of the reactions of gluconeogenesis pathway are the reversible steps of glycolysis except the steps 1, 3 and 9 which are the irreversible steps of glycolysis (Figure 9.2).

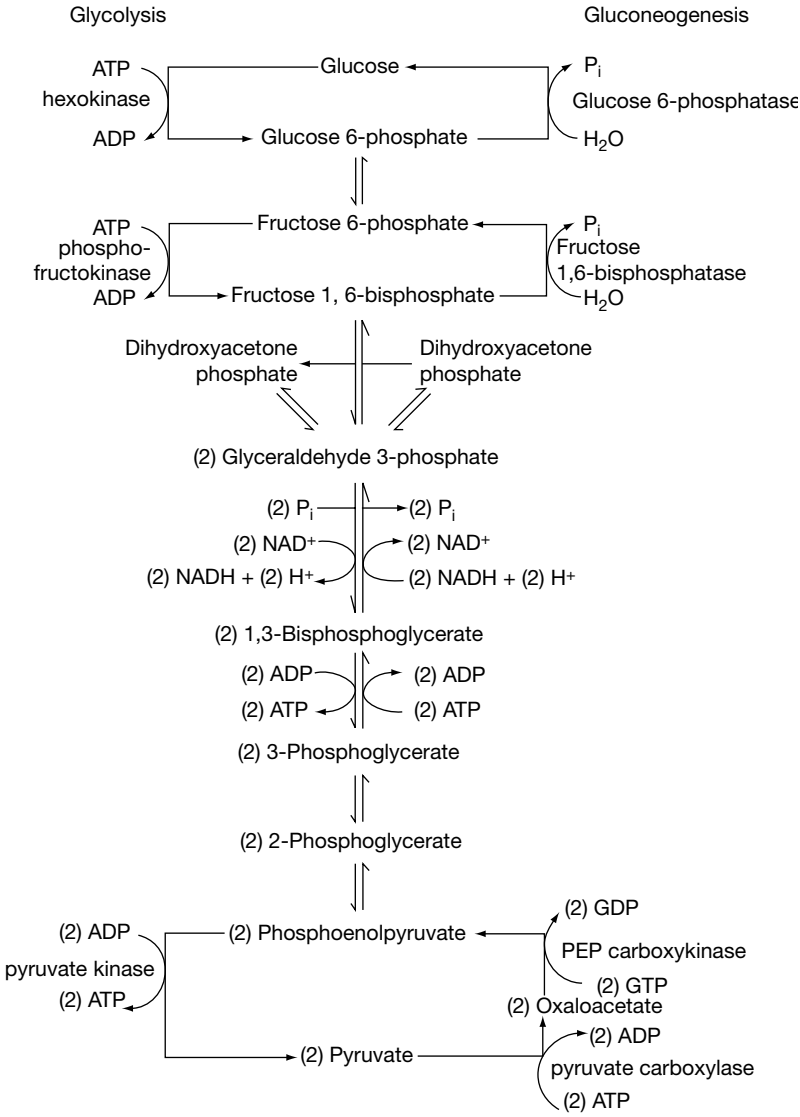


Figure 9.2 Gluconeogenesis pathway with key molecules and enzymes is the same as that of glycolysis in reverse direction. Steps (1), (3) and (7) of glycolysis pathway are irreversible and cannot be used in gluconeogenesis. Rest of the steps of gluconeogenesis are the same as that of glycolysis in reverse direction

1. Gluconeogenesis begins in the mitochondria due to the presence of the enzyme pyruvic carboxylase in mitochondria, leads to the formation of oxaloacetate through carboxylation of pyruvic acid and also use one molecule of ATP. To pyruvic carboxylase, acetyl-CoA acts as a positive modulator and ADP as a negative modulator.
2. Using NADH oxaloacetate is reduced to malate, this is the step needed for transport out of the mitochondria.
3. Malate is oxidized to oxaloacetate using NAD^+, gluconeogenesis occurs in the cytoplasm, where the remaining steps of gluconeogenesis occur.
4. Phosphoenolpyruvate is produced by decarboxylation and phosphorylation to oxaloacetate by the enzyme phosphoenolpyruvate carboxykinase, the reaction also undergoes hydrolysis of one molecule of GTP to GDP.
5. The next steps in the pathways are the same as that of reverse pathway of glycolysis. Fructose-1,6-bisphosphate gets converted to fructose-6-phosphate, by the enzyme fructose-1,6-bisphosphatase, this reaction requires one molecule of water and one phosphate molecule is released. This step is the rate-limiting step of gluconeogenesis.

   Fructose diphosphate phosphatase is a regulatory enzyme which together with phosphofructokinase plays a key role in regulating the flow of carbon up and down the glycolytic sequence. In this oligomeric protein, the number of monomers depends on the source. The phosphatase is strongly inhibited by AMP.
6. Enzyme phosphoglucoisomerase converts fructose 6-phosphate to glucose-6-phosphate.

   The production of glucose from glucose-6-phosphate requires a second phosphatase that catalyses the conversion of glucose-6-phosphate to glucose, which is an exergonic reaction.
7. Glucose-6-phosphatase characteristically associated with the endoplasmic reticulum. Glucose is transported into the cytosol by glucose transporters located in the endoplasmic reticulum membrane.

9.3.2 Regulation of Gluconeogenesis

Gluconeogenesis pathways are the same as that of glycolysis in reverse direction, except the three enzymes which are different.

In gluconeogenesis the three enzymes of glycolysis, hexokinase/glucokinase, phosphofructokinase and pyruvate kinase are replaced with glucose-6-phosphatase, fructose-1,6-bisphosphatase and PEP carboxykinase. The reciprocal control between the two cycles, glycolysis and gluconeogenesis allows inhibiting each other.

In cytoplasm, the majority of the enzymes responsible for gluconeogenesis are found, the exceptions are mitochondrial pyruvate carboxylase, and, phosphoenolpyruvate carboxykinase (in animals). The latter exists as an isozyme located in both the mitochondrion and the cytosol. The enzyme fructose-1,6-bisphosphatase plays a key role in controlling the rate of gluconeogenesis.

The activity of the gluconeogenesis is regulated by inhibiting the activity of the key enzymes of the pathway. The gluconeogenesis enzymes the pyruvate carboxylase and fructose-1,6-bisphosphatase both are activated by acetyl CoA and citrate, respectively. The acetyl-CoA and citrate also inhibits the activity of pyruvate kinase due to the reciprocal control of the cycle.

The release of the hormone glucagon leads to increased levels of cAMP and therefore activates gluconeogenesis, whereas the hormone insulin has the opposite effect, it counteracts the action of glucagon. Glucagon is released when blood glucose level is low and activates gluconeogenesis, it triggers phosphorylation of enzymes and regulatory proteins by protein kinase A, and results in inhibition of glycolysis and stimulation of gluconeogenesis, thus bringing blood glucose levels up.

9.4 CORI CYCLE

The process of partial oxidation of glucose to lactate in muscle, then conversion of lactate back to glucose in liver and then supply of glucose back to muscles, this co-operative cycle which utilizes both the muscle and liver tissues is known as the Cori cycle.

The requirement of energy for muscular activity is provided by the skeletal muscles, where the glycogen is broken down. Glycogenolysis is the pathway in which breakdown of glycogen takes place. Glycogenolysis releases glucose in the form of glucose-6-phosphate. Glucose-6-phosphate is readily fed into glycolysis for further oxidation. During exercise, the store of ATP gets exhausted and needs to be constantly stocked up. Under aerobic conditions, this energy comes from feeding pyruvate (Figure 9.3).

Under anaerobic (partial) condition like under intense muscular activity, energy must be released through anaerobic respiration. Anaerobic respiration converts pyruvate to acetyl-CoA and CO_2 within the mitochondria by a process called pyruvate decarboxylation. Most importantly, fermentation regenerates NAD^+, maintaining the NAD^+ concentration so that additional glycolysis reactions can occur.

Lactate produced by anaerobic fermentation in muscle cells is transferred to liver. This initiates the other half of the Cori cycle. In the liver, gluconeogenesis reverses both glycolysis and fermentation by converting lactate first into pyruvate, and finally back to glucose. The glucose is then supplied to the muscles through the bloodstream; it is ready to be fed into further glycolysis reactions. If muscle activity has stopped, the glucose is used to replenish the supplies of glucose through glycogenesis.

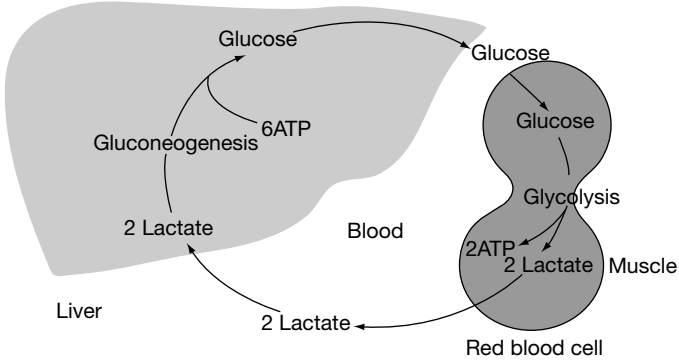


Figure 9.3 Cori's cycle. The degradation of glucose and its resynthesis during muscular activity

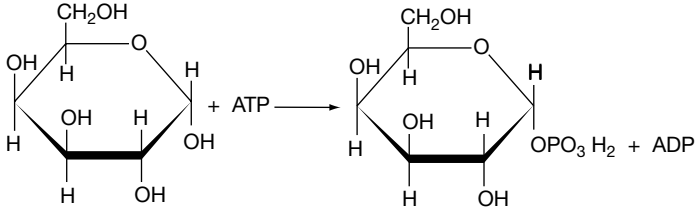
9.5 UTILIZATION OF OTHER CARBOHYDRATES

Sugar, other than glucose, is metabolized in the glycolytic sequence following their conversion by auxiliary enzymes to intermediates in that sequence. Thus, fructose and mannose can be phosphorylated by ATP in the presence of hexokinase and be converted into fructose-6-phosophate and mannose-6-phosphate. The former is an intermediate in glycolysis; mannose-6-phosphate is converted to fructose-6-phosphate by the enzyme phospho-mannose isomerase in a reaction analogous to that catalysed by phosphoglucoisomerase.

Disaccharides such as lactose and sucrose are extremely common sources of carbohydrate in the diet of animals. The initial steps in their utilization involve hydrolysis to the component monosaccharides by specific glycosidases, lactase and sucrase (invertase) found in the animal's digestive tract. The metabolism of galactose formed (together with glucose) on the hydrolysis of lactose is an interesting story.

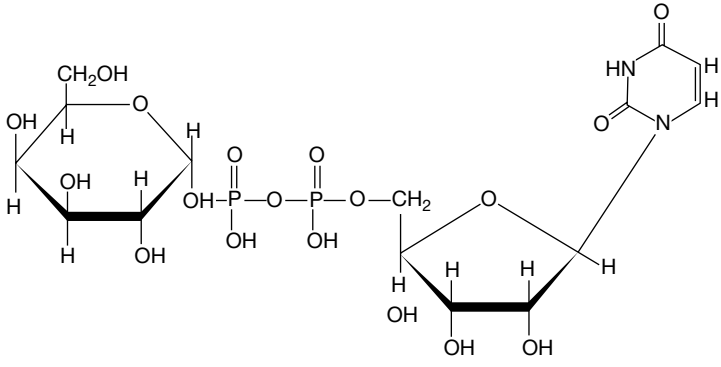
9.5.1 Utilization of Galactose

The initial reaction with galactose involves phosphorylation by ATP in the presence of a specific galactokinase that produces galactose-1-phosphate. This enzyme is present in both yeast and animal liver cells.



Galactose — Galactose-1-phosphate (9.1)

Further metabolism of galactose-1-phosphate involves uridine triphosphate (UTP) and a uracil derivative of that sugar known as uridine diphosphate galactose (UDP galactose):



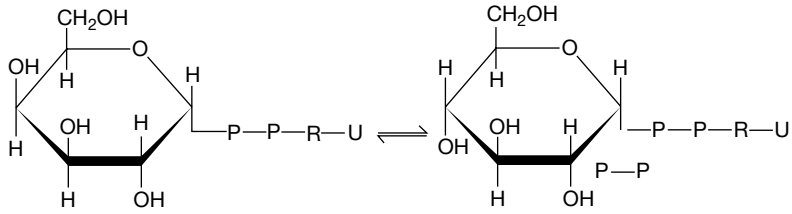
Uridine diphosphate galactose
(UDP-galactose)

The galactose-1-phosphate formed in reaction (reaction 9.1) is converted to UDP galactose by the enzyme UDP galactose pyrophosphorylase (reaction 9.2), which is present in the liver of adult humans:

$$\underset{\text{Galactose-1-phosphate}}{\text{Gal-P}} + \underset{\text{UTP}}{\text{U-R-P-P-P}} \rightleftharpoons \underset{\text{UDP-galactose}}{\text{U-R-P-P-Gal}} + \underset{\text{Pyrophosphate}}{\text{P-P}} \qquad (9.2)$$

The various components of the UTP and sugar phosphate molecules have been identified (R = ribose; P = phosphate) to indicate the nature of the reaction. The reaction is readily reversible, as could be anticipated, since the one (interior) pyrophosphate bond is utilized to form the pyrophosphate in the sugar nucleotide; the number of energy-rich structures in the reactants and products is consequently the same. This reaction is a model one for forming these nucleoside diphosphate sugars or sugar nucleotides. As another example, ADP glucose would be formed from ATP and glucose-1-phosphate in the presence of the specific pyrophosphorylase.

In the next step, the galactose moiety in UDP galactose is isomerized to a glucose moiety, thereby forming UDP glucose. The enzyme that catalyses this reaction is known as UDP glucose epimerase (reaction 9.3).



(9.3)

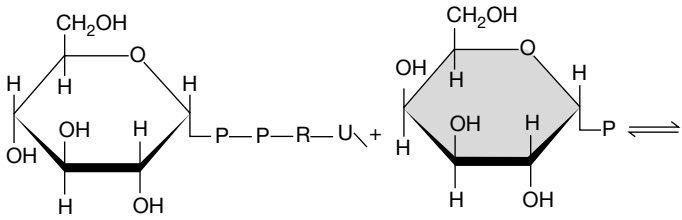
Finally, the action of the third enzyme UDP-glucose pyrophosphorylase liberates the glucose (formerly the galactose) moiety from UDP-glucose as glucose-1-phosphate (reaction 9.4).

$$\underset{\text{UDP-glucose}}{\text{U-R-P-P-Glu}} + \underset{\text{UTP}}{\text{P-P}} = \underset{}{\text{U-R-P-P-P}} + \underset{\text{Glucose-1-phosphate}}{\text{Glu-P}} \qquad (9.4)$$

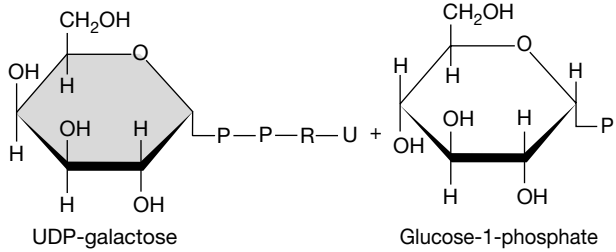
Note that this reaction is the same as reaction 2 except that the sugar involved is glucose. The sum of reactions (in the reactions 9.2 and 9.4) is the conversion of galactose-1-phosphate into glucose-1-phosphate. The metabolism of the latter by glycolysis accounts for the metabolism of galactose in adult humans.

The enzyme catalysing the formation of UDP-galactose from galactose-1-phosphate is found only in the liver of adults. How, then, does an infant metabolize galactose? This is a pertinent question, because one of the major energy sources that an infant has is the sugar lactose in the milk which it consumes.

Studies have shown that an infant liver tissue contains the enzyme phosphogalactose uridyl transferase:



(9.5)



The coupling of this reaction with reaction (9.3) accounts for the net conversion of galactose-1-phosphate into glucose-1-phosphate and is the normal route for galactose metabolism in infants. This series of reactions has attracted much attention because of a hereditary disorder known as galactosemia. Infants that have this defect cannot metabolize galactose and they exhibit a high level of galactose in the blood. The sugar is excreted in the urine and, if the condition is not attended to, the infant can develop cataracts and may become mentally retarded. The simple remedy, once the condition is identified, is to remove the source of galactose, usually the milk in the infant's diet, and supply a galactose-free diet.

Galactosemic individuals lack the enzyme uridyl transferase (reaction 9.5), and this accounts for their failure to metabolize galactose. Only after the individual has reached puberty does an adequate amount of UDP-galactose pyrophosphorylase appears in the liver, thereby providing him or her with the capacity to metabolize galactose.

It should be pointed out that the sugar nucleotides (e.g., UDP-glucose, UDP-galactose, etc.) are precursors of important cellular constituents such as glycogen, cell wall components and hyaluronic acids. Since the galactosemic infant needs a source of UDP-galactose to produce these cellular constituents, it will convert glucose-1-phosphate to UDP-galactose by reversing reactions 9.4 and 9.3. The adult human, on the other hand, will have available the pyrophosphorylase (reaction 9.2) for the synthesis of UDP-galactose.

9.6 BIOSYNTHESIS OF SOME CARBOHYDRATES

A limited number of homopolysaccharides such as insulin, a fructosan found in antichoke, are made by specific transglycosidases which transfer fructosyl units directly from donor such as sucrose to an acceptor such as the growing chain of insulin. However, the majority of the important disaccharides and polysaccharides found in nature, such as sucrose, glycogen, starch and cellulose, are synthesized by the transfer of glycosyl units from nucleoside diphosphate sugars to suitable acceptors.

9.6.1 The Role of Sugar Nucleotides

Two important general equations constitute the basic mechanism for this synthetic process. The first of these involves the formation of the nucleoside diphosphate sugar (or sugar nucleotide):

$$\underset{\substack{\text{(XTP)}\\\text{Nucleoside}\\\text{tri-Phosphate}}}{\text{X-R-P-P-P}} + \underset{\substack{\text{Glycosyl-1}\\\text{Phosphate}}}{\text{P-Gly}} \rightleftharpoons \underset{\substack{\text{Nucleoside}\\\text{di-phosphate sugar}\\\text{(sugar nucleotide)}}}{\text{X-R-P-P-P-P-Gly}} + \underset{\text{Pyrophosphate}}{\text{P-P}} \qquad (9.1)$$

This reaction is readily reversible since the reaction involves formation of a new pyrophosphate bond at the expense of the internal pyrophosphate bond of XTP. However, the equilibrium may be displaced far to the right in the presence of a pyrophosphatase that can hydrolyse the pyrophosphate formed. The second reaction involves the transfer of the sugar or glycosyl moiety to an acceptor.

$$\underset{\text{Sugar nucleotide}}{\text{X-R-P-P-Gly + Acceptor}} \longrightarrow \underset{\text{Nucleotide diphosphate}}{\text{X-R-P-P + Gly + Acceptor}} \qquad (9.2)$$

The equilibrium for reaction (9.2) is usually far to the right since the linkage between the C-1 atom of the monosaccharide and phosphate in the sugar nucleotide molecule is an energy-rich structure ($\Delta G'$ of hydrolysis of UDP-glucose to UDP and glucose is –8000 cal/mole). In the case of sucrose structure has a higher $\Delta G'$ of hydrolysis, the reaction is reversible.

A supply of glycosyl-1-phosphates and nucleoside triphosphate for reaction (9.1) is obviously required for the synthesis of polysaccharide.

$$\text{Glucose + ATP} \xrightarrow{\text{Glucokinase}} \text{Glucose – 6phosphate} \xrightarrow{\text{Phosphoglucomutase}} \text{Glucose-1 phosphate}$$

Glucose-1-phosphate can also be formed from a glycogen or starch by the action of phosphorylase a:

$$(\text{Glucose})_n + H_3PO_4 \rightleftharpoons (\text{Glucose})_{n-1} + \text{Glucose-1-phosphate}$$

The nucleoside triphosphates are generated by a widespread enzyme called nucleoside diphosphate kinase:

$$\text{NDP} + \text{ATP} \rightleftharpoons \text{NTP} + \text{ADP}$$

The enzymes responsible for the formation of the sugar nucleotide donor (reaction 9.1) are known as nucleoside diphosphate sugar pyrophosphorylases.

$$\underset{\text{Glucose-1-phosphate}}{\text{Glu-P}} + \underset{\text{UTP}}{\text{U-R-P-P-P}} \rightleftharpoons \underset{\text{UDP – glucose}}{\text{U-R-P-P-Glu}} + \underset{\text{Pyrophosphate}}{\text{P-P}}$$

Similarly, GDP-mannose is synthesized by a specific GDP-mannose pyrophosphorylase:

$$\underset{\text{Mannose-1-phosphate}}{\text{Man-P}} + \underset{\text{GTP}}{\text{G-R-P-P-P}} \rightleftharpoons \underset{\text{GDP-mannose}}{\text{G-R-P-P-Man}} + \underset{\text{Pyrophosphate}}{\text{P-P}}$$

Once the correct nucleoside diphosphate sugar has been synthesized, the sugar moiety is transferred in the presence of the appropriate nucleoside diphosphate sugar transferase to the suitable acceptor by the general reaction (9.2).

A few examples will suffice to illustrate the general scheme of synthesis. Two enzymes discovered by Leloir are concerned with the synthesis of sucrose in plants, UDP-glucose fructose transglycosylase catalyses the reaction:

$$\underset{\text{UDP – glucose}}{\text{U-R-P-P-Glu + Fructose}} \rightleftharpoons \underset{\text{Sucrose}}{\text{Glu-Fructose}} + \underset{\text{UDP}}{\text{U-R-P-P}} \qquad (9.3)$$

There is evidence that this enzyme, although it synthesizes sucrose because of its favourable K_{eq}, is actually involved in the degradation of sucrose with the formation of UDP-glucose (reaction going to the left) and the preservation of glycosidic bond energy. This provides a mechanism whereby sucrose can be broken down to UDP-glucose which can then enter other synthetic pathways.

A second enzyme concerned with sucrose synthesis, UDP-glucose fructose-6-phosphate-transglycosylase, catalyses the reaction:

$$\underset{\text{UDP – glucose}}{\text{U–R–P–P–Glu + Fructose–6–phosphate}} \rightleftharpoons \underset{\text{Sucrose-phosphate}}{\text{Glu–fructose–6–phosphate}} + \underset{\text{UDP}}{\text{U–R–P–P}}$$

In the presence of a specific phosphatase, sucrose-phosphate is dephosphorylated to form sucrose.

$$\underset{\text{Sucrose-phosphate}}{\text{Glu-fructose-6-phosphate} + H_2O} \rightleftharpoons \underset{\text{Sucrose}}{\text{Glu-fructose}} + H_3PO_4$$

This enzyme is responsible for the synthesis of sucrose in plants, since it involves the phosphatase step which is irreversible.

9.7 PENTOSE PHOSPHATE PATHWAY: HEXOSE MONO PHOSPHATE (HMP) SHUNT

The pentose phosphate pathway, to be described now contains two reactions capable of producing the reductant NADPH. Furthermore, this pathway also produces a number of different sugar phosphates (Figures 9.4a and 9.4b).

The existence of an alternate route for the metabolism of glucose is indicated by the fact that in some tissues the classical inhibitors of glycolysis, iodoacetate and fluoride, have no effect on the utilization of glucose.

9.7.1 Enzymes of the Pentose Phosphate Pathway

1. **Glucose-6-phosphate dehydrogenase:** The oxidative irreversible part of the pathway starts with the reaction catalysed by the enzyme glucose-6-phosphate dehydrogenase and the coenzyme $NADP^+$. The enzyme catalyses the following reaction:

$$\text{Beta D-Glucose – 6-phosphate} + NADP^+ \longleftarrow \underset{NADPH + H^+}{\text{6-Phosphoglucono-δ-lactone +}} \qquad (9.1)$$

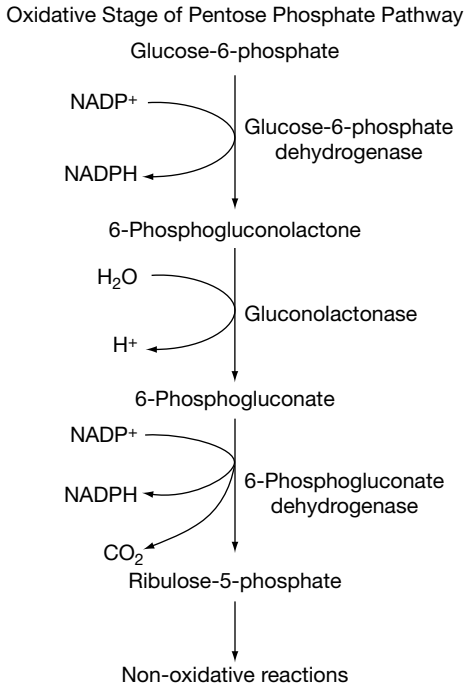


Figure 9.4(a) Oxidative stage of pentose phosphate pathway

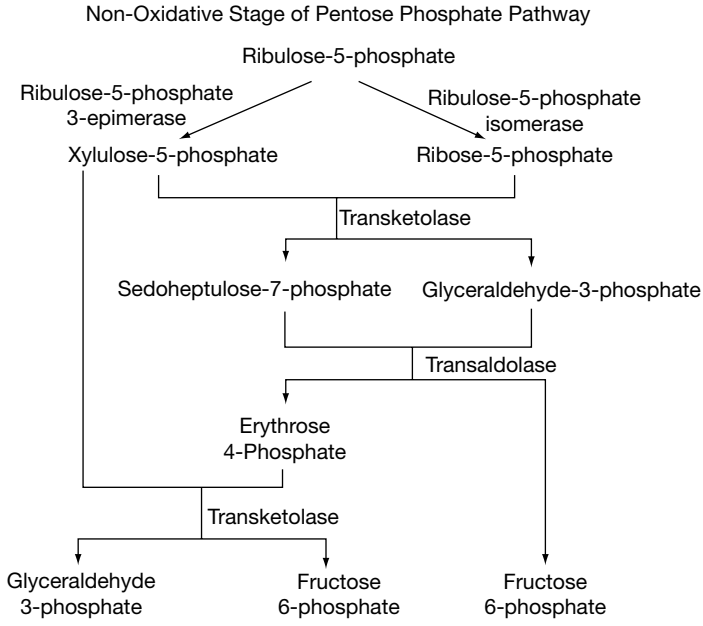


Figure 9.4(b) Non-oxidative stage of pentose phosphate pathway

Although the product was believed initially to be phosphogluconic acid, the δ-lactone of this acid is known to be the first product. The reaction as written is reversible because the oxidation of NADPH will proceed in the presence of the enzyme and the lactone. It is easy to visualize that the oxidation of the pyranosyl form of the substrate involves the removal of two hydrogen atoms to form the lactone. The lactone is unstable and hydrolyses spontaneously to 6-Phosohogluconic acid.

Not surprisingly, this reaction is subject to metabolic control; the $NADP^+$ required in the forward reaction is competitively inhibited by NADPH. The enzyme is also inhibited by fatty acids. Both types of inhibition are meaningful in terms of one of the functions of the pentose phosphate pathway.

2. **6-Phosphogluconolactonase:** Hydrolysis of the 6-phosphogluconic-δ-lactone produced in reaction (9.1) occurs readily in the absence of any enzyme. However, a lactonase that ensures rapid hydrolysis of the lactone also exists.

$$\text{6-Phosphoglucono–δ–lactone} + H_2O \xrightarrow{Mg^{2+}} \text{6-Phosphogluconic acid} \qquad (9.2)$$

The ΔG′ for the hydrolysis of the lactone is large; therefore, the overall oxidation of glucose-6-phosphate to phosphogluconic acid is irreversible.

Moreover, the next reaction also is irreversible, and together with reactions (9.1) and (9.2) constitute the irreversible phase of the pentose phosphate pathway.

3. **6-Phosphogluconic acid dehydrogenase:** This dehydrogenase was also included in the early work of Warburg, who showed that CO_2 was a product of a crude yeast extract which contained glucose-6-phosphate dehydrogenase.

Because the reaction involves both an oxidation and decarboxylation, it was suggested that a 3-keto-6-phosphogluconic acid might be an intermediate product prior to decarboxylation. No direct evidence in support of such a compound has been offered, and the reaction is hence believed to be a single step oxidative decarboxylation resulting in the formation of ribulose-5-phosphate. The $NADP^+$ dehydrogenase, which is widely distributed, requires Mn^{2+} or other divalent cations for activity. The reaction is not reversible.

$$\text{6-Phosphogluconic acid} + NADP^+ \xrightarrow{Mn^{2+}} \text{3-keto-6-phosphogluconic acid} + \text{D-ribulose-5-phosphate} + NADPH + H \qquad (9.3)$$

4. **Phosphoriboisomerase:** At the level of ribulose-5-phosphate, the carbon atoms of glucose enter the second or reversible part of the pentose phosphate pathway; however, all subsequent reactions of this part are readily reversible.

Initially, ribulose-5-phosphate undergoes two isomerization reactions to form products subsequently utilized in the pathway. Phosphoriboisomerase catalyses the interconversion of the keto sugar and the aldopentose phosphate, ribose-5-phosphate. This reaction is analogous in its action to the phosphohexose isomerase encountered in glycolysis. The K_{eq} for the reaction from left to right is approximately 3:

$$\text{D-ribulose 5-phosphate} \longleftrightarrow \text{D-ribose-5-phosphate} \qquad (9.4)$$

5. **Phosphoketopentose epimerase:** The second isomerization involving ribulose-5-phosphate is catalysed by the enzyme phosphoketopentose epimerase.

The K_{eq} is 0.8:

$$\text{D-ribulose 5-phosphate} \longleftrightarrow \text{D-xylulose-5-phosphate} \quad (9.5)$$

The mechanism for this reaction is not known although it probably involves the enediol as an intermediate.

6. **Transketolase:** Up to this point, the pathway has dealt with the oxidative degradation of the hexose chain of glucose-6-phosphate and the subsequent interrelations of the pentose phosphates produced. During the period in which these reactions were being studied it was apparent that other sugars, including heptoses, tetroses and trioses, were also formed. Some clarification of the relations between the pentoses and these other sugars resulted when the enzyme transketolase was discovered and described. This enzyme catalyses the transfer of the ketol group from a donor molecule to an acceptor aldehyde.

   In the specific instance, transketolase catalyses the transfer of a ketol group from xylulose-5-phosphate to ribose-5-phosphate to form sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate.

$$\text{D-xylulose-5-phosphate} + \text{D-ribose-5-phosphate} \xleftrightarrow[Mg^{2+}]{\text{TPP}} \text{D-sedoheptulose-7-phosphate} + \text{D-glyceraldehydes-3-phosphate} \quad (9.6)$$

   Transketolase consists of two identical subunits and utilizes both thiamin pyrophosphate (TPP) and Mg^{2+} as cofactors.

7. **Transaldolase:** This enzyme, like transketolase, functions as a transferring enzyme by catalysing the transfer of the dihydroxy acetone moiety of fructose-6-phosphate or sedoheptulose-7-phosphate to a suitable aldose. As represented in the scheme for pentose phosphate metabolism, the acceptor aldose may be glyceraldehyde-3-phosphate or, in the reverse direction, erythrose-4-phosphate:

$$\text{D-sedoheptulose-7-phosphate} + \text{D-glyceraldehyde-3-phosphate} \longleftrightarrow \text{D-fructose-6-phosphate} + \text{D-erythrose-4-phosphate} \quad (9.7)$$

   Ribose-5-phosphate may also be an acceptor, in which case an octose, octulose-8-phosphate, is formed. The enzymatic mechanism involves formation of an intermediate Schiff base between the carbonyl of the transferred dihydroxy moiety and an ε-amino group in a lysine residue in the enzyme. This mechanism is similar to that of aldolase in glycolysis. However, transaldolase cannot use free dihydroxy-acetone or its phosphate as substrates.

   Finally, to complete the pentose phosphate pathway, the erythrose-4-phosphate produced in reaction (9.7) can accept a C_2-unit from xylulose-5-phosphate in a reaction also catalysed by transketolase to form fructose-6-phosphate and glyceraldehyde-3-phosphate:

$$\text{erythrose-4-phosphate} + \text{D-xylulose-5-phosphate} \longleftrightarrow \text{D-fructose-6-phosphate} + \text{D-glyceraldehyde-3-phosphate} \quad (9.8)$$

9.7.2 Significance of the Pentose Phosphate Pathway

When the details of the pentose phosphate pathway were being clarified, it frequently was considered an alternate route for metabolism of glucose. For this pathway to function to any extent,

a mechanism was needed for reoxidizing the NADPH reduced in reactions (9.1) and (9.3). Since there were no coupled reactions for the reoxidation of NADPH (analogous to those in glycolysis where the reoxidation of NADH occurs), the oxidation was presumed to be carried out by the electron transport chain of mitochondria. When it was subsequently shown that NADPH, in contrast to NADH, is not readily oxidized by the respiratory chain, other roles for the NADPH produced and its reoxidation were sought.

It is now established that NADPH, in contrast to NADH, plays an important role as a reductant in many biosynthetic reactions. Whenever a biosynthetic step involves a reduction with a nicotinamide nucleotide, the coenzyme is, with few exceptions, NADPH. The pentose phosphate pathway in turn is a major mechanism for production of the NADPH.

As examples of this function, NADPH is specifically utilized in the biosynthesis of long chain fatty acids and steroids. It is the reducing agent employed in the reduction of glucose to sorbitol, the reduction of dihydrofolic acid to tetrahydrofolic acid and the reduction of glucuronic acid to L-gulonic acid. In addition, NADPH is used in the reductive carboxylation of pyruvic acid to malic acid by the malic enzyme. Finally, NADPH plays a unique role in hydroxylation reactions involved in the formation of unsaturated fatty acids, the conversion of phenylalanine to tyrosine and the formation of certain steroids. Evidence of the role of pentose phosphate pathway in producing NADPH for biosynthetic purposes is found in the fact that the 'pathway enzymes' are especially prominent in tissues such as adipose tissues, mammary gland or adrenal cortex that carry out these biosynthesis.

It is conceivable that an organism might have greater need for NADPH produced by the oxidative phase of the pentose phosphate pathway than for the pentoses that are simultaneously formed. The ready conversion of the pentoses so produced to hexose and triose clearly obviates any difficulties due to excess pentose. But it should be pointed out that one of the pentoses, ribose, is of course required by all cells for the synthesis of nucleic acids. Note that the pentose phosphate pathway needs no extra ATP, it is not dependent on metabolites from the Krebs cycle, and it may be regulated primarily by the demand for NADPH for biosynthesis, as NADPH is probably not oxidized tó any extent through the respiratory chain.

In addition, erythrose-4-phosphate (reaction 9.7) is required in the first step of a biosynthetic pathway in plants and microorganisms that leads to shikimic acid and, subsequently to several amino acids.

While the ribose-5-phosphate and erythrose-4-phosphate can be produced from glucose-6-phosphate by the irreversible oxidative phase, they can also be formed from fructose-6-phosphate and glyceraldehyde-3-phosphate by reversal of reactions (9.8), (9.7) and (9.6) thus the cell can use either an oxidative or non-oxidative process to form these important intermediates. We shall see that this latter process is also utilized by photosynthetic organisms to generate essential intermediates of the CO_2 reduction cycle of photosynthesis.

9.7.3 Entner-Doudoroff Pathway

Some bacteria (e.g., *Pseudomonas*, *Azotobacter* spp.) lack phosphofructokinase and therefore cannot degrade glucose by the glycolytic sequence. These organisms instead initiate glucose catabolism by producing 6-phosphogluconic acid by reactions (9.1) and (9.2). The acid then undergoes a dehydration and rearrangement to form an α-ketodeoxy sugar phosphate which in turn is cleaved by an aldolase-type enzyme into pyruvate and glyceraldehyde-3-phosphate.

Modification of this scheme permits other sugars (galactose) and sugar acids (D-glucuronic acid, D-galacturonic acid) to be metabolized, but an essential feature is the production of a 2-keto-3-deoxy intermediate which can be cleaved after phosphorylation.

9.8 CITRIC ACID CYCLE

In glycolysis, under aerobic conditions the end product is pyruvic acid. The citric acid cycle is also known as the Krebs cycle or the tricarboxylic acid cycle.

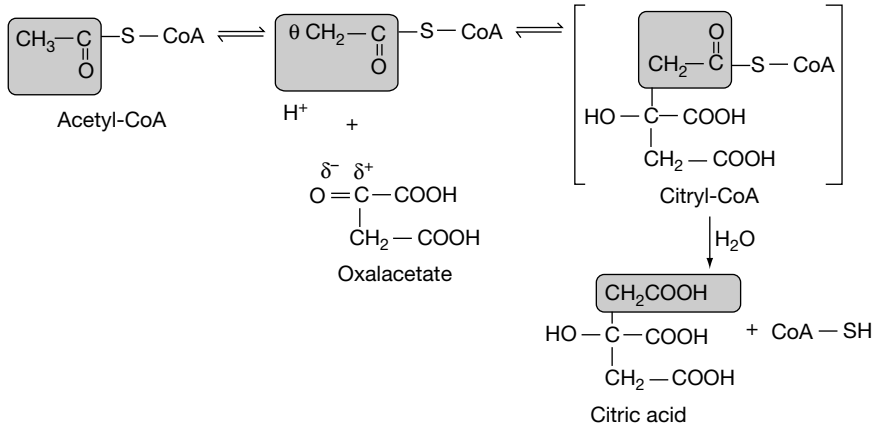
Pyruvic acid is not an intermediate in tricarboxylic acid cycle. The alpha-keto acid is first converted to acetyl-CoA by the multienzyme complex known as the pyruvic dehydrogenase complex. This conversion, which is an alpha-oxidation decarboxylation is carried on in the mitochondrion following the formation of pyruvic acid in the cytosol during glycolysis. The reaction involves six cofactors, namely coenzyme A, NAD^+, lipoic acid, FAD, Mg^{2+} and thiamine pyrophosphate (TPP).

$$CH_3COCOOH + CoA\text{-}SH + NAD^+ \xrightarrow[\text{TPP, FAD}]{\text{Lipoic acid, } Mg^{2+}} CH_3COSCoA + NADH + H^+ CO_2 \qquad (9.1)$$

$$\Delta G' = -8000 \text{ cal(pH 7.0)}$$

9.8.1 Reactions of the Tricarboxylic Acid Cycle (Citric Acid Cycle)

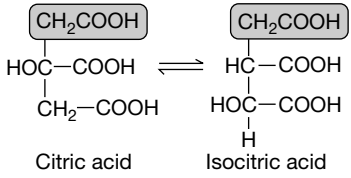
1. **Citrate synthase:** The enzyme that catalyses the entry of acetyl-CoA into the tricarboxylic acid cycle is known as citrate synthase (formerly condensing enzyme) and is found in the matrix of the mitochondrion. The two carbon atoms which originate from the acetyl-CoA are shaded in the reaction shown here and in subsequent reactions. The equilibrium constant for the reaction is 3×10^5; therefore, the equilibrium is far in the direction of citrate synthesis (as shown in reaction 1 that there is a formation of a carbon–carbon bond and free coenzyme A at the expense of the thioester). Indirect evidence indicates that citryl-CoA is formed as an intermediate on the enzyme but does not dissociate as such until it is cleaved to free citrate and coenzyme A.



(9.2)

The synthesis of citric acid is the first reaction in the Krebs cycle, thus it is the 'committed step' and subject to regulation. Citrate synthase is inhibited by high concentrations of NADH and succinyl-CoA. Both of these compounds bind to the mammalian enzyme to decrease the affinity of the enzyme for one of its substrates acetyl-CoA and thereby slow the reaction. ATP apparently is inhibitory due to its action on the accumulation of succinyl-CoA in the succinic thiokinase reaction; however, at high ATP concentration, the formation of GDP from GTP and ADP will be reversed. The product formed by citrate synthase, citric acid is in turn a regulator of enzymes in the glycolytic sequence.

2. **Aconitase:** The reaction of interest that is catalysed by aconitase is the interconversion of citric and isocitric acid.



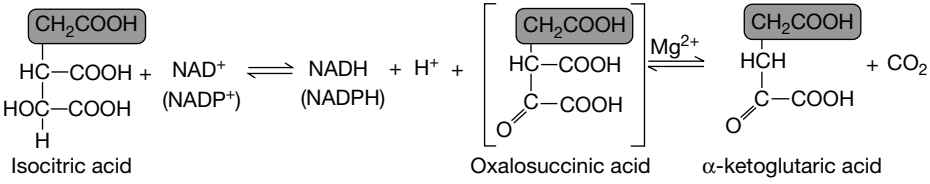
(9.3)

At equilibrium, the ratio of citric acid to isocitric acid is about 15.

Aconitase, which requires Fe^{2+} also catalyses an isomerization between citric acid, isocitric acid and a third acid, cis-aconitic acid. Indeed, cis-aconotic acid is frequently indicated as an intermediate in the conversion of citric to isocitric acid. The requirement for Fe^{2+} ion by the enzyme suggests that its role is in the formation of the carbonium ion by promoting the dissociation of the hydroxyl group.

Note that when isocitric acid is formed from citric acid (reaction 9.3) the symmetric molecule citric acid is acted upon in an asymmetric manner by the enzyme aconitase. By this we mean that the hydroxyl group in isocitric acid is located on a carbon atom derived initially from oxalacetate rather than the methyl group of acetyl-CoA.

3. **Isocitric dehydrogenase:** Isocitric dehydrogenase catalyses the oxidative beta-decarboxylation of isocitric acid to beta-ketoglutaric acid and CO_2 in the presence of a divalent cation (Mg^{2+} or Mn^{2+}); in this reaction, a nicotinamide nucleotide is the oxidant. It would be logical to consider this reaction as a result of an initial oxidation which produces oxalosuccinic acid and then a decarboxylation reaction on this beta-keto acid to form CO_2 and alpha-ketoglutarate.

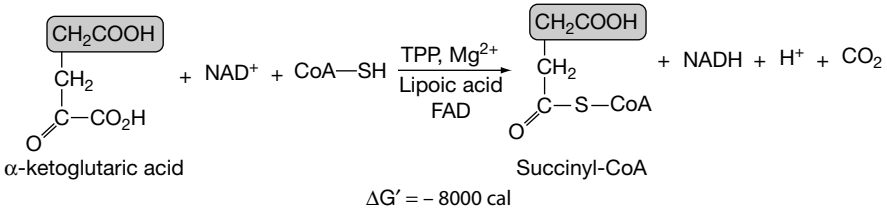


(9.4)

The evidence, however, indicates that oxalosuccinate, if formed is firmly bound to the surface of the enzyme and is not released as a free intermediate in either the oxidative decarboxylation of isocitrate or the reverse reaction, the reductive carboxylation of alpha-ketoglutarate. For this reason, the name isocitric enzyme has been proposed in analogy with the malic enzyme.

Most tissues contain two kinds of isocitric dehydrogenases. One of these requires NAD^{2+} and Mg^{2+} and is found only in the mitochondria. The other enzyme requires $NADP^{+}$ and occurs both in mitochondria and in the cytoplasm. The NAD^{+} specific enzyme is involved in the functioning of the tricarboxylic acid cycle; the mitochondrial $NADP^{+}$ requiring enzyme is associated with other, anabolic activities of the cycle.

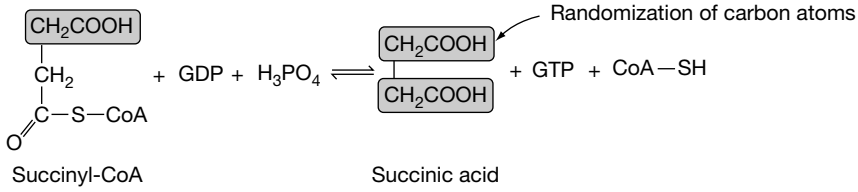
4. **Alpha-ketoglutaric acid dehydrogenase:** The next step of the tricarboxylic acid cycle involves the formation of succinyl-CoA by the oxidative alpha-decarboxylation of alpha-ketoglutaric acid. This reaction is catalysed by the alpha-ketoglutaric dehydrogenase complex, which requires TPP, Mg^{2+}, NAD^{+}, FAD, lipoic acid and coenzyme A as cofactors. The mechanism is analogous to that of the pyruvic acid dehydrogenase complex.



(9.5)

The reaction as a whole is not readily reversible because of the decarboxylation step. Succinyl CoA and NADH, produced in the reaction are inhibitory to the enzyme that produces them. The molecular weight of the *E. coli* enzyme complex is 2×10^6. Again, the transferase serves as the core protein.

5. **Succinic thiokinase:** In the preceding reaction, the high-energy bond of a thioester has been formed as the result of an oxidative decarboxylation. The enzyme succinic thiakinase catalyses the formation of a high-energy phosphate structure at the expense of the thioester (reaction 9.6).



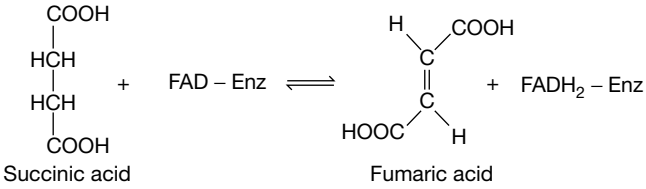
(9.6)

Since reaction (9.6) involves the formation of a new high-energy phosphate structure and the utilization of a thioester, the total number of high-energy structures on each side of the reaction is equal.

Therefore, the reaction is readily reversible, and the K_{eq} is 3.7. The GTP formed in reaction (9.6) can in turn react with ADP to from ATP and GDP in a reaction catalysed by a nucleoside diphosphokinase. Since the pyrophosphate linkages in GTP and ATP have approximately the same $\Delta G'$ of hydrolysis, the reaction is readily reversible with a K_{eq} of about 1:

$$GTP + ADP \rightleftharpoons GDP + ATP$$

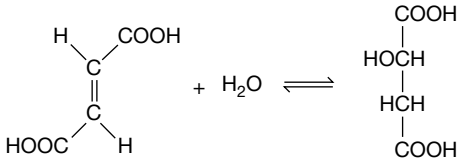
6. **Succinic delydrogenase:** This enzyme catalyses the removal of two hydrogen atoms from succinic acid to form fumaric acid.



Succinic acid Fumaric acid (9.7)

The immediate acceptor (oxidizing agent) of the electrons is a flavin coenzyme (FAD) which in contrast to other flavin enzymes, is bound to succinic dehydrogenase through a covalent bond. Succinic dehydrogenase is firmly associated with the inner mitochondrial membrane and is rendered soluble only with difficulty. The 'solubilized' preparations from beef heart and yeast contain 1 mole of flavin per mole of enzyme (200,000 mol. wt.) and four atoms of iron described as non-heme iron. Since the iron is not associated with a heme, as in the cytochromes, it is spoken of as 'non-heme' iron. Several such proteins are known now, and it is clear that they function in oxidation–reduction reactions, the iron atom being alternately oxidized and reduced. Succinic dehydrogenase is competitively inhibited by malonic acid.

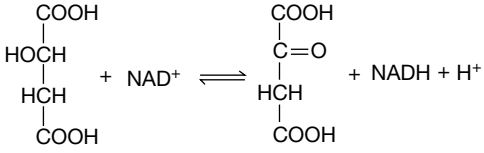
7. **Fumarase:** The next reaction is the addition of H_2O to fumaric acid to form L-malic acid.



Fumaric acid L-Malic acid (9.8)

The equilibrium for this reaction is about 4.5. The enzyme that catalyses the reaction, fumarase, has been crystallized (200,000 mol. wt.) from pig heart. It is a tetramer of four identical polypeptide chains.

8. **Malic dehydragenase:** The tricarboxylic cycle is completed when the oxidation of L-malic acid to oxalactic acid is accomplished by the enzyme malic dehydogenase. The reaction is the fourth oxidation–reduction reaction to be encountered in the cycle; the oxidizing agent for the enzyme from pig heart is NAD^+:



L-Malic acid (9.9)

At pH 7.0, the equilibrium constant is 1.3×10^{-5}; thus, the equilibrium is very much to the left. On the other hand, the further reaction of oxalacetate with acetyl-CoA in the condensation reaction (reaction (9.2)) is strongly exergonic, in the direction of citrate synthesis. This tends to drive the conversion of malate to oxalacetate by displacing the equilibrium through the continuous removal of oxalacetate.

The malic dehydrogenase of the mitochondrial matrix described above is distinct from its isoenzyme counterpart in the cytosol. The cytosolic malic dehydrogenase plays an important role in the production of NADPH from NADH in the cytosol.

Stoichiometry

The balanced equation for the complete oxidation of pyruvate to CO_2 and H_2O may be written as:

$$CH_3-\underset{\underset{O}{\|}}{C}-COOH + 2\tfrac{1}{2}\,O_2 \longrightarrow 3CO_2 + 2H_2O \qquad (9.10)$$

Since this is accomplished in a stepwise manner by the reactions of the tricarboxylic acid cycle (reactions 9.1 through reaction 9.9), it is useful to examine the stoichiometry in detail.

1. There are five oxidation steps: reactions 9.1, 9.4, 9.5, 9.7 and 9.9. In each of these, a pair of hydrogen atoms is removed from the substrate and transferred to either a nicotinamide to either a nicotinamide coenzyme or a flavin coenzyme.
   The reoxidation of these reduced coenzymes, five in all, by means of the cytochrome electron-transport system results in the reduction of five atoms or 2½ moles of oxygen.
2. When the five electron pairs are used to reduce O_2, 5 moles of H_2O are formed:

   $$½O_2 + 2H^+ + 2e^- \longrightarrow H_2O$$

   By inspection, one can see that 2 moles of H_2O have been consumed directly in reactions 9.2 and 9.8. To account for the net production of only 2 moles of H_2O in pyruvate oxidation (reaction 9.10), a third mole of H_2O must be accounted for. This is done by noting that GTP is produced from GDP and H_3PO_4 in reaction (9.6) of the cycle and that, in order to write the overall reaction of (9.10) as corresponding to the sum of reactions (9.1) through (9.9) the GTP produced in reaction (9.6) must be balanced out—it does not appear in reaction (9.10) by consuming a third mole of H_2O to convert the GTP back to GDP and H_3PO_4.
3. Finally, 3 moles of CO_2 are produced in the tricarboxylic acid cycle. These are equivalent to the three carbon atoms in the pyruvic acid, but not that only the CO_2 produced in reaction (9.1) arises directly from the pyruvic acid. The other two moles of CO_2 (reactions 9.4 and 9.5) have as their origin the two carboxylic groups of oxalacetate (note shading).
   All the reactions of the tricarboxylic acid cycle are reversible except the oxidative alpha-decarboxylation of alpha-ketoglutarate reaction (9.5). As pointed out earlier, this reaction is entirely analogous to the irreversible oxidation alpha-decarboxylation of pyruvic acid. This then means that the cycle cannot be made to proceed in a reverse direction, although individual sections are reversible (from oxalacetate to succinate or from alpha-ketoglutarate to citrate, for example). Similarly, acetyl-CoA and CO_2 cannot be converted to pyruvate by a reversal of reaction (9.1).

9.8.2 Regulation of the Tricarboxylic Acid Cycle

A continuing supply of oxidized NAD^+ is required to permit the Krebs cycle to operate. The enzymes of the electron-transport sequence carryout this vital activity and the concomitant process of oxidative phosphorylation. When these processes are inhibited, generally speaking, the Krebs cycle cannot function. But the rate at which it functions is under much finer control.

Pyruvic dehydrogenase, which provides a supply of acetyl-CoA for oxidation via the Krebs cycle, is inhibited when the level of NADH or acetyl-CoA builds up. Additional control may also be exerted by cyclic AMP produced when the concentration of ATP increases, for there is evidence that mammalian pyruvic dehydrogenase can be phosphorylated by a cyclic AMP-activated protein kinase. Phosphorylation of a seryl-group in one of the non-identical peptide chains of the pyruvate dehydrogenase inactivates the whole enzyme complex. Reactivation occurs when the ATP and thus the cyclic AMP concentration decreases, resulting in dephosphorylation of the enzyme by means of a phosphatase. The control mechanism would be analogous to that of glycogen synthetase where the phosphorylated form of the enzyme (phospho-pyruvic dehydrogenase) is the inactive form of the enzyme.

Citrate synthase is under fine control; both ATP and NADH can inhibit this initial reaction of the Krebs cycle. The inhibition of ATP and NADH on isocitric dehydrogenase have also been noted (point no. 3 of tricarboxylic cycle). Thus, the energy charge of the cell can readily affect the rate at which the tricarboxylic acid cycle operates.

Krebs cycle is the primary source of certain key biosynthetic intermediates of the cell. A prominent example is alpha-ketoglutarate formed in reaction (9.4). Alpha-ketoglutarate provides the carbon skeleton for the biosynthesis of glutamic acid, glutamine, ornithine (and therefore 5/6 of the carbon of citrulline and arginine) proline and hydroxyproline. Another essential intermediate is succinyl-CoA which is utilized in the synthesis of the porphyrins found in haemoglobin, myoglobin and the cytochromes. Other more specialized examples can be cited in this regard. For example, the citric acid that accumulates in the vacuoles of citrus species, or the isocitric and malic acids that are found in high concentration in certain sedums and in apple fruit would have their origin in the cycle.

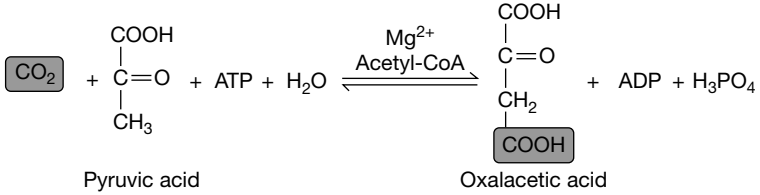
In order for alpha-ketoglutarate, or any of the other Krebs cycle intermediates just mentioned to function in an anabolic role, one simple requirement must be met. Both the C_2 unit (acetyl-CoA) and the C_4 unit (oxalacetate) that combine and give rise in the Krebs cycle to alpha-ketoglutarate (or other intermediate) must be provided in a stoichiometric amount equivalent to the alpha-ketoglutatate (or other intermediate) being removed for anabolic purposes. Thus, if a cell over a period needs to make 5.76 μmoles of glutamic acid from alpha-ketoglutarate, it must provide 5.76 μmoles of acetyl-CoA and 5.76 μmoles of oxalacetate.

This consideration immediately introduces the questions of the 'normal' sources of the C_2 and C_4 units the acetyl-CoA can be derived from pyruvate (reaction (9.1)) and therefore have its origin in carbohydrates that give rise to pyruvate in glycolysis. The C_2 unit can also be derived from fatty acids during beta-oxidation. The C_4 unit oxalacetate can be derived from a number of sources; we have considered its production from pyruvate through the action of pyruvic carboxylase (reactions in which conversion of pyruvic acid to oxaloacetic acid takes place, and in reaction 9.11). It could also be produced by the action of PEP-carboxykinase (reactions in which conversion of oxaloacetic acid to phosphoenol pyruvic acid takes place and reaction 9.12) although physiologically this reaction appears to take carbon atoms out of the Krebs cycle rather than into it. A very important source of oxalacetate is from the intermediates of the Krebs cycle itself. Thus, succinate, produced in the glyoxylic acid cycle soon to be described could provide the oxalacetate by its conversion to the C_4 unit via reactions 9.7, 9.8 and 9.9. Finally, oxalacetate can be produced by the transamination of aspartic acid.

9.8.3 Anaplerotic Reaction

The preceding section on the anabolic nature of the Krebs cycle has raised the questions of how the level of intermediates can be replenished when, for example, certain of those intermediates are removed for anabolic purposes. H. L. Kornberg has proposed the term anaplerotic for these replenishing or 'filling-up' reactions.

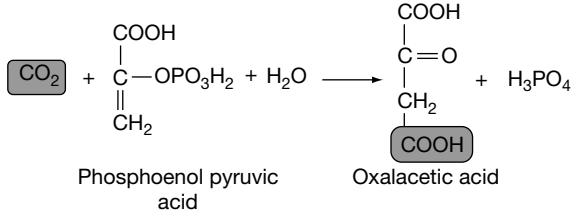
1. **Pyruvic carboxylase:** The single most important anaplerotic reaction in animal tissues is the one catalysed by pyruvic carboxylase, a mitochondrial enzyme.



(9.11)

The reaction it catalyses links intermediates of the glycolytic sequence and the tricarboxylic acid cycle.

2. **Phosphoenol pyruvic acid carboxylase (PEP-carboxylase) catalyses reaction (9.12).**



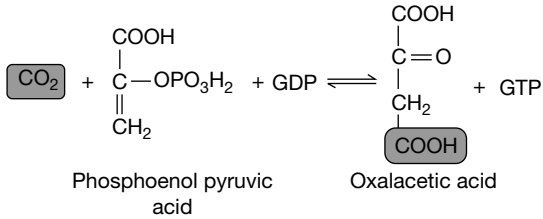
(9.12)

The enzyme requires Mg^{2+} for activity and the reaction is irreversible. Phosphoenol pyruvic acid carboxylase occurs in higher plants, yeast and bacteria (except *Pseudomonas*) but not in animals. It presumably has the same function as pyruvic carboxylase, namely, to ensure that the Krebs cycle has an adequate supply of oxalacetate.

Phosphoenol pyruvate → Oxalacetate → Aspartic acid

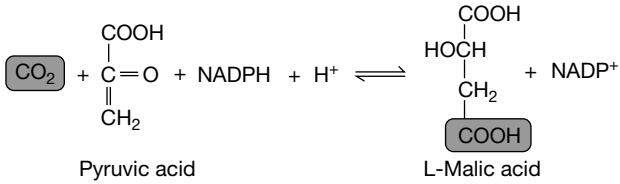
The reaction shown above is a simple means for synthesizing aspartate from PEP, and aspartate can control its own production by inhibiting the first step in the sequence.

3. **Phosphoenolpyruvic acid carboxykinase** in theory could catalyse the replenishment of oxalacetate from phosphoenol pyruvate. However, the affinity of the enzyme for oxalacetate is very great (Km = 2 × 10 − 6) while that for CO_2 is low. Thus, the enzyme favours phosphoenol pyruvate formation.



(9.13)

4. **Malic enzyme catalyses the reversible formation of L-malate from pyruvate and CO_2 and the K_{eq} for the reaction at pH 7 is 1.6.**



(9.14)

Malic enzyme is found in plants, in several animal tissues and in some bacteria grown on malic acid. The enzyme is believed to be significant because of its ability to produce NADPH required for biosynthetic purposes.

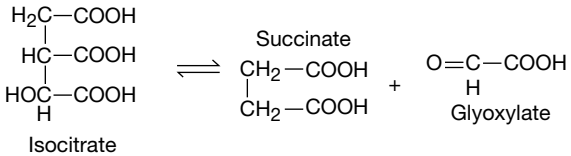
CO_2 fixation reactions: Reactions 9.11 through 9.14 are examples of 'CO_2- fixation reactions.' The initial observations that stimulated work on these reactions were made by Wood and Werkman in 1936. They observed that when propionic acid bacteria fermented glycerol to propionic and succinic acid, more carbon was found in the products than had been added as glycerol. Carbon dioxide, moreover, proved to be the source of the extra carbon atoms or the carbon that was 'fixed.' Today, the physiological significance of CO_2 fixation extends beyond the metabolism of propionic acid bacteria and includes not only the anaplerotic reactions listed above but also such enzymes as acetyl-CoA carboxylase propionyl CoA carboxylase and ribulose-1,5-diphosphate carboxylase.

9.9 THE GLYOXYLIC ACID CYCLE

Two major roles of the tricarboxylic acid cycle have now been described: the complete oxidation of acetyl CoA (and compounds convertible to acetyl-CoA) and multiple anabolic activities, e.g., the synthesis of glutamic acid succinyl-CoA aspartic acid. Since the reaction of the cycle (reactions 9.2 to 9.9) can only degrade acetate, there remains the basic questions of how some organisms (many bacteria, algae and some higher plants at a certain stage in their life cycle) can utilize acetate as the only carbon source for all the carbon compounds of the cell. Or put another way, since acetate can only be oxidized to CO_2 and H_2O by the Krebs cycle, how can acetate, in some organisms, give rise to carbohydrates as well as amino acids derivable from the tricarboxylic acid cycle?

This challenging problem was most successfully pursued by H. L. Kornberg, who together with others showed that acetate undergoes, in those organisms that convert acetate to carbohydrate, an anabolic sequence called the glyoxylate cycle (Figure 9.6). In effect, the glyoxylate cycle bypasses reactions 9.4 through 9.8 of the Krebs cycle, thereby omitting the two reactions in which CO_2 is produced (reactions 9.4 and 9.5). The bypass consists of two reactions whereby, (1) isocitrate is split into succinate and glyoxylate and (2) glyoxylate reacts with another acetyl-CoA to form malate.

Consider first the two reactions. Instead of isocitrate being oxidized, it is cleaved by the enzyme isocitritase (isocitrate lyase) to form succinic acid and glyoxylic acids.



(9.15)

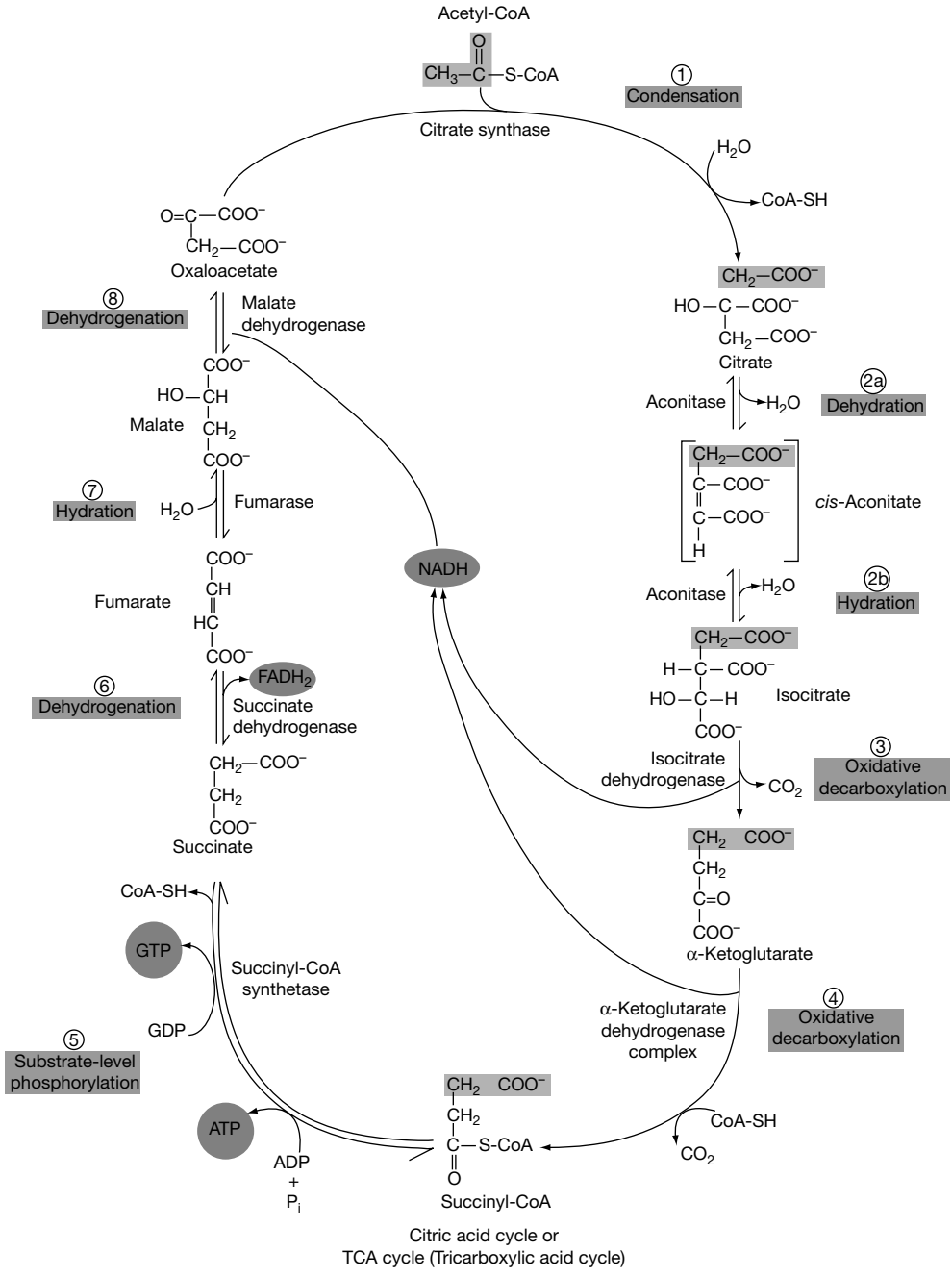


Figure 9.5 Citric acid cycle: Citric acid cycle oxidation takes place in mitochondria

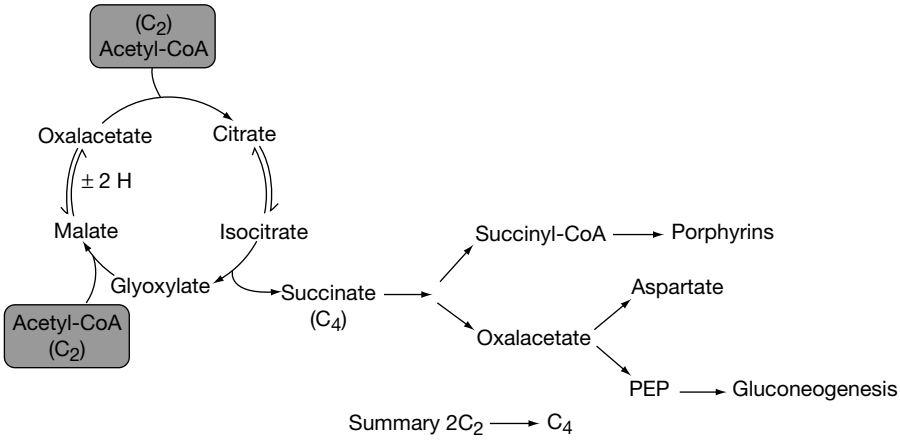
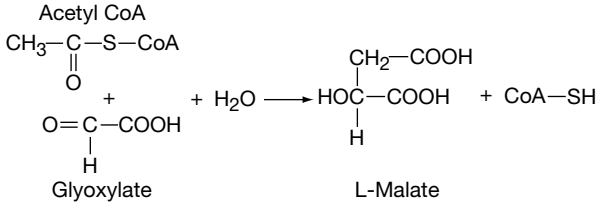


Figure 9.6 The glyoxylate cycle, showing the formation of one mole of succinate from two of acetate (as acetyl-CoA)

The glyoxylic acid formed is then condensed with 1 mole of acetyl-CoA to produce L-malic acid in a reaction analogous to that of citrate synthase (reaction 9.2) discussed earlier. The enzyme involved is called malate synthase:



(9.16)

While these two reactions bypass the decarboxylation steps of the Krebs cycle, this itself does not constitute a cycle until they are written together with the reactions catalysed by malic dehydrogenase (reaction 9.9), citrate synthase (reaction 9.2) and aconitase (reaction 9.3). Together the five enzymes constitute the glyoxylate cycle and accomplish the conversion of 2 moles of acetate (as acetyl-CoA) to succinic acid (Figure 9.6).

The full significance of the glyoxylate cycle can now be appreciated by realizing that succinate, as a product of the cycle, can undergo reactions which have been previously described. For example, the succinate can be converted to succinyl-CoA (reaction 9.6) and serve as a precursor of porphyrins. The succinate can be oxidized to oxalacetate via reactions 9.7 through 9.9 and can be utilized for the synthesis of aspartic acid and other compounds (e.g., pyrimidines) derived from aspartic acid. The oxalacetate can be converted to PEP and can undergo the reactions of gluconeogenesis. Finally, the oxalacetate could condense with acetyl-CoA (reaction 9.2) and meet the requirements for the Krebs cycle to function in an anabolic manner.

Those tissues of higher plants that have a functioning glyoxylate cycle contain organelles (glyoxysomes) having the five enzymes required for the cycle to operate. It is interesting to note that the glyoxysome appears in the cotyledons of high-lipid seeds shortly after germination begins and at a time when lipids are being utilized as the major source of carbon for carbohydrate

synthesis. Thus the high-lipid seed (e.g., peanut, castor bean, etc.) can convert lipid to carbohydrate synthesis which animals and most plants are incapable of performing since they lack the glyoxylate cycle.

9.10 GLYCOGENESIS

The process used in the synthesis of glycogen is called as glycogenesis. In this process of glycogen synthesis, addition of glucose molecules to the chains of glycogen takes place for storage. During rest glycogenesis is activated and follows the Cori cycle in the liver. Glycogenesis is also activated by insulin.

9.10.1 Steps of Glycogenesis Pathway

- The enzyme glucokinase converts glucose into glucose-6-phosphate.
- The enzyme phosphoglucomutase converts glucose-6-phosphate to glucose-1-phosphate, passes through an intermediary step of glucose-1,6-bisphosphate.
- The next step in glycogenesis is that the enzyme uridyl transferase or UDP-glucose pyrophosphorylase converts glucose-1-phosphate to uridine diphosphate glucose and pyrophosphate. The enzyme pyrophosphatase hydrolyses pyrophosphate into two molecules of Pi.
- The enzyme glycogen synthase assembles glucose molecules in a chain and promotes the transfer of glucose molecule from uridine diphosphate glucose to the non-reducing end of branched glycogen molecule. Glycogen synthase alone cannot initiate the synthesis of new glycogen chain, it requires 'glycogenin' (intriguing protein) a primer to initiate a new chain of glycogen.

The initial step in the glycogen chain synthesis is the transfer of a glucose molecule from uridine diphosphate glucose to the hydroxyl group of tyrosine of glycogenin and this reaction is catalysed glucosyltransferase activity (this enzyme is a protein intrinsic in glycogenin). The extension of the new chain takes place by glycogenin (by its chain extension activity) by addition of seven more glucose molecules each from uridine diphosphate glucose and further extension of the chain takes place by the enzyme glycogen synthase (Figure 9.7).

The enzyme glycogen synthase cannot make α (1:6) bonds present at branching point of the glycogen chain. For branching, the branching enzymes are present known as amylo-α (1:4)$\rightarrow\alpha$ (1:6) transglycosylase. This branching enzyme act on the chain after it has been sufficiently lengthened by the synthetase. In the growing glycoside chain with glucose units linked by 1,4-glycosidic linkage, this enzyme will remove chain fragments of 6 or 7 glucose units and reattach the fragment, at spaces of 8 to 12 glucose units apart on the original chain by α-1:6 linkage to form branches (Figure 9.8).

9.11 GLYCOGENOLYSIS

Glycogenolysis is the breakdown of glycogen polymers to glucose monomers.

9.11.1 Reaction

Three steps are involved in glycogenolysis as discussed hereunder.

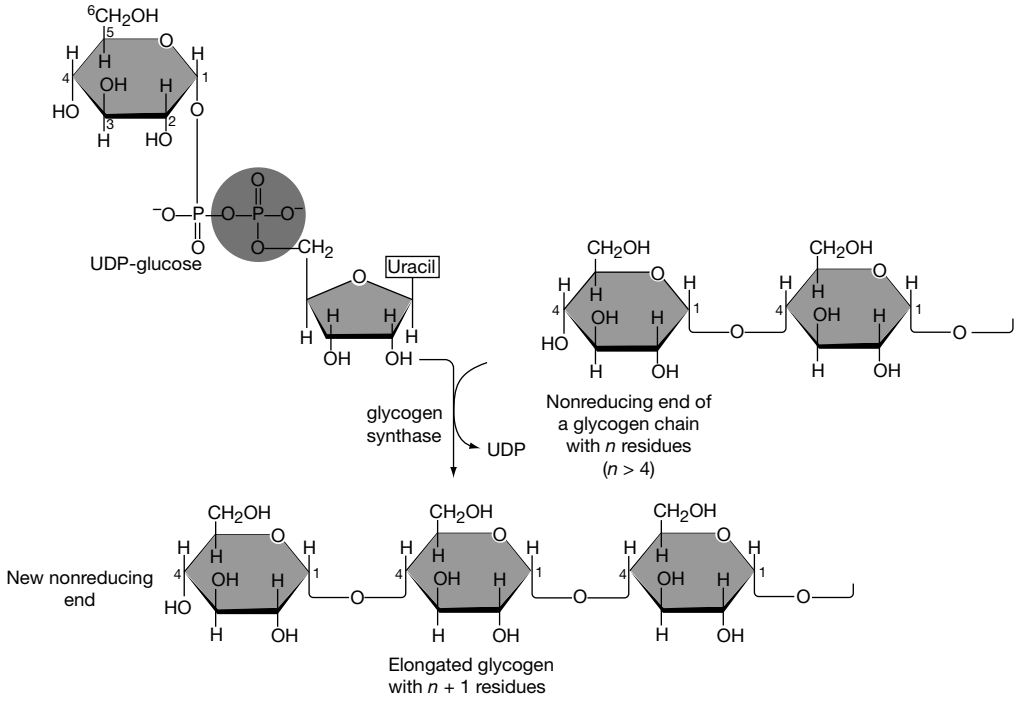


Figure 9.7 Glycogenesis pathway leads to glycogen synthesis by addition of glucose mole to the non-reducing end of a glycogen branch to make a new (α1–4) linkage with the help of the enzyme glycogen synthesis

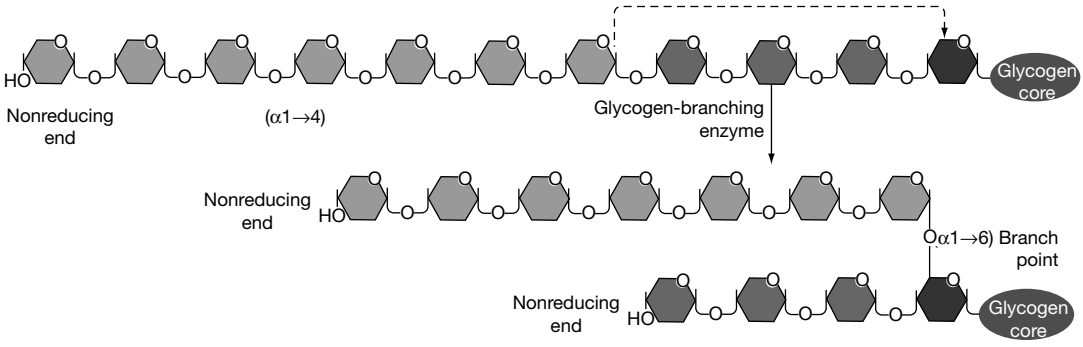


Figure 9.8 Glycogenesis is the process of glycogen synthesis, in which glucose molecules are added to chains to form glycogen for storage

First step: The overall reaction for the first step is:

Glycogen (n residues) + Pi ↔ Glycogen (n-1 residues) + G1P

In this step glycogen phosphorylase catalyzes the reaction in which α-1-4 glycosidic linkages between two glucose molecules is attacked by Pi at non-reducing end of glycogen and cleaves the terminal glucose molecule as alpha-D-glucose-1-phosphate. In this reaction pyridoxal phosphate is an essential cofactor because its phosphate group act as acid catalyst to promote attack on glycosidic bond by Pi (inorganic phosphate) (Figure 9.9). The glycogen phosphorylase enzyme can act on non-reducing ends of glycogen branches up to four glucose molecules are left before the alpha 1-6 branching point reached, then it stops its activity.

Second step: The 2nd step involves the debranching enzyme oligo α1-6 to α1-4 glucan transferase catalyzes two succeeding reactions. First the transferase activity of the enzyme which transfers the three remaining glucose residues from branch to another 1,4 terminal of glycogen, where they reattached in α1-4 linkage. Then enzyme α1-6 glucosidase activity cleaves the single glucose residue remaining at the branch point as free glucose. Once these branches are transferred, the remaining glucose attached at the α[1→6] branch gets hydrolysed by this enzyme.

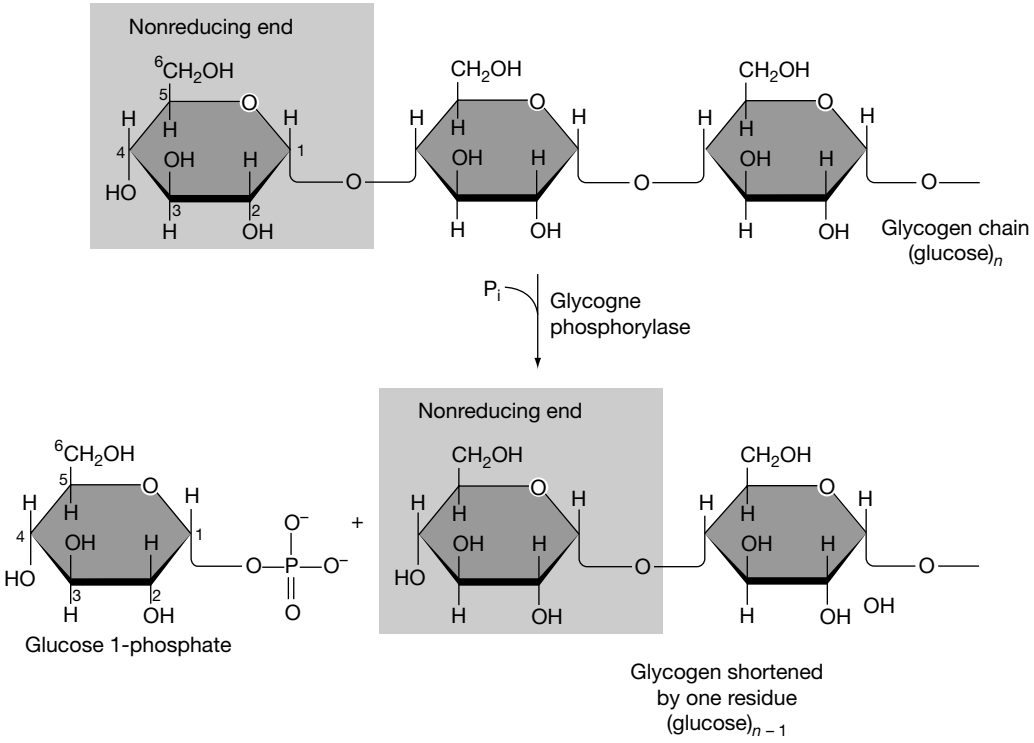


Figure 9.9 Glycogenolysis (also known as 'glycogenlysis') is the conversion of glycogen polymers to glucose monomers

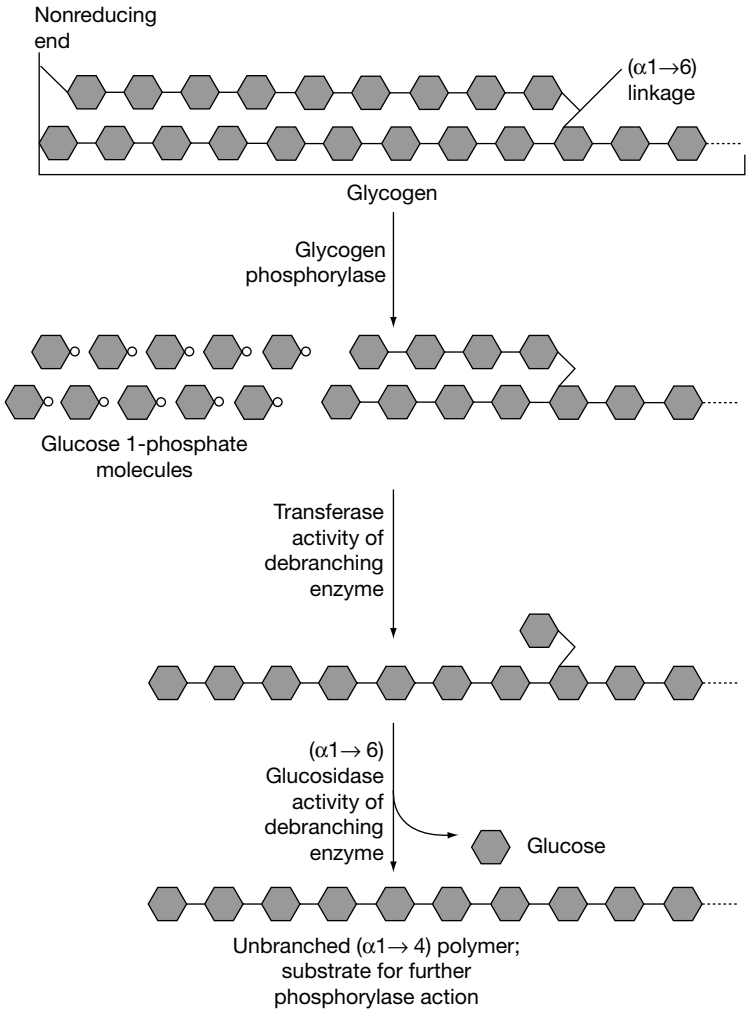


Figure 9.10 Glycogen breakdown near an (<1–6) branch point

After these activities of debranching enzymes, again the glycogen phosphorylase activity starts (Figure 9.10).

Third step: In the last step, the end product of the glycogen phosphorylase enzyme which is G1P (glucose-1-phosphate) is converted to G6P (glucose-6-phosphate) by the enzyme phosphoglucomutase. The reaction begins with the enzyme phosphorylated on the serine residue, first the enzyme donates its phosphoryl group to G1P producing G6P, then in next step the phosphoryl group at C-1 of glucose-1-6-bisphosphate is transferred back to the enzyme reforming the phosphoenzyme and producing G6P (Figure 9.11).

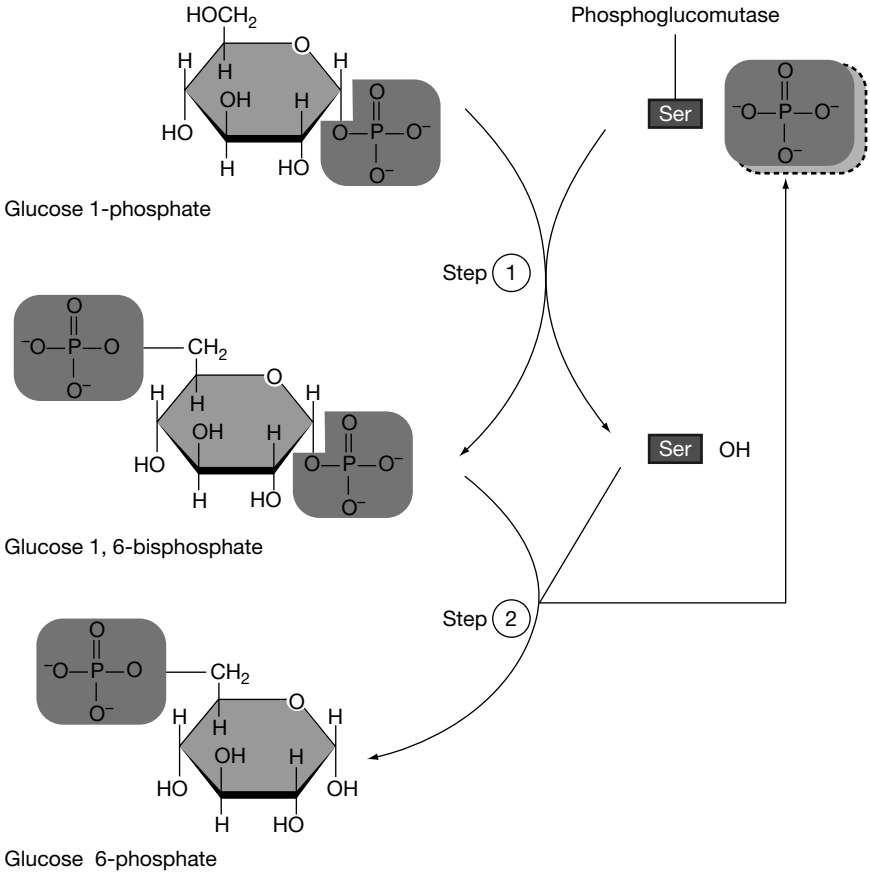


Figure 9.11 Last step of glycogenolysis which converts glucose-1-phosphate to glucose-6-phosphate by the enzyme phosphoglucomutase

9.11.2 Regulation

The key regulatory enzyme of the process of glycogenolysis is glycogen phosphorylase: Phosphorylation → activation

Dephosphorylation → inhibition

9.12 REGULATION OF BLOOD GLUCOSE

Carbohydrate Tolerance: The students will realize from the previous discussion of carbohydrate metabolism that the capacity of an animal to metabolize carbohydrate is dependent upon many factors. This capacity for carbohydrate utilization is commonly evaluated by the so-called carbohydrate tolerance tests, and the results are expressed in the form of tolerance curves. There

are a number of variations in the procedure of conducting tolerance tests, but all depend upon the administration of a definite quantity of glucose, with blood sugar determinations at definite time intervals thereafter. The blood sugar is determined previous to sugar administration in order to provide a basal value. The blood sugar values are then plotted against time to give the tolerance curve.

The so-called standard glucose tolerance test is conducted on patients as follows. The patient, who for three days has eaten a diet containing at least 300 g of carbohydrate per day, reports in the morning without breakfast (post-absorptive state). A sample of venous blood is taken for analysis and immediately 100 g of glucose in 300 ml of water is given by mouth. Blood samples for sugar analysis are taken at one-half-, one-, two- and three-hour intervals after the sugar is given. In general the post-absorptive blood sugar of a normal individual is below 100 mg per cent before the sugar is given, does not rise above 160 mg per cent and falls to normal values within two hours. The diabetic generally has a post-absorptive blood sugar above 100 mg per cent, which rises higher than normal after sugar is given and remains elevated for a longer period of time. Minor individual variations in both normal and diabetics are observed. Tolerance curves run on arterial blood from the fingertip are similar to those on venous blood, except that all the sugar values are higher. The difference between the arterial and venous curves is a measure of the rate of sugar utilization in the tissues. This difference decreases with decreased capacity for utilization.

The blood sugar values of a tolerance test are determined by the balance between the rate of sugar absorption from the intestine and the rate at which sugar is removed from the blood by the metabolic processes. As previously indicated, the absorption rate may be increased in hyperthyroidism and decreased in hypothyroidism, and tests run under these conditions must be interpreted accordingly. The difficulty may be circumvented by administering the sugar intravenously, which, however, gives a different type of curve.

Some of the major factors, other than diabetic states, which influence carbohydrate tolerance, are discussed hereunder.

Carbohydrate starvation: It is well established that starvation or the ingestion of high-fat diets lowers the capacity of the body to utilize carbohydrate, and the ingestion of sugar or high-carbohydrate meals after prolonged carbohydrate, and deprivation may cause hyperglycaemia and glucosuria. Tolerance tests conducted under such conditions may give typical diabetic curves. Accordingly, a patient should have a liberal supply of carbohydrate in his or her diet for a few days before a tolerance test is run. Since it has been demonstrated that secretion of insulin by the pancreas is related to blood sugar level, increasing with hyperglycaemia, it would appear reasonable to assume that the decrease in capacity to utilize carbohydrate as a result of carbohydrate starvation is related to decreased insulin secretion. Although this may be part of explanation, other factors are concerned. Starvation diabetes represents not only some decreased capacity to secrete insulin, but also decreased efficiency of enzyme systems concerned with carbohydrate metabolism.

Effect of exercise: Severe exercise for an hour and a half immediately after the ingestion of glucose decreases the hyperglycaemia and hastens return of the blood sugar to normal levels. This effect appears to be due to increased carbohydrate utilization as the result of the exercise. Violent exercise just before ingestion of glucose, however, exaggerates the hyperglycaemia. The exaggerated hyperglycaemic response due to exercise before glucose is abolished by the ingestion of large amounts of carbohydrate on the preceding day.

Hyperinsulinism: Greatly accelerated utilization of carbohydrate is observed in cases of so-called hyperinsulinism, which may be due to benign or malignant tumours of the islets of Langerhans or to functional hypertrophy and hyperplasia of the islets. Excessive amounts of insulin are secreted under these conditions. Such cases may suffer severe hypoglycaemic attacks, which can be alleviated by the administration of carbohydrate. However, in the long-term treatment it has been found best to provide dietary carbohydrate in slowly assimilable forms (starches) and to give a rather high protein diet so that glucose may be slowly formed from the absorbed amino acids. The objective is to avoid stimulation of the pancreas by alimentary hyperglycaemia as much as possible.

Diseases of the liver: Cases of acute yellow atrophy, infectious hepatitis and other extensive diseases of the liver exhibit abnormal carbohydrate metabolism because of failure of liver function. In such cases the reduced capacity of the liver to form and store glycogen is likely to result in hypoglycaemia. The tolerance curves are characterized by a low initial post-absorptive blood sugar level (hypoglycaemia) which after glucose is taken, may resemble the curves of the diabetes mellitus. The deficiency here resides in the failure of the liver to convert glucose to glycogen and not in the general capacity to oxidize it in the peripheral tissues.

Some types of glucagon storage disease show abnormal glucose tolerance curves.

Acute and chronic infections: That a great variety of infections may decrease carbohydrate tolerance is well established. Hyperglycaemia, glucosuria and reduced carbohydrate tolerance characteristic of moderately severe diabetes may be associated with pyodermic infections such as carbunculosis and furunclosis. The sugar tolerance curve in most infections is generally more or less of the diabetic type, but becomes normal when the infection is cleared up.

Thiamine deficiency: Although various vitamin deficiencies may contribute to some extent to decreased carbohydrate tolerance, only in case of severe thiamine deficiency is a direct effect apparent. Under these conditions the glucose tolerance curves are abnormally elevated and prolonged. Such an effect is probably related to the function of thiamine pyrophosphate as coenzyme in the oxidation of pyruvic acid.

Nervous disorders: Claude Bernard discovered that puncture of the floor of the fourth ventricle of the brain induces glucosuria, and this condition is referred to as piqure diabetes. However, a great variety of other injuries to the brain have been found to cause hyperglycemia and glucosuria. In hypophyseal cachexia or Simmond's disease, which is caused by atrophy or degeneration of the anterior lobe of the pituitary, the blood sugar falls to hypoglycaemic levels. This effect would be expected as a result of decreasing the anti-insulin effects of the anterior pituitary hormones.

Hypoglycaemia and abnormal carbohydrate tolerance may be associated with certain disturbances of the sympathetic nervous system such as neurocirculatory asthenia, vagotonia and various neuroses. The glucose tolerance curves are abnormally flat (plateau curves) and fall to mild hypoglycaemic levels several hours after glucose is given.

Effects of anaesthetics and other drugs: General anaesthetics and ether in particular raise the blood sugar and diminish carbohydrate utilization. This hyperglycaemic effect appears to be due to largely to increased epinephrine secretion with resultant increased liver glycogenolysis. Morphine also causes hyperglycaemia through stimulation of the adrenal medulla. As expected, ephedrine and other drugs resembling adrenaline exert a similar effect upon carbohydrate metabolism, though they generally have less hyperglycaemic action when given in therapeutic doses.

Dinitrophenol, which exerts a general calorigenic action upon metabolism, has been found to increase blood sugar and lactic acid and to increase the rate of carbohydrate oxidation.

9.13 CARBOHYDRATE METABOLIC DISORDER AND DISEASES

9.13.1 Diabetes Mellitus

Primary Idiopathic or Essential Diabetes

Diabetes mellitus means hyperglycaemia due to lack of insulin. This also takes place due to the overproduction of other hormones, for example, glucagons hormones of the anterior pituitary, adrenal and thyroid which are antagonists to insulin or due to increased production of insulinase which inactivates insulin. In India, males are more prone to diabetes mellitus than females.

Treatment of Diabetes Mellitus

Administration of oral anti-diabetic drugs like diabenese—which are not polypeptides—in moderate diabetes and insulin in severe diabetes.

High protein and low carbohydrate and fat diet are advisable. High protein meal serves as a prolonged source of carbohydrate without rapid hyperglycaemic effect and it has protective effect on liver.

Exercise lowers the blood sugar level stimulating the β-cells of pancreas to liberate more insulin to act on blood sugar for glycogenesis and glycolysis.

The patient suffering from diabetes mellitus should have normal psychological affairs. The psychological stress and strain stimulate the alpha cells, of pancreas and adrenal medulla causing the liberation of glucagons and epinephrine, respectively, which have glycogenolytic effect resulting in increased blood sugar (hyperglycaemia).

A diabetic patient requiring more than 200 units/day of insulin is regarded as insulin resistant which is due to antigenic effect of insulin.

9.13.2 Hereditary Disorders of Glycogen Metabolism

von Gierke in 1929 first reported an anatomopathological description of glycogen storage disease, in which large amounts of glycogen are stored in the liver.

The glycogen storage diseases arise because of the congenital absence or deficiency or enzymes involved in glycogen metabolism. In type 1, von Gierke's disease proper, glucose-6-phosphatase is absent, leading to the deposition of excess glycogen of normal structure in the liver and kidneys.

There are various clinically recognized types of glycogen storage disease, each due to specific enzyme deficiency. In several types of the disease, the structure of the glycogen present is abnormal. For example, a deficiency of amylo-1, 6-glucosidase produces glycogen with abnormally short branches, and a deficiency of amylo-(1,4 $\rightarrow$ 1,6)-transglucosylase (branching enzyme) forms glycogen with few branch points.

Table 9.1 gives the types of glycogen storage disease and their enzyme deficiencies. A case of Hers' disease has been reported with a deficiency of both liver phosphorylase and glucose-6-phosphatase.

Table 9.1 Types of Glycogen Storage Disease

Cori Type	Name of the Disease	Glycogen Structure	Enzyme Defect	Organs Involved
I	von Gierkes disease	Normal	Glucose-6-Phosphatase	Liver, kidney Generalized
II	Pompe's disease	Normal	Acid maltase ?	
III	Cori's, Forbes disease, limit Dextrinosis	Short outer branches	Amylo-1, 6-Glucosidase (debrancher)	Liver, heart, muscle
IV	Andersen's disease	Few branch points	Amylo-(1,4 to 1, 6)-Transglucosylase (brancher)	Liver, heart, Muscle, erythrocytes Others
V	McArdle's. disease	Normal	Muscle Phosphorylase	Muscle
VI	Hers' disease	Normal	Liver phosphorylase	Liver

9.14 SUMMARY OF CARBOHYDRATE METABOLISM

The pathways used in carbohydrate metabolism are shown in the following diagram.

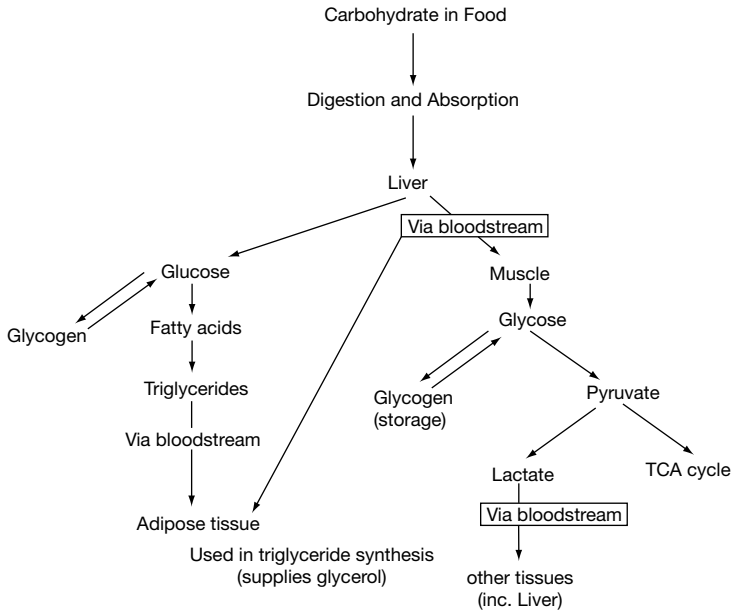


Figure 9.12 Summary of carbohydrate metabolism

EXERCISES

1. Write the fates of pyruvate after glycolysis.
2. Explain in detail the anabolic nature of the Krebs cycle.
3. Write the importance of **Entner-Doudoroff Pathway.**
4. Write the utilization/metabolism of galactose by yeast and animal liver cells.
5. How the glycolysis is regulated by hexokinase?
6. Explain Cori's cycle with the help of the diagram.
7. Draw a flow diagram showing the reactions of Krebs cycle. Discuss its importance.
8. What is Krebs cycle? Why is it so called? What is the alternate name of the cycle? Justify the name. State very briefly the steps of the Krebs cycle.
9. Discuss the various steps of TCA cycle and point out where energy, water and CO_2 are formed. How many molecules of ATP are produced in the cycle?
10. Describe schematically the citric acid cycle.
11. Describe the pentose phosphate pathway of carbohydrate metabolism and the significance of it.
12. Give an account of the citric acid cycle and explain why it is called a common metabolic pathway.
13. Discuss the oxidation of glucose in the tissues up to the stage of pyruvic acid with particular reference to the energy rich phosphate bonds.
    (a) Glucogenesis
    (b) Glycogenesis
    (c) Glycogenolysis

MULTIPLE CHOICE QUESTIONS

1. Glycogen synthetase activity is decreased by
   (a) Glucose
   (b) Insulin
   (c) Cyclic AMP
   (d) Fructokinase

   Ans. c
2. Glucose is removed from the blood following a meal by
   (a) Hexokinase
   (b) Glucokinase
   (c) Both (a) and (b)
   (d) None of these

   Ans. b
3. The branching enzyme acts on the glycogen when the glycogen chain has been lengthened between glucose units.
   (a) 1 and 6 (b) 2 and 7
   (c) 3 and 9 (d) 6 and 11

   Ans. d
4. Hexokinase has a high affinity for glucose than
   (a) Fructokinase
   (b) Galactokinase
   (c) Glucokinase
   (d) All of these

   Ans. c

5. Citrate is converted to isocitrate by aconitase which contains
   (a) Ca^{++} (b) Fe^{++}
   (c) Zn^{++} (d) Mg^{++}

   Ans. b

6. This process of partial oxidation of glucose to lactate in muscle, then it is converted back to glucose in liver and then again supplying it to muscles. This cooperative cycle which utilizing both the muscle and liver tissues is known as the
   (a) Cori cycle
   (b) Glycolysis
   (c) Gluconeogenesis
   (d) None of these

   Ans. a

7. The enzyme glycogen synthase cannot make α–(1:6) bonds present at branching point of the glycogen chain. For branching, the branching enzymes are present known as
   (a) Amylo-α (1:4)->α (1:6) transglycosylase
   (b) Amylo-α (1:4)->α (1:6) transketolase
   (c) Amylo-α (1:4)->α (1:6) synthetase
   (d) None of these

   Ans. a

8. Thus, if a cell over a period needs to make 5.76 μmoles of glutamic acid from alpha-ketoglutarate, it must provide 5.76 μmoles of acetyl-CoA and
   (a) 5.56 μmoles of oxalacetate
   (b) 5.76 μmoles of oxalacetate
   (c) 5.66 μmoles of oxalacetate
   (d) None of these

   Ans. b

9. Exercise lowers the blood sugar level stimulating the β-cells of pancreas to liberate more insulin to act on blood sugar for
   (a) Glycogenesis and glycolysis
   (b) Glycogenolysis and glycolysis
   (c) Glycogenesis and glycogenolysis
   (d) None of these

   Ans. a

10. Thus the high-lipid seed (e.g., peanut, castor bean, etc.) can convert lipid to carbohydrate synthesis which animals and most plants are incapable of performing since they lack
    (a) Glyoxylate cycle (b) Krebs cycle
    (c) Glycolysis (d) None of these

    Ans. a

11. Transketolase consists of two identical subunits and utilizes both thiamine pyrophosphate (TPP) and
    (a) Ca^{2+} as cofactors
    (b) Mg^{2+} as cofactors
    (c) Mn^{2+} as cofactors
    (d) None of these

    Ans. b

12. Morphine also causes hyperglycaemia through stimulation of the
    (a) Adrenal medulla
    (b) Adrenal cortex
    (c) Thymus
    (d) None of these

    Ans. a

13. Gluconeogenesis begins in the mitochondria due to the presence of the enzyme
    (a) Pyruvic carboxylase
    (b) Pyruvic synthetase
    (c) Both (a) and (b)
    (d) None of these

    Ans. a

14. von Gierke's disease is due to deficiency of the enzyme
    (a) Glucose-6-Phosphatase
    (b) Acid maltase
    (c) Muscle phosphorylase
    (d) Liver pshosphorylase

    Ans. a

15. Pompe's disease is due to deficiency of the enzyme
    (a) Glucose-6-Phosphatase
    (b) Acid maltase
    (c) Muscle phosphorylase
    (d) Liver phosphorylase

    Ans. b

10 Metabolism of Lipids

CHAPTER OBJECTIVES

10.1 Introduction
10.2 Functions of Tissues in Lipid Metabolism
10.3 Fatty Acid Transport into Mitochondria
10.4 Fatty Acid Oxidation
10.5 Fatty Acid Biosynthesis
10.6 Cholesterol Metabolism
10.7 Classification of Plasma Lipoproteins and the Systemic Pathway of Lipids
10.8 Biosynthesis of Triacylglycerols
10.9 Glycerophospholipid Biosynthesis
10.10 Eicosanoids
10.11 Metabolic Disorder of Lipids: Disturbances in Lipid Metabolism

10.1 INTRODUCTION

The metabolism of lipids embraces the metabolism of free fatty acids, fats, phosphatides, sterols and other complex lipids. Although a great deal of information on lipid metabolism has been gained in recent years, much is still unknown on this subject.

The fats are of particular importance, because they represent the bulk of stored energy-producing food in the body. Fats are stored in the adipose tissues or fat depots, of which the fatty subcutaneous layer (panniculus adiposus) is generally the most important. Other depots include the omentum, mesentery, intermuscular connective tissues and connective tissues around organs such as kidneys and heart. These adipose tissues may contain as much as 90 per cent fat (triglycerides). Since the storage of carbohydrate (as glycogen)and of proteins in tissues is always accompanied by much water, and since the caloric value of fats is more than twice that of carbohydrate or protein, the storage of fats as a source of energy to the body is most efficient. Subcutaneous fat and fat about organs (perirenal fat, etc.) serve to protect against mechanical trauma and also to insulate against heat loss from the body.

Phospholipids, cholestrol and glycolipids are not stored in the adipose tissues but are found in the various organs of the body as essential structural constituents.

10.2 FUNCTIONS OF TISSUES IN LIPID METABOLISM

Role of the Liver

The liver is an organ that is actively concerned with lipid metabolism. The lipid content of the liver of most animals averages about 5 per cent but is dependent on the diet of the animal and the presence or absence of a number of factors. Robinson has summarized the role of liver in terms of the effects on specific lipids.

Effect on FFA: Plasma free fatty acids (FFA) are rapidly taken up by the liver. In the fasting state, between 30 and 50 per cent of the circulating FFA may be removed. The uptake is directly related to the level of circulating FFA; however, the liver does not utilize these acids for energy to the same extent as does muscle tissue but converts the FFA to triglycerides and phosphatides. These lipids are stored or released to the circulation.

Effects on triglycerides: Some 30 to 40 per cent of the triglycerides circulating in the blood after the meal are taken up by the liver. Plasma triglycerides have a short half-life as do free fatty acids. Most of the triglyceride taken up by the liver is by the parenchymal rather than reticulo-endothelial cells. When triglycerides labelled with H^3 in the fatty acids and C^{14} in the glycerol are injected, the C^{14}/H^3 ratio is maintained for only a short time: by 20 min the ratio has fallen to about one-half of its initial value. These results probably mean that chylomicron triglycerides taken up by the liver are in part hydrolysed, but then triglycerides are again formed by reesterification. The triglycerides released from the liver a short time after injection of labelled FFA are generally in association with the low density lipoproteins. The lipemia that occurs after eating is due to absorbed glycerides and to the contribution of the liver.

Effect on phospholipids: Phospholipids are added to liver tissue when chylomicrons and lipoproteins are removed from the blood by liver. Development of elegant chromatographic techniques has resulted in the study of metabolism of specific liver phospholipids. These studies are complicated by the fact that the phosphatides are unique in their high content of unsaturated fatty acids and in possessing phosphorous and nitrogenous- or carbohydrate-like residues.

It seems clear that although the glyceride concentration of liver responds rapidly to dietary changes and toxic substances, phospholipid concentration changes relatively little. The liver is an important source of plasma phosphatides. These are synthesized by the liver tissue but liver also modifies and breaks down phosphatides. It has been calculated that rat liver breaks down 23 mg lecithin and 26 mg phosphatidylethanolamine per 100 grams of liver per hour.

Effect on cholesterol metabolism: The liver is active in modifying lipoproteins. Associated with the lipoproteins are cholesterol esters and free cholesterol. The liver may well be the chief organ concerned with the regulation of the total body content of cholesterol and with the control of plasma cholesterol levels. It is stated that the liver (1) supplies endogenous cholesterol and cholesterol esters to plasma; (2) regulates plasma cholesterol levels; (3) governs bile acid production from cholesterol and (4) is the chief organ concerned with cholesterol homeostatis.

Fatty Livers

Mammalian liver normally contains about 5 per cent lipid. This is found in the hepatic cells and in the Kupffer cells in the form of small droplets. Under the influence of various pathological and physiological disturbances, the lipid content may rise to 25 or 30 per cent. Depending on the magnitude of the fatty infiltration, fat droplets increase in size from 2 μ to 10 μ. A number of these may join together to produce one large drop which replaces almost the entire cytoplasm of the hepatic cell. Fatty cysts up to 100 μ in diameter may also develop. There appears to be some difference in the extent to which hepatic and Kupffer cells take up fat in different conditions.

Functions of Adipose Tissue

Adipose tissue lipid storage is directed toward energy storage during calorie excess and toward the release of FFA during stress of energy deficit. Free fatty acids of blood which enter adipose tissue leave the protein of the blood to which they were associated. Fatty acids of blood triglycerides leave the blood after lipolysis by lipoprotein lipase at the cell surface and become adipose tissue FFA. These fatty acids may be returned to the circulation by union with protein at the cell surface or may be stored as triglycerides. Adipose tissue glycerides are subject to lipolysis by enzymes that can be stimulated by a number of hormonal factors.

A number of hormones influence adipose tissue function. Epinephrine, norepinephrine, adrenocorticotropic hormone (ACTH), growth hormone, glucagon and the thyrotropic hormone (TSH) accelerate the release of FFA from adipose tissue. Insulin induces rapid incorporation of glucose into adipose tissue and storage as fat. Both fatty acid and triglyceride syntheses are stimulated by insulin.

10.3 FATTY ACID TRANSPORT INTO MITOCHONDRIA

The enzymes of fatty acid oxidation are present in the mitochondrial matrix (in animal cells). The fatty acids with the carbon chain length of 12 carbon or less can enter mitochondria without the mitochondrial membrane transporters. The fatty acids with the carbon chain length of 14 carbon or more carbons can enter mitochondria only with the help of mitochondrial membrane transporters.

The free fatty acids which are obtained in the diet or released from adipose tissue are majority and are of carbon length 14 or more. Therefore, they undergo 3 enzymatic reactions of carnitine shuttle to enter the mitochondrial matrix.

Step 1: The first reaction is catalysed by the enzyme acyl-CoA synthetase which is present in the outer mitochondrial membrane.

$$\text{Fatty acid} + \text{CoA} + \text{ATP} \leftrightarrow \text{Fatty acyl-CoA} + \text{AMP} + \text{PP}_i$$

This enzyme catalyse the formation of thioester linkage between fatty acids carbonyl group and thiol group of CoA to yield fatty acyl-CoA, coupled to the cleavage of ATP to AMP and PP_i.

Fatty acyl-CoA esters found at the cytosolic side of the outer mitochondrial membrane has two fates: either it can be used in the cytosol to synthesis of membrane lipids or can be transported in the mitochondria and oxidized to produce ATP.

Step 2: The fatty acids which enters the mitochondria for oxidation are attached to the hydroxyl group of carnitine to found fatty acyl-carnitine catalyze by the enzyme carnitine acyltransferase (in the outer mitochondrial membrane). The fatty acyl carnitine passes to intermembrane space through large core of outer mitochondrial membrane. Fatty acyl carnitine enters the mitochondrial matrix by facilitated diffusion through the acyl-carnitine/carnitine transporter of the inner mitochondrial membrane.

Step 3: The enzyme carnitine acyltransferase II (present in inner mitochondrial membrane) transfers fatty acyl group from carnitine to intramitochondrial, regenerates free fatty acyl-CoA and releases carnitine free into the mitochondrial matrix. Carnitine is free to re-enter the intermembrane space via acyl-carnitine.

The two separate pools of CoA and fatty acyl-CoA are present one in cytosol and the other in mitochondria. These pools have different functions, cytosol CoA is used in the biosynthesis of fatty acids, whereas mitochondrial matrix CoA is used in oxidative degradation of pyruvate, fatty acids and amino acids (Figure 10.1).

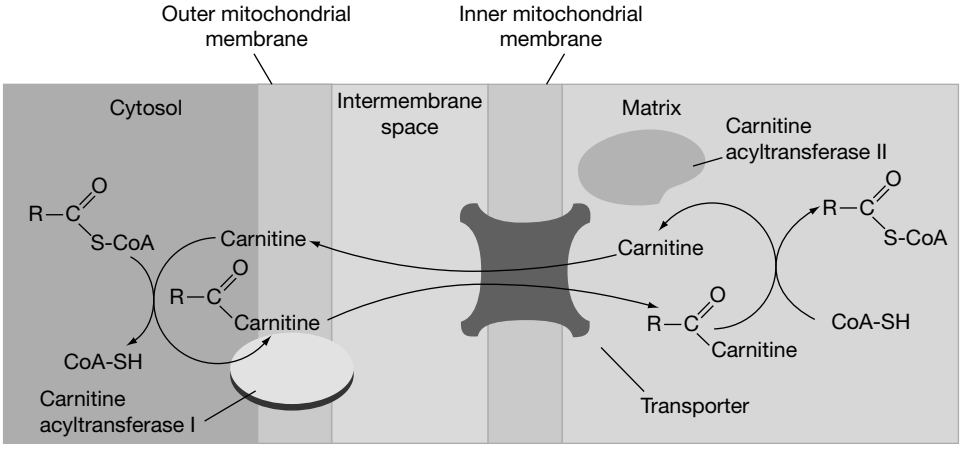


Figure 10.1 Entry of fatty acids into mitochondria via the carnitine transporter. Fatty acyl carnitine is formed at the outer mitochondrial membrane or in the intermembrane space it moves into the matrix of mitochondria through the transporter in the inner mitochondrial membrane by facilitated diffusion. In the mitochondrial matrix, the acyl group is transferred to mitochondrial coenzyme A and liberating carnitine free to return to the intermembrane space through the same transporter present in the inner mitochondrial membrane

10.4 FATTY ACID OXIDATION

β-oxidation of fatty acids: The mitochondrial oxidation of fatty acids takes place in three stages.

Stage 1: β-oxidation of fatty acids leads to the successive removal of two carbon moiety from the carbonyl end of the fatty acyl chain in the form of acetyl-CoA, e.g., β–oxidation of palmitic acid (C-16) undergoes seven cycles. However, in each cycle loses two carbons as acetyl-CoA.

At the end of the seven cycles, the last two carbons of the palmitic acid remain as acetyl-CoA. Formation of each acetyl-CoA requires removal of four hydrogen atoms from the fatty acyl moiety by the enzyme dehydrogenase.

Stage 2: Acetyl-CoA generated from fatty acid oxidation, from glucose via glycolysis and pyruvate oxidation enters the citric acid cycle. In citric acid cycle, the acetyl groups of acetyl-CoA are oxidized to CO_2.

Stage 3: Stage 1 and stage 2 of fatty acid oxidation generate the reduced electron carriers NADH and $FADH_2$. In the stage 3 these electron carriers donate electrons to respiratory chain through which electrons are transferred to oxygen with the simultaneous phosphorylation of ADP to ATP. The energy released by fatty acid oxidation is conserved in the form of ATP (Figure 10.2).

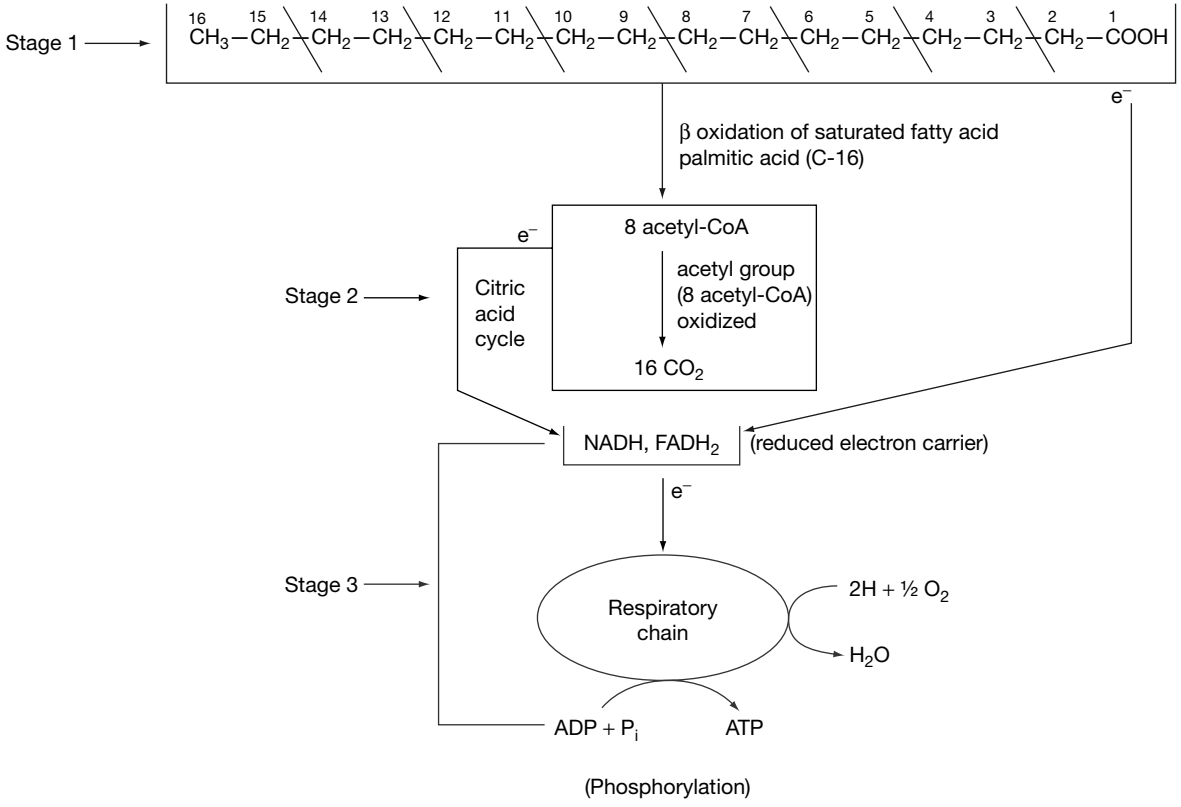


Figure 10.2 β-oxidation of fatty acids. In stage1, β-oxidation of palmitic acid (C-16). In stage 2 acetyl-CoA generated from fatty acid oxidation, enters the citric acid cycle. In the stage 3 electron carrier donate electrons to respiratory chain

10.4.1 β-Oxidation of Saturated Fatty Acids

The mitochondrial β-oxidation of saturated fatty acids is via four enzyme-catalysed reactions.

Step 1: The hydrogenation of fatty acyl-CoA by the enzyme acyl-CoA dehydrogenase produces a double bond between the α- and β-carbon atom, i.e., C-2 and C-3, yielding a trans Δ^2-enyol-CoA (Δ^2 represents the position of the double bond).
The enzyme acyl-CoA dehydrogenase is specific for a range of fatty acyl chain length.

1. Very long acyl-CoA dehydrogenase acts on fatty acids of 12 to 18 carbon length.
2. Medium acyl-CoA dehydrogenase acts on fatty acids of 4 to 14 carbon length.
3. Short chain acyl-CoA dehydrogenase acts on fatty acids of 4 to 8 carbon length.

All three isozymes are flavoproteins with FAD as a prosthetic group. FAD is the electron acceptor, and FAD is reduced to $FADH_2$. Reduced FAD transfers the electron to respiratory chain, ultimately to oxygen with the simultaneous phosphorylation of ATP about 1.5 ATP molecules synthesized per electron pair.

Step 2: In the second step of β-oxidation of saturated fatty acids, water is added to the double bond of trans Δ^2 Enoyl-CoA to form L-β-hydroxyacyl-CoA catalysed by the enzyme enyol-CoA hydratase.

Step 3: L-β-hydroxyacyl-CoA is dehydrogenated to form β-ketoacyl-CoA by the action of the enzyme β-hydroxyacyl-CoA dehydrogenase. In this step NAD is the electron acceptor, and NAD is reduced to NADH. Reduced NAD transfers the electron to respiratory chain, ultimately to the oxygen with the simultaneous phosphorylation of ADP.

Step 4: The last step of β-oxidation of saturated fatty acids leads to the conversion of the β-keto-acyl-CoA to acyl-CoA by the action of the enzyme acyl-CoA acetyltransferase. β-ketoacyl-CoA with the molecule of free coenzyme A is promoted by the enzyme Acyl-CoA acetyltransferase, to split off the two-carbon fragment of the original fatty acids at the carboxyl-terminal to acetyl-CoA. The reaction's other product is the coenzyme A thioester of the fatty acid, reduced by two carbon atoms. This reaction is called thiolysis because β-ketoacyl-CoA is cleaved by reaction with thiol group of coenzyme A.

The overall process continues until the complete chain is cleaved into acetyl-CoA units. The final cycle produces to separate acetyl-CoAs, instead of one acyl-CoA and one acetyl-CoA. The acyl-CoA unit is edited by two carbon atoms in every cycle. Concurrently, one molecule of $FADH_2$, NADH and acetyl-CoA are formed (Figure 10.3).

10.4.2 β-Oxidation of Unsaturated Fatty Acids

β-oxidation of unsaturated fatty acids possess one or more double bonds. The bonds are in cis-configuration, whereas in saturated fatty acids these bonds are in trans-configuration. A trans-Δ^2 bond formation is prevented because of the position of a cis-bond, therefore the β-oxidation of unsaturated fatty acids requires additional two enzymes, an isomerase and a reductase.

Oleate (18 Carbon) is a monounsaturated acid with cis-double bond between C-9 and C-10. Oleate is taken as an example of beta-oxidation of unsaturated fatty acids as shown in Figure 10.4.

β-oxidation of unsaturated fatty acids of oleate: Upon oxidation, oleate is converted to oleoyl-CoA. Oleoyl-CoA undergoes three passes through the fatty acids to yield three molecules of acetyl-CoA and coenzyme A ester of a cis Δ^3 Dodecenoyl-CoA (12 carbon unsaturated fatty acids).

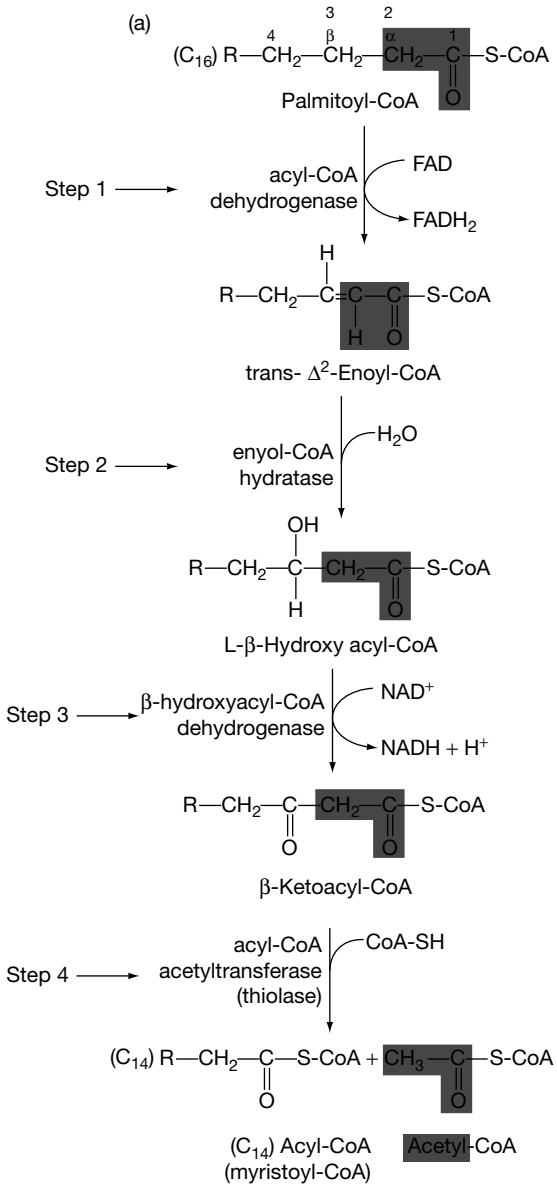


Figure 10.3 β-oxidation of saturated fatty acids: b-oxidation of palmitic acid undergoes seven cycles, four cycles are shown. In each cycle, one acetyl residue is removed (shaded). In each cycle, two carbon moieties are removed and at last palmitic acid (16 carbons) is left with 2 carbons, i.e., acetyl-CoA. In all, 8 molecules of acetyl-CoA are formed

Cis-Δ^3 Dodecenoyl-CoA cannot serve as a substrate for enoyl-CoA hydratase, which act only on trans-double bond cannot on cis-double bond (as shown in the step 2 of β-oxidation of saturated fatty acids). The enzyme, Δ^3, Δ^2-enyol-CoA isomerase isomerizes the cis-Δ^3-enyol-CoA to trans-Δ^2

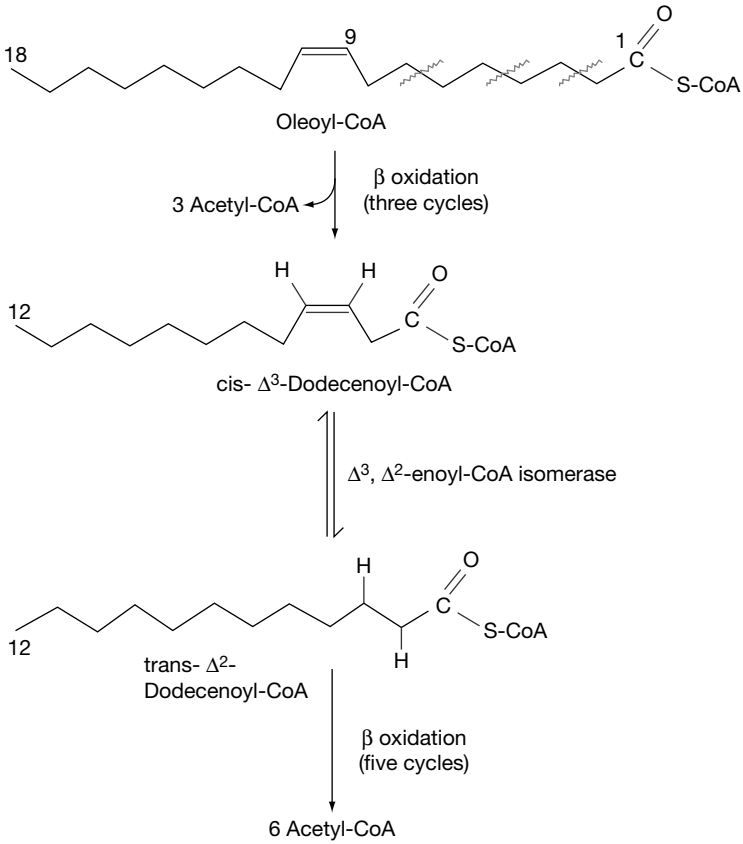


Figure 10.4 Oxidation of monounsaturated fatty acid. Oleic acid as oleoyl-CoA (Δ^9) is shown as an example for the oxidation of a monounsaturated fatty acid. For this oxidation additional enzymes are used for converting the cis-isomer to trans-isomer and to reposition the double bond

Dodecenoyl-CoA. Trans-Δ^2 Dodecenoyl-CoA serves as a substrate for the enzyme enoyl-CoA hydratase and converted to L-β-hydroxiacyl-CoA. The residual enzymes of β-oxidation act on this intermediate to produce acetyl-CoA and the coenzyme A ester of 10 carbon saturated fatty acids, decanoyl-CoA. To produce five more molecules of acetyl-CoA, the decenoyl-CoA has to undergo four more passes through the pathway. In all, nine acetyl-CoAs are produced from one molecule of oleate (18 carbon).

The enzyme cis-Δ^3-Enoyl CoA isomerase converts the cis-Δ^3 bond to trans-Δ^2 bond if the acyl CoA contains a cis-Δ^3 bond (Figure 10.4).

10.4.3 β-Oxidation of Polyunsaturated Fatty Acid

β-oxidation of polyunsaturated fatty acid is different from the beta-oxidation unsaturated fatty acids because polyunsaturated have more than one double bond. To explain the β-oxidation of polyunsaturated fatty acid, linoleate (C-18) is taken as an example. The 18 carbon linoleate has two double bonds, i.e., one Δ^9 and another Δ^{10} and both the double bonds are in cis-configuration.

Linoleoyl-CoA (cis-Δ^9, cis-Δ^{12}) undergoes three passes through the β-oxidation sequence to yield three molecules of acetyl-CoA and co-enzyme A ester of 12 carbon polyunsaturated fatty acids with a cis-Δ^3, cis-Δ^6 configuration. This intermediate cannot be used by the enzymes of β-oxidation pathway because its double bonds are at wrong position and have cis-configuration, whereas the β-oxidation enzymes act on trans-configuration. Therefore, the combined action of enoyl-CoA isomerase and 2, 4-dienoyl-CoA reductase as shown in the Figure 10.5, rearranges the position and configuration of the double bond. Enoyl-CoA isomerase enzyme rearranges the position and the configuration of the double bond. The combined action of both these enzymes (enoyl-CoA isomerase and 2, 4-dienoyl-CoA reductase) allows re-entry of this intermediate into β-oxidation pathway and its degradation to 6 acetyl-CoA. This pathway leads to the overall conversion of linoleate to 9 molecules of acetyl-CoA.

An intermediate 2, 4-dienoyl intermediate, is produced upon dehydrogenation, if the acyl CoA contains a cis-Δ^4 double bond, 2, 4-dienoyl intermediate is not a substrate for enoyl CoA hydratase. However, an intermediate 2, 4-dienoyl is reduced by the enzyme 2,4-Dienoyl CoA reductase to cis-Δ^3-enoyl CoA, and in this reaction NADPH is used up (Figure 10.5).

To Summarize

Odd numbered double bonds are handled by the enzyme isomerase and even numbered double bonds are handled by the enzyme reductase (which creates an odd numbered double bond).

10.4.4 β-Oxidation of Odd-Numbered Chains

Odd number of carbon fatty acids is generally found in the plant lipids and lipids of some marine organisms.

Chains with an odd number and even number of carbons are oxidized in the same manner, but the final products of odd number fatty acids are propionyl-CoA and acetyl-CoA. The acetyl-CoA can be oxidized in citric acid cycle, but propionyl-CoA enters the different pathway involving three enzymes as shown in Figure 10.5.

An enzyme propionyl-CoA carboxylase along with ATP and biotin, carboxylated propionyl-CoA into D-stereoisomer of methylmalonyl-CoA, using bicarbonate ion.

The enzyme methylmalonyl-CoA epimerase converts D-methylmalonyl-CoA's D-conformation enzymatically into the L-conformation of L-methylmalonyl-CoA.

Then L-methylmalonyl-CoA undergoes intramolecular rearrangement, which is catalysed by the enzyme methylmalonyl-CoA mutase (requires coenzyme-B_{12} as its coenzyme) to form succinyl-CoA. The succinyl-CoA formed can then enter the citric acid cycle (Figure 10.6).

10.4.5 Oxidation in Peroxisomes

When the carbon chain of fatty acid is too long say greater than C-22, then the oxidation cannot takes place in mitochondria. Therefore, the oxidation of long chain fatty acids first takes place in peroxisomes then followed by mitochondria. However, the oxidation ceases at octanyl CoA.

One important difference between the mitochondrial and peroxisomes oxidation is that peroxisomes' oxidation is not coupled to ATP synthesis. Rather, the high-potential electrons are transferred to O_2 and lead to the formation of H_2O_2. In peroxisomes, the catalase enzyme is present which converts the hydrogen peroxide into water and oxygen.

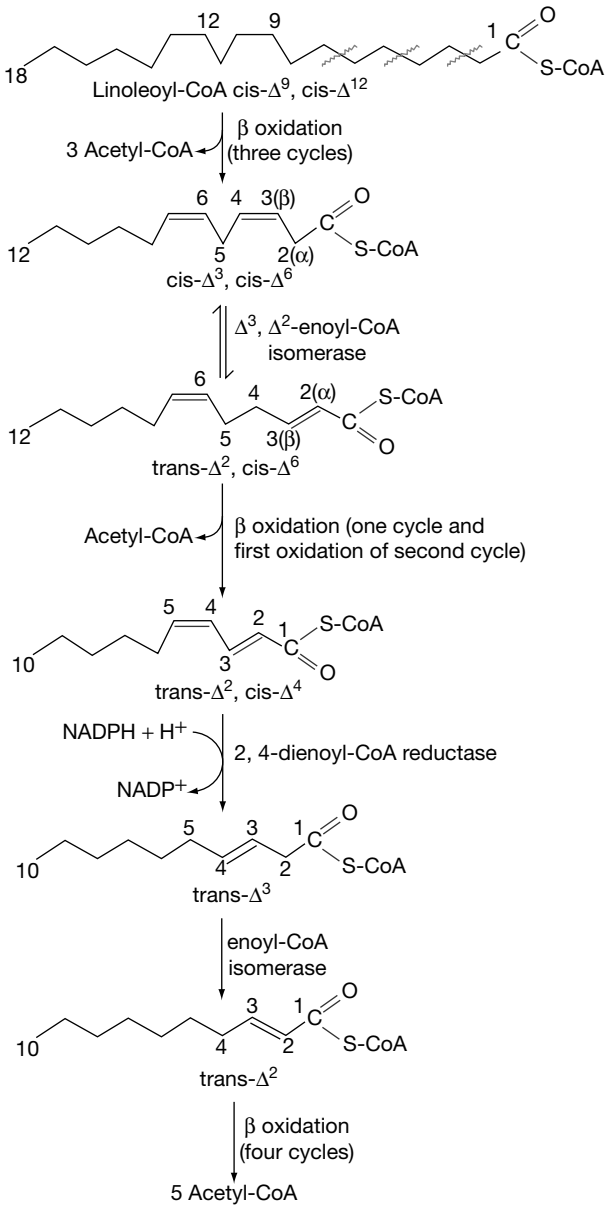


Figure 10.5 Oxidation of a polyunsaturated fatty acid. The linoleic acid as linoleoyl-CoA (cis-Δ^9, cis-Δ^{12}) is taken as an example to show the oxidation of a polyunsaturated fatty acid. Oxidation required other auxiliary enzyme (a reductase) in addition to isomerase enzyme. The combined action of these two enzymes converts trans–Δ^2, cis-Δ^4-dienoyl-CoA intermediate to the trans-Δ^2, -enoyl-CoA

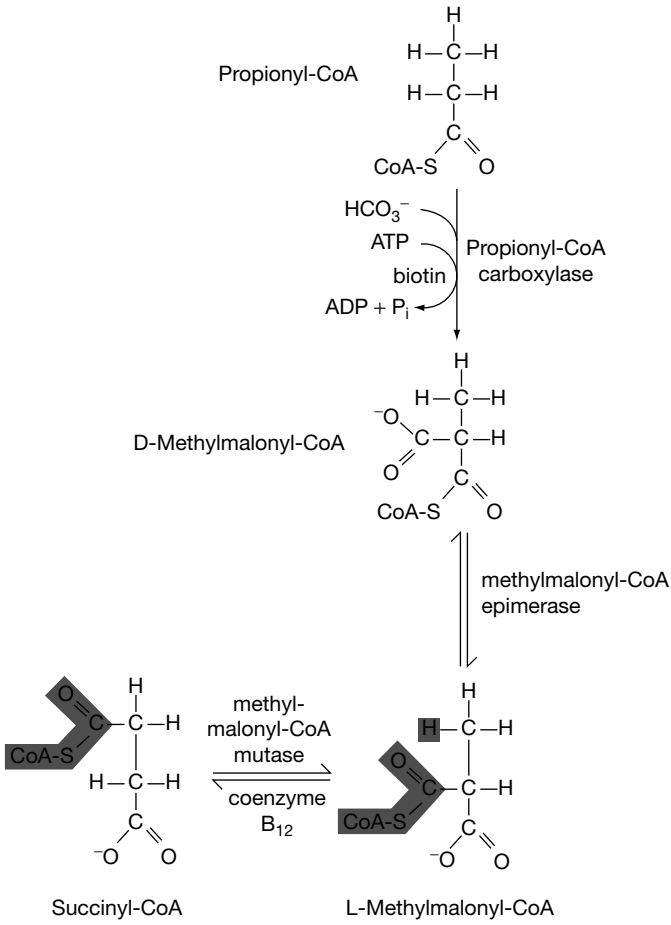


Figure 10.6 Oxidation of odd number fatty acid. The product of β-oxidation of odd number fatty acids is the propionyl-CoA. Propionyl-CoA does not enter the citric acid cycle therefore, it is first carboxylated to form D-methylmalonyl-CoA then D-methylmalonyl-CoA epimerized to L-methylmalonyl-CoA then undergoes an intramolecular rearrangement to form succinyl-CoA which is a substrate to citric acid cycle

Peroxisome needs specific enzymes for β-oxidation of long chain fatty acids in peroxisome. The enzymes used in the β-oxidation of peroxisome and mitochondria has three main differences.

1. β-oxidation in the peroxisome is different from the mitochondria, the enzymes used for β-oxidation in the peroxisome are peroxisomal carnitine acyltransferase for transport of the activated acyl group into the peroxisome. The enzymes used for β-oxidation in mitochondria are carnitine acyltransferase I and II.
2. Acyl-CoA oxidase is the enzyme which catalyses the first oxidation step in the peroxisome.
3. The enzyme β-ketothiolase used for β-oxidation in peroxisome has a changed substrate specificity, which is different from the enzyme β-ketothiolase used for β-oxidation in mitochondria (Figure 10.7).

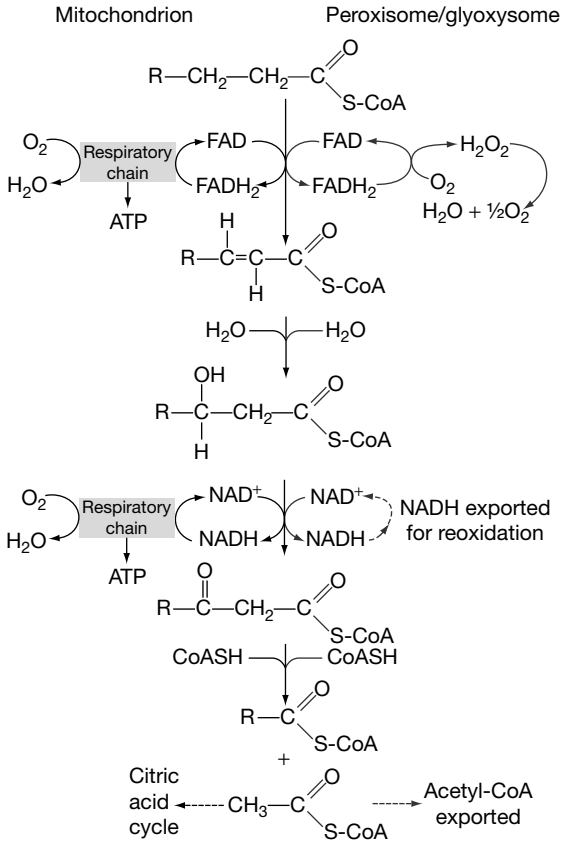


Figure 10.7 Oxidation in peroxisomes. The fatty acid oxidation in peroxisomes differs from the mitochondrial system in two ways. In the first way, oxidative step electrons pass directly to oxygen, generating H_2O_2. In the second way, oxidative step NADH is formed but cannot be reoxidized in the peroxisomes; so, it finally enters mitochondria. The acetyl-CoA produced by peroxisomes is also exported. Acetyl-CoA produced in mitochondria is further oxidized in citric acid cycle

10.5 FATTY ACID BIOSYNTHESIS

A considerable body of evidence implicated acetate as a precursor of fatty acids. We hereby provide a list of this information hereunder.

1. Fatty acids are formed from acetate and metabolites that are converted to acetate.
2. Fatty acids can be formed from non-fat material in the diet.
3. All of the carbons of straight chain, even numbered fatty acids arise from acetate.
4. Acetate is incorporated as an intact two-carbon unit.
5. The acetate units are aligned in a head-to-tail sequence in fatty acids.
6. Two reductive steps appear to be required for each acetate unit added.
7. Fatty acids can be formed outside of the mitochondria, while β-oxidation is largely mitochondrial.

8. Possible C4, C6, C8, etc., intermediates of biosynthesis do not accumulate and acetyl-CoA forms long chain fatty acids.
9. The carbon of tracer HCO_3^- is not found in the fatty acid synthesized.

The fatty acid synthesis takes place when **acetyl-CoA** is carboxylated to malonyl-CoA (Figure 10.8). This reaction is catalysed by acetyl-CoA carboxylase. This enzyme has three separate polypeptide subunits. The acetyl-CoA carboxylase enzyme contains a biotin prosthetic group covalently bound in amide linkage to the ε-amino group of a Lys-residue in one of the three polypeptides of the enzyme molecule. The two-step reaction is catalysed by this enzyme.

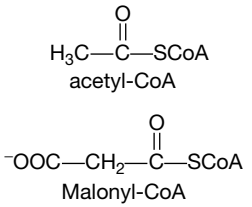


Figure 10.8 Structure of Acetyl-CoA and Malonyl-CoA

Step 1: The carboxyl group, derived from bicarbonate, is transferred to biotin, and this is an ATP-dependent reaction.

Step 2: The biotinyl group serves as a temporary carrier of CO_2, transferring it to acetyl-CoA to yield malonyl-CoA.

$$HCO_3^- + ATP + \text{acetyl-CoA} \rightarrow ADP + Pi + \text{malonyl-CoA}$$

Synthetase systems: The seven enzymic reactions required for fatty acid synthesis are thought to be related to the seven proteins suggested to be present in the particle by the finding of seven N-terminal amino acids. Lynen proposed that the synthetase consists of 21 subunits of average molecular weight of 100,000 and that the seven proteins are arranged around a central sulphydryl group.

Fatty acids synthase complex: The fatty acids synthase complex consists of seven polypeptides.

- Acyl carrier protein (ACP)
- Acetyl-CoA-ACP transacetylase (AT)
- β-ketoacyl-ACP synthase (KS)
- Malonyl-CoA-ACP transferase (MT)
- β-ketoacyl-ACP reductase (KR)
- β-Hydroxyacyl-ACP dehydratase (HD)
- Enoyl-ACP reductase (ER)

To catalyse the formations of fatty acids from acetyl-CoA and malonyl-CoA, all these proteins act together. During the process of fatty acids synthesis, the intermediates remain covalently attached as thioester to SH group (thiol) of Cys-residue in one of the seven synthase proteins (β-ketoacyl-ACP synthase) and the other is -SH group of ACP (acyl carrier protein).

ACP (acyl carrier protein): ACP is a small protein containing 4′phosphopantetheine as the prosthetic group. The 4′phosphopantetheine prosthetic group of ACP serves as a flexible arm. The amino acid composition of the protein has been determined and it is now recognized that the reactions of the fatty acid synthesis occur while the acyl groups are covalently linked to the sulphur of acyl carrier protein ACPSH. The 4-phosphopantetheine is linked to acyl carrier protein by a phosphodiester linkage through the hydroxyl group of a serine residue. Acyl-CoA derivatives are the chief intermediates in catabolic reactions for fatty acids and that acyl-ACP derivatives participate only in synthesis reactions (Figure 10.9).

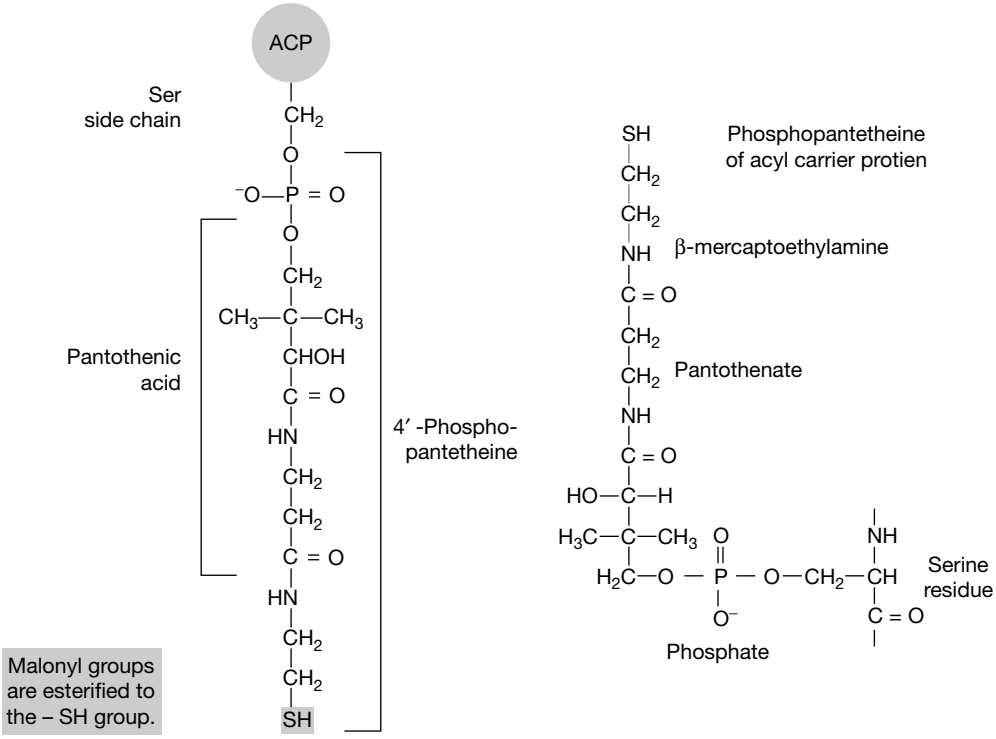


Figure 10.9 Acyl carrier protein (ACP). The prosthetic group is 4′ phosphopantetheine is covalently attached to the hydroxyl group of a serine residue in ACP

Fatty Acid Synthesis, Individual Steps

Step 1: In the first reaction, in acetyl-CoAs, acetyl group is transferred to the Cys-SH group of the β-ketoacyl-ACP synthase. This reaction is catalysed by the enzyme acetyl-CoA ACP transacetylase.

Step 2: In the second reaction, the enzyme malonyl-CoA ACP transferase catalyses the transfer of the malonyl group from malonyl-CoA to the ACP, SH group. The acetyl and malonyl group are close to each other in charged fatty acid synthase complex and are activated for chain extension.

Step 3: Condensation: The third step is the condensation of acetyl ACP and malonyl ACP. This results in the formation of acetoacetyl ACP, an acetoacetyl group bound to ACP through the phosphopantethine-SH group with the evolution of CO_2. This reaction is catalysed by β-keto-acyl-ACP synthase (KS). The acetyl group is transferred from the Cys-SH group of the enzyme to the malonyl group on the -SH of ACP.

Step 4: Reduction of acetoacetyl ACP: In this step, acetoacetyl ACP undergoes reduction of the carbonyl group and C-3 to form D-β-Hydroxybutyryl-ACP. This reaction is catalysed by the enzyme β-ketoacyl-ACP reductase, wherein NADPH is the electron donor. The double bond is reduced to a hydroxyl group. However, only the D-isomer is formed.

Step 5: Dehydration of D-β-Hydroxybutyryl ACP: In this step, water is drawn out from C-2 and C-3 of D-β-Hydroxybutyryl-ACP to generate a double bond in the product, trans-Δ^2–butenoyl-ACP. This enzyme is catalysed by the enzyme β-hydroxyacyl-ACP dehydratase.

Step 6: Reduction: Finally, the double bond of trans-Δ^2–butenoyl-ACP is reduced to form butyryl ACP, NADPH being the electron donor. This reaction is catalysed by the enzyme enoyl-ACP reductase.

Step 7: Transfer: The growing fatty acid is transferred from phosphopantetheine to the cysteine sulphydryl of the condensing enzyme. The new cycle starts with another malonyl-CoA.

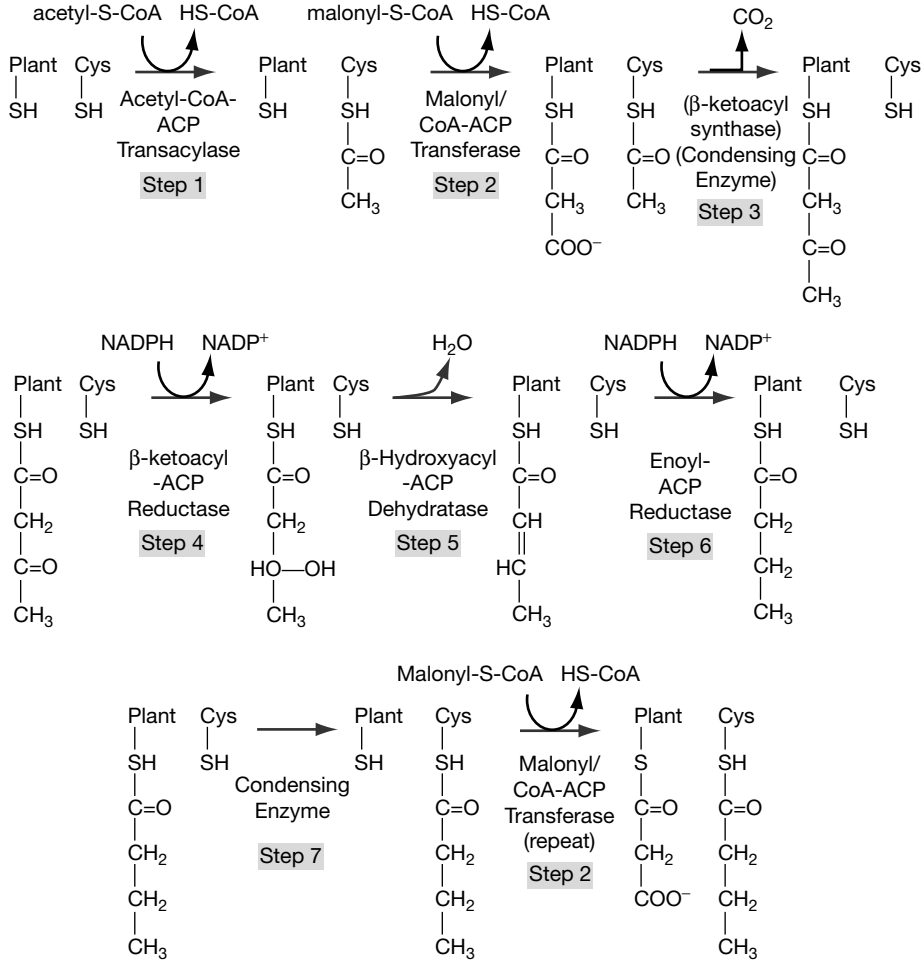


Figure 10.10 Fatty acid biosynthesis shows that the individual steps of the reaction pathway are catalysed by different enzymes

A thioesterase domain catalyses the hydrolysis of the thioester connecting the fatty acid to phosphopantetheine, when the fatty acid becomes 16 carbon atoms long. The final product of the fatty acid synthase complex is the palmitate which is 16-Carbon long saturated fatty acid.

The two carbon are synthesized at a time in the fatty acid molecule. The synthesis of fatty acids starts from the methyl end and moves toward the carboxylic acid end. Thus, the addition of C-16 and C-15 takes place first and followed up by the addition of C-2 and C-1.

Summary of Fatty Acid Synthesis

$$7\text{acetyl-CoA} + 7CO_2 + 7\text{ATP} \rightarrow 7\text{malonyl-CoA} + 7\text{ADP} + 7P_i$$

Then, seven cycles of condensation and reduction:

$$\text{Acetyl-CoA} + 7\text{malonyl-CoA} + 14\text{NADPH} + 14H^+ \rightarrow \text{Palmitate} + 7CO_2 + 14\text{NADP}^+ + 8\text{CoA} + 6H_2O$$

The overall reaction of fatty acid synthesis:

$$8\text{ acetyl-CoA} + 14\text{NADPH} + 7\text{ATP} + 14H^+ \rightarrow \text{Palmitate} + 14\text{NADP}^+ + 8\text{CoA} + 7\text{ADP} + 7P_i + 6H_2O$$

Acetyl-CoA generated in the mitochondria is transported to the cytosol via a shuttle mechanism involving citrate (acetyl-CoA reacts with oxaloacetate to give citrate). A tricarboxylate translocase transports citrate from mitochondria to cytosol. Fatty acid synthesis occurs in the cytosol.

Chain elongation of fatty acids: The enzymes present in endoplasmic reticulum catalyse beyond the 16-carbon length of the palmitate product of fatty acid synthase. The chain elongation of fatty acids which are either produced by fatty acyl synthase or polyunsaturated fatty acids from diet is done by endoplasmic reticulum enzymes. The initial condensation step is catalysed by fatty acid elongases.

10.5.1 Desaturases of Fatty Acid

The function of desaturases is to introduce double bonds in a fatty acid chain at specific position. Desaturase enzyme is absent in mammals; therefore, mammals fails to introduce cis-double bonds at carbons beyond C9 in the fatty acid chain. Hence, mammals fails to synthesize linoleate (18:2 Δ9,12) and linolenate (18:3 Δ9,12,15). But plants have desaturases enzyme to produce linoleate and linolenate. Linoleate and linolenate are the essential fatty acids that cannot be synthesized in the body, therefore they need to be obtained from diet.

Mammals can synthesize arachidonic acid, but plants cannot. Mammals take linoleic acid (obtained from the diet) and convert it into arachidonic acid (20:4 Δ5, 8, 11, 14). The biosynthesis occurs in the endoplasmic reticulum. For leukotrienes, proacyclins, thromboxanes and prostaglandins, arachidonic acid is the important precursor.

The palmitoleic (16:Δ9) acid and oleic acid (18:Δ9) are the two naturally occurring monosaturated fatty acids. Oleic acid is produced by the dehydrogenation of stearic acid. The production of oleic acid takes place by the dehydrogenation of stearic acid, this reaction is catalysed by the enzyme stearoyl CoA desaturase. The substrate used in this reaction is stearoyl CoA.

A nonheme iron centre, NADH and an oxygen-binding site are present in stearoyl CoA desaturase. Two flavoprotein proteins are also required, which are cytochrome b5 and cytochrome b5 reductase. All of these proteins are associated with the endoplasmic reticulum membrane. Cytochrome b5 reductase transfers electrons one at a time from NADH through FAD to cytochrome b5 then an electron is transferred by cytochrome b5 to reduce the non-heme iron to the ferrous state from the ferric state and then the ferrous iron coordinates to an O_2 molecule. The formation of cis-double bond takes place at 9, 10 positions of the substrate at this iron centre.

In this fatty acid desaturation cycle, O_2 is the terminal electron acceptor. In this process per oleoyl CoA, two molecules of water are produced and that means in the overall process four electrons were transferred. Out of the four electrons, two electrons came from the NADH and the other two electrons form stearoyl CoA (Figure 10.11).

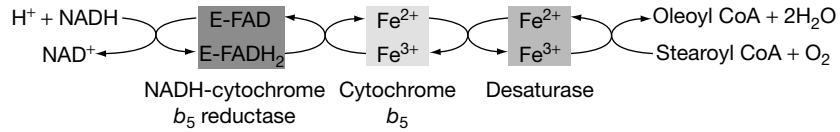


Figure 10.11 Desaturases of fatty acid

10.5.2 Differences between Fatty Acid Synthesis and β-oxidation Pathways

Table 10.1 shows the differences between fatty acid synthesis and β-oxidation pathways.

Table 10.1 Differences between Fatty Acid Synthesis and β-oxidation Pathways

	β-Oxidation pathway	Fatty acid synthesis
Location of the pathway	Mitochondrial matrix	Cytosol
Acyl carriers (thiols)	Coenzyme-A	Phosphopantetheine (ACP) and cysteine
Electron acceptors/donor	FAD and NAD^+	NADPH
Hydroxyl intermediate (L- or D- forms)	L	D
2-Carbon product/donor	Acetyl-CoA	Malonyl-CoA (and acetyl-CoA)

10.5.3 Regulation of FA Metabolism

- FA synthesis and is coordinately regulated.
- Three hormonal signals determine the state of FA metabolism. The fatty acid synthesis is inhibited by the two hormones glucagon and epinephrine and stimulates fatty acid oxidation, whereas insulin counters the effect of these two hormones as it stimulates FA biosynthesis and inhibits fatty acid oxidation.

Hormonal Regulation

The effects of phosphorylation of acetyl-CoA carboxylase (ACC). A variety of protein kinases may phosphorylate 8 to 12 residues that acetyl-CoA carboxylase contains. These protein kinases are controlled by hormones. When the blood glucose levels are low, hormone glucagon is the signal. Then, upon the binding of glucagon to the cell receptors, an intracellular phosphorylation cascade gets activated which in turn activates protein kinases which phosphorylate acetyl-CoA carboxylase (ACC). Once the ACC gets phosphorylated, it gets inactivated. The enzyme ACC can be activated only by a specific phosphatase which dephosphorylates the enzyme. Once the enzyme ACC gets dephosphorylated it becomes active. To produce fatty acids from triacylglycerols stored in the tissue, the adipose cell receptors triggers a phosphorylation cascade that activates the lipases. Serum albumin carries these fatty acids to the needy tissues.

The hormone glucagon stops the fatty acid biosynthesis by initiating the transportation of fatty acid from the adipose tissue. Insulin counteracts the effects of glucagon. If the blood glucose levels are high, then insulin gets activated. The enzyme phsphodiesterase is activated by insulin to hydrolyse cAMP to terminate phosphorylation cascade of glucagon. However, insulin starts its own phosphorylation cascade that activates a variety of phosphatases. To stop the liberation of fatty acids from adipose tissues, phosphatases are required to dephosphorylate the phosphorylated lipase in the adipose tissue. Further, ACC gets dephosphorylated by another insulin-activated phosphatase and there by activating it. Insulin thereafter concurrently turns on fatty acid biosynthesis and stops the liberation of fatty acid from the adipose tissue (Figure 10.12).

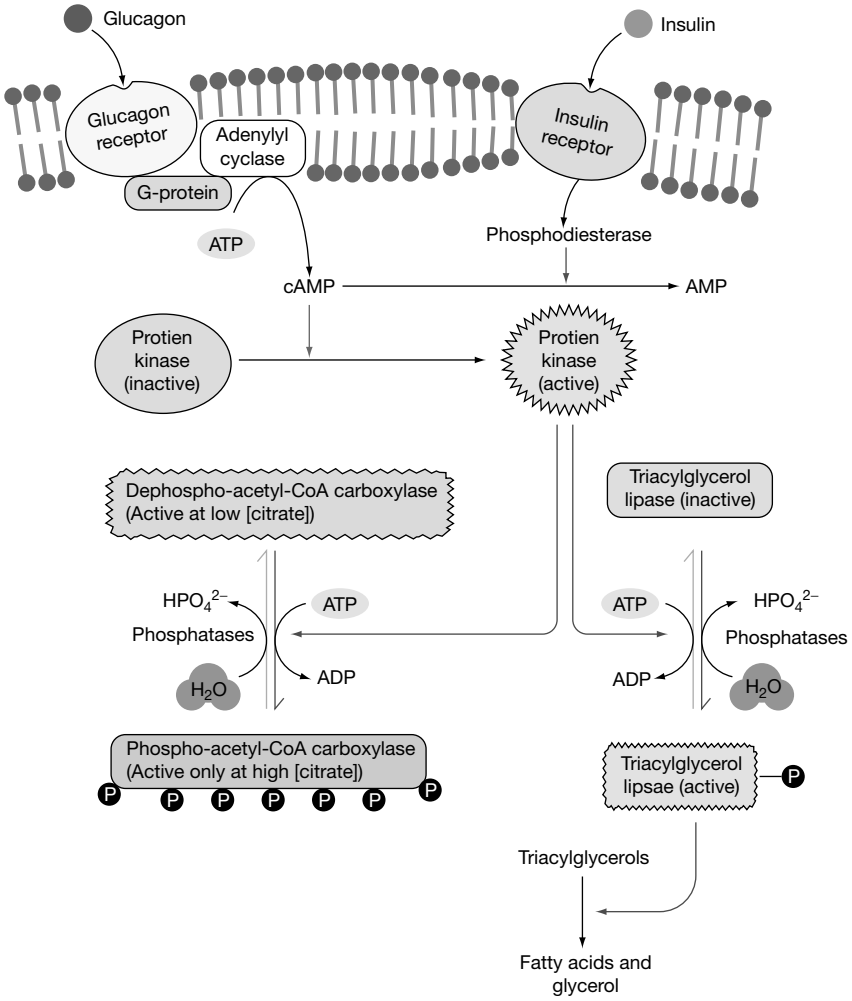


Figure 10.12 Hormonal Regulation of fatty acid biosynthesis: The effects of glucagon are counteracted by the hormone insulin

- Transcription factors include upstream stimulatory factors (USFs) and **sterol regulatory element-binding protein (SREBP-1)** that provide a stimulatory effect of insulin. In regulating the cholesterol synthesis, the role of SREBPs (sterol regulatory element-binding proteins) was first identified.
- The transcription of the Fatty Acid Synthase gene in liver cells are lessen by polyunsaturated **fatty acids**, by suppressing the production of SREBPs.
- The mechanism of hormonal regulation is covalent phosphorylation of acetyl-CoA carboxylase, the rate-limiting step of FA biosynthesis.
- Acetyl-CoA carboxylase is inhibited by phosphorylation. Phosphorylated acetyl-CoA carboxylase can regain partial activity by allosterically binding citrate.

Allosteric Regulation of FA Metabolism

- Acetyl-CoA carboxylase is the rate-limiting step of FA biosynthesis. Acetyl-CoA carboxylase is allosterically inhibited by palmitoyl-CoA and activated by citrate.

  Citrate shuttle is also inhibited by palmitoyl-CoA and thus slows down FA biosynthesis.
- β-oxidation is inhibited by malonyl-CoA and acetyl CoA.

  The final step of β-oxidation which is catalysed by 3-ketoacyl thiolase is inhibited by acetyl-CoA.
- Malonyl-CoA inhibits the transport of acyl-CoA to mitochondria via inhibition of carnitine-acyl transferase.
- Additionally, ATP and NADH also inhibit β-oxidation.

10.6 CHOLESTEROL METABOLISM

10.6.1 The Biosynthesis of Cholestrol

Prior to the introduction of isotopic techniques, it was known that the animal body could synthesize cholestrol. Deuteroacetate (CH_3^2 –COOH) was found to be converted by yeast to ergosterol in high yield, and in 1942 Bloch and Rittenberg showed this acetate to be converted to cholesterol by rats and mice. In vitro preparations of tissues synthesize cholesterol activity residing in microsomal and soluble supernatant fractions.

A number of established facts regarding cholesterol synthesis is provided hereunder.

1. Cholesterol synthesis is observed in essentially all tissues from cholesterol, liver, skin, adrenal glands and gonads being the most active. Adipose tissue, muscle, aorta and adult brain have a low order of synthesis.
2. Acetate is the principal precursor of cholesterol.
3. Of the 27 carbons of cholesterol, 15 arise from the methyl and 12 from the carboxyl of acetate.
4. The first phases of synthesis involve activation of intermediates through union with CoASH.

5. Later stages of synthesis involve participation of phosphates rather than coenzyme A.
6. Some of the early stages of synthesis appear to be common to the extra mitochondrial synthesis of fatty acids.
7. Refined yeast and liver preparations of a microsomal plus a soluble protein fraction, require ATP, NADPH, Mg^{++} and Mn^{++}.
8. Mevalonic acid and squalene are prime intermediates in the pathway.
9. Cholestrol synthesis is one example of a basic biosynthetic process in nature that involves condentation of isoprenoid (C_5) units and the formation of the active intermediate isopentenyl pyrophosphate.
10. Several mechanisms are available for the formation of the active C_5 units, and multiple pathways are detectable for interconversion of biosynthesized sterols.

A general description of the overall reaction for the formation of the squalene by one of the recognized pathway is provided hereunder.

$$18CH_3COSCoA + 24ATP + 13NADPH + 7H^+ \rightarrow C_{30}H_{50} + 6HCO_{3^-} + 13NADH^+ + 18CoASH + 24ADP + 24Pi$$

Details of the mechanisms of cholesterol biosynthesis and condensation of acetate to form the C_{27} sterol is provided via the following steps.

1. Activation of acetate to acetyl-CoA.
2. Condensation of two acetyl-CoA to acetoacetyl CoA.
3. Formation of β-hydroxy-β-methylglutaryl CoA.
4. Formation of mevalonic acid.

   Popjak has proposed that the reductive step takes place with the glutryl compound attached to enzyme (E). An alternative pathway for the formation of mevalonic acid has been proposed. In this pathway acetoacetyl CoA is neither an intermediate in the biosynthesis of HMG-CoA, nor is this latter compound an obligatory intermediate. Rather, acetyl CoA reacts with malonyl CoA to yield acetoacetyl–E, which reacts with another acetyl CoA to yield enzyme bound HMG. HMG can be transferred to CoA or reduced directly to mevalonic acid. At this time, it cannot be determined which of the pathway is quantitatively most significant.
5. Mevalonic acid is phosphorylated by ATP to form the 5-phosphate. The 5-phosphomevalonic acid is converted to 5-diphosphomevalonic acid (mevalonic acid-5-pyrophosphate).
6. Diphosphomevalonate loses CO_2 and H_2O to form isopentenyl pyrophosphate. This reaction requires ATP but the proposed 3-phospho-5-diphosphomevalonate has not been isolated. The proposed intermediate is probably unstable and decarboxylated, and formation of inorganic phosphate occurs simultaneously.
7. Isopentyl pyrophosphate is isomerized to 3,3-dimethylallyl pyrophosphate by enzymes present in yeast and in liver.
8. A molecule of the 3,3-dimethylallyl pyrophosphate then reacts with a molecule of the isopentenyl pyrophosphate to form geranyl pyrophosphate.
9. A molecule of isopentenyl pyrophosphate (C_5) reacts with geranyl pyrophosphate (C_{10}) to form farnesyl pyrophosphate (C_{15}).

10. Two molecules of farnesyl pyrophosphate condense to form squalene. The enzyme in liver that carries out this reaction is firmly bound to microsomes, and the system requires reduced pyridine nucleotide and Mg^{++}, Mn^{++} or CO^{++}.
11. Squalene undergoes cyclization to the primary animal sterol lanosterol by squalene oxydocyclase I. NADH is required as a coenzyme as is molecular oxygen; the O atom of the 3-β-hydroxyl group is derived from molecular oxygen and not from water. Popjak suggests that the following shifts occur during cyclization: migration of H from position 17α to 20β; from 13α to 17α a double methyl shift from position 14 to 13 and one from position 8 to 14 with elimination of a proton from position 9.
12. The primary animal sterol lanosterol is converted to cholesterol through a number of intermediates (Figures 10.13 and 10.14).

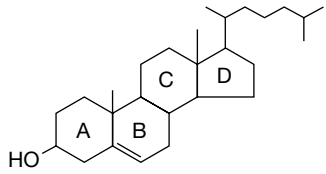


Figure 10.13 Structure of Cholesterol

The conversion of lanosterol to cholesterol at this time is not understood with certainty. For some time, it was believed that a single pathway existed for this conversion. In this scheme, three methyl groups were removed from lanosterol, the Δ^8 bond was shifted to Δ^5, and as a final step, the Δ^{25} bond was reduced. The possible intermediates are shown hereunder.

Lanosterol → 4,4-dimethyl-cholesta-8,24-diene-3-β-ol → 4,4-dimethyl-cholesta-8,24-diene-3-one → $\Delta^{8.24}$ -cholestadienol (zymosterol) → $\Delta^{5.24}$-cholestadienol (desmosterol) → cholesterol

The final step in this series is the reduction of the Δ^{24} bond, and the $\Delta^{5.24}$ compound desmosterol is an obligatory intermediate.

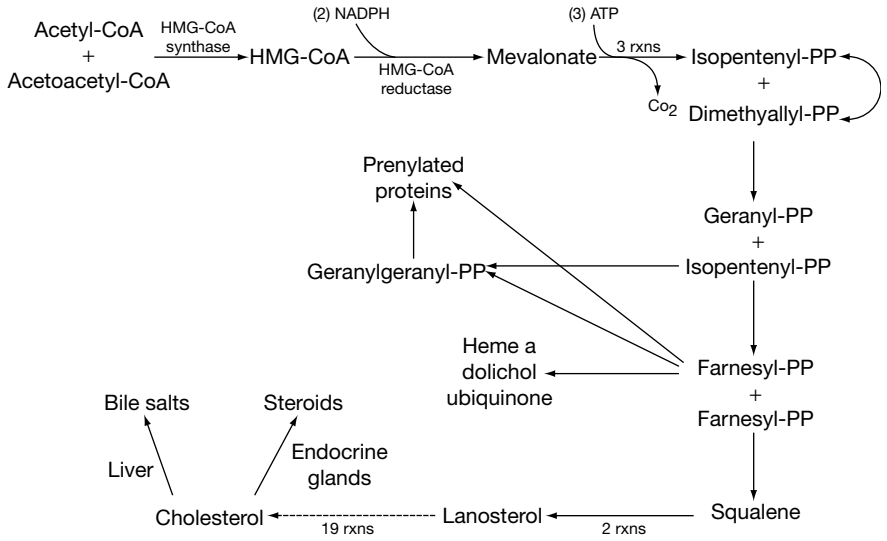


Figure 10.14 Pathway of cholesterol biosynthesis: Synthesis begins with the transport of acetyl-CoA from the mitochondrion to the cytosol. The 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase is the rate-limiting step

10.6.2 Regulation of Cholesterol Biosynthesis

Cholesterol synthesis is regulated in two ways:

1. Cholesterol production is regulated by intracellular cholesterol concentration.
2. Cholesterol production is regulated by the hormones glucagon and insulin.

The rate-limiting step in cholesterol biosynthesis is the conversion of HMG-CoA to mevalonate catalyzed by the enzyme HMG-CoA reductase. Regulation in response to cholesterol levels is mediated by transcriptional regulation of a gene which codes for HMG-CoA reductase enzyme. This gene which codes for HMG-CoA reductase along with 20 other genes encoding enzymes that mediate the synthesis of cholesterol and unsaturated fatty acids is controlled by SREBPs. These proteins, when newly synthesized, are embedded in endoplasmic reticulum (ER).

The transcriptionally active domain of SREBP is separated from rest of the SREBP by proteolytic cleavage. The transcriptionally active domain of SREBP activates transcription of the gene which codes for HMG-CoA reductase and other genes. **When cholesterol levels are high,** then SREBPs are inactive, and SREBP in a complex with another protein SCAP (SREBP cleavage activating protein) remain secured to ER. **When the cholesterol levels are low,** the SCAP leads to a conformation change and causes release of the SCAP–SREBP complex from the ER and moves to Golgi complex and the SREBP is cleaved. The SREBPs are active and activate the transcription of the gene which codes for HMG-CoA reductase and other genes, leads to the biosynthesis of cholesterol (Figure 10.16).

Cholesterol production is regulated by the hormones glucagon and insulin. Hormonal control is mediated by covalent modification of HMG-CoA reductase enzyme itself. When this enzyme is phosphorylated it exists in inactive form, when this enzyme is dephosphorylated it exists in active form. Glucagon stimulates phosphorylation; therefore, leads to the inactivation of the enzyme HMG-CoA reductase and stops the cholesterol biosynthesis. Insulin promotes the phosphorylation; therefore, leads to the activation of the enzyme HMG-CoA reductase and starts the biosynthesis of cholesterol (Figure 10.15).

10.6.3 Catabolism of Cholesterol

Bile Acids' Synthesis: Excretion and catabolism of cholesterol. Cholesterol is formed by many tissues, but it is catabolized by only a few. The chief catabolic fate of cholesterol is its oxidation to cholanic acids (cholic acids), and the chief route of excretion is into the gastrointestinal tract via the bile or through mucosal cells. The gonads and the adrenals utilize cholesterol in the synthesis of hormones, but this is not a quantitatively significant fate and does not account for the loss of much cholesterol from the body. Cholesterol is also found in skin fat or sebum. This loss is highly variable but may amount to 100 mg per day. Squalene is also lost in the sebum.

Formation of bile acids: The formation of bile acids from cholesterol in the liver represents the quantitatively most important fate of cholesterol some 80 to 90 per cent of the body cholesterol being ultimately metabolized to bile acids. In this transformation, the following reactions occur: inversion of the 3-β-hydroxy group, additional hydroxylation at C-7 and C-12, oxygenation at C-24, reduction of the Δ^5 double bond activation and cleavage of the terminal isopropyl group of side chain and activation and final conjugation of the resulting cholanic acids.

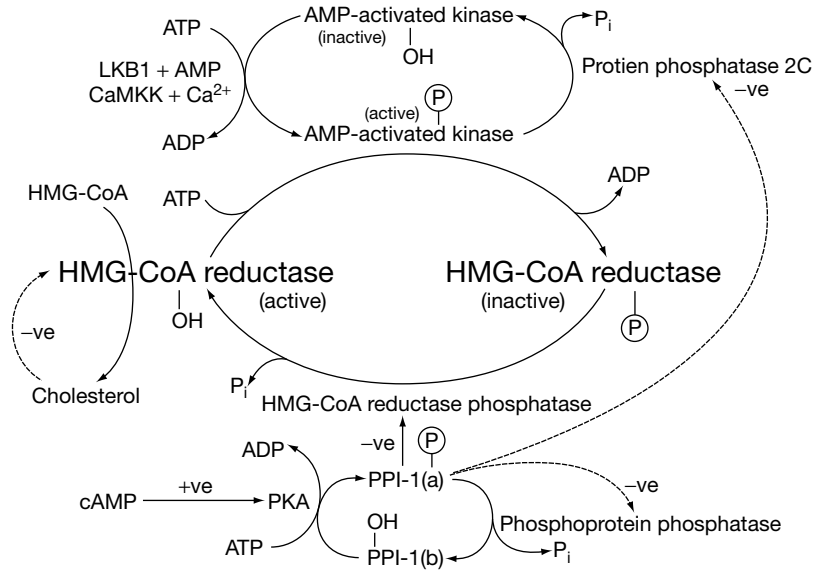


Figure 10.15 HMGR is regulated by covalent modification. HMGR is phosphorylated by the enzyme HMGR kinase, which is AMP-activated protein kinase, AMPK. The enzyme HMGR kinase is regulated by phosphorylation. The two enzymes, LKB1 and CaMKK catalyse the phosphorylation of AMPK. Cholesterol biosynthesis is inhibited by glucagon and epinephrine by increasing the activity of the inhibitor of phosphoprotein phosphatase inhibitor-1, PPI-1. Conversely, cholesterol biosynthesis is stimulated by insulin, because it removes phosphates and activates HMGR activity. HMGR in its dephosphorylated state is active and inactive in phosphorylated state

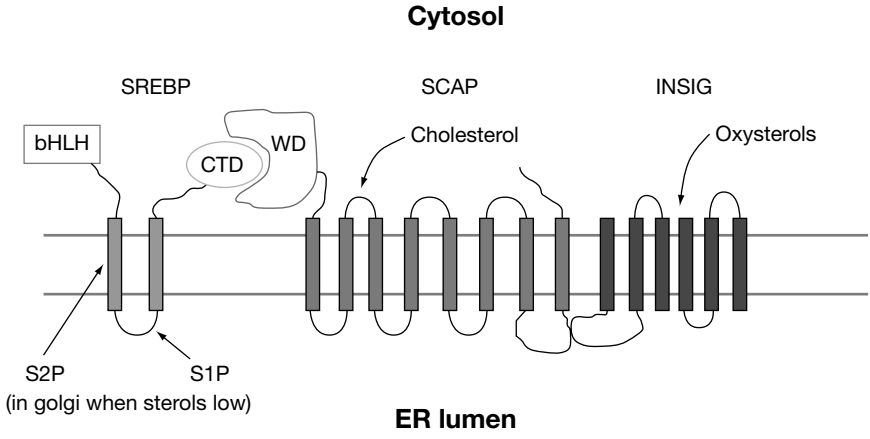


Figure 10.16 Diagramatically depicted the regulation of cholesterol synthesis. In the ER membrane, interactions between SREBP, SCAP and INSIG when sterols are high

The two primary bile acids formed from cholesterol in most mammalian species are cholic and chenodeoxycholic acids. No intermediates in the conversion of cholesterol to cholic acid have been isolated, the pathway is shown in Figure 10.17.

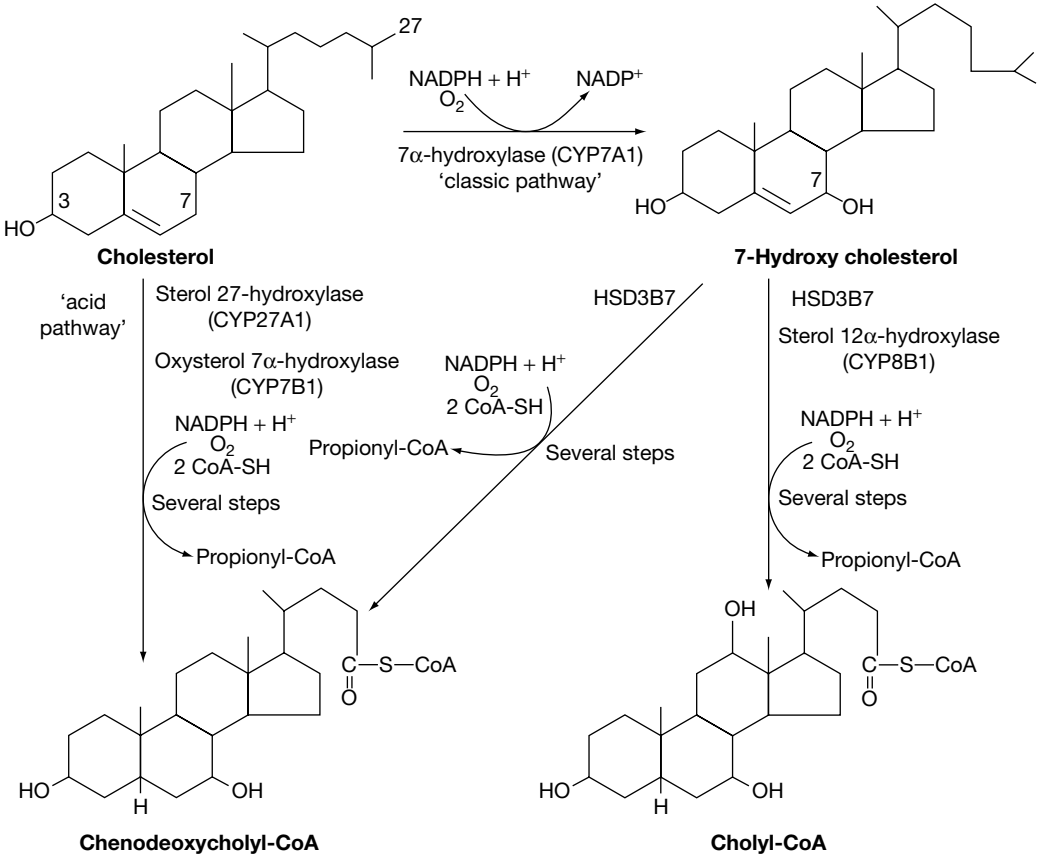


Figure 10.17 Synthesis of the two primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA). The conversion of cholesterol to 7-hydroxycholestrol is catalysed by the enzyme 7α-hydroxylase (CYP7A1), in bile acid synthesis this is the rate-limiting step. Expression of CYP7A1 occurs only in the liver. Conversion of 7α-hydroxycholesterol to cholyl-CoA is catalysed by the enzyme sterol 12α-hydroxylase (CYB8B1)

In the rat cholic and chenodeoxycholic acids are present in bile in the proportion 8:2 and are conjugated predominantly with taurine. The structures of glycocholic acid and taurocholic acid are shown in Figure 10.18.

Metabolism of bile acids: Bergstrom summarizes the relationships of the faecal excretory products of the bile acids as provided hereunder.

Faecal sterols: Faecal sterols arise from unabsorbed dietary sterols and from materials excreted into the intestine**.** Absorption of dietary cholesterol is generally less than 50 per cent. Normally in man, 0.5 g of cholesterol is secreted into the intestine per day. It has been estimated that

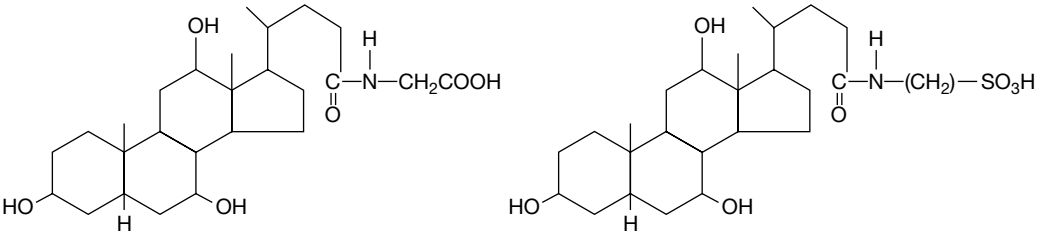


Figure 10.18 Structure of the conjugated cholic acids

70 per cent of the faecal sterols found during a cholesterol free diet study, arose from intestinal secretions and 30 per cent from biliary secretion. The faecal sterols include cholesterol, cholestanol, coprostanol, epicoprostanol, cholestanone, coprostanone, lathosterol, 7-dehydrocholesterol and methostenol, -4α-methyl-5α cholest-7-en-3β-ol. These last three sterols and cholesterol are known tissue constituents. It is recognized that both cholesterol and the bile acids undergo an enterohepatic circulation with absorption and secretion of each of these materials. This enterohepatic circulation has made it most difficult to quantitate any one single route.

10.7 CLASSIFICATION OF PLASMA LIPOPROTEINS AND THE SYSTEMIC PATHWAY OF LIPIDS

The transportation of lipids to the cells and from the cells is done by some carriers known as lipoproteins, these carriers are required because lipids are not soluble in blood (plasma). The size of the lipoproteins ranges from 10 to 1000 nm. These particles are spherical in shape and possess high molecular weight. The structure of lipoproteins shows a non-polar core and a hydrophilic surface. The lipoproteins contain hydrophilic surface due to which they are soluble in plasma and the non-polar core, contains hydrophobic lipids, triglycerides and cholesterol esters, bounded by a hydrophilic surface coat of phospholipids, unesterified cholesterol and apoproteins. The classification of lipoproteins is provided hereunder.

10.7.1 Types of Lipoprotein

- Chylomicrons
- Very low density lipoproteins (VLDL)
- Intermediate density lipoproteins (IDL)
- Low density lipoproteins (LDL) and lipoprotein (a)
- High density lipoproteins

10.7.2 Chylomicrons

The size of the chylomicrons is about 1000 nm, and they are the largest among all of the lipoproteins. **Right after the digestion of fat,** chylomicrons are seen in the blood. Dietary cholesterol and triglycerides are transported by chylomicrons to muscles, fat tissue and to breasts. Chylomicrons are constituted of 1–2 per cent protein, 85–88 per cent triglycerides, ~8 per cent

phospholipids, ~3 per cent cholesteryl esters and ~1 per cent cholesterol. Chylomicrons are mainly constituted of triglycerides, whose percentage is around 85 to 88.

10.7.3 Very Low Density Lipoproteins (VLDL)

The size of VLDL is very small as compared to chylomicrons, its size is approximately 25–90 nm. The percentage of protein, triglycerides, phospholipids, cholesteryl esters and cholesterol in VLDL is 5–12 per cent, 50–55 per cent, 18–20 per cent, 12–15 per cent and 8–10 per cent respectively and also contains different types of apolipoproteins. VLDL particles synthesized by the liver that transport triglycerides to muscles and to fat tissue.

10.7.4 Intermediate Density Lipoproteins (IDL)

The size of IDL is 40 nm and smaller than VLDL. The percentage of protein, triglycerides, phospholipids, cholesteryl esters and cholesterol in IDL is 10–12 per cent, 24–30 per cent, 25–27 per cent, 32–35 per cent and 8–10 per cent respectively and also contains same apolipoproteins as that of VLDL. IDLs are either converted to LDLs or taken up by the liver.

10.7.5 Low Density Lipoproteins (LDL) and Lipoprotein(a)

The size of LDL is 26 nm and smaller than IDL. The percentage of protein, triglycerides, phospholipids, cholesteryl esters and cholesterol in IDL is 20–22 per cent, 10–15 per cent, 20–28 per cent, 37–48 per cent and 8–10 per cent respectively and also contains apolipoproteins. LDL particles are the carriers of cholesterol and are also known as the 'bad cholesterol.' They are called bad cholesterol because when LDL cholesterol is more in the blood then it combines with other substances and forms plaques which block the arteries which carries blood to the heart and brain. This condition is called atherosclerosis. If a clot formation takes place in the plaque region in arteries, then the flow of blood is stopped; if the blood flow is blocked to heart muscle then it can lead to heart attack, and if the blood flow is blocked to the brain, it can cause a stroke.

10.7.6 High Density Lipoproteins

The size of HDL is 6–12.5 nm and smaller than LDL. The percentage of protein, triglycerides, phospholipids, cholesteryl esters and cholesterol in HDL is 55 per cent, 3–15 per cent, 26–46 per cent, 15–30 per cent and 2–10 per cent respectively and contains several types of apolipoproteins. HDL is also known as the 'good cholesterol' because it removes the cellular cholesterol and takes away cholesterol from body cells and tissues to the liver for excretion. If the HDL levels are high, then the risk for coronary heart disease (CHD) is less.

10.8 BIOSYNTHESIS OF TRIACYLGLYCEROLS

Biosynthesis of triacylglycerols depends upon the energy needs of an organism. The fatty acids synthesized or ingested by an organism have two fates, either fatty acids are stored in the form of triacylglycerol for the storage of metabolic energy or incorporated into the phospholipid components of membranes.

When the organism is growing rapidly, synthesis of new membranes requires the production of membrane phospholipids; when an organism has plenty of food supply and not growing then it shunts most of the fatty acid into storage fat as triacylglycerols.

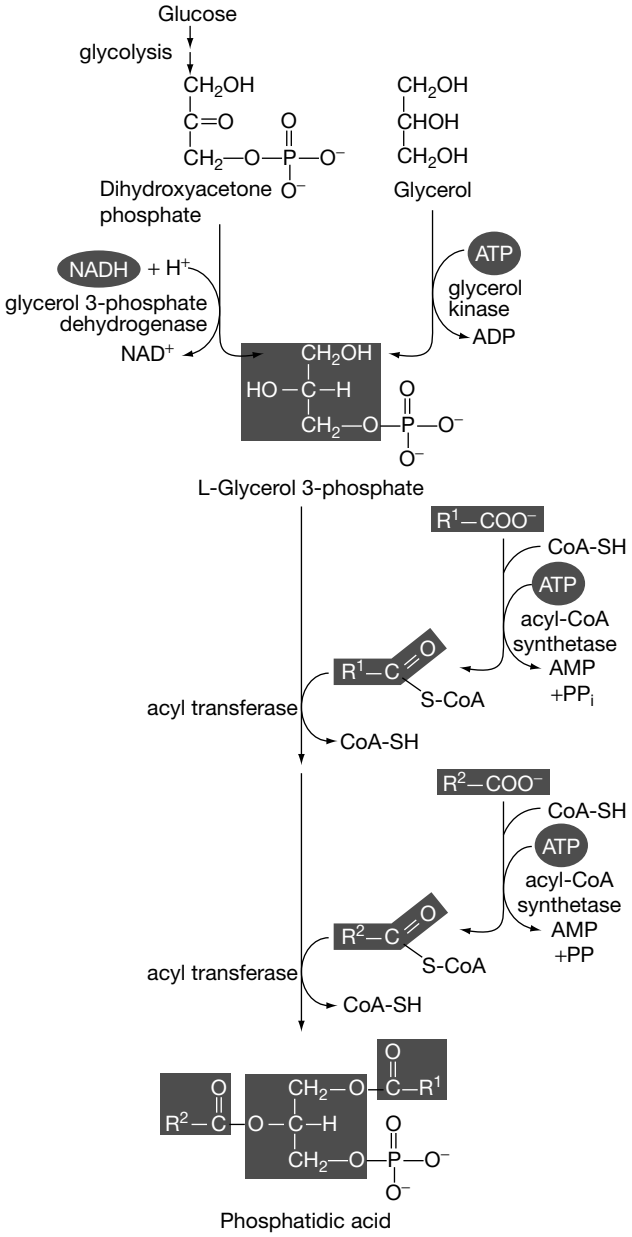


Figure 10.19 Continued

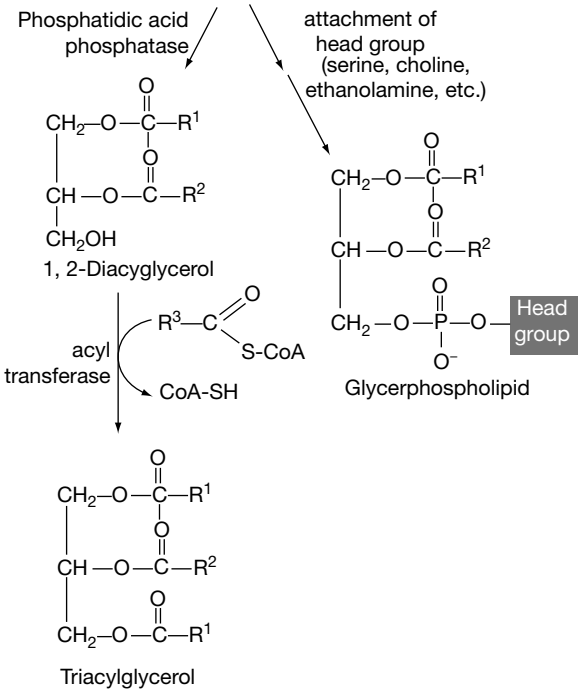


Figure 10.19 Biosynthesis of Triacylglycerol and Glycerophospholipid from Phosphatidic acid

The first step in the biosynthesis of triacylglycerols involves the formation of L-α-glycerophosphate which may be found by the action of a glycerol kinase enzyme on ATP and glycerol. The glycerol kinase enzyme is active in liver and kidney, but inactive in adipose tissue and many other tissues. In these tissues, L-glycerol-3-phosphate must be supplied by the glycolytic pathway. Triacylglycerols are found from glycerol-3-phosphate and acyl-CoA. The first product is a diacylglycerol-3-phosphate more commonly called phosphatidic acid which is either hydrolysed by phosphotase to form a diacylglycerol, the diacylglycerol reacted with another acyl-CoA to yield triacylglycerols, or phosphatidic acid gets converted to a glycerophospholipid (Figure 10.19).

10.9 GLYCEROPHOSPHOLIPID BIOSYNTHESIS

Phosphatidic acid can be converted into phospholipids. Phospholipids are the main components of biological membranes.

10.9.1 Phosphatidyl Choline and Phosphatidyl Ethanolamine Biosynthesis

The activation of choline or ethanolamine is the first step in the biosynthesis of these two glycerophospholipids. The activation is a two-step process as discussed hereunder.

The first step is phosphorylation of phosphoethanolamine or phosphocholine with ATP.

The second step synthesizes cytosine diphosphate ethanolamine or cytosine diphosphate choline. This reaction is catalysed by cytidylyl transferase. This reaction yields pyrophosphate which is hydrolysed by pyrophosphatase which makes this activation irreversible.

Diacylglycerol is formed from phosphatidic acid when phosphatidic acid gets dephosphorylated by the enzyme phosphatidic acid phosphatase dephosphorylate.

CDP-ethanolamine or CDP-choline reacts with diacylglycerol to form phosphatidylethanolamine or phosphatidylcholine (Figure 10.20).

The enzymes that catalysed these two reactions are given hereunder.

CDP ethanolamine: 1, 2-diacylglycerol phosphoethanolamine transferase and CDP choline: 1, 2-diacylglycerol phosphocholine transferase.

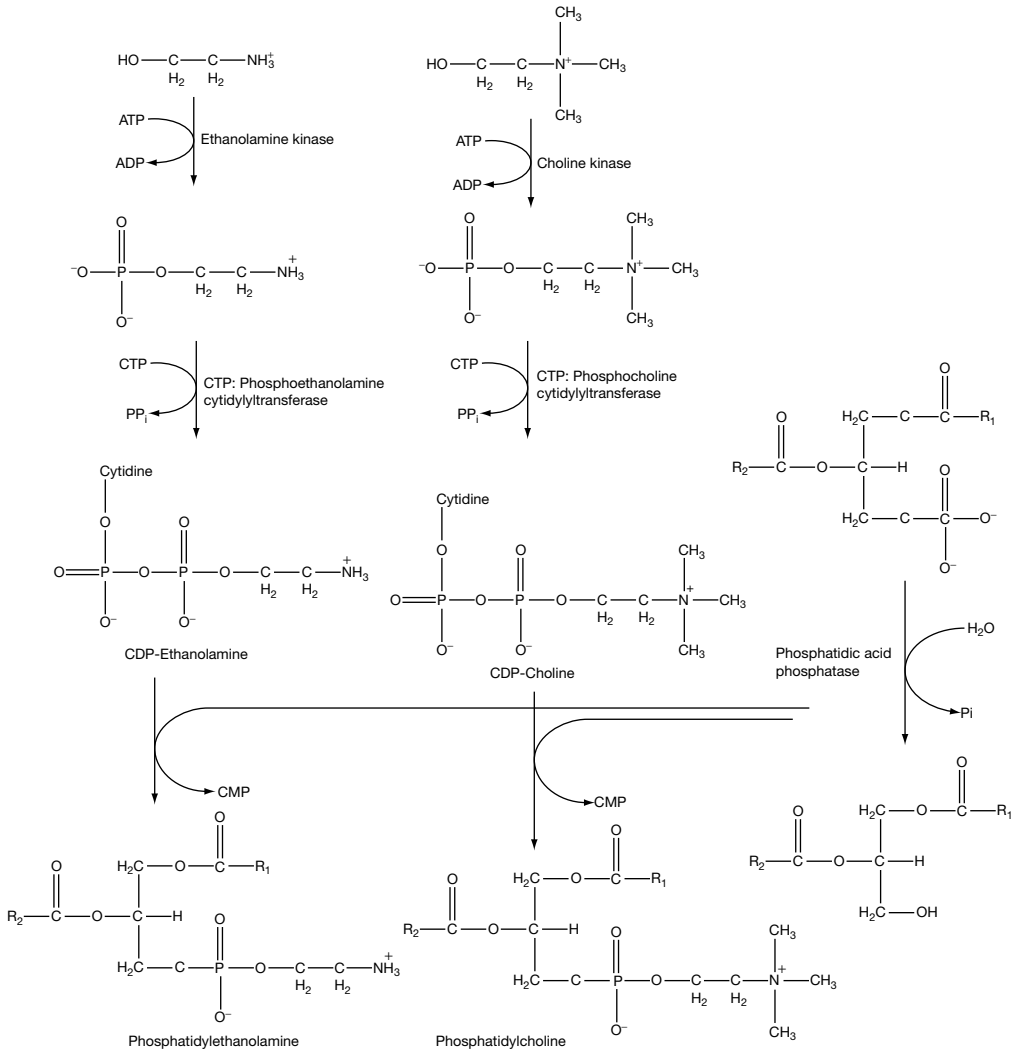


Figure 10.20 Continued

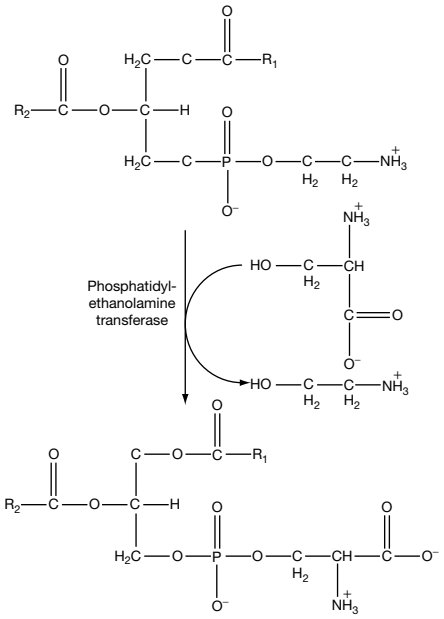


Figure 10.20 Biosynthesis of Phosphatidyl Choline and Phosphatidyl Ethanolamine

10.9.2 Phosphatidylserine

Phosphatidylserine is synthesized in mammals by phosphatidylethanolamine transferase. In this enzyme the hydroxyl group of serine attacks the phosphate group of phosphatidylethanolamine eliminating ethanolamine to form phosphatidyl serine (Figure 10.21).

10.9.3 Phosphatidylinositol, Phosphatidylglycerol and Cardiolipin Biosynthesis

To synthesize these phospholipids, the phosphatidic acid needs to be activated in order to be condensed with an alcohol to produce the corresponding phospholipids. Activation of phosphatidic acid is in the same manner as ethanolamine and choline.

CDP: CDP reacts with phosphatidate to form cytidine diphosphodiacylglycerol using phosphatidate cytidylyltransferase. This activation reaction produces pyrophosphate which is hydrolysed by pyrophosphatase which makes this activation irreversible (Figure 10.22).

10.10 EICOSANOIDS

Twenty carbon length fatty acids produced upon the breakdown of phospholipids are known as eiconsanoids. In response to hormonal signals or other stimuli activate phospholipase A2 which attacks phospholipids, and arachidonate is released from the middle carbon of glycerol. Arachidonate is converted to prostaglandins by the enzymes of smooth endoplasmic reticulum starting the formation of prostaglandins H_2 (PGH_2), which is the precursor of many other prostaglandins and thromboxanes.

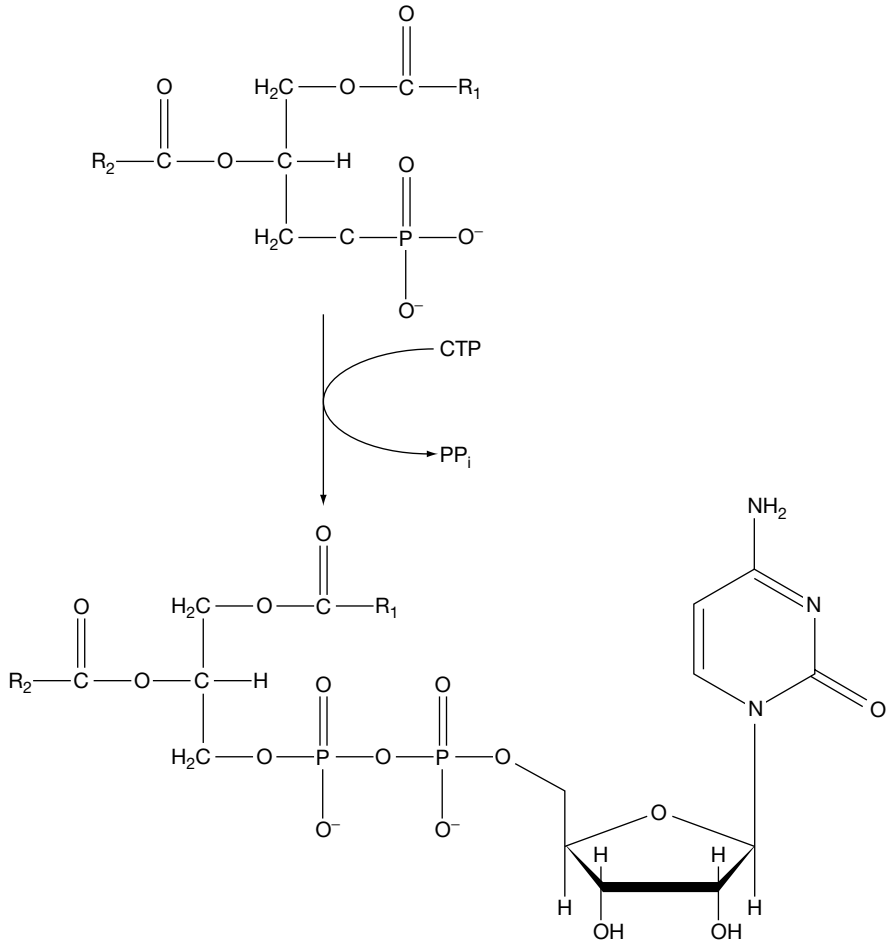


Figure 10.21 Biosynthesis of Phosphatidylserine

10.10.1 Characterstics of Eicosonaids

1. Eicosonaids have short lifetimes (between 30 and 200 s).
2. Eicosanoids are effective at very low concentrations (10–14 M).
3. Eicosanoids act at sites near their biosynthesis.
4. Eicosanoids include thromboxanes (Tx), leukotrienes, prostaglandins (PGF), etc.
5. Eicosanoids are synthesized from arachidonic acid in the endoplasmic reticulum.

10.10.2 Eicosanoid Synthesis

The first step of eicosanoid synthesis is catalysed by the enzyme cyclooxygenase (COX) also known as prostaglandins H_2 synthase.

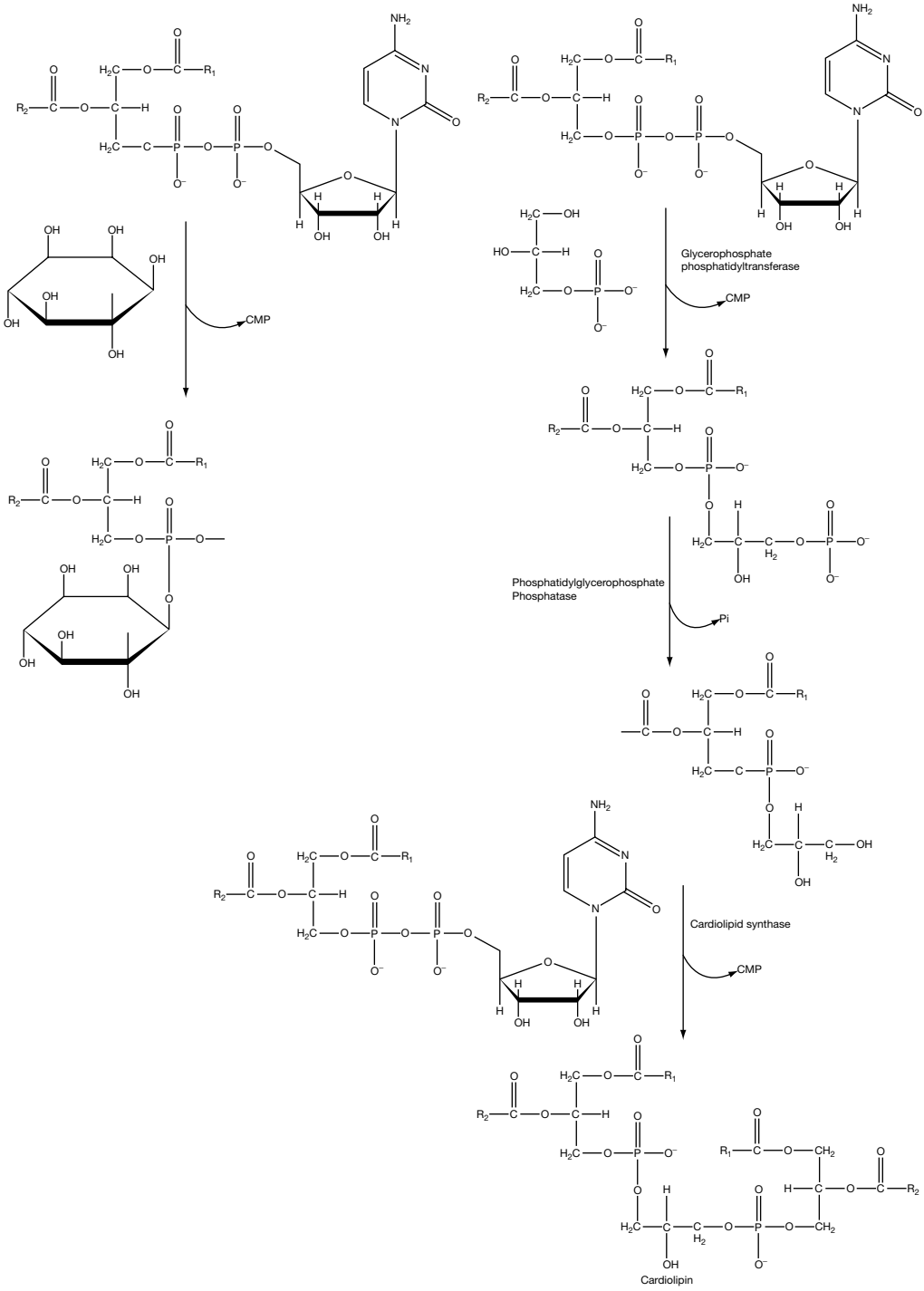


Figure 10.22 Biosynthesis of Phosphatidylinositol, Phosphatidylglycerol and Cardiolipin

The release of arachidonate is activated by variety of stimuli and the conversion into eicosanoids. Platelets produce thromboxane A2 to stimulate platelet aggregation. The main roles of eicosanoids include participation in tissue injury, inflammation and pain sensitivity. Whenever there is a tissue injury, the injured tissues are invaded by special inflammatory cells, monocytes and neutrophils, and interact with the smooth muscle cells and fibroblasts. The arachidonate release and eicosanoid production is stimulated by this interaction. Some of the examples of tissue injury due to which eicosanoid biosynthesis gets stimulated are heart attacks, rheumatoid arthritis and ulcers.

Aspirin inhibits COX activity and reduces pain sensitivity. This is done by transferring an acetyl group (from acetylsalicylic acid) to serine residue, which prevents the first step of eicosanoid formation. Large number of eicosanoids formation is prevented by aspirin, which leads to number of side effects, like preventing aggregation of platelets to form blood clots and stops the mucin secretion in the stomach which protects the gastric wall.

The drugs, ibuprofen (motrin) and acetaminophen (tylenol) are competitive inhibitors for the arachidonate. These drugs are nonsteroidal anti-inflammatory, anti-fever and pain relievers.

There are two isozymes of enzyme cyclooxygenase (COX) called COX-1 and COX-2. Both COX-1 and COX-2 are reversibly inhibited by ibuprofen and acetaminophen and irreversibly inhibited by aspirin. The important functions of COX-1 are platelet aggregation maintenance of renal blood flow, mucin secretion and regulation of gastric acid secretion. The expression of COX-2 is induced by inflammatory mediators such as interleukin-1. Eicosanoids are produced by COX-2 which promotes inflammation, fever and pain (Figure 10.23).

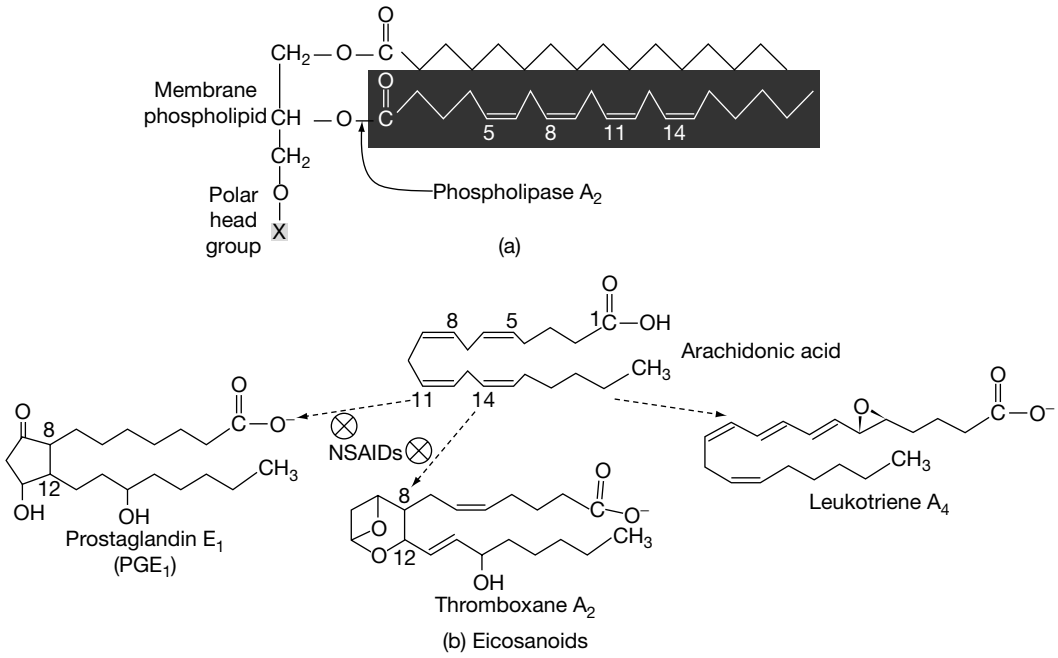


Figure 10.23 Eicosanoid synthesis is the simultaneous epoxidation and cyclization catalysed by the enzyme cyclooxygenase (COX) also known as prostaglandin H_2 synthase

10.11 METABOLIC DISORDER OF LIPIDS: DISTURBANCES IN LIPID METABOLISM

Normally the various kinds of lipids of the various tissues are synthesized, deposited mobilized and catabolized at rates that tend to maintain a relatively steady state. The most common disturbances in lipid metabolism involve the deposition and mobilization of triglycerides in the fat depots, leading to obesity when deposition exceeds mobilization and ultimately to cachexia when mobilization exceeds deposition.

Obesity: The amount of fat in the storage depot is determined by the balance between the rates of deposition and mobilization. An animal becomes fat when the rate of deposition exceeds the rate of mobilization and lean when the process is reversed. It has been established that obesity results purely from the ingestion of more food than is necessary to meet energy requirements. In other words, it is a question of the appetite being improperly balanced (or controlled) with energy needs. Alteration in the energy demand of an animal without simultaneous change in appetite may occur for various reasons. This may be true with decreased activity and to lead obesity. Castration is often associated with obesity because of lowered activity hypothyroidism, which depresses the rate of food oxidation. In some cases, neither of these conditions causes obesity because the appetite is proportionately lowered. Certain pituitary deficiencies may lead to abnormal distribution of body fat without changing the total quantity. Some cases of hyperinsulinism become obese because of the greatly stimulated appetite. It has been demonstrated that the hypothalamic region of the brain is concerned with regulation of appetite. Injuries to the region in experimental animals may lead to great obesity due to appetite stimulation.

Cachexia: There are a number of conditions that lead to abnormally high rates of mobilization from fat depots relative to the rates of deposition, causing in severe cases, disappearance of most of the adipose tissue. Such situations may be observed in carcinoma, malnutrition, certain chronic infectious diseases, hyperthyroidism and severe uncontrolled diabetes. The greatly increased mobilization of body fat to supply the increased energy demand is the primary cause of the wasting away or cachexia of hyperthyroidism. Two processes operate to cause the cachexia of severe uncontrolled diabetes. In the first place due to inability to utilize appreciable carbohydrate, energy is supplied through the mobilization and utilization of excessive amounts of depot fat. In the second place, the capacity of the diabetic to synthesized fat for deposit is drastically reduced.

Severe thiamine deficiency leads to decreased capacity to convert carbohydrate to fat and also decreased appetite with depletion of fat depots. Of course, in plain starvation the net effect is fat mobilization to supply energy without deposition.

Idiopathic hyperlipemia (Buerger-Grutz disease): This hereditary disease is characterized by very high serum lipid levels. Total fatty acids, largely as fat may be 10 times the normal. Also, there are marked elevations of cholesterol and phospholipids. The fasting serum is milky white. Widely distributed cutaneous xanthomas, consisting of soft yellow papules and nodules are a prominent feature of the disease. The principal complications are episodes of relapsing pancreatitis and atherosclerosis. Many patients in the teens and twenties suffer too.

Little is known about the cause of the disease though there is some evidence that the turnover of plasma fatty acids is decreased and that blood lipase is deficient.

Gaucher's disease: Gaucher first observed this hereditary disease in a patient in whom the splenic pulp had been replaced entirely by large pale cells, which now are known as 'Gaucher cells.' The Gaucher cells are found particularly in spleen, brain and bone marrow. Gaucher cells have found to contain abnormally large amount of (a) a cerebroside composed of galactose, sphingosine and lignoseric acid (kerasin); (b) a cerebroside containing glucose instead of galactose and (c) a water-soluble glycolipid made up of sphingosine or a sphingosine-like base, long chain saturated fatty acids and one or more hexose groups. This substance has been named 'polycerebroside.'

Thannhauser considers that in Gaucher's disease there is an imbalance between the rates of cerebroside formation and catabolism, leading to the accumulation of cerebrosides in certain cells to form Gaucher cells.

Niemann-Pick disease: This disease has many clinical features in common with Gaucher's disease. It is characterized by a very generalized distribution of large pale cells (Niemann-Pick cells) having a foamy appearance of vacuolated cytoplasm. The spleen, lung, lymph nodes, liver and bone marrow are primarily involved, though organs in general are infiltrated. The ganglion cells of the central nervous system and elsewhere undergo degeneration. There is an immense accumulation of lipid in the Niemenn-Pick cells, of which sphingomyelin is the major component. Thannhausar has postulated that in Niemann-Pick disease the conversion of ceramide (acylsphingosine) to sphingomeyelin is normal but the reverse process is defective. A hereditary condition of infants which in many respects resembles Neimann-Pick disease is **Tay-Sachs disease**. Both conditions occur most frequently in individuals of the Jewish race; in both conditions, there is a cherry red spot in the macula and macular degeneration; the histological changes in the brain are similar in both conditions and cases of Niemann-Pick and Tay-Sachs disease have been observed in siblings of the same families. In Tay-Sachs disease, the chief pathology is related to degeneration of ganglion cells of the central nervous system and retina. Apparently, this degeneration is caused by the accumulation of the lipid in the cells. However, sphingomyelin does not appear to be prominent component of this lipid.

There are a number of other pathological states associated with abnormal lipid metabolism as discussed hereunder.

Atherosclerosis: Atherosclerosis disease is associated with a variable combination of changes in the intima of arteries with focal accumulation of lipids, carbohydrates blood and blood products, fibrous tissue and calcium. It can be seen that lipids are only one of the several of factors concerned with the disease. Atherosclerosis occurs in the aorta, coronary, cerebral, renal, iliac and femoral arteries. There is a gradual narrowing of the artery leading to a reduction in blood supply, and there may be a sudden occlusion of a vessel due to the supreme imposition of a thrombus on the atherosclerotic lesion. In the earliest stages of the disease, there is a focal accumulation of fat-laden cells under the endothelium. Subsequently, fibrous tissue is added, and there are ulceration and other changes.

EXERCISES

1. What are lipids? How are they classified? Discuss the characteristics and functions of a class of lipids having nitrogen base and phosphoric acid as constituents.
2. Give the classification and characteristics of lipids. Discuss briefly the chemistry and importance of phospholipids.

3. Write short notes on:
   (a) Essential fatty acids
   (b) Sterol
   (c) Phospholipids
   (d) Saponification number
   (e) Hydrogenated fats
   (f) Lecithin
   (g) lodine number
   (h) Lipoproteins
   (i) Unsaturated fats
   (j) Saponification
   (k) Rancidity
   (l) Cholesterol
4. How can the lipids be classified? Enumerate some neutral fats present in our body.
5. Name and with test and reactions show the presence of characteristics functional group occurring in glycerol.
6. With structures, give examples of two unsaturated fatty acids essential for our body.
7. Give an account of the metabolism of fat with particular reference to β-oxidation.
8. What is lipogenesis? Describe the cytoplasmic system of fatty acid synthesis.
9. Describe the oxidation of fatty acids in the body.
10. How are fatty acids synthesized and degraded in the body?
11. Describe the fate of lipids in our body. What are essential fatty acids?
12. Describe the complete oxidation of oleic acid in the body. State how many molecules of ATP are formed during the process.
13. Describe briefly the chemistry of cholesterol and its physiological importance. What is the normal blood cholesterol level?
14. Describe metabolism of cholesterol with reference to atherosclerosis. Discuss the role of lipoprotein in it.
15. What are ketone bodies? Describe how ketosis is produced in the body.
16. What is ketosis? How are the ketone bodies produced in the body? How does body try to combat ketosis?
17. Discuss the following:
   (a) β-oxidation of fatty acids
   (b) Transport of lipid
18. Write in detail about the biosynthesis of cholesterol.
19. Define lipids and write in detail about the classification of lipids and their physiological function.
20. Short notes on:
   (i) Essential fatty acids
   (ii) Cholesterol
   (iii) Lipoprotein
   (iv) Phospholipids

(v) Phosphorylation
(vi) Ketosis
(vii) Ketone bodies
(viii) Ketogenesis
(ix) Unsaturated fatty acids

MULTIPLE CHOICE QUESTIONS

1. Fats are solids at
   (a) 10°C (b) 20°C
   (c) 30°C (d) 40°C
   Ans. b
2. Esters of fatty acids with higher alcohols other than glycerol are said to be
   (a) Waxes (b) Fats
   (c) Both (a) and (b) (d) None of these
   Ans. a
3. The combination of an amino-alcohol, fatty acid and sialic acid form
   (a) Phospholipids (b) Sulpholipids
   (c) Glycolipids (d) Aminolipids
   Ans. c
4. Hydrolysis of a fat by an alkali is called
   (a) Saponification number
   (b) Saponification
   (c) Both (a) and (b)
   (d) None of these
   Ans. b
5. The rate of fatty acid oxidation is increased by
   (a) Phospholipids (b) Glycolipids
   (c) Amino lipids (d) All of these
   Ans. a
6. Cardiolipin found in mitochondria is formed from
   (a) Lipositol
   (b) Phosphatidyl ethanolamine
   (c) Phosphatidyl glycerol
   (d) None of these
   Ans. c
7. Lecithin contains a nitrogenous base named as
   (a) Ethanolamine (b) Choline
   (c) Inositol (d) All of these
   Ans. b
8. Phosphatidyl inositol is found in
   (a) Cabbages (b) Soya beans
   (c) Cauliflowers (d) Apples
   Ans. b
9. The alkyl radical in plasmalogen is an _____ alcohol
   (a) Saturated (b) Unsaturated
   (c) Both (a) and (b) (d) None of these
   Ans. b
10. The concentrations of sphingomyelins are increased in
    (a) Gaucher's disease
    (b) Fabry's disease
    (c) Febrile disease
    (d) Niemann-Pick disease
    Ans. d
11. Sphingomyelins contain a complex amino alcohol named as
    (a) Serine (b) Lysolecithin
    (c) Sphingosine (d) Glycol
    Ans. c
12. Glycolipids contain an amino alcohol
    (a) Sphingosine
    (b) Iso-sphingosine
    (c) Both (a) and (b)
    (d) None of these
    Ans. c
13. Cerebrosides may also be classified as
    (a) Sphingolipids (b) Sulpholipids
    (c) Aminolipids (d) Glycolipids
    Ans. a

14. Gangliosides are the glycolipids occurring in
    (a) Liver (b) Brain
    (c) Kidney (d) Muscle
    Ans. b
15. The protein moiety of lipoprotein is known as
    (a) Apoprotein (b) Pre-protein
    (c) Post-protein (d) Pseudoprotein
    Ans. a
16. The iodine number of essential fatty acids of vegetable oils
    (a) High (b) Very high
    (c) Very low (d) Low
    Ans. d
17. The reduction product of cholesterol by bacteria in the intestine occurs in faeces
    (a) Ergosterol (b) Demosterol
    (c) Coprosterol (d) Lanosterol
    Ans. c
18. Fatty acids are oxidized by
    (a) α-Oxidation (b) β-Oxidation
    (c) ω-Oxidation (d) All of these
    Ans. d
19. Long chain fatty acids are first activated to acyl-CoA in the
    (a) Cytosol (b) Mitochondria
    (c) Microsomes (d) Lysosomes
    Ans. a
20. Long chain acyl-CoA penetrates mitochondria in the presence of
    (a) Palmitate (b) Carnitine
    (c) Sorbitol (d) DNP
    Ans. b
21. For the activation of long chain fatty acids the enzyme thiokinase requires the cofactor
    (a) Mg^{++} (b) Ca^{++}
    (c) Mn^{++} (d) K^{+}
    Ans. a
22. The synthesis of prostaglandins is inhibited by
    (a) Aspirin (b) Arsenite
    (c) Fluoride (d) Cyanide
    Ans. a
23. Prostaglandins lower cyclic AMP in
    (a) Thyroid (b) Adipose tissue
    (c) Platelets (d) Lung
    Ans. b
24. Chylomicrons and VLDL both are released from the intestine or hepatic cell by reverse
    (a) Pinocytosis
    (b) Diffusion
    (c) Osmosis
    (d) Passive diffusion
    Ans. a
25. Cyclooxygenase is also termed as
    (a) Inhibiting enzyme
    (b) Prostaglandins H_2 synthase
    (c) Oxidizing enzyme
    (d) Reducing enzyme
    Ans. b
26. Leukotrienes are formed by only
    (a) 5-Lipoxygenase (b) Lipase
    (c) Phospholipase (d) None of these
    Ans. a

11 Metabolism of Amino Acids

CHAPTER OBJECTIVES

11.1 Introduction
11.2 Overview of Nitrogen Metabolism
11.3 Nitrogen Balance
11.4 Some Reactions Play Important Role in Biosynthesis of Amino Acids
11.5 Metabolism of Ammonia
11.6 Urea Cycle
11.7 Biosynthesis of Amino Acids
11.8 Catabolism of Amino Acid
11.9 Plant and Animal Pigments
11.10 Metabolic Disorder of Amino Acids

11.1 INTRODUCTION

The amino acids share with the purine and pyrimidine nucleotides the fact that they are nitrogen-containing building blocks of large, informational molecules—the proteins and nucleic acids. Higher plants and many microorganisms regularly obtain the nitrogen required for biosynthesis of these compounds in the form of nitrate ion. Plants and most microorganisms will also utilize NH_3 when it is available as a source of nitrogen for synthesis of amino acids, proteins, and nucleic acids. While the higher animal can also utilize NH_3 for synthesis of its nitrogen-containing compounds, the animal's principal source of nitrogen is the protein it consumes in its diet. The protein is hydrolysed to amino acids by enzymes in the gastrointestinal tract, and these are absorbed into the blood and transported to the liver. This organ will remove a portion of the amino acids for specific biosynthetic tasks, while the remainder pass on to extra-hepatic tissues where they can be synthesized into proteins. The liver is the site of synthesis of several blood proteins (plasma albumin, the globulins, fibrinogen and prothrombin). It also metabolizes any amino acids in excess of hepatic needs for protein synthesis, converting the nitrogen atoms into urea and the carbon skeleton into intermediates previously encountered in the metabolism of carbohydrates

and lipids. While much is known about the detailed metabolism of the 20 amino acids found in most proteins, we have space only to treat those reactions which, in general, apply to all amino acids and to discuss the role of NH_3 in the formation of urea and the purines and pyrimidines.

11.2 OVERVIEW OF NITROGEN METABOLISM

Nitrogen is the important requirement for the biosynthetic pathways leading to amino acids and nucleotides. Because soluble, biologically useful nitrogen compounds are generally scant in natural environments, organisms are economic in their use of ammonia, amino acids and nucleotides. Free amino acids, purines and pyrimidines formed during metabolic turnover of nucleic acids and proteins are often salvaged and reused. How nitrogen is introduced into biological systems from the environment is discussed below.

11.2.1 Biologically Available Nitrogen Pool is Maintained by Nitrogen Cycle

Air is the source of nitrogen. Only few species can convert atmospheric nitrogen into useful forms to living organisms. Different species, metabolic processes in the biosphere function interdependently to save and use again biologically available nitrogen in a huge **nitrogen cycle** (Figure 11.1).

Nitrogen Fixation: Fixation (reduction) of atmospheric nitrogen by nitrogen fixing bacteria to yield ammonia (NH_3 or NH_4^+) is the first step of nitrogen cycle.

Nitrification: Although, most living organisms can use ammonia, ammonia is oxidized to nitrite (NO_2^-) and then to nitrate (NO_3^-) by soil bacteria known as nitrifying bacteria, and this process is known as **nitrification**.

Denitrification: Nitrate and nitrite are taken up by plants and several bacteria and reduce them by nitrate and nitrite reductases. Plants incorporate ammonia into amino acids. Therefore, plants are the source of amino acids for animals, both nonessential and essential, to build their proteins. Proteins of dead organisms undergo microbial degradation and return ammonia back to the soil, this ammonia is converted back to nitrite and nitrate by nitrifying bacteria. The bacteria under anaerobic conditions convert nitrate to N_2, and maintains the balance between fixed nitrogen and atmospheric nitrogen, and this process is known as **denitrification** (Figure 11.1).

Oxygen is the final electron acceptor of these soil bacteria used in a series of reactions, generates a transmembrane proton gradient, which is used to synthesize ATP.

11.3 NITROGEN BALANCE

Our knowledge of the intermediary metabolism of amino acids and proteins has its foundations in early nutritional investigations. Osborne and Mendel demonstrated in 1914 that the growing rat required tryptophan and lysine in its diet. Subsequently, W. C. Rose showed that eight other amino acids were required by the rat for growth and development. World War II provided the stimulus and the research funds for identifying the amino acids required by man in experiments which involved the feeding or gram quantities of highly purified amino acids to male volunteers.

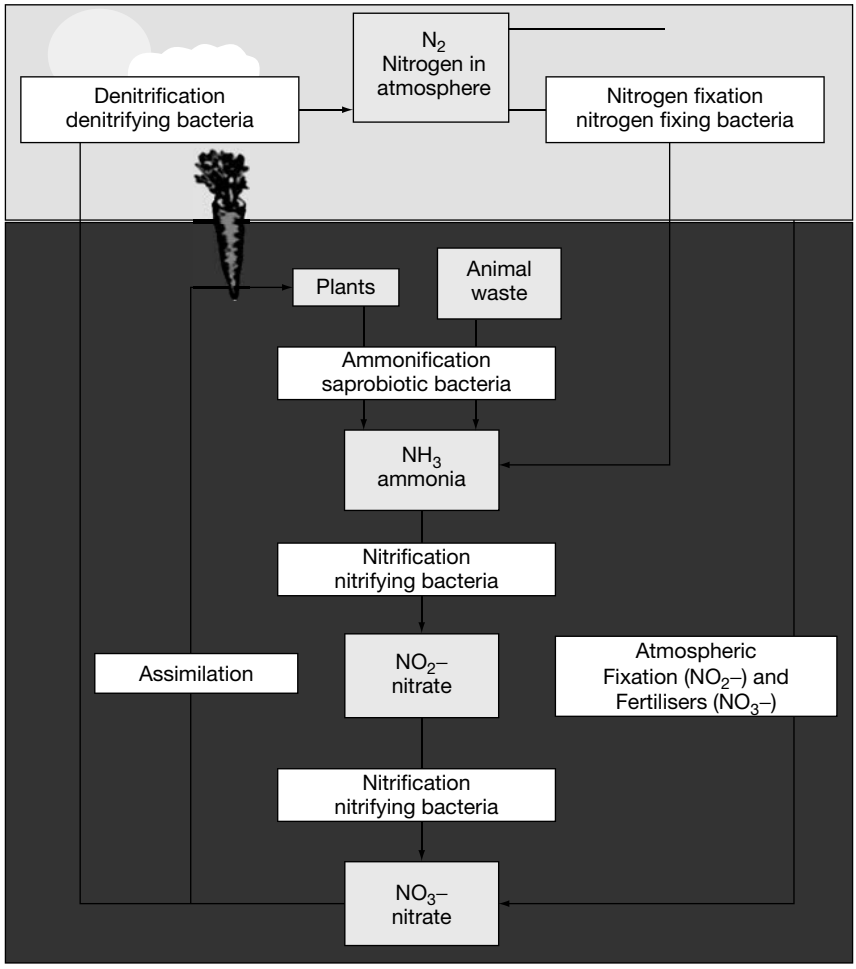


Figure 11.1 Nitrogen Cycle

These experiments, which were performed by keeping the subjects in nitrogen equilibrium, demonstrated that lysine, tryptophan, phenylalanine, threonine, valine, methionine, leucine and isoleucine were indispensable.

An individual (man or other animal) is said to be in nitrogen equilibrium when the nitrogen consumed per day in the diet is equal to the amount of nitrogen excreted. The former is easily measured, especially if the diet is a synthetic one consisting of a mixture of amino acids; the nitrogen excreted is that found in the urine and faeces.

An adult animal can be maintained in nitrogen equilibrium provided an amount of nitrogen is supplied which is adequate to meet its minimum metabolic needs. If one of these amino acids is omitted from the diet, the animal will degrade tissue proteins to meet its requirements and will go into negative nitrogen balance. That is, the nitrogen excreted in the urine and faeces

exceeds that in the diet. When the omitted amino acid is restored to the diet, the individual attains equilibrium again.

Fevers and wasting diseases place an individual in negative nitrogen balance as does inadequate dietary nitrogen. On the other hand, and growing animal which is continually increasing the amount of its body protein will be in positive nitrogen balance, that is, it takes in more nitrogen than it excretes.

There are two important consequences of the nutritional work on the indispensable amino acids we have described. First, it is clear that the animal cannot make these amino acids at least in the amounts it requires. We may ask, then, whether the animal lacks the ability to make the carbon skeleton of the indispensible amino acid. The answer apparently is yes, for if an animal which is being furnished a diet which is deficient in phenylalanine for example, is supplied with phenylphruvic acid, the keto analogue of phenylalanine, and extra nitrogen in the form of the other indispensable amino acids, it goes into equilibrium. These results are interpreted as meaning that the problem is not one of supplying nitrogen but rather one of synthesis of the carbon skeleton. In the case of phenylalanine, the difficulty is in the synthesis of the aromatic ring the amino acid possesses. Thus, it may be concluded that certain types of carbon skeleton are not readily synthesized by higher animals.

Since only about half of the amino acids that occur naturally in proteins are indispensable to animals, it is clear that animals can synthesize the remaining amino acids. These amino acids which can be synthesized are known as dispensable amino acids. Their synthesis involves not only the manufacture of the carbon skeleton, but also includes the aspartic acid, to complete the dispensable amino acid. This transfer of the nitrogen atom is accomplished by transamination, a general reaction of amino acids that is involved both in the breakdown and the synthesis of many of aminoacids.

11.4 SOME REACTIONS PLAY IMPORTANT ROLE IN BIOSYNTHESIS OF AMINO ACIDS

Transamination: The transfer of the amino group of an amino acid to an alpha-keto acid to form a new amino and keto acid, this reaction is catalysed by the enzymes transaminases or aminotransferases, known as transamination (Figure 11.2). This reaction uses pyridoxal phosphate as coenzyme by its reversible conversion to pyridoxamine phosphate. The reaction is given hereunder (Figure 11.3).

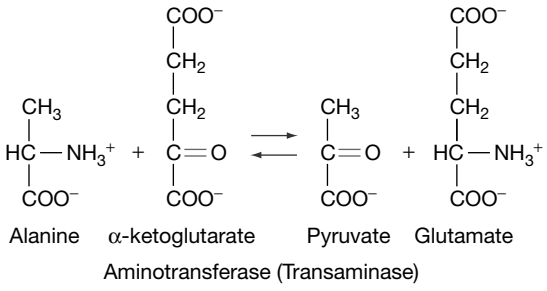


Figure 11.2 Transamination reaction

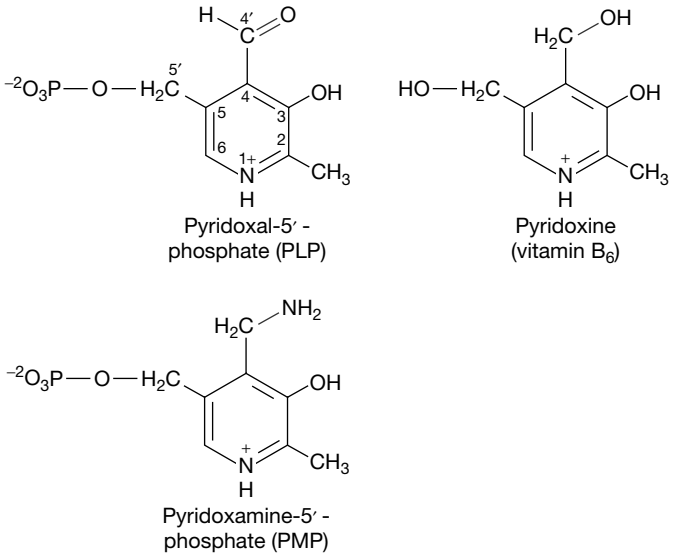


Figure 11.3 Structure of Pyridoxal phosphate, Pyridoxine and Pyridoxamine

Transamidation: In transamidation, the transfer of the amide nitrogen of glutamine to a keto group (in a same way as that in transamination) takes place. If amide nitrogen of glutamine is transferred to an α-keto acid, an amino acid can be formed. If it is transferred to the keto group of fructose, glucosamine is formed. Both the reactions, transamination and transamidation, serve in the interconversion and synthesis of the nonessential amino acids. Both transamination and transamidation reactions are active in liver and therefore liver serves as a control role in amino acid metabolism.

Oxidative Deamination of Amino acids: Oxidative deamination is the mechanism by which amino group is removed from the alpha-carbon of amino acid to form a keto group and free ammonia. D- and L-amino acid oxidases, present in liver, kidney and in other tissues, are active in deamination of the D- or L-amino acids, respectively. Normally, most of the deamination occurs in the liver. D- and L-amino acid oxidases contain FAD or FMN as the prosthetic group. The deamination mechanism is given hereunder.

$$R\,CH\,NH_2\,COOH + FP \xrightarrow{\text{L–amino acid oxidase}} \underset{\text{Imino acid}}{RC = NH\,COOH} + FPH_2$$

$$\underset{\text{Imino acid}}{RC = NH\,COOH} + H_2O \xrightarrow{\text{Spontaneous}} R\,CO\,\underset{\alpha\text{-keto acid}}{COOH} + NH_3$$

$$FPH_2 + O_2 \longrightarrow FP + H_2O_2$$

$$H_2O_2 \longrightarrow H_2O + \frac{1}{2}O_2$$

L-glutamic acid is deaminated by L-glutamic acid dehydrogenase which requires NAD or NADP as coenzyme; in this case, the deamination is reversible, whereas the deamination

by L-amino acid oxidase is not reversible. Actually, most amino acids are synthesized and deaminated by transamination reactions with glutamic acid which alone is capable of taking up ammonia direct while in the form of alpha-ketoglutarate.

Glycine is acted upon by a specific enzyme 'glycine oxidase.' The amino acids serine and threonine are deaminated nonoxidatively by dehydrases.

$$\frac{1}{2}O_2 + \underset{\text{Glycine}}{CH_2NH_2COOH} \xrightarrow{\text{Glycine oxidase}} \underset{\text{Glyoxylic acid}}{CHO \cdot COOH} + NH_3$$

$$\underset{\text{Serine}}{CH_2OH \cdot CHNH_2COOH} \xrightarrow{\text{Serine dehydrase}} CH_3 \cdot C = \underset{\text{Imino acid}}{NH \cdot COOH} + H_2O$$

$$CH_3C = NH \cdot COOH + H_2O \longrightarrow \underset{\text{Pyruvic acid}}{CH_3CO\,COOH} + NH_3$$

Decarboxylation: Tissues like liver and microorganisms of the intestinal tract contains enzymes called decarboxylases (which require pyridoxal phosphate as coenzyme) that remove CO_2 from the carboxylic group and convert the amino acid to its corresponding amine. This process is confined to putrefaction in intestines and produces toxic amines like tyramine, tryptamine, putrescine and cadaverine from tyrosine, tryptophan, ornithine and lysine, respectively.

$$R \cdot CH \cdot NH_2COOH \xrightarrow[\text{pyridoxalphosphate}]{\text{Decarboxylase}} R \cdot CH_2 \cdot NH_2 + CO_2$$

11.4.1 Metabolism of the Carbon Skeleton

After removal of the ammonia, the alpha-keto acid can follow one of these pathways, i.e., reamination to form original amino acid, oxidation to CO_2 and H_2O and formation of glucose (glucogenic amino acids) or ketone bodies (ketogenic amino acids).

11.5 METABOLISM OF AMMONIA

Deamination reactions liberate ammonia. Liver is the main site of ammonia metabolism. The normal levels of ammonia in blood is 10–12 μg/100 ml. Some amount of ammonia is also produced in the intestines due to bacterial action on food amino acids and may be absorbed. Hepatic diseases, lead to increase in blood ammonia levels with depression of the central nervous system (CNS), a condition called hepatic coma.

Methods Available for Removal of Ammonia

1. Amination of α-keto acids to form amino acids.
2. Amidation of glutamic acid to form glutamine

$$\text{Glutamic acid} + \text{Ammonia} + \text{ATP} \xrightarrow[\text{Glutamic synthetase, } Mg^{++}]{\text{Glutaminase}} \text{Glutamine} + H_2O + \text{ADP} + \text{Pi}$$

   The ammonia formed is removed by the formation of glutamine. Glutamine enters the blood stream and is transported to the kidney, where the reverse reaction is brought about by the enzyme glutaminase. The ammonia liberated is utilized to replace sodium and potassium of glomerular filtrate and is excreted as ammonium chloride or phosphate.
3. Formation of urea in the liver is the most important method for metabolism of ammonia as discussed in detail in the section on urea cycle.

11.6 UREA CYCLE

The urea cycle was discovered by the scientists Krebs and Henseleit, hence the name of the cycle is coined after their name. The urea cycle takes place in the liver. During liver disease such as liver cirrhosis, when liver functions are very poor in that case blood urea decreases with the increase in ammonia. Enzyme studies have revealed that arginase enzyme is present only in liver, which is required in the last step of urea cycle, which produces urea from arginine.

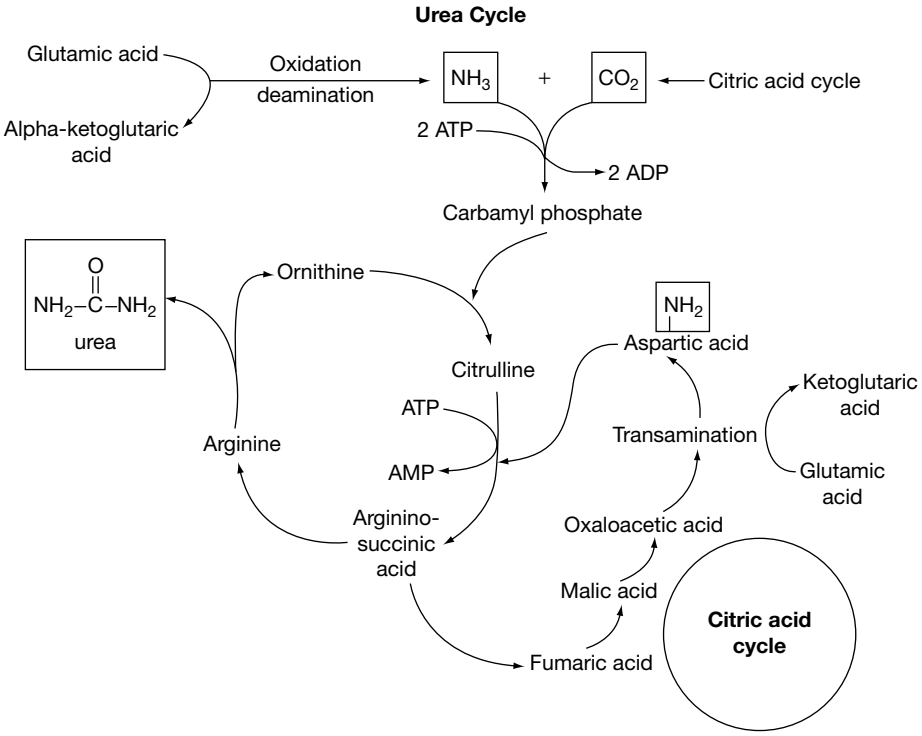


Figure 11.4 Urea Cycle and its interrelation with Citric acid cycle

Steps of urea cycle are listed hereunder.

1. **First step, formation of carbamyl phosphate:** The first step in the urea cycle is the formation of carbamyl phosphate. Carbon dioxide is first activated and the active CO_2 reacts with NH_3 to form carbamyl phosphate. Both reactions requires ATP and the presence of N-acetyl glutamate and catalysed by the enzyme carbamyl phosphate synthase I (present in the mitochondria).

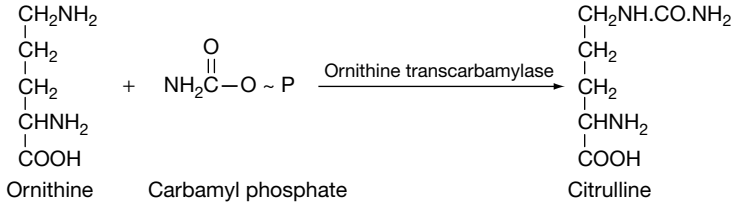
$$CO_2 + NH_3 + 2ATP \xrightarrow[\text{N-Acetyl glutamic acid, } Mg^{++}]{\text{Carbamyl phosphate synthase}} \underset{\text{Carbamyl phosphate}}{NH_3C(=O)\cdot O \sim P} + 2ADP + P$$

The urea cycle is effectively controlled by N-acetyl glutamic acid, formed by a combination of acetyl-CoA and glutamic acid.

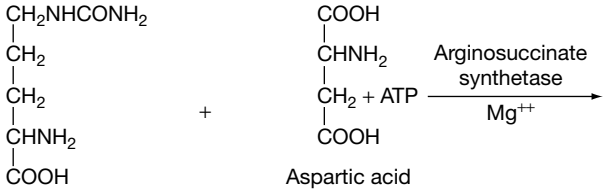
$$\text{Glutamate} + \text{Acetyl-CoA} \longrightarrow \text{N-acetyl glutamic acid} + \text{HS-CoA}$$

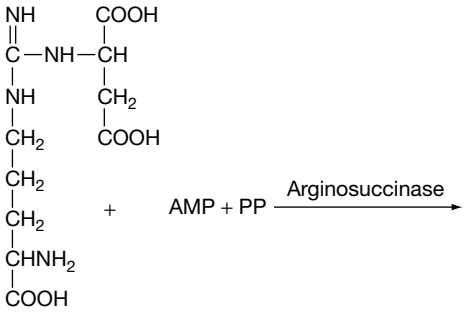
Increased levels of amino acid lead to increased formation of N-acetyl glutamic acid which allosterically stimulates the enzyme carbamyl phosphate synthase and drives the urea cycle forwards. When glutamate, aspartate, glutamine and aspargine are available in abundance, glutamate hydrogenase acts in the direction of deamination. When these amino acids are in short supply, the enzyme functions in the direction of amination.

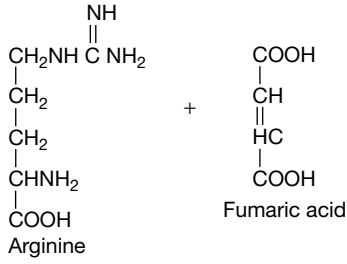
2. **Second step, formation of citrulline from ornithine:** This is catalysed by the enzyme ornithine transcarbamylase.



3. **Third step, formation of arginine from citrulline:** In this step, condensation of citrulline with aspartic acid takes place to form arginosuccinic acid by arginosuccinate synthetase which further splits into two molecules—arginine and fumaric acid by the enzyme arginosuccinase.

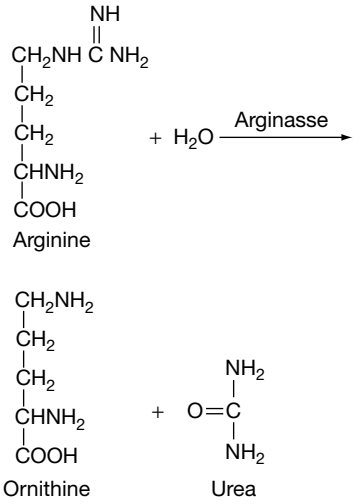






Fumaric acid can be converted to malic acid then to oxaloacetate in the citric acid cycle. The oxaloacetate upon amination again forms aspartic acid.

4. **Fourth step, formation of urea from arginine:** Arginase is the enzyme present in the liver, which hydrolyses arginine to form urea and ornithine. Mammalian liver arginase require cobalt or manganese ions as activator. Although little amounts of arginase are present in the kidney, brain, mammary gland, testis and skin, they have no role in urea formation.



Ornithine regenerated in the fourth step, re-enters the cycle by entering at second step. In the third step of urea cycle, aspartic acid has lost its ammonia in converting the ureido group of citrulline to the guanido group of arginine; however, it can be reformed by passing through citric acid cycle and later gets aminated.

In the mitochondrial matrix occurs the formation of carbamyl phosphate and the transfer of carbamyl group to ornithine to form citrulline. After the formation of citrulline, the remaining steps of urea cycle occurs in the cytosol. The ornithine now re-enters the mitochondrial matrix to repeat the cycle again.

Thus, carbamyl phosphate contributes the carbon and one of the amino nitrogens of urea. The second amino nitrogen is derived from the alpha-amino group of aspartic acid.

Deficiency may occur due to any of the enzymes used in the urea cycle.

1. Deficiency of carbamyl phosphate synthetase and ornithine transcarbamylase will result in accumulation of ammonia in blood and leads to hyperammonemia, types I and II.
2. Deficiency of arginosuccinate synthetase causes citrullinemia.
3. Deficiency of arginosuccinase results in accumulation of arginosuccinate in blood and leads to arginosuccinicaciduria.
4. Deficiency of arginase causes hyperarginemia.

Blood urea: Normal levels of urea in blood in an individual is 15 to 40 mg/100 ml. Urea is mainly excreted by the kidney. The urea clearance value is about 75 ml/min (maximal clearance, i.e., when urine formation is 2.0 ml/min or more). When urine formation is below 2.0 ml/min, the clearance rate is only about 54 ml/min (standard clearance).

11.7 BIOSYNTHESIS OF AMINO ACIDS

All amino acids are synthesized in the body from alpha-keto acids formed during carbohydrate metabolism by substituting the keto group by an amino group.

Mammals cannot synthesize all 20 amino acids, whereas most bacteria and plants can synthesize all the 20 amino acids. The amino acids which cannot be synthesized in the body and has to be obtained from food are called **essential amino acids**. The **nonessential amino acids** are not needed in the diet. The biosynthetic pathways for the 20 amino acids are discussed hereunder.

11.7.1 Glutamate and Aspartate

Glutamate is synthesized from widely distributed α-KG by a simple one-step transamination reaction catalysed by glutamate dehydrogenase (GDH) as shown hereunder (Figure 11.5).

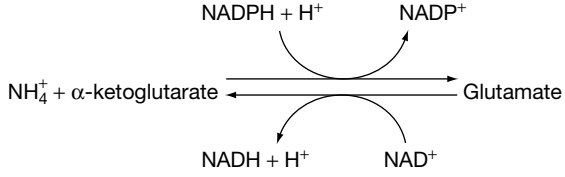
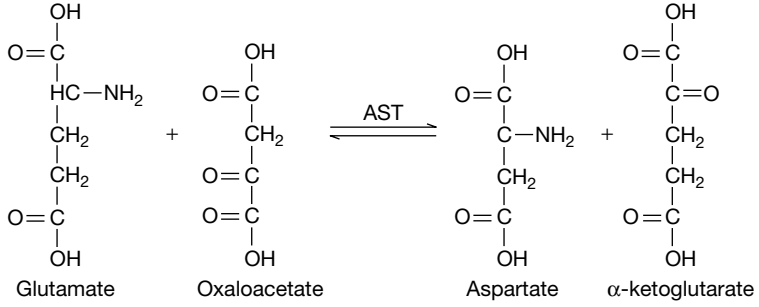


Figure 11.5 Reaction of glutamate dehydrogenase

Aspartate is synthesized by a transamination reaction catalysed by aspartate aminotransferase, AST, (earlier known as serum glutamate-oxalate transaminase, SGOT) as shown hereunder.



Reaction of Aspartate Aminotransferase

Aspartate can also be synthesized from asparagine by removal of ammonia by the action of asparaginase as shown hereunder (Figure 11.6).

Reaction by alanine transaminase: Alanine can be synthesized from pyruvate by alanine transaminase (Figure 11.7).

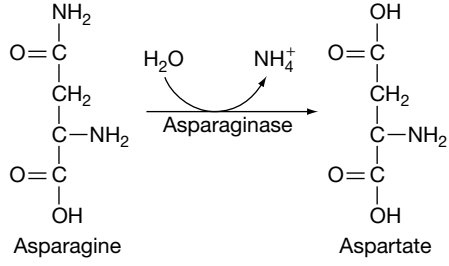


Figure 11.6 Reaction of Asparaginase

11.7.2 Glucose–Alanine Cycle

Mainly muscles and several other tissues transfer alanine to the circulation. Glucose is released from liver by glycogenolysis and gluconeogenesis during muscular contraction. Glucose is

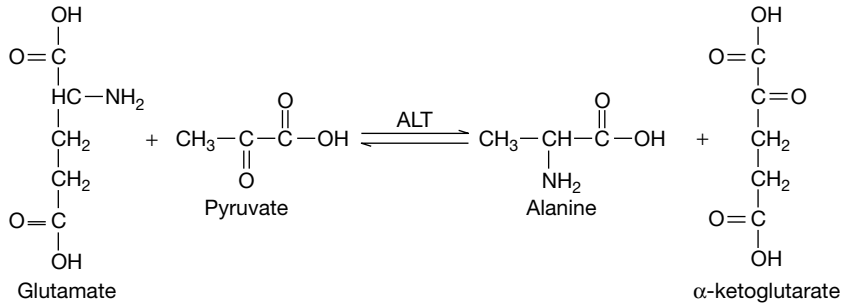


Figure 11.7 Transamination of pyruvate by alanine transaminase

utilized by muscle by glycolysis, producing pyruvate. While part of this pyruvate is converted to lactate, the rest is aminated to form alanine. In glucose–alanine cycle, the transport of glucose from liver to muscles and alanine from muscle to liver takes place (Figure 11.8).

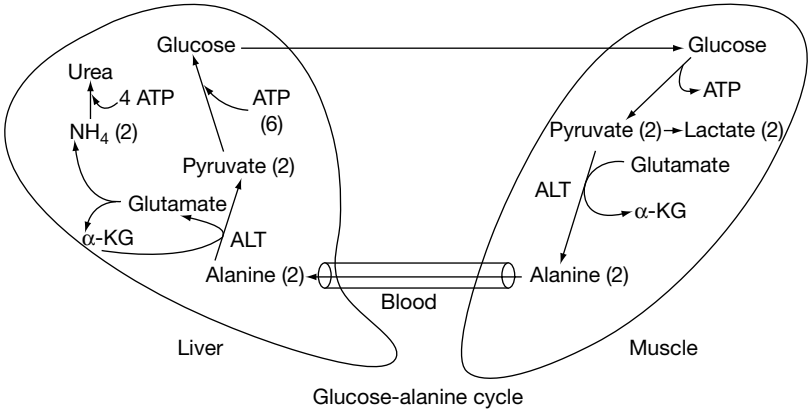


Figure 11.8 Glucose–Alanine Cycle

11.7.3 Proline Biosynthesis

In biosynthesis of proline, all five carbon atoms of proline are derived from glutamate and proline is a cyclized derivative of glutamate (Figure 11.10). In the first step, ATP reacts with the γ-carboxyl group of glutamate to form an acyl phosphate, which is reduced by NADPH or NADH to glutamate γ-semialdehyde by the enzyme glutamate dehyrogenase. The glutamate γ-semialdehyde in proline pathway undergoes a rapid, reversible cyclization delta[1] pyrroline 5-carboxylate.This intermediate delta[1] pyrroline 5-carboxylate undergoes quick spontaneous cyclization and is then reduced by the enzyme pyrroline carboxylate reductase to yield proline.

11.7.4 Arginine Biosynthesis

From glutamate, arginine is synthesized, and arginine by the action of arginase is converted to ornithine and urea. In principle, ornithine could also be synthesized from glutamate γ-semialdehyde by transamination, but the spontaneous cyclization of the semialdehyde in the proline pathway

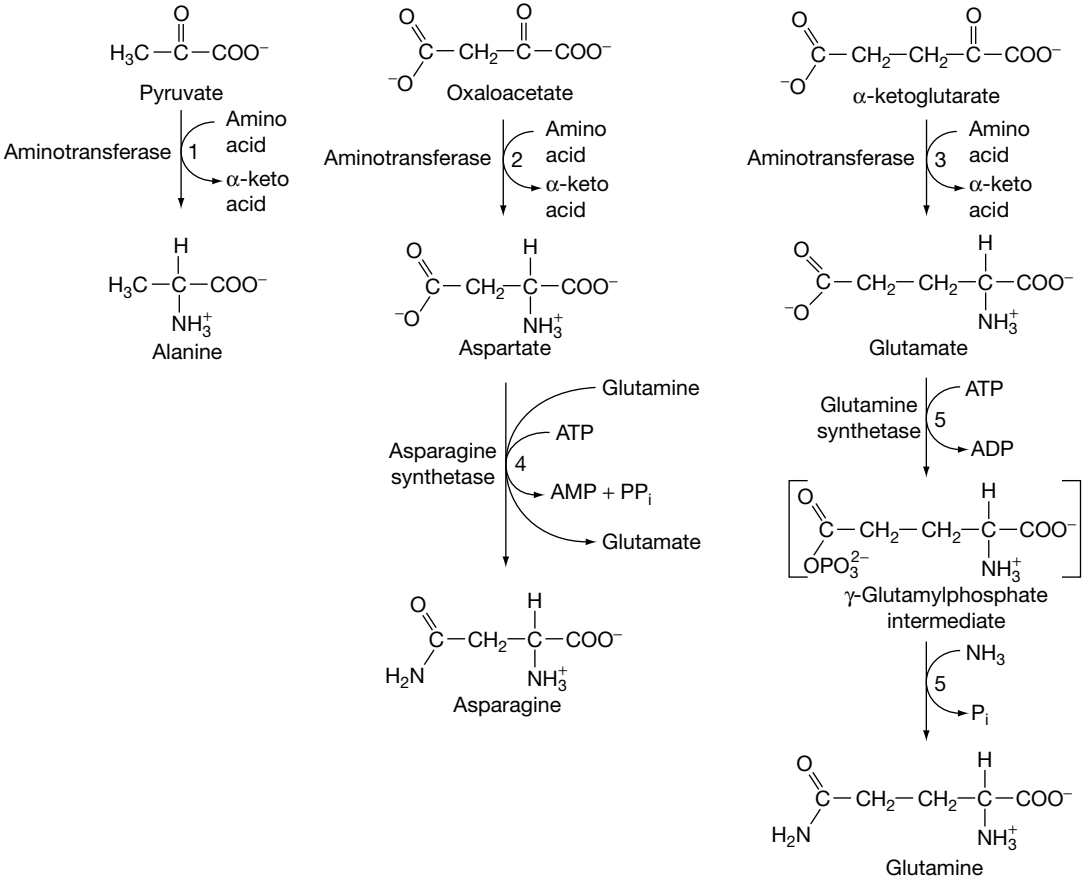


Figure 11.9 Synthesis of 1. Alanine (by transamination of pyruvate), 2. Aspartate (by transamination of oxaloacetate), 3. Glutamate (by transamination of alpha-ketoglutarate), 4. Asparagine (by amidation of aspartate) and 5. Glutamine (by amidation of glutamate)

precludes a sufficient supply of this intermediate for ornithine synthesis. In bacteria, for biosynthesis of ornithine, there is a de novo biosynthetic pathway, which has some common steps as that of the proline pathway, but has additional two steps that circumvent the difficulty of the impulsive cyclization of glutamate γ-semiaidehyde. First, acetyation blocks the α-amino group of glutamate, and this reaction requires acetyl + CoA. Then, after the transamination step, ornithine is produced after the removal of the acetyl group.Mammals have somewhat different pathways to synthesize proline and arginine. Proline can also be synthesized from arginine obtained from tissue protein or diet protein. A urea cycle enzyme, arginase converts arginine to ornithine and urea (Figure 11.4). The enzyme ornithine δ-aminotransferase converts ornithine to glutamate γ-semialdehyde (Figure 11.11). The semi-aldehyde cyclizes to delta^{1}-pyrroline-5-carboxylate, which is then converted to prolline (Figure 11.10). The pathway for arginine synthesis shown in Figure 11.12 is absent in mammals. When arginine is not sufficient for protein synthesis, the ornithine δ-aminotransferase reaction operates in the direction of ornithine formation. Ornithine

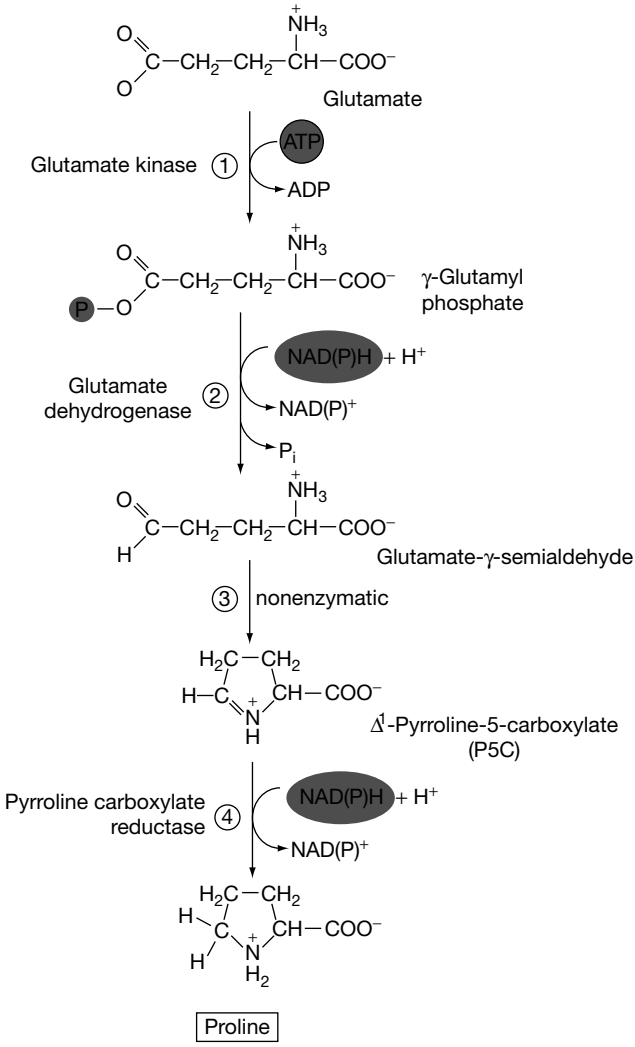


Figure 11.10 Proline Biosynthesis

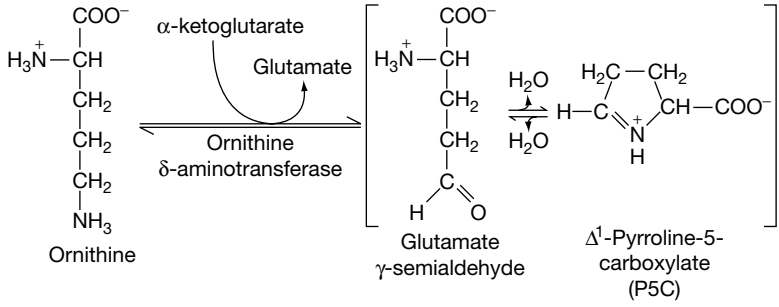


Figure 11.11 Mammalian pathway to Proline by ornithine δ-aminotransferase reaction

is then converted to citrulline and arginine in the urea Cycle. The enzyme δ-aminotransferase is found in mitochondrial matrix of most tissues. The reverse reaction of ornithine to P5C is possible only in mammalian pathway for synthesis of ornithine (Figure 11.11).

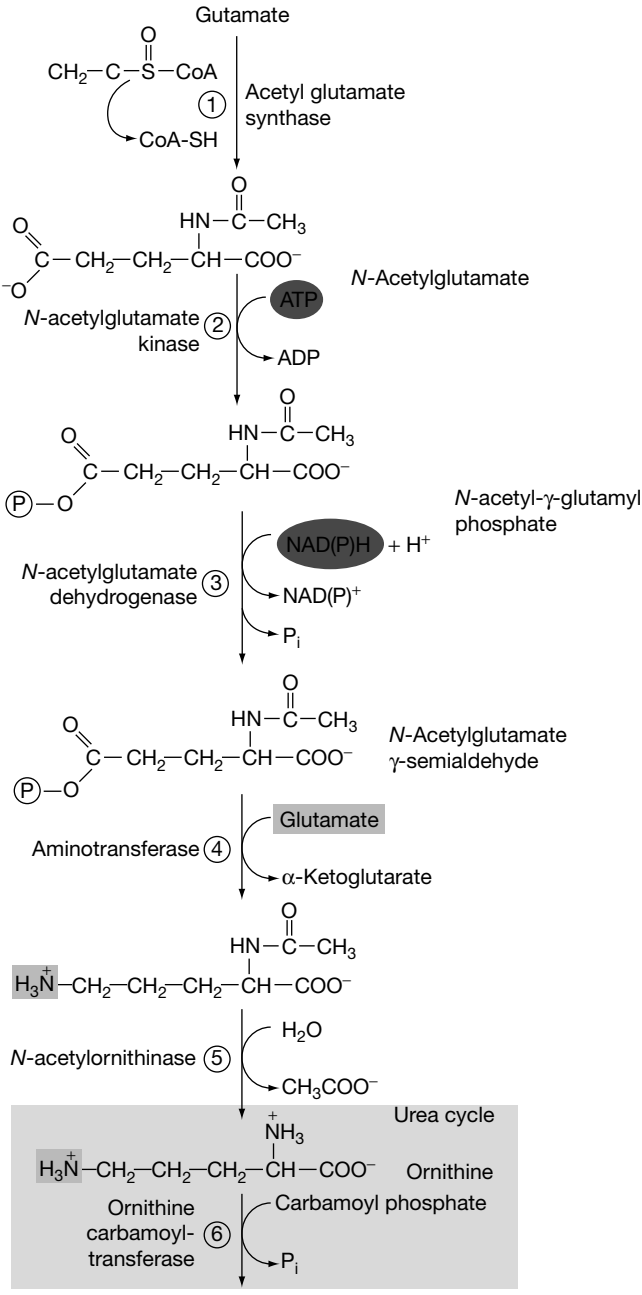


Figure 11.12 (Continued)

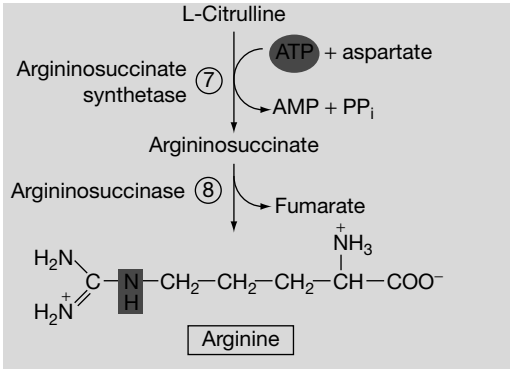


Figure 11.12 Biosynthesis of Arginine from Glutamate in Bacteria

11.7.5 Serine Biosynthesis

The main pathway to de novo biosynthesis of serine starts from the glycolytic intermediate 3-phosphoglycerate (PGA). An NADH-linked dehydrogenase converts 3-PGA into 3-phosphopyruvate which undergoes transamination reaction to produce 3-phosphoserine, which is converted to serine by phosphoserine phosphatase (Figure 11.13).

11.7.6 Glycine Biosynthesis

The enzyme glycine hydroxymethyltransferase catalyses the formation of glycine from serine and the enzyme serine hydroxymethyltransferase catalyses the formation of serine from glycine. This reaction is reversible as it uses THF as the cofactor, for the formation of glycine and N^5, N^{10}-methylene-THF it transfers the hydroxymethyl group of serine to the tetrahydrofolate (THF) (Figure 11.14).

Glycine is a nonessential, glycogenic amino acid. Glycine can be synthesized from CO_2 and NH_3 by glycine synthase. Pyridoxal phosphate is required as a coenzyme.

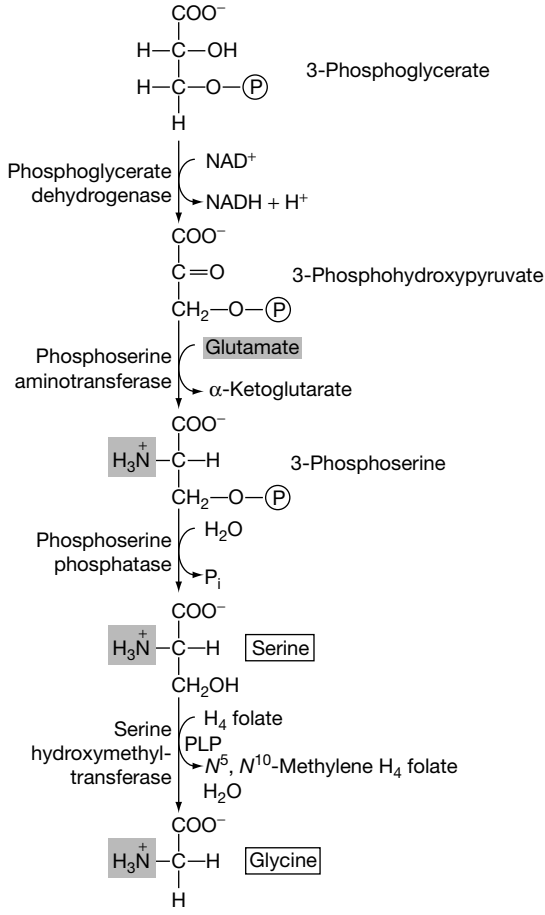


Figure 11.13 Biosynthesis of Serine from 3-phosphoglycerate and Biosynthesis of Glycine from serine in all Organisms

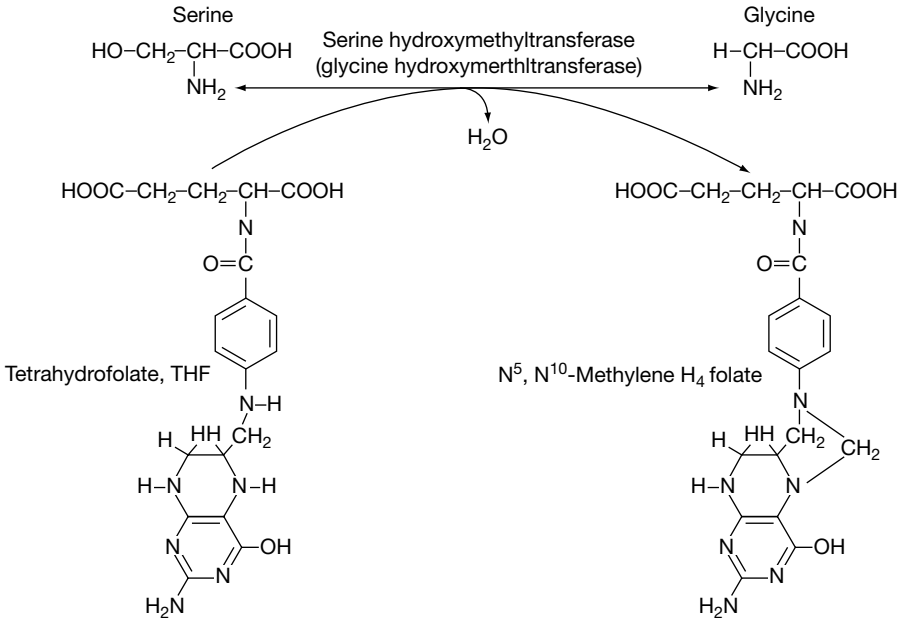


Figure 11.14 Glycine Biosynthesis with N^5, N^{10}-methylenetetrahydrofolate as methyl group donor

The plants with C3 pathway (Calvin cycle) of photosynthesis produce large amounts of glycolic acid in high light intensities, low CO_2 and high O_2 concentration. A part of glycolic acid is converted to glycine with glutamate.

11.7.7 Cysteine Biosynthesis

Methionine, cysteine and cystine are sulphur-containing amino acids. Methionine is essential while the other two can be readily synthesized. All are glycogenic. Methionine is important as a methyl group donor in reactions known as transmethylation reactions and the enzyme is known as methyl transferase or methylferase.

The homocysteine interacts with serine to form cysteine and alpha-ketobutyrate. A compound, cystathionine (a complex of serine and homocysteine) is formed as an intermediate (Figure 11.15).

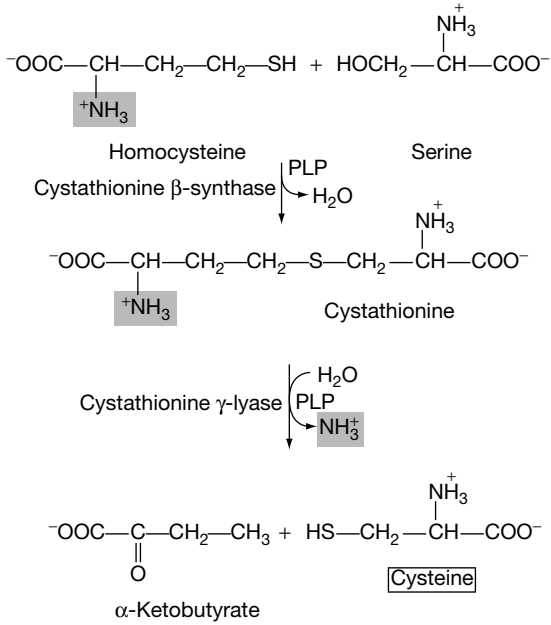


Figure 11.15 Biosynthesis of Cysteine from Homocysteine and Serine in Mammals

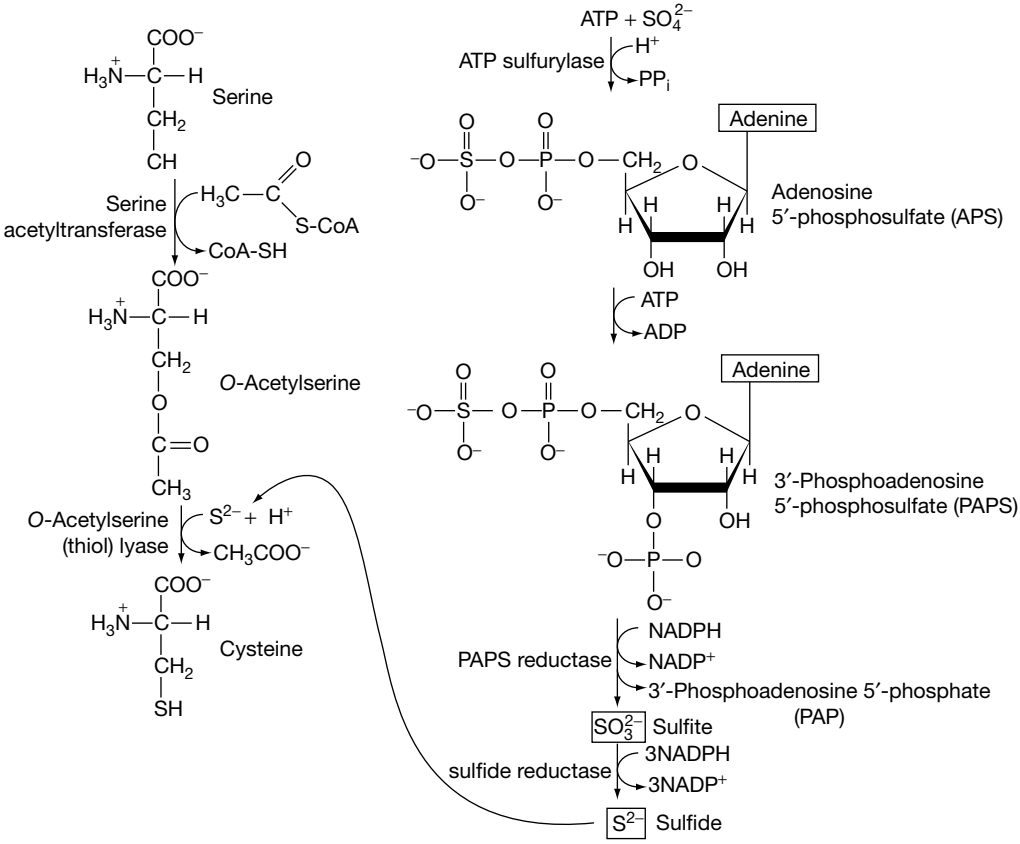


Figure 11.16 Biosynthesis of Cysteine from Serine in Bacteria and plants. The origin of reduced sulphur is shown here.

For the synthesis of cysteine, reduced sulphur is required which is produced from the environmental sulphates by plants and bacteria (Figure 11.16).

11.7.8 Biosynthesis of Six Essential and Three Nonessential Amino Acids are Synthesized from Oxaloacetate and Pyruvate

Methionine, threonine and lysine are synthesized from aspartate, whereas isoleucine, valine and leucine are synthesized from pyruvate (Figure 11.17). Alanine and aspartate are synthesized from pyruvate and oxaloacetate, respectively by transamination from aspartate. By amidation of aspartate, with glutamine donating the NH_4^+, asparagine is synthesized.

Biosynthesis of Methionine, Threonine, Lysine. Isoleucine, Valine and Leucine

The six essential amino acids are methionine, threonine, lysine, isoleucine, valine and leucine and have interconnected biosynthetic pathways.

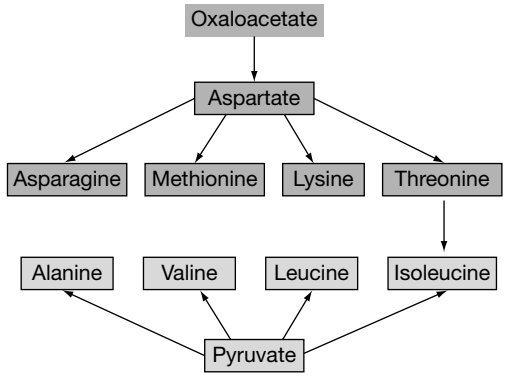


Figure 11.17 Biosynthesis of six essential and three nonessential amino acids are synthesized from oxaloacetate and pyruvate

Threonine and isoleucine: Homoserine derived from aspartate through aspartate β-semialdehyde is the precursor for thereonine also. In fact, thereonine is an isomer of homoserine in which alcohol group is in secondary position. The conversion of homoserine to thereonine, however, involves two steps. In the first step, the –OH group of homoserine is phosphorylated in the presence of the enzyme homoserine kinase. Then, this product is reacted upon by the enzyme homoserine phosphate metaphosphate or O-phosphoserine lyase, which transfers alcohol group to the secondary position. This enzyme has also been called as thereonine synthase, since the product is L-thereonine (Figure 11.18). The enzyme threonine dehydratase (serine dehydratase) converts threonine to alpha-ketobutyrate which is the beginning of isoleucine biosynthesis, this reaction also requires pyridoxal phosphate, therefore, threonine is one of the precursors of isoleucine (Figure 11.21).

The valine and isoleucine pathways share four enzymes, that is, enzymes 2, 3, 4 and 5 are the same in the valine and isoleucine biosynthetic pathways. Pyruvate gives rise to valine and isoleucine in pathways that begin with condensation of two carbons of pyruvate hydroxyethyl thiamine pyrophosphate with another molecule of pyruvate or with α-ketobutyrate (isoleucine path).

Valine and Leucine: Knowledge about the synthesis of valine and leucine from aspartate has come out of studies with *E. coli* and other microorganisms. An acetaldehyde molecule derived from pyruvate condenses with another molecule of pyruvate to yield alpha-acetolactate in the first step. The enzyme isomeroreductase rearranges the product molecule involving the reduction of carboxyl group. Further, alpha-beta-dihydroxy isovalerate is dehydrated by dehydratase enzyme subsequently to produce alpha-keto isovalerate. Alpha-keto isovalerate is transaminated by the enzyme aminotransferase to produce valine (Figure 11.22). An intermediate in the valine pathway, α-ketoisovalerate, is the starting point for a four-step branch pathway leading to leucine (Figure 11.23). In the synthesis of leucine, alpha-keto isovalerate is acylated first by a molecule of acetyl-CoA to yield isopropyl malate by the enzyme alpha-isopropylmalate synthase. Alpha-isopropyl malate is then rearranged to beta-isopropyl-malate. This rearrangement actually proceeds into two steps. In the first step, the molecule is dehydrated and in the next, it is hydrated again. Beta-isopropyl malate undergoes oxidative decarboxylation to yield alpha-ketoisocaproate which is transaminated to produce leucine (Figure 11.23).

Methionine: Aspartic acid is converted to aspartate β-semialdehyde via its phosphate. Aspartate β-semialdehyde is the common intermediate in the synthesis of methionine, thereonine and isoleucine and lysine (Figures 11.19, 11.18, 11.21 and 11.20). Homoserine is the branching point for the synthesis of these amino acids. Homoserine is synthesized by the reduction of aspartyl phosphate by the enzyme homoserine dehydrogenase. The dehydrogenase can use either NADH or NADPH as a coenzyme (Figure 11.19).

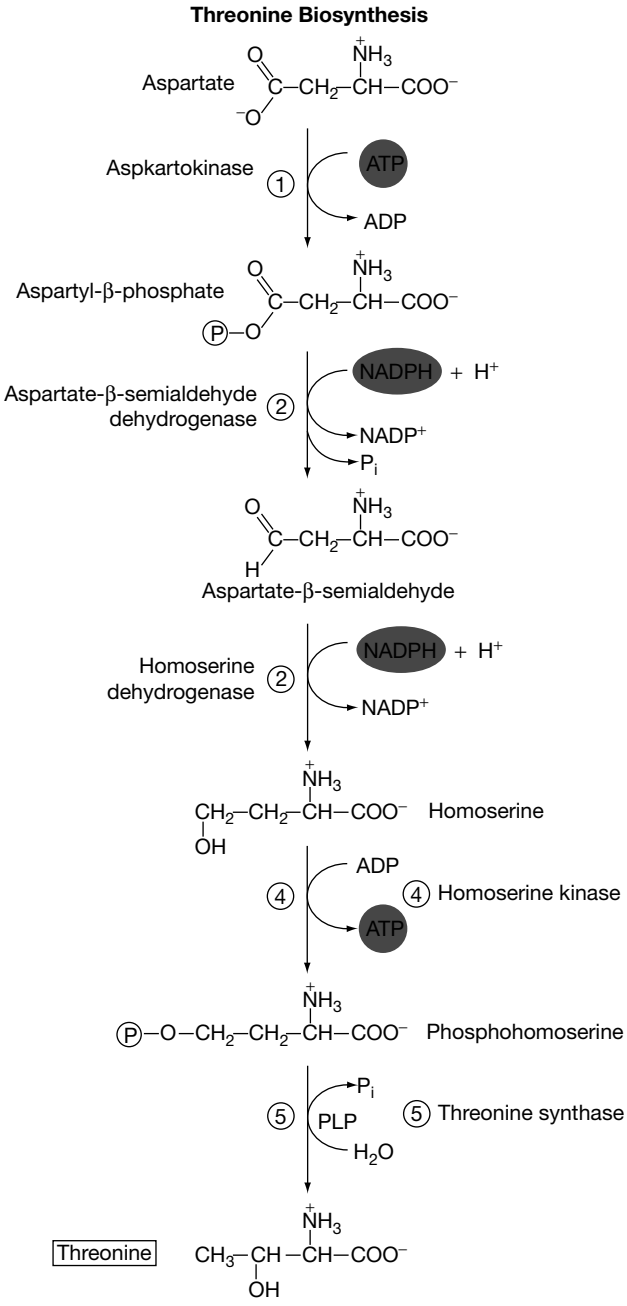


Figure 11.18 Biosynthesis of Threonine, an essential amino acid, from Aspartate

Lysine: Synthesis of lysine in bacteria involves aspartic acid which is then converted to aspartate β-semialdehyde, which is an intermediate in the synthesis of lysine. The number of steps involved in the synthesis of lysine from meso-α, ε-Diaminopimelate is shown in Figure 11.20.

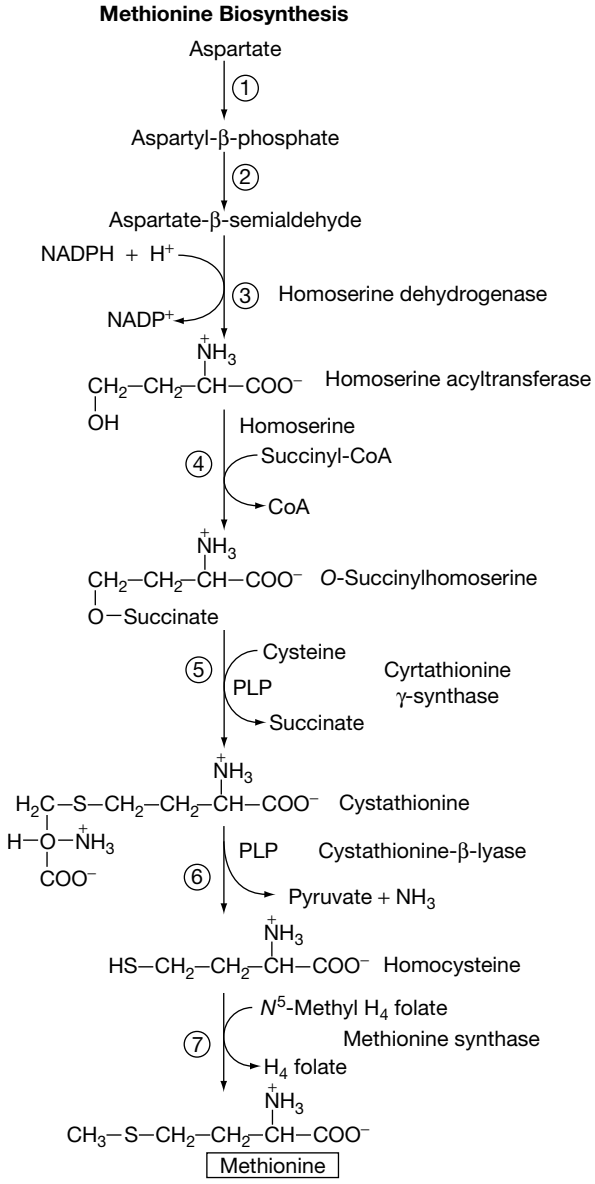


Figure 11.19 Biosynthesis of Methionine, an essential amino acid, from Aspartate

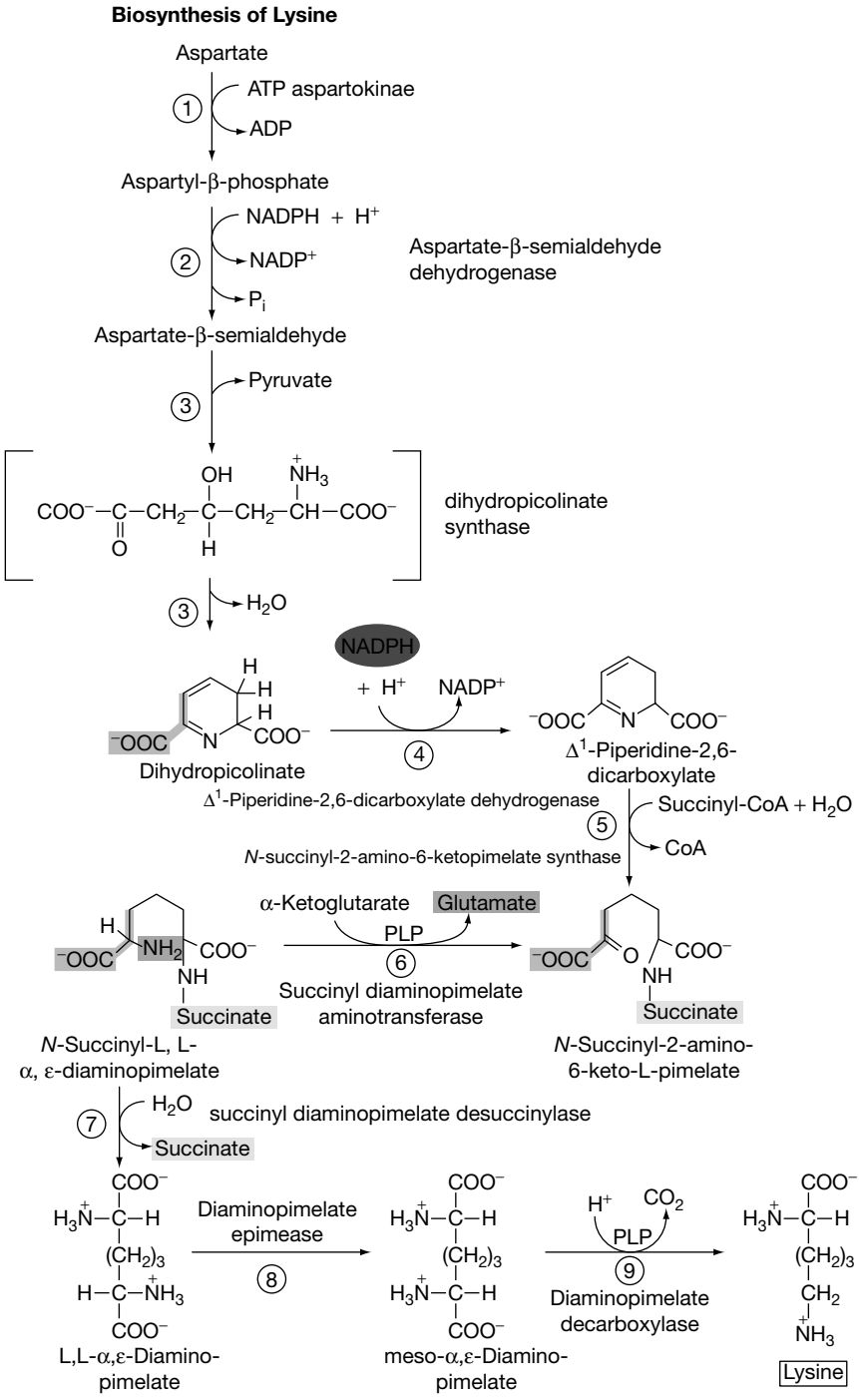


Figure 11.20 Biosynthesis of Lysine, an essential amino acid, from Aspartate

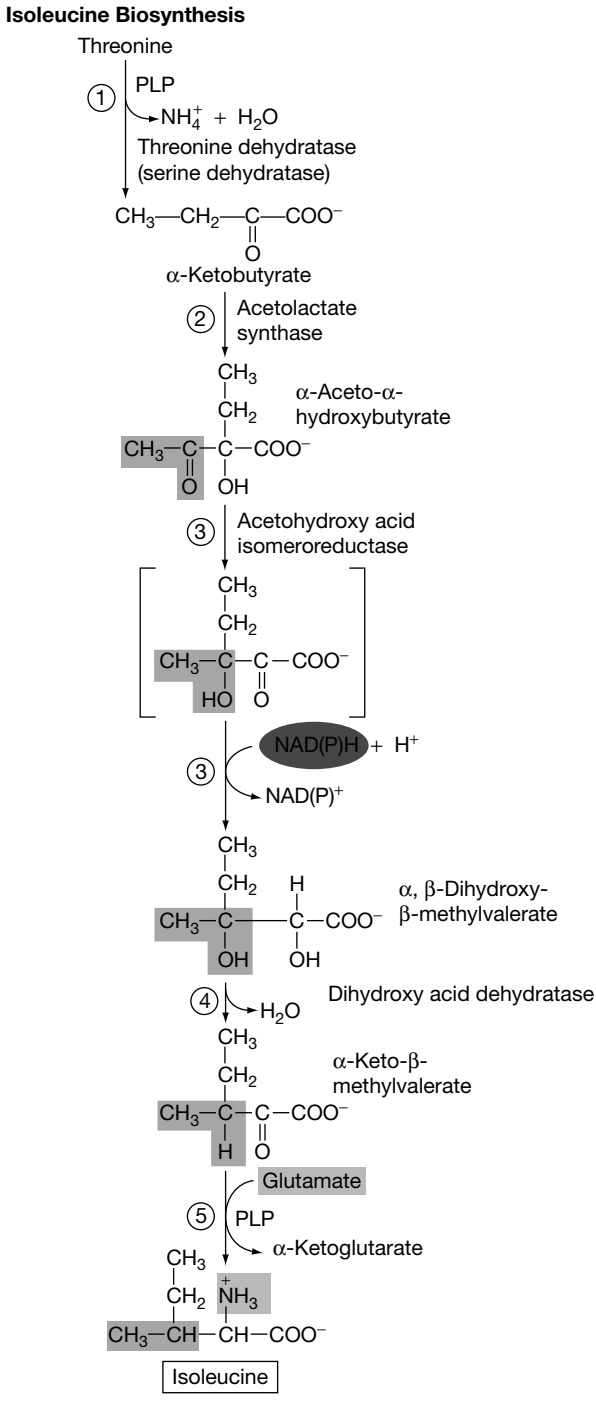


Figure 11.21 Biosynthesis of Isoleucine, an essential amino acid, from Pyruvate

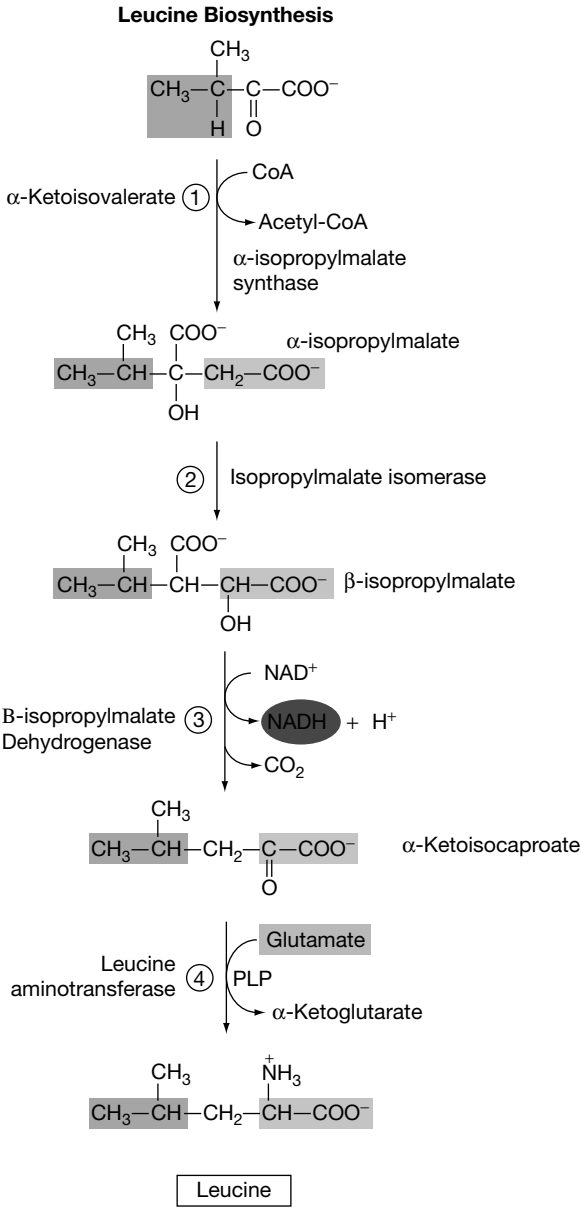


Figure 11.22 Biosynthesis of leucine, an essential amino acid, from Pyruvate

11.7.9 Biosynthesis of Tryprophan, Phenylalanine and Tyrosine

The branched pathway to tryprophan, phenyialanine and tyrosine, occurring in bacteria, fungi and plants, is the main biological route of aromatic ring formation. It proceeds through ring closure of an aliphatic precursor followed by stepwise addition of double bonds. The biosynthesis of

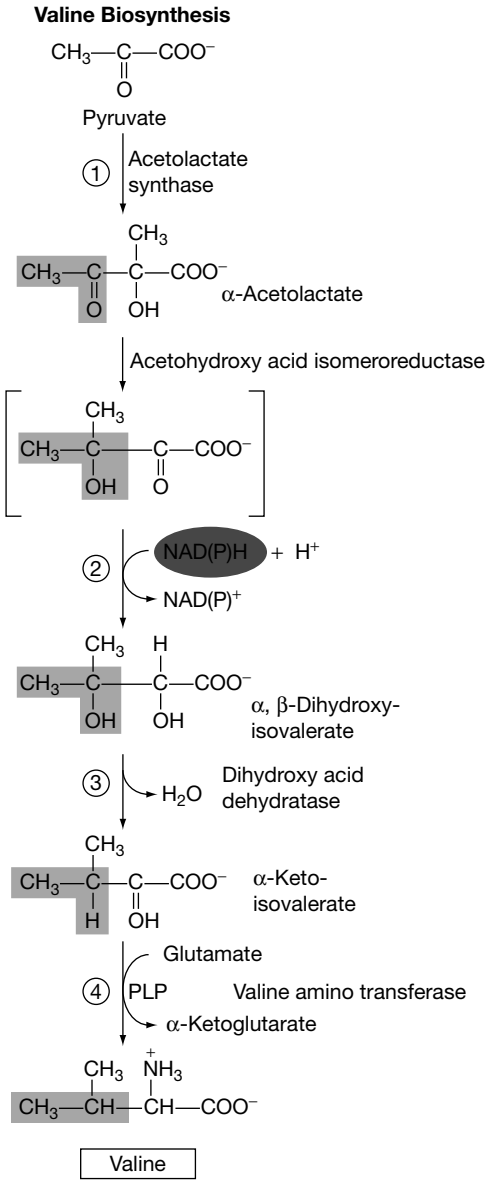
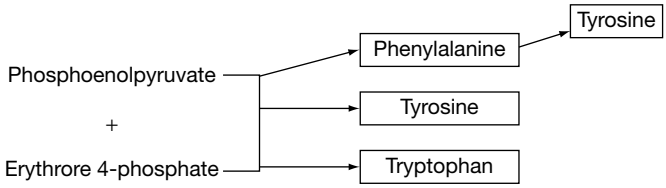


Figure 11.23 Biosynthesis of Valine, an essential amino acid, from Pyruvate

tryptophan begins with the reaction between erythrose-4-phosphate and phosphoenolpyruvate, and in steps 1 to 4 it produces shikimate, which is made up of seven carbons (Figure 11.24). In the next three steps, that is steps 5, 6 and 7, shikimate is converted to chorismate by the addition of three more carbons from another molecule of PEP (see step 6). The branching pathway to tryptophan, phenylalanine and tyrosine starts from chorismate (Figure 11.24).



The synthesis of trytophan from chorismate starts with the reaction with glutamine. The product of the reaction is anthranilic acid and the enzyme, anthrinilate synthetase. Anthranilic acid reacts with phosphoribosyl pyrophosphate (PRPP) to produce N-(5′-phosphoribosyl)-anthranilate in the presence of the enzyme anthranilate phosphoribosyl transferase. In the next step, it is decarboxylated with the formation of side ring also, to produce indoleglycerol phosphate.

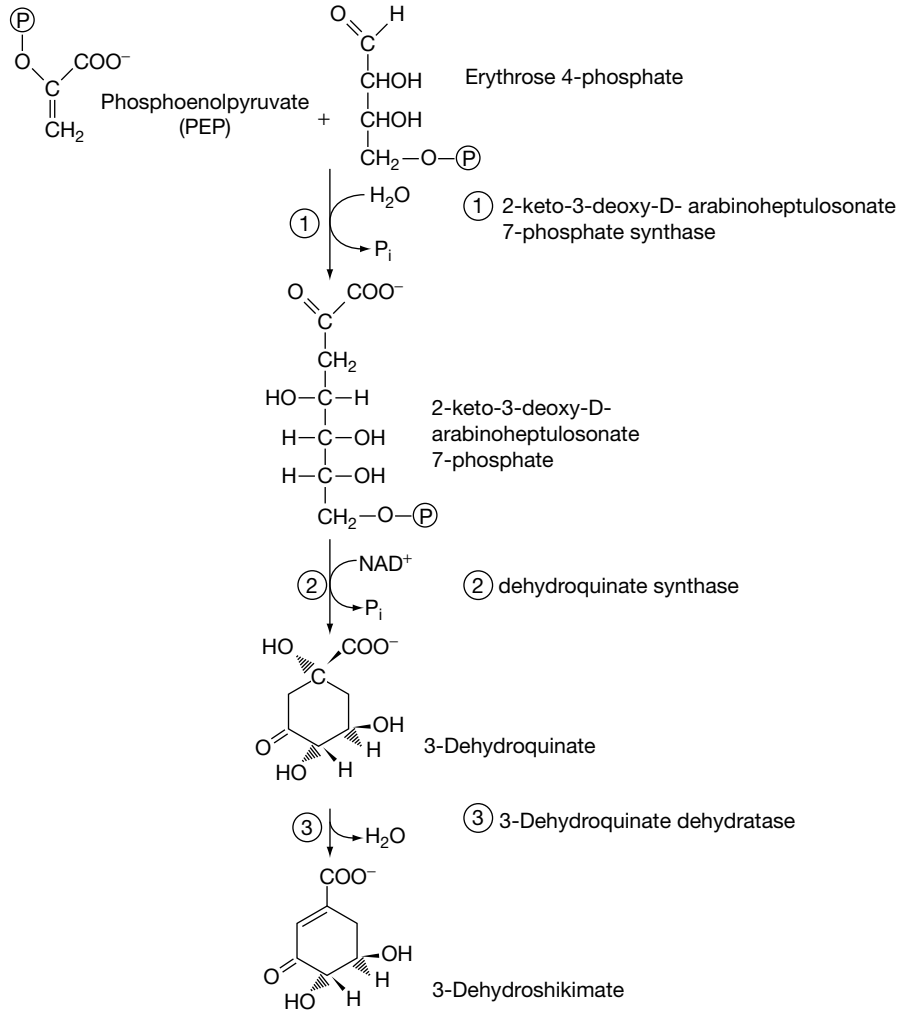


Figure 11.24 (Continued)

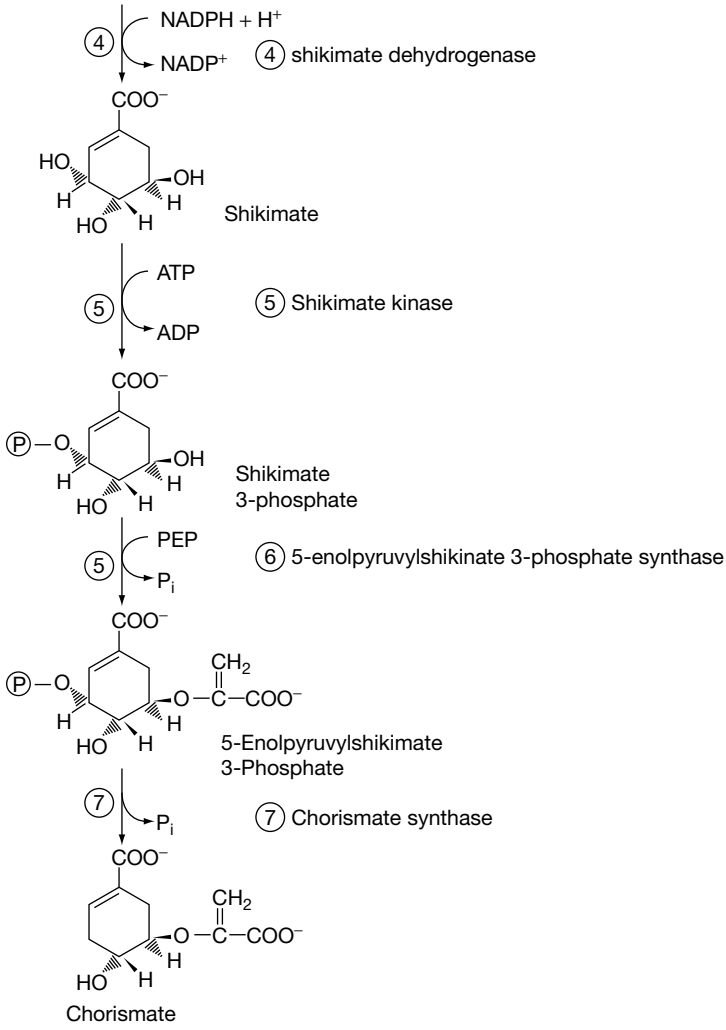


Figure 11.24 Biosynthesis of Chorismate, an intermediate in the biosynthesis of tryptophan in Bacteria and plants

Synthesis of tryptophan from indoleglycerol phosphate has been demonstrated in *E. coli*, *Neurospora* and higher plants. The enzyme trytophan synthase has been crystallized from bacteria and the fungi. The enzyme trytophan synthase catalyses the last reaction to produce trytophan. Serine condenses with indole glycerol phosphate in the presence of pyridoxal phosphate to produce trytophan (Figure 11.25). This enzyme has an $\alpha_2\beta_2$ subunit structure and can be dissociated into two α-subunits and a β_2-subunit that catalyse different parts of the overall reaction:

$$\text{Indole-3-glycerol phosphate} \longrightarrow \text{Indole} + \text{glyceraldehyde-3-phosphate}$$

$$\text{Indole} + \text{Serine} \xrightarrow[\beta_2\text{-subunit}]{\alpha\text{-subunit}} \text{Tryptophan} + H_2O$$

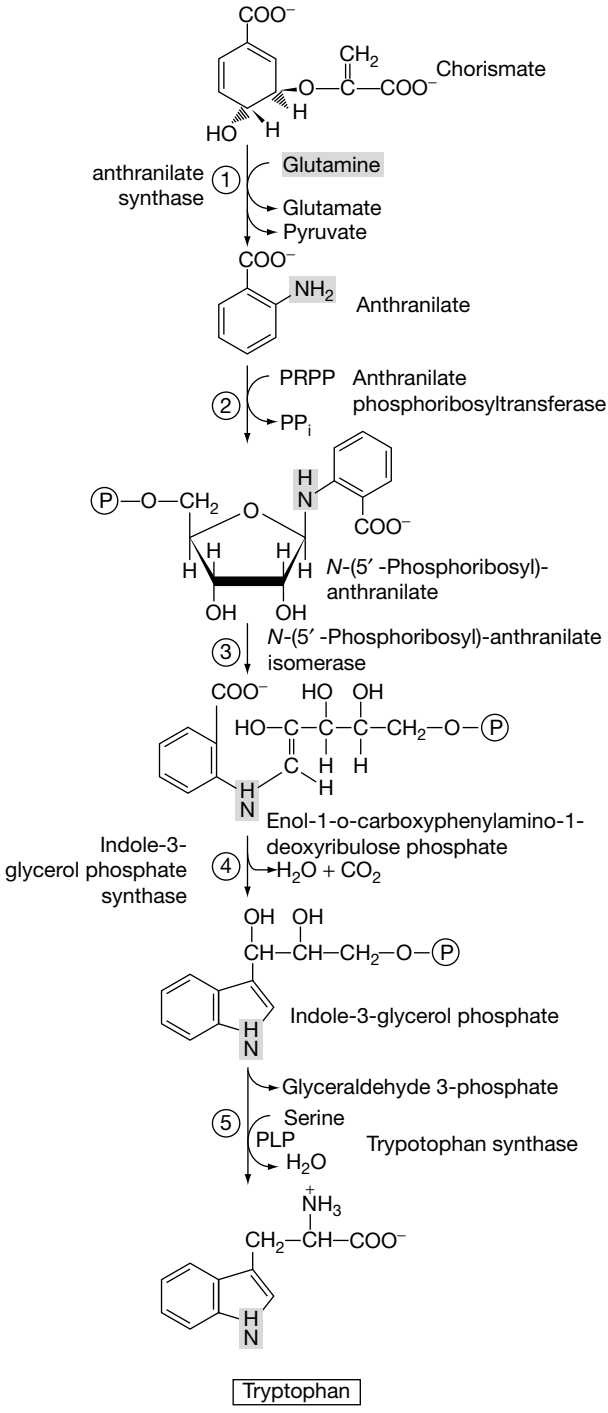


Figure 11.25 Biosynthesis of tryptophan from Chorismate in Bacteria and plants

The pathways of phenylalanine and tyrosine are synthesized from chorismate in plants and bacteria, is less complex than the tryptophan pathway. Chorismate is converted to prephenate by the rearrangements of side groups. This reaction is catalysed by the enzyme chorismate mutase. Prephenate is the common precursor for biosynthesis of both phenylalanine and tyrosine (Figures 11.27 and 11.28). In the synthesis of phenylalanine, it is decarboxylated and dehydrated to a compound called phenylpyruvic acid. Phenylpyruvic acid after transamination produces phenylalanine.

For tyrosine synthesis, prephenic acid undergoes oxidative decarboxylation first in the presence of enzyme prephenate dehydrogenase, produces hydroxyphenylpyruvic acid. This compound is then transaminated to tyrosine.

Tyrosine can be produced directly from phenylalanine in animals, through hydroxylation at C-4 of the phenyl group by phenylalanine hydroxylase; however, this enzyme also participates in the degradation of phenylalanine. Tyrosine is considered a conditionally essential amino acid, or as nonessential insofar as it can be synthesized from the essential amino acid phenylalanine.

Hydroxylation of phenylalanine generates tyrosine (Figure 11.26). Half of the phenylalanine required goes into the production of tyrosine, phenylalanine is converted to tyrosine by hydroxylation, this reaction is catalysed by the enzyme phenylalanine hydroxylase and is a mixed-function oxygenase. In this reaction one atom of oxygen is integrated into H_2O and the other oxygen is integrated into the OH of tyrosine. TATHF related cofactor **tetrahydrobiopterin** controls the reducing power of the reaction. The enzyme dihydropteridine reductase maintains the tetrahydrobiopterin in reduced state, and this enzyme is NADH-dependent (Figure 11.26).

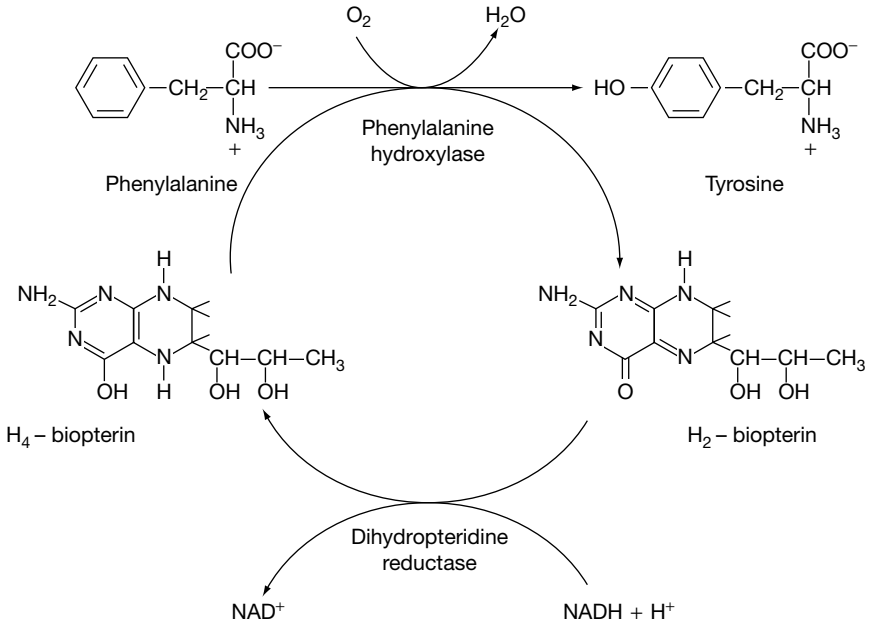


Figure 11.26 Biosynthesis of Tyrosine from Phenylalanine

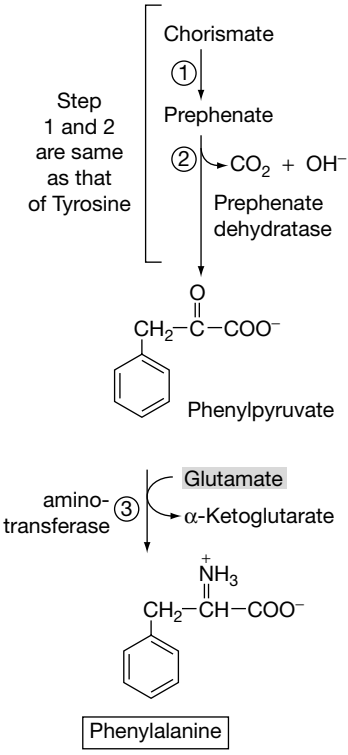


Figure 11.27 Biosynthesis of Phenylalanine from Chorismate in Bacteria and plants

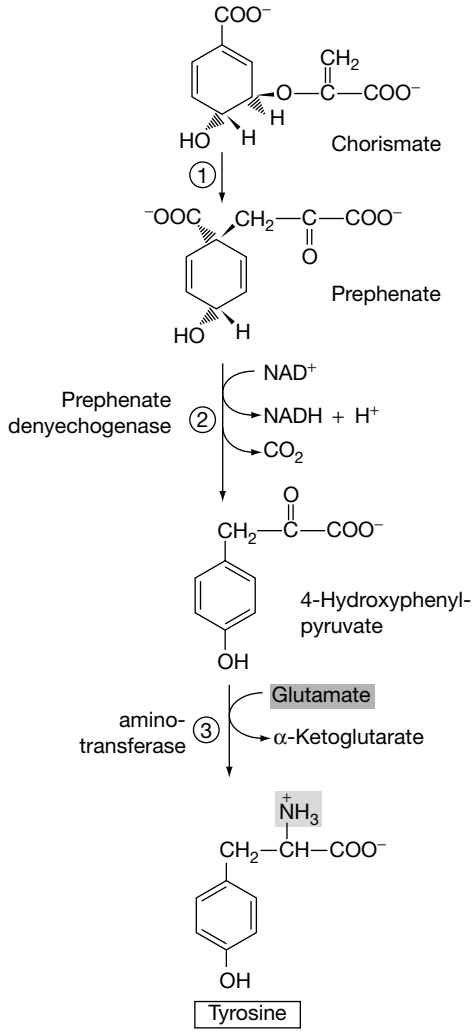


Figure 11.28 Biosynthesis of Tyrosine from Chorismate in Bacteria and plants

11.7.10 Biosynthesis of Histidine

Histidine is a hetrocyclic amino acid with a unique biosynthetic pathway derived from ATP and the phosphoribosyl pyrophosphate (PRPP). One of the products in the reaction 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) is a precursor of ATP. In this way, ATP is recycled during the synthesis of histidine. The synthesis of histidine from imidazole glycerol 3-phosphate. The biosynthetic pathways of histidine differ in several respects from other amino acid biosynthetic pathways in all plants and bacteria. There are three precursors of histidine (1). PRPP contributes five carbons, (2). the purine ring of ATP contributes nitrogen and a carbon and (3).

glutamine supplies the second ring of nitrogen. The key steps in histidine biosynthesis are condensation of ATP and PRPP, in which N-1 of the purine ring is linked to the activated C-1 of the ribose of PRPP (step 1 in Figure 11.29). Opening of purine ring that ultimately leaves N-1 and

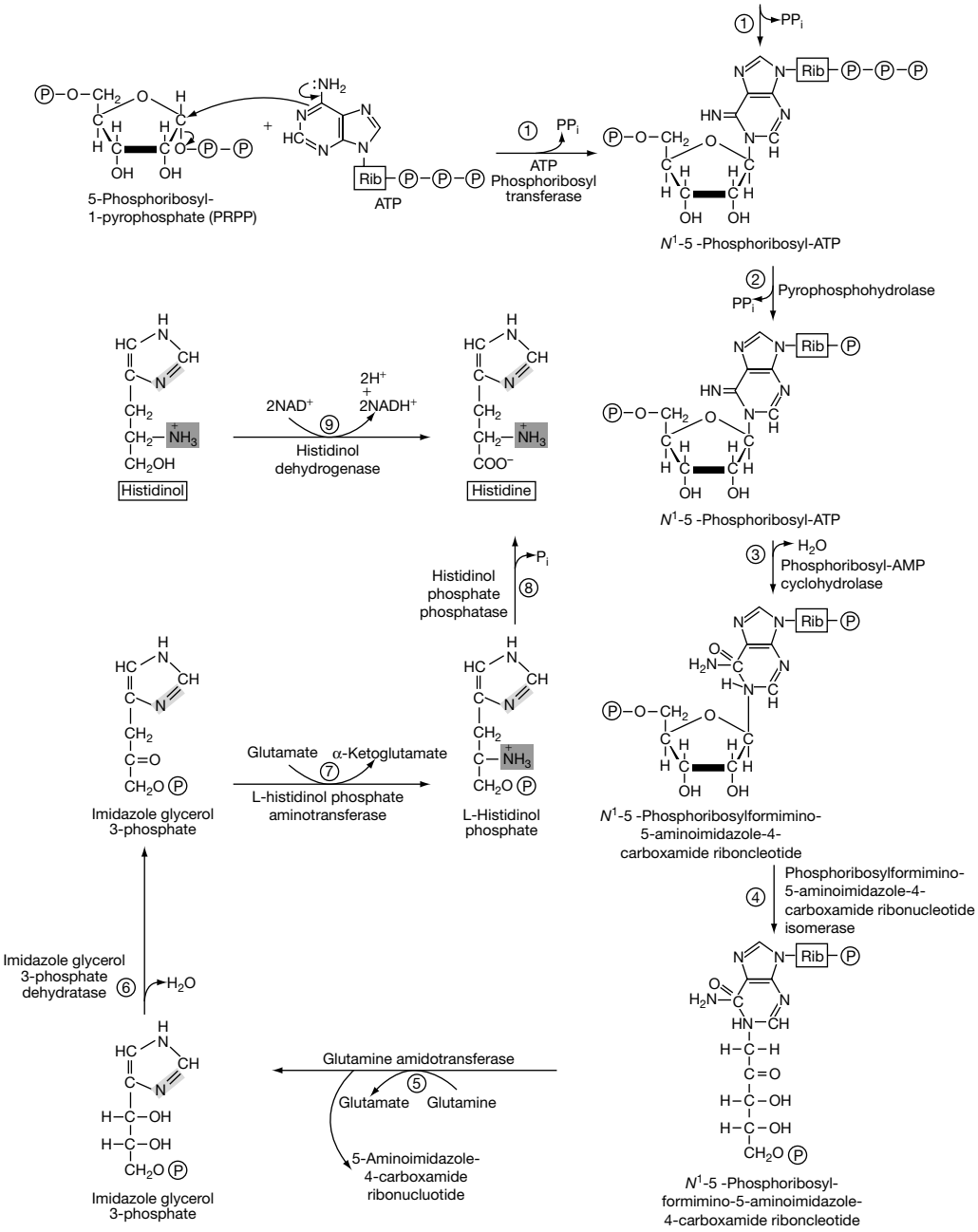


Figure 11.29 Biosynthesis of Histidine in bacteria and plants

C-2 of adenine linked to the ribose (step 3) and leads to the formation of the imidazole ring, a reaction in which glutamate donates a nitrogen (step 5). The use of ATP used in this reaction as a metabolite rather than a high energy cofactor is strange but not uneconomical because it interconnects with the purine biosynthetic pathway. The 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an intermediate of purine biosynthesis, is rapidly recycled to ATP.

11.8 CATABOLISM OF AMINO ACID

Amino acids catabolism is not as active as glycolysis and fatty acid oxidation. Amino acids catabolism accounts only to 10 to 15 per cent of human body's energy production. The catabolic pathway of 20 amino acids forms six major products, and all of them enter the citric acid cycle. Then, from here, carbon skeleton of amino acids are destined to either glucogenesis or ketogenesis or are oxidized completely to CO_2 and H_2O.

All or part of carbon skeleton of these seven amino acids, leucine, lysine, phenylalanine, isoleucine, tyrosine, thereonine and tryptophan are ultimately broken down to acetyl-CoA. Five amino acids glutamine, glutamate, arginine, histidine and proline are broken down to α ketoglutarate. Four amino acids isoleucine, methionine thereonine, and valine are broken down to succinyl-CoA. Two amino acids phenylalanine and tyrosine are broken down to fumarate. Two amino acids asparagine and aspartate are broken down to oxaloacetate. Parts or all of six amino acids, Alanine, cysteine, glycine, serine, thereonine, tryptophan are broken down to pyruvate which can be converted either to acetyl-CoA or oxaloacetate. As shown in Figure 11.30, all twenty amino acids enters the citric acid cycle through a specific point.

Some amino acids shown more than once show different fate for their different parts of carbon skeleton of amino acids. Seven amino acids that are degraded fully or in part to acetoacetyl-CoA or to acetyl-CoA are leucine, lysine, phenylalanine, isoleucine, tyrosine, thereonine and tryptophan can yield ketone bodies, and are ketogenic in nature. In uncontrolled diabetes mellitus, liver produces more ketone bodies from these ketogenic amino acids as well as from fatty acids. The amino acids that are degraded to succinyl-CoA, alpha-ketoglutarate, fumarate and to pyruvate and or oxaloacetate can be converted to glucose and glycogen. These amino acids are glucogenic in nature. Five amino acids tryptophan, phenylalanine, isoleucine, tyrosine, thereonine and isoleucine are both glucogenic and ketogenic in nature.

11.8.1 Importance of One Carbon Pool in Amino Acid Catabolism

We have already discussed transamination reactions requiring pyridoxal phosphate. Another important reaction in amino acid catabolism is one-carbon transfer, which involves one of the three cofactors, biotin, tetrahydrofolate or S-adenosylmethionine (for structures, see Chapter 15).

1. Biotin transfers carbon in its most oxidized state, CO_2.
2. THF, the one-carbon derivative of tetrahydrofolic acid is of much biological importance in reactions involving amino acids, purines, pyrimidines, etc. Figure 11.31 shows the relationship of tetrahydrofolic acid derivatives in the synthesis of or breakdown of a number of biologically important compounds. The one-carbon derivatives of tetrahydrofolic acid are both utilized in the synthesis of compounds such as purines, methionine and histidine, and are formed in the breakdown of these compounds. In other cases, the breakdown of substances only yields one carbon **FH4** derivatives (tryptophan, betaine, acetone, etc.).

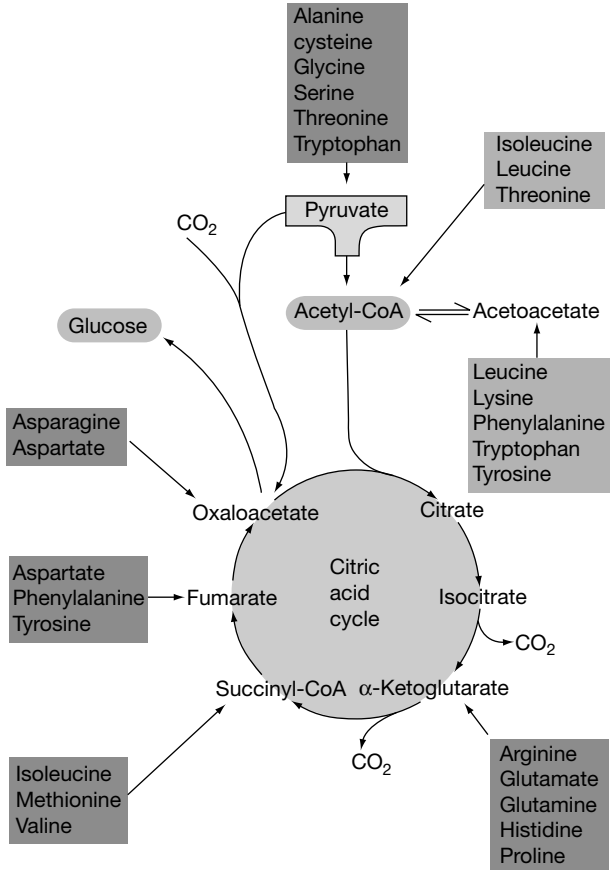


Figure 11.30 Grouping of amino acids according to their degradative end product. In the Figure, some amino acids are written more than once as different parts of their carbon skeleton are degraded to different end products. Glucogenic amino acids and ketogenic amino acids

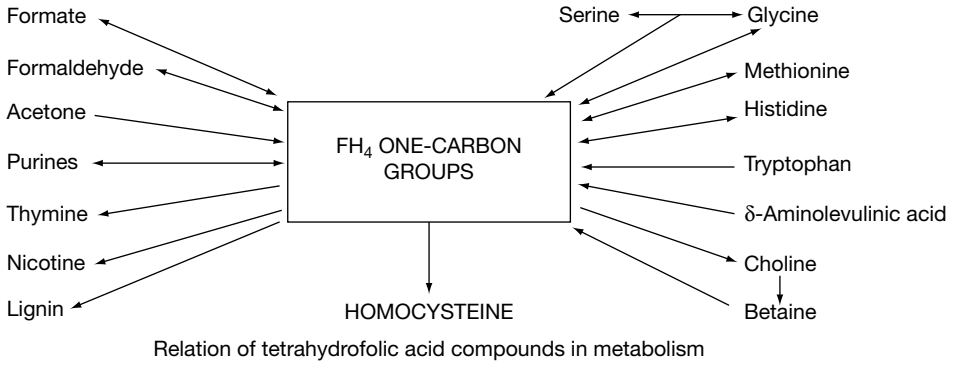


Figure 11.31 Relation of THF compounds in metabolism

Further, **FH4** derivatives are utilized in the synthesis of some compounds but apparently are not formed directly in their breakdown (thymine, choline and nicotine). Thus, it is seen that many compounds, in conjunction with one carbon tetrahydrofolic acid derivatives, constitute a mobile active 'one carbon pool' that is involved in many biological reactions.

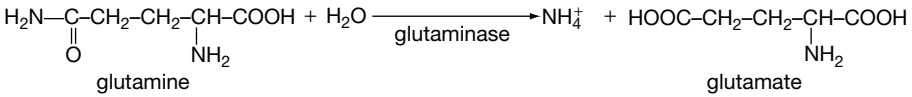
3. S-adenosylmethionine transfers methyl groups, the most reduced state of carbon.

11.8.2 Glutamic and Aspartatic Acid Catabolism

These amino acids are nonessential and glycogenic. N-acetyl glutamate and aspartate participate in the urea cycle. Both help in the transport of ammonia by forming corresponding amides asparagine and glutamine.

On decarboxylation, glutamic acid forms gamma-aminobutyric acid. Glutamic acid is one of the constituent of glutathione which is important for the activity of many sulphydral enzymes. Glutamate is a constituent of folic acid.

Aspartic acid takes part in the synthesis of the purine and pyrimidine rings.



11.8.3 Alanine Catabolism

Alanine is a glycogenic nonessential amino acid. Deamination or transamination of alanine forms pyruvic acid which can be converted to glucose or oxidized in citric acid cycle. Beta-alanine is the constituent of pantothenic acid.

11.8.4 Arginine, Ornithine and Citrulline Catabolism

Arginine is metabolized to ornithine and urea by the action of the enzyme arginase. Ornithine on transamination is converted to glutamic acid semialdehyde which is oxidized to glutamate (Figure 11.32). Arginine is an essential amino acid, whereas ornithine and citrulline are nonessential. However, arginine is glycogenic.

11.8.5 Proline Catabolism

Hyroxyproline is formed from proline after its incorporation into protein. Proline is metabolized by the reverse of its synthetic reactions, forming glutamic acid and alpha-ketoglutarate.

11.8.6 Serine Catabolism

Serine is converted to glycine, this reaction is catalysed by the enzyme glycine hydroxymethyltransferase and THF (Figure 11.14). Serine is a glycogenic and nonessential amino acid. Serine produces pyruvic acid by the action of the enzyme dehydrase. The beta-carbon of serine serves as a source of one-carbon fragment.

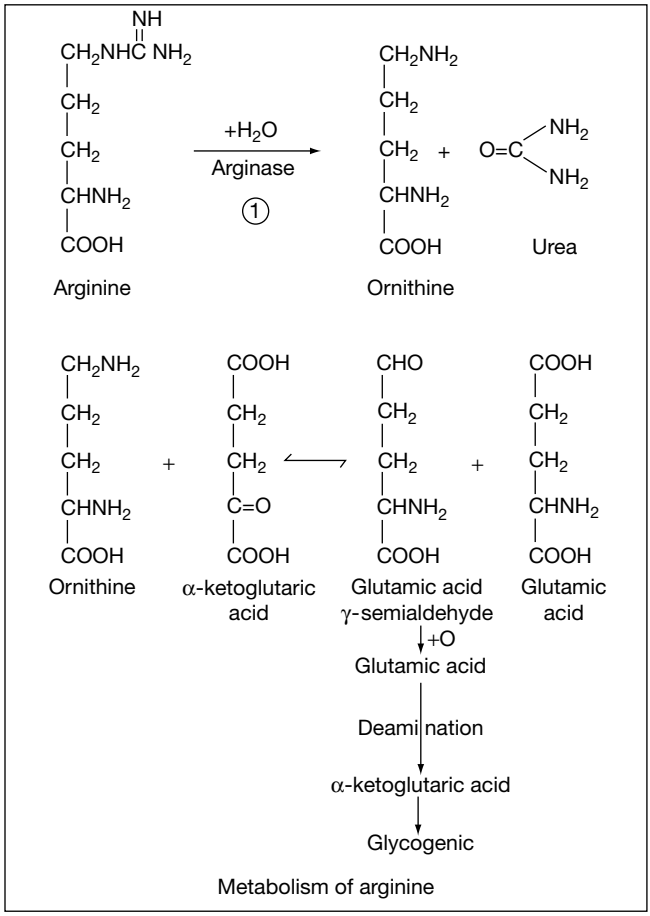


Figure 11.32 Catabolism of arginine

11.8.7 Threonine Catabolism

Threonine is broken down by the enzyme threonine aldolase into acetaldehyde and glycine as shown in Figure 11.33. The acetaldehyde is converted to acetate. In vertebrates, threonine is converted to alpa-amino-beta-ketobutyrate by dehydratase enzyme, which is similar to serine dehydratase, which on oxidative decarboxylation gives propionyl CoA. The other pathway is the formation of 2-ketopropanol which is converted to pyruvate. Both the pathways are glycogenic and threonine is an essential amino acid.

11.8.8 Glycine Catabolism

Glycine is converted to serine, this reaction is catalysed by the enzyme serine hydroxymethyl-transferase and THF (Figure 11.14). The formate formed from glycine can be taken up by tetra-hydrofolic acid and can participate in all reactions involving a one-carbon pool. Glycine moiety is incorporated into purine ring in C4, C5 and N7 position. Glycine upon reacting with benzoic acid forms hippuric acid.

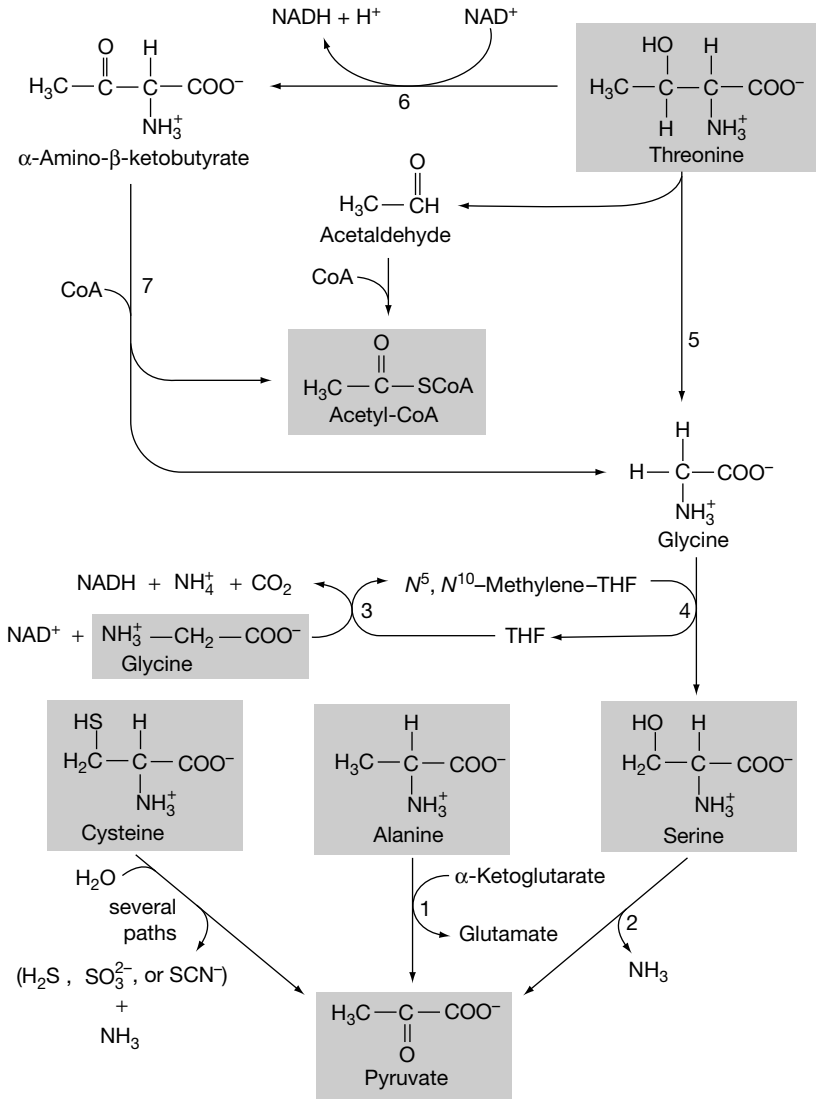


Figure 11.33 Catabolism of Threonine

Metabolic Disorder of Glycine

Glycinuria: In kidneys, decreased glycine tubular reabsorption.

11.8.9 Cysteine Catabolism

The main pathway of catabolism of cysteine is pyruvate formation by desulphydration and deamination. A subsidiary pathway of oxidation of the -SH to sulphate followed by decarboxylation yields taurine. Taurine is used in conjugation of cholic acid to form one of the bile acids,

taurocholic acid. Taurine produced from cysteine is a constituent of the bile acid taurocholic acid. The reversible oxidation–reduction reaction cystine ↔ to cysteine helps in keeping the sulphydral enzymes in the reduced and active state.

Metabolic Disorder of Cysteine

Cystinuria: Cystine, lysine, arginine and ornithine in large amounts are excreted in urine. Cystine being the most insoluble of all is precipitated and crystals can be seen under a microscope. This condition is called cystinuria.

Homocystinuria: In this genetic defect, homocysteine is excreted in the urine and plasma methionine levels are increased. The defect is due to a block in further metabolism of homocysteine formed after transmethylation.

11.8.10 Methionine Catabolism

Catabolism of methionine to cysteine and succinyl-CoA

The condensation reaction of methionine with adenosine triphosphate is catalysed by the enzyme methionine adenosyl transferase and produce **S-adenosyl methionine** (SAM) as shown hereunder (Figure 11.34).

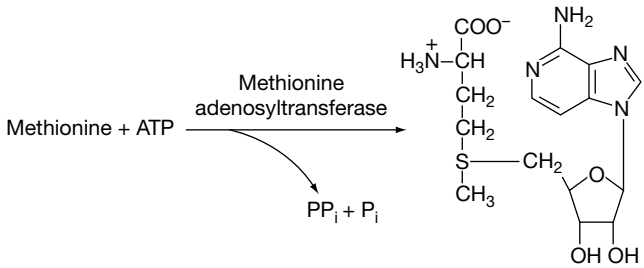


Figure 11.34 Synthesis of S-adenosyl methionine (SAM)

By the transulfuration reaction (requires pyridoxal phosphate as a coenzyme), the SAM is converted to S-adenosylhomocysteine by the enzyme methylase. S-adenosylhomocysteine is further metabolized to homocysteine by the enzyme adenosylhomocysteinase, then homocysteine by the enzyme cystathionine beta synthase form an intermediate compound, cystathionine. Then this intermediate compound, cystathionine forms cysteine and α ketobutarate which is then converted to propionyl-CoA, methylmalonyl-CoA and finally succinyl-CoA as shown in the Figure 11.35.

11.8.11 Valine, Leucine and Isoleucine Catabolism

All the above mentioned three amino acids by transamination are converted to alpha-keto acid (valine to alpha-ketoisovalerate, leucine to alpha-ketoisocaproate and isoleucine to alpha-keto beta-methylvalerate). Then these corresponding keto acids by the enzyme alpha-keto acid dehydrogenase complex are converted to corresponding alpha, beta-unsaturated acyl-CoA thioesters (valine to isobutyryl CoA, leucine to isovaleryl CoA and isoleucine to alpha-methylbutyryl CoA). Then alpha, beta-unsaturated acyl-CoA thioesters by the enzyme acyl-CoA dehydrogenase are converted to corresponding CoA derivatives. During the catabolism of valine, it is first converted to methylacrylyl CoA, then to propionyl CoA, then to methylmalonyl CoA and at last to glucose.

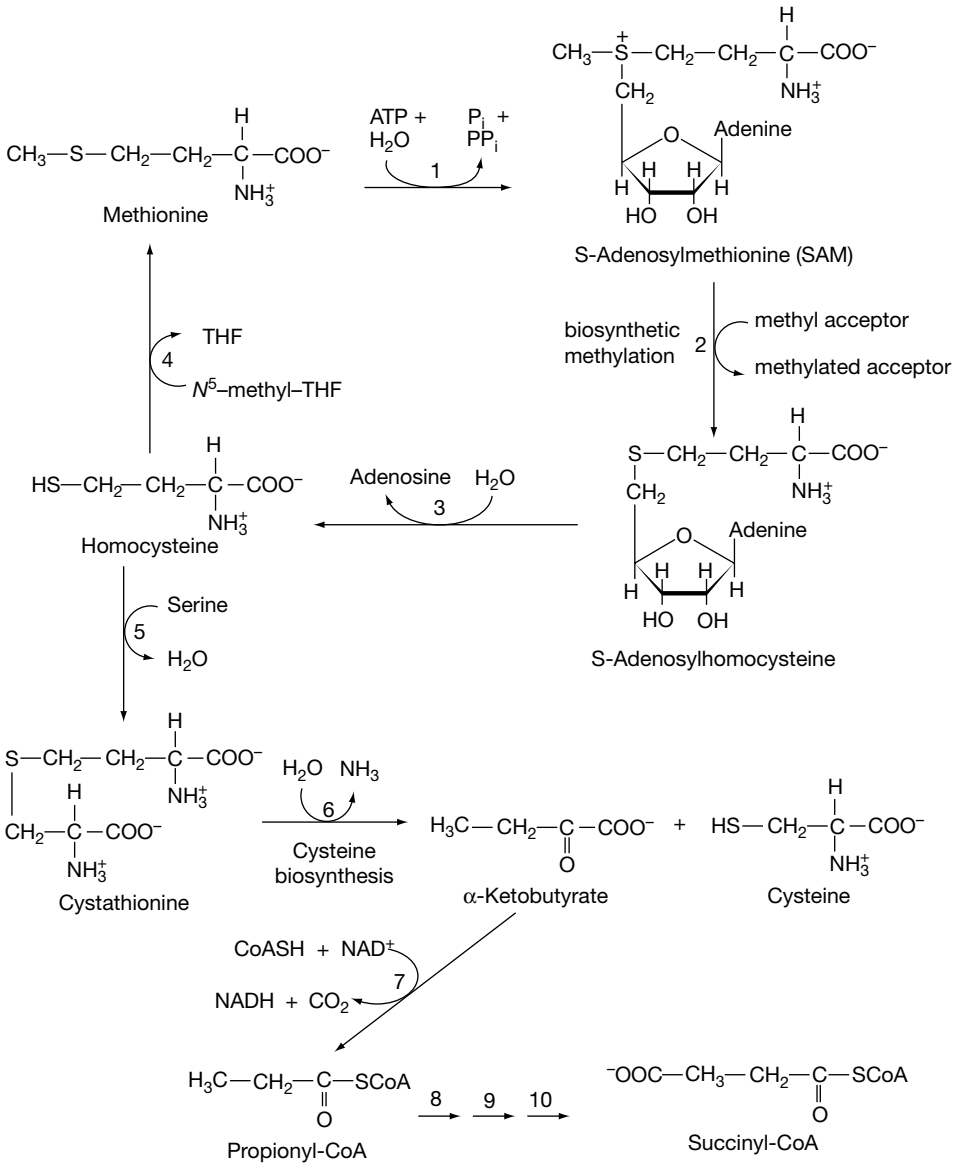


Figure 11.35 Catabolism of Methionine to cysteine and succinyl-CoA. The enzymes involved in the pathway are 1. Methionine adenosyl transferase, 2. methylase, 3. adenosylhomocysteinase, 4. homocystein methyl transferase, 5. Cystathionine beta synthase, 6. Cystathionine gamma-lyase, 7. Alpha-keto acid dehydrogenase, 8. Propionyl-CoA carboxylase, 9. Methylmalolyl-CoA racemase and 10. Methylmalonyl-CoA mutase

Therefore, valine is glycogenic. During the catabolism of leucine, it is degraded to β-methylcrotonyl CoA, then to HMG CoA, then to aceyl CoA or to actoacetate both at last are converted to fat, therefore leucine is ketogenic (Figure 11.36). During the catabolism of isoleucine, it degraded to triglyl CoA, then to methylacetoacetyl CoA, then to aceyl CoA or to propionyl CoA then propionyl CoA is converted to glucose and acetyl CoA is converted to fat. Therefore isoleucine is both glycogenic and ketogenic. Valine, leucine and isoleucine are essential aminoacids.

Metabolic disorder of the branched chain amino acid: Defect in the enzyme alpha-keto acid dehydrogenase lead to the disease, maple syrup urine disease. In this disease, the branched chain amino acids will result in the excretion of their keto acids in the urine.

11.8.12 Phenylalanine and Tyrosine Catabolism

Both phenylalanine and tyrosine are aromatic amino acids. While phenylalanine is an essential amino acid, tyrosine is a nonessential amino acid because tyrosine can be synthesized from phenylalanine by the enzyme hydroxylase.

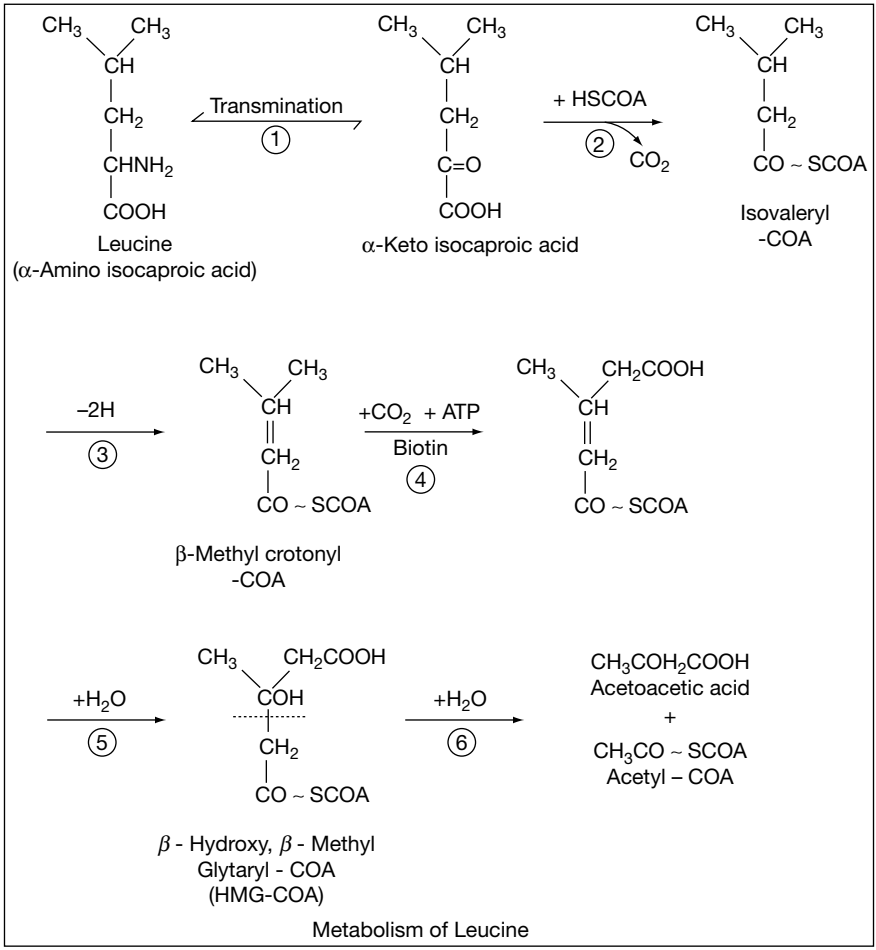


Figure 11.36 Catabolism of Leucine

Phenylalanine is metabolized to tyrosine and tyrosine upon deamination is converted to P-hydroxy phenyl pyruvic acid, which upon enzymic conversion forms homogentisic acid, which in the liver is further converted to fumaric acid and acetoacetic acid by opening of the benzene ring. Fumaric acid is oxidized by citric acid cycle and is therefore glycogenic. The acetoacetic acid, a ketone body leaves the liver and utilized in the tissues (Figure 11.37).

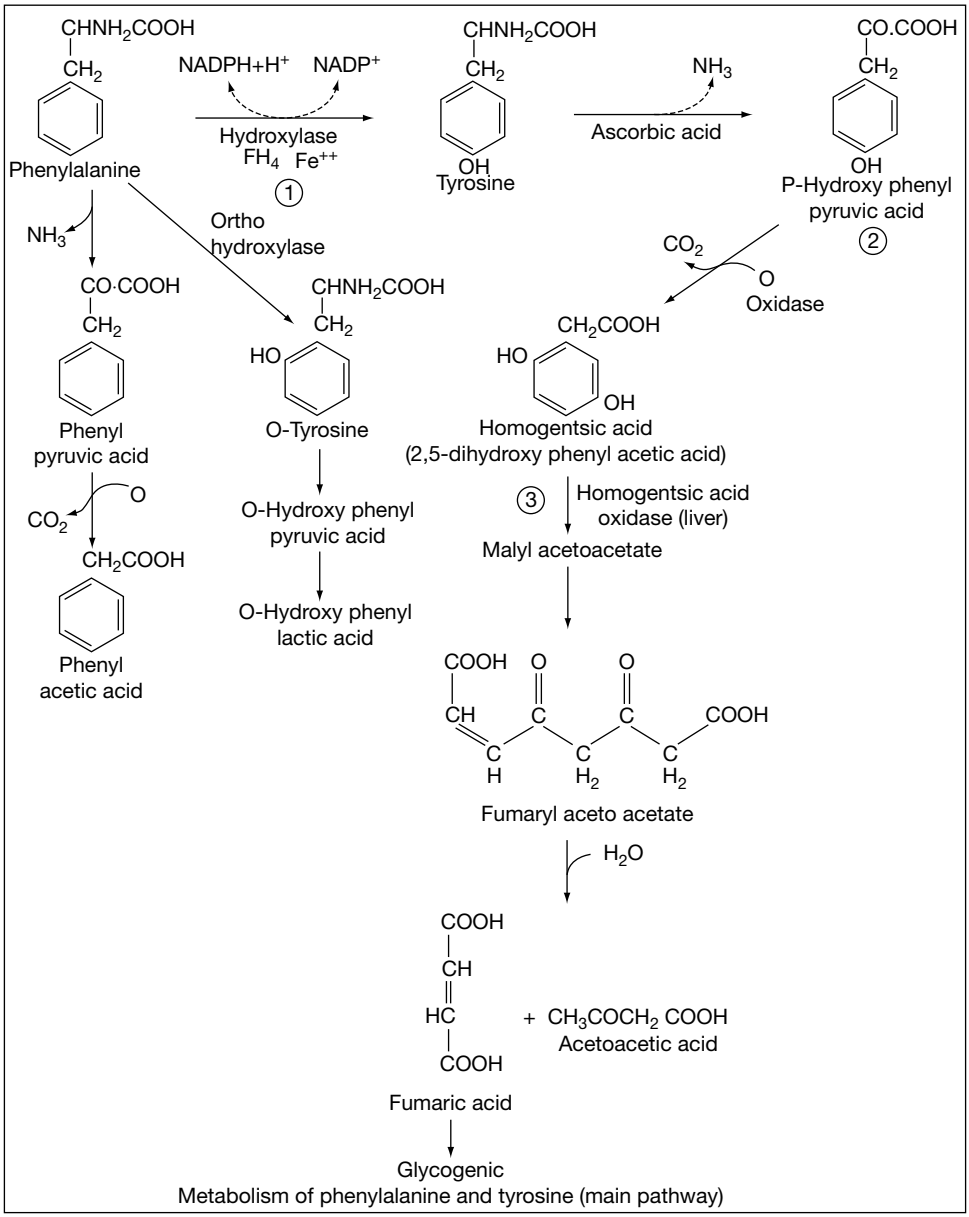


Figure 11.37 Catabolism of Phenylalanine and tyrosine

These amino acids upon oxidation form the compound DOPA (3,4 dihydroxyphenylalanine), which upon decarboxylation is converted to dopamine. Then, dopamine is converted to hormones, norepinephrine and epinephrine. The pigment melanin is also synthesized from DOPA. Tyrosine also leads to the synthesis of thyroxine. Thyroxine is synthesized by iodination and coupling of two molecules of iodinated tyrosine. Both amino acids are glycogenic and ketogenic (Figure 11.38).

Metabolic Disorder of Phenylalanine and Tyrosine

Phenylketonuria: This condition occurs as an inborn error of metabolism in children and is associated with mental symptoms. The hydroxylation of phenylalanine in the para position to form tyrosine is mediated by the enzyme phenylalanine hydroxylase, requiring Fe^{++} and NADPH + H^+ as coenzymes.

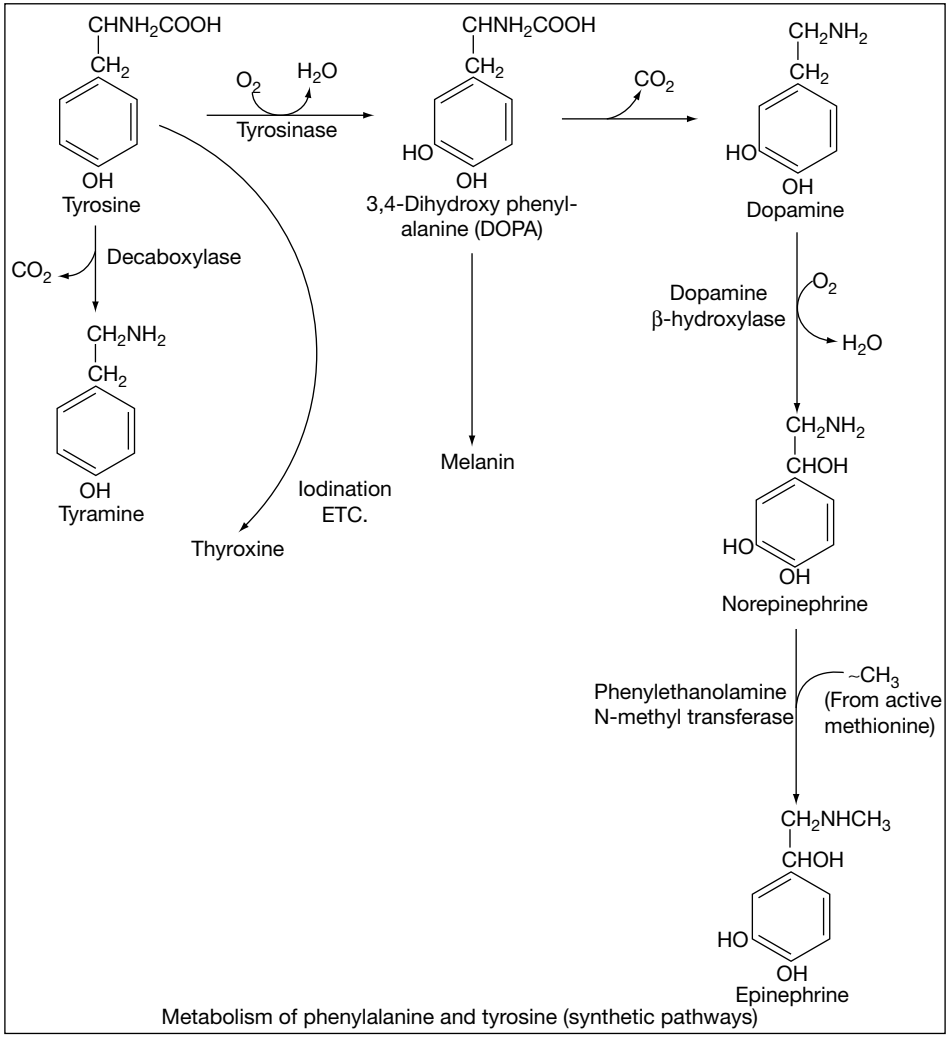


Figure 11.38 Catabolism of tyrosine to norepinephrine and epinephrine

The enzyme phenylalanine hydroxylase is present in liver but in phenylketonuria this enzyme is absent in liver. In this disease, phenlalanine is converted to phenylpyruvic, lactic and acetic acids which cannot be metabolized further and are excreted. Also, an abnormal orthohydroxy derivative is formed whose metabolites may also be found in the urine.

Tyrosinosis: Due to the lack of the enzyme parahydroxyphenylpyruvic acid oxidase, homogentisic acid cannot be formed and lead to the accumulation of tyrosine in tissues and blood and excreted in urine.

Alkaptonuria: Due to the lack of enzyme homogentisic acid oxidase in liver, the homogentisic acid is excreted in urine. On standing, the urine turns black in colour due to further oxidation of homogentisic acid.

Ochronosis: A condition in which deposition of homogentisic acid in the cartilages of ear and other exposed places and may darken them.

Alkaptonuria may occur in infants due to deficiency of vitamin C and can be cured by administration of vitamin C.

Albinism: Due to the lack of enzyme tyrosinase, DOPA cannot be converted to melanin. In this disease, there is no pigment in the skin, hair and iris and lead to white colour.

11.8.13 Lysine Catabolism

When lysine condenses with a molecule of alpa-ketoglutaric acid, it forms saccharopine with the removal of water molecule. Saccharopine first undergoes dehydrogenation and then followed by hydration, splits to form glutamic acid and alpha-aminoadipic acid semialdehyde. The eta amino group of alpha-aminoadipic acid semialdehyde is deaminated and also transaminated with another molecule of alpha-ketoglutaric acid to form glutamate and alpha-keto adapic acid. This undergoes decarboxylation to form glutaryl-CoA, then to crotonyl CoA and finally to acetoacetyl CoA. Lysine is an essential amino acid and is both glycogenic and ketogenic.

Metabolic disorder of lysine: Due to the deficiency of α-aminoadipic semialdehyde synthase enzyme, large amounts of lysine and saccharopine were excreted in urine.

11.8.14 Histidine Catabolism

Histidine is metabolized to urocanaic acid after deamination by the enzyme histidase, with the addition of a double bond into the product. Urocanic acid by the enzyme urocanase is converted to 4-imidazolone-5-propionic acid. The 4-imidazolone-5-propionic acid is catalysed by the enzyme hydrolase to N-formiminoglutamtamic acid, this is later converted to glutamate and N^5-formimino THF by the enzyme glutamate formiminotransferase and this enzyme transfers the fomimino group to THF (Figure 11.40). N^5-formiminoTHF is a 1-carbon THF intermediate.

Decarboxylation of histidine yields histamine in the intestine by bacteria and in many tissues. This reaction is catalysed by the enzyme histidine decarboxylase (Figure 11.40). Histamine is a toxic amine and causes a rise in blood pressure.

The metabolic disorder of histidine: The deficiency of the enzyme histidase leads to the disease histidinemia. Histidase is the first enzyme of histidine metabolism, and due to its deficiency urocanic acid is not formed. Therefore, urocanic acid is absent from skin and sweat, which is normally present in skin and sweat.

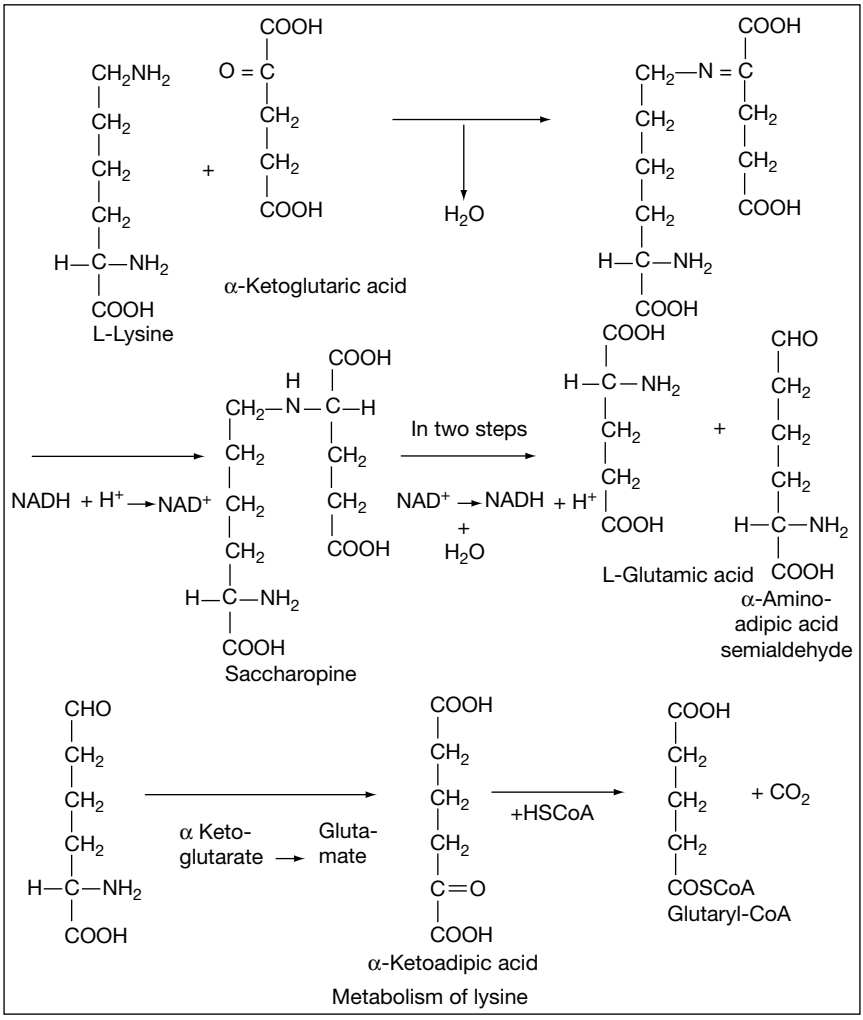


Figure 11.39 Catabolism of Lysine

11.8.15 Tryptophan Catabolism

Tryptophan is catabolized to acetoacetate through a number of side reactions. Tryptophan is converted to formyl Kynurenine by the enzyme tryptophan pyrrolase which is iron porphyrin oxygenase, which opens the indole ring. This is the first enzyme of tryptophan catabolism. Formylkynurenine is converted to kynurenine, which upon deamination forms kynurenic acid. Kynurenic acid acts as antiexcitotoxics and anticonvulsives. The metabolic disorder of tryptophan is schizophrenia, and in this defect large amount of kynurenic acid is excreted in urine. Kynurenic acid is a non-competitive antagonist at the glycine binding site of the of N-methyl-D-aspartate (NMDA) receptor, NMDA is ionotropic receptor of glutamate.

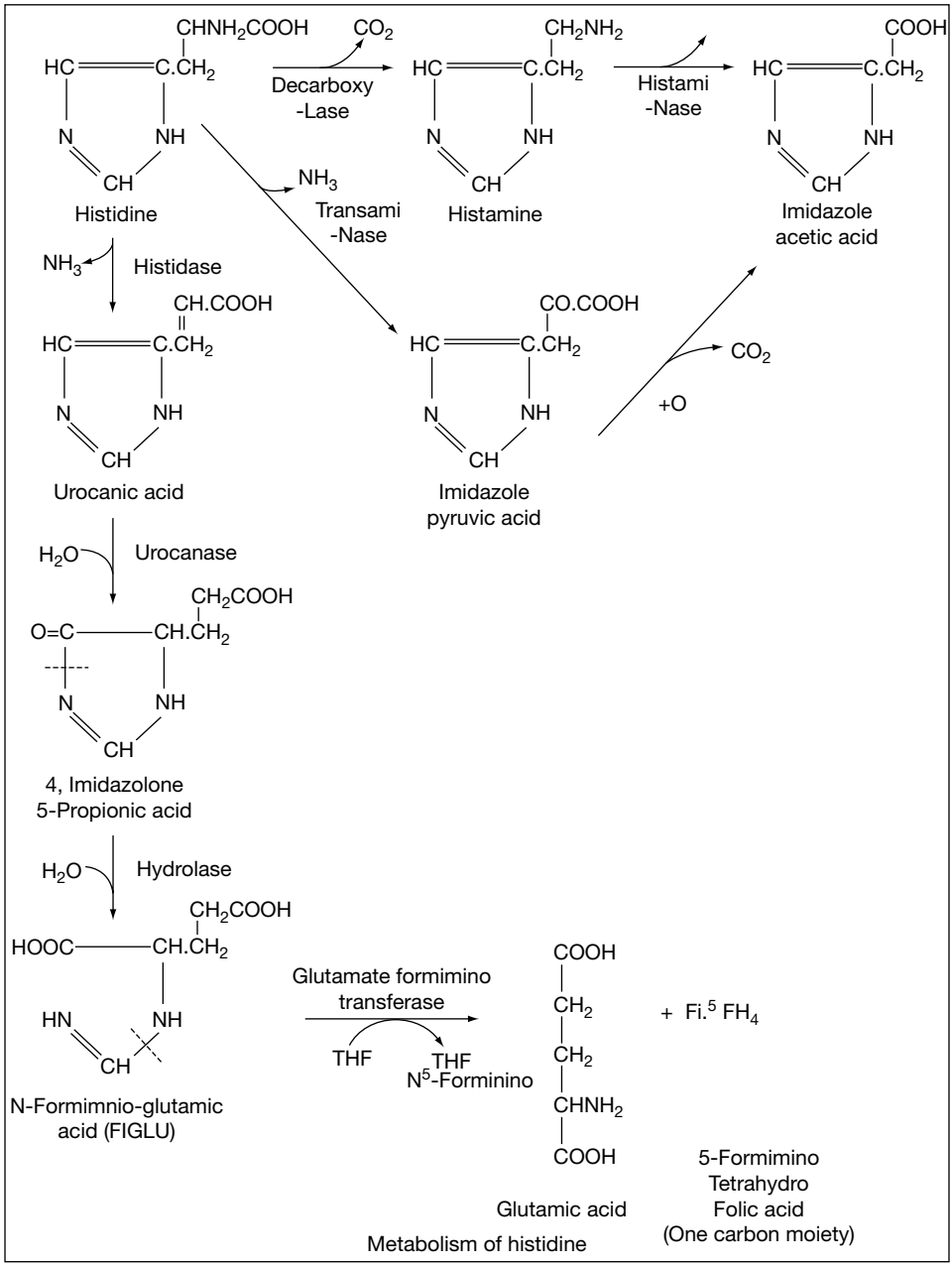


Figure 11.40 Catabolism of Histidine

Kynurenine by the enzyme kynurenine hyroxylase is converted to 3-hydroxy kynurenine, which is further catalysed by the enzyme kynureninase to form 3-hydroxyanthranilic acid and alanine (Figure 11.41). Alanine leads to the formation of glucose; therefore, tryptophan is a

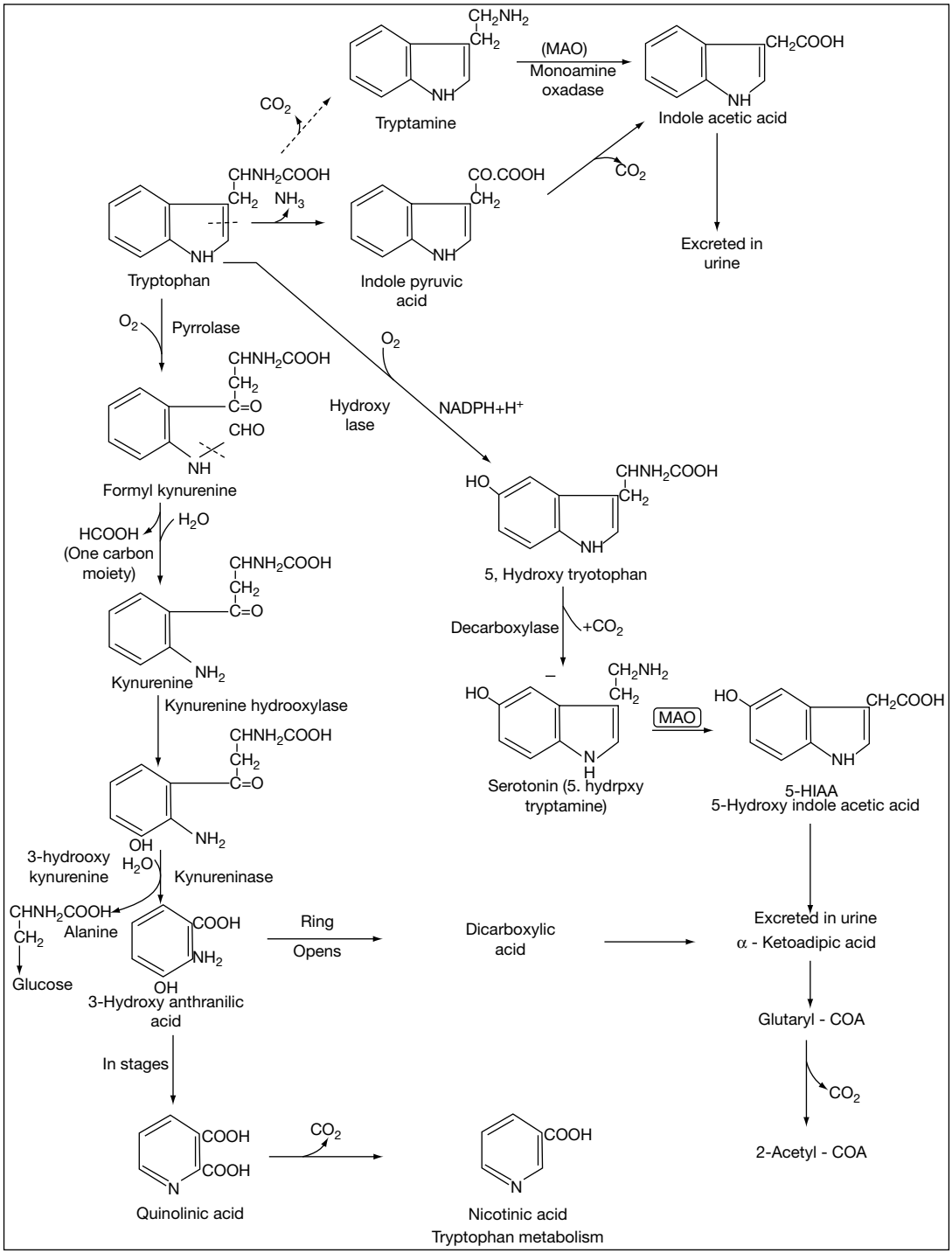


Figure 11.41 Catabolism of Tryptophan

glucogenic amino acid. 3-hydroxyanthranilate upon oxidation forms 2-amino-3-carboxymuconic 6-semialdehyde, this product undergoes two fates. This intermediate leads to acetoacetate, therefore tryptophan is also a ketogenic. Another reaction in liver forms quinolinic acid upon cyclization, which is a non-enzymic reaction. Then, quinolate by transamination reaction and after undergoing many rearrangements, forms nicotinic acid which further leads to the formation of coenzyme NAD^+ and $NADP^+$.

Tryptophan upon hydroxylation at position 5 by hydroxylase enzyme is converted to 5-hydroxy tryptophan; then, this compound upon decarboxylation of side chain by decarboxylase enzyme forms serotonin. 5-hydroxy tryramine (serotonin) is a potent vasoconstrictor (Figure 11.42). Serotonin is converted to 5-HIAA (5-hydroxyindole acetic acid) by the enzyme MAO (monoamine oxidase). The drug which inhibit the MAO is iproniazid-isopropyl isonicotinyl hydrazide, and stops the conversion of serotonin to HIAA and prolong serotonin action on brain producing psychic stimulation due to increased cerebral activity. In brain, serotonin is in bound form. The anti-hypertensive drug reserpine, acts by releasing the serotonin from its bound form and makes free and available to the enzyme MAO action. Hence, reserpine decreases the cerebral activity.

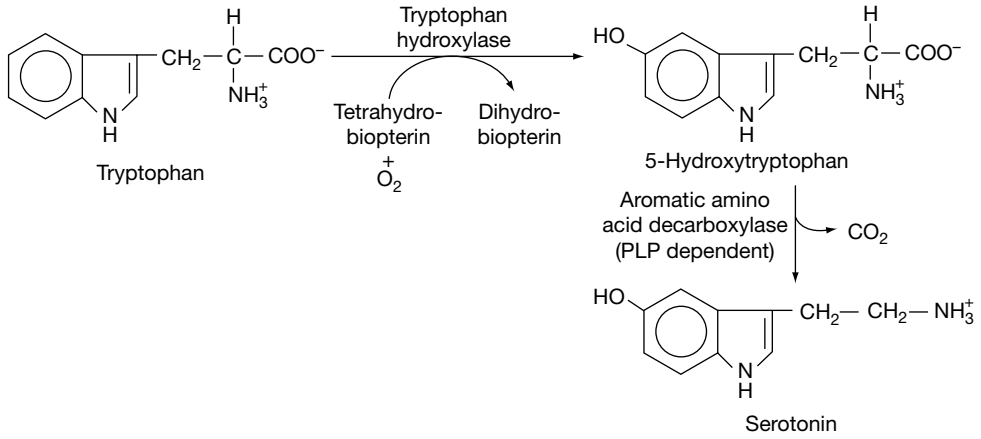


Figure 11.42 Synthesis of Serotonin from Tryptophan

11.9 PLANT AND ANIMAL PIGMENTS

Pigments are a heterogeneous group of chemicals found in both plants and animals. They are coloured substances and absorb light in the visible range. Besides imparting a characteristic colour to the particular tissue or organ where they exist, the pigments are also involved in trapping light for photosynthesis and many other physiological processes.

11.9.1 Plant Pigments

Chlorophylls

Chlorophylls are green pigment of plants, which absorb light for the process of photosynthesis. The most important type of chlorophyll is chlorophyll a, which is present in almost all green plants including some bacteria. Chlorophyll b is present in higher plants and green algae,

chlorophyll c in diatoms and brown algae and chlorophyll d in some red algae. Purple bacteria contain another type of chlorophyll—the bacteriochlorophyll, which resembles chlorophyll a.

Chlorophylls in higher plants are contained within the chloroplasts. The chloroplasts are disc like or oval bodies and their average diameter is about 4 to 6 μ. The chloroplast is surrounded by a two-layered membrane, and the pigment is located in particulate structures called grana (singular-granum). The grana are embedded in the hydrophilic matrix of the chloroplast (see Chapter 3 for structure of chloroplast). The size of the granum ranges from species to species, but the average range is 0.3 to 0.7 μ. A granum is made up of about 40 to 50 disc-like sacs—the thylakoids. Adjacent grana are connected by tubular structures called lamella. The non-granal portion of the chloroplast matrix is called stroma. Grana are lacking in algal cells but free grana or chromatophores are found in bacteria.

The chlorophylls have a porphyrin structure or a tetra-pyrrole nucleus with an Mg atom in the centre of the nucleus. A phytyl group and a methyl group are invariably attached to the nucleus. Various chlorophylls differ in their side chain (Figure 11.43). The structure of chlorophyll a was established by Hans Fischer in 1940. Later on, the structure was confirmed by R. B. Woodward, who could carry on the total synthesis of chlorophyll in 1960.

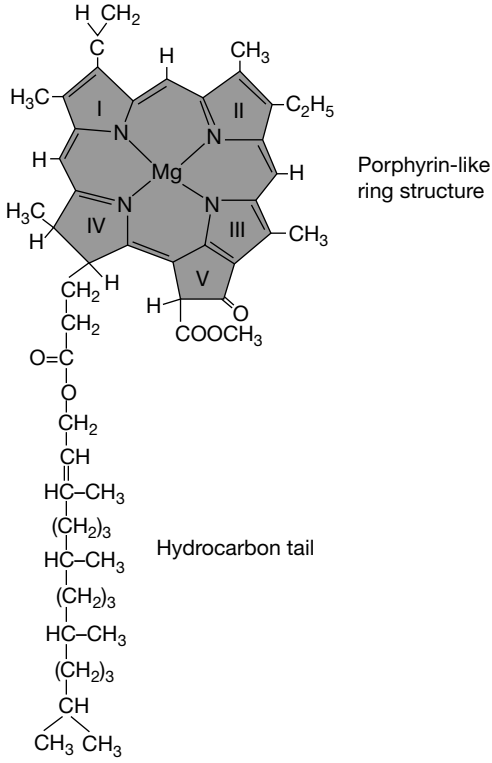
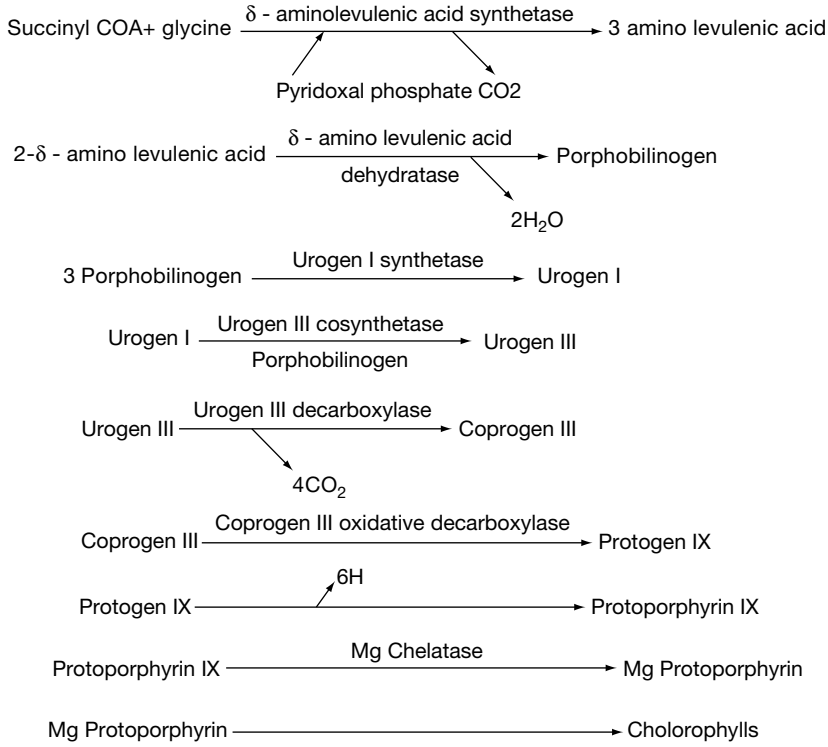


Figure 11.43 Structure of Chlorophyll Pigment

Chlorophylls absorb visible light efficiently because of their many conjugated double bonds. The absorption of light by chlorophylls is the most important biophysical event as the light energy trapped by the pigments is converted into chemical energy. In higher plants, both chlorophyll a and chlorophyll b work in close co-operation for the conversion of light into chemical energy.

Biosynthesis of chlorophylls: The pathway for the biosynthesis of chlorophylls is similar to that of the synthesis of heme pigment of animals, up to the synthesis of protoporphyrin. The various components of the pathway have been analysed using cell-free extracts of photosynthetic bacteria and chloroplast preparations from higher plants.



Phycobilins

Phycobilins are algal pigments, which also absorb light. However, the light absorbed by these pigments is transferred as excitation energy to chlorophylls where it can be used for photosynthesis. Therefore, phycobilins help chlorophylls in the trapping of light energy and they are called 'accessory pigments.'

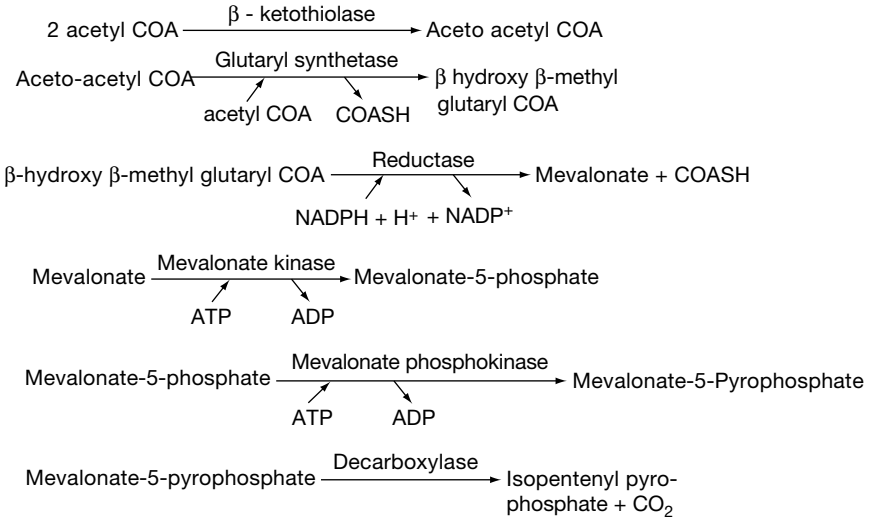
The two important phycobilins are phycoerythrobilin (a red phycobilin) and phycocyanobilin (a blue phycobilin). They are the most important pigments of red- and blue-green algae and occur in the cell as protein conjugates. The phycobilins are linear tetra-pyrroles and unlike chlorophylls they do not have Mg^{++}.

Carotenoids

Carotenoids are also accessory pigments of plants and are located inside the chloroplasts. They transfer light energy after capturing it to the chlorophylls for photosynthesis, although their principal function appears to be the protection of chlorophylls against photo-oxidation. They are long polyisoprenoid molecules having conjugated double bonds. There are two major classes of carotenoids—the carotenes which are isoprenoid hydrocarbons and contain no oxygen and the xanthophylls which are very similar in structure to carotenes, but contain oxygen.

β-carotene (for the structure, see Chapter 15) is the most abundant carotenoid. It is yellow orange in colour and its absorption maxima is 430–480 nm.

Carotenoids are synthesized stepwise in the small units of C5 chains. The reactions for the synthesis of carotenoids, starting from acetyl-COA are given hereunder.



Isopentenyl pyrophosphate is common to the biosynthesis of carotenoids and sterols. In the synthesis of carotenoids, eight isopentenyl units condense to form carotene skeleton. The intermediate compounds involved in this series or reaction are geranyl pyrophosphate geranyl (C_{10}), farnesyl pyrophosphate (C_{15}), geranyl gcranyi pyrophosphate (C_{20}) followed by a condensation of two C_{20} intermediates (geranyl pyrophosphate) to produce a C_{40} compound phytoene. Phytoene is a colourless compound and its successive dehydrogenation produces different carotenes.

Flavonoids

The flavonoids are responsible for mostly red, blue and violet colour of the plant organs. Chemically, the flavonoids are phenolic compounds and most of them have a C_6—C_3—C_6 skeleton. Some important coloured flavonoids are discussed hereunder.

The distribution of flavonoids in plant kingdom is of taxonomic significance. Algae, fungi and bacteria do not have any kind of flavonoid, white mosses possess a few types of them. Ferns and gymnosperms contain several types of simple flavonoids while in angiosperms a whole range of flavonoids are found. Highly complex forms of flavonoids are present in the highly evolved families such as compositae.

Biosynthesis: The precursors of flavonoid biosynthesis are shikimic acid, phenylatanine, cinnamic acid and p-coumaric acid. Shikimic acid is an intermediate in the biosynthesis of aromatic amino acids. The pathway for the biosynthesis of flavonoids may be outlined as follows:

Shikimic acid + Phosphoenol pyruvic acid → Prephenic acid

Prephenic acid → p-hydroxy phenyl pyruvic acid

p-hydroxy phenyl pyruvic acid → Tyrosine

Tyrosine → p-coumaric acid

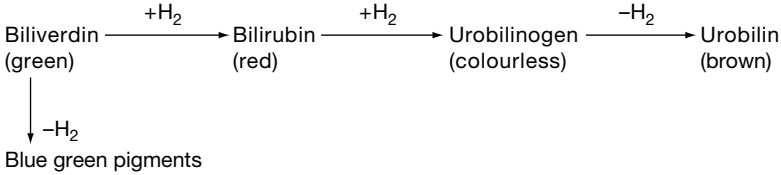
p-coumaric acid → Chalone

Chalones → Different flavonoids

11.9.2 Animal Pigments

Bile Pigments

Golden or brownish yellow colour of human bile secreted by the liver is due to two pigments, bilirubin and biliverdin. These two pigments are called bile pigments. These two pigments are interconvertible involving oxidation–reduction process. Biliverdin is a tetra-pyrrole compound, lacking a metallic atom and it is green in colour. The oxidation of this pigment produces blue green pigment while reduction produces biliverdin which is red in colour.



The bile pigments are derived from the heme of haemoglobin from aged red blood cells and other heme proteins such as myoglobin, catalase, cytochromes, peroxidase, etc. The bile pigments are degraded further by the action of micro-organisms present in the intestine to a brown pigment called stercobilin, which is the chief pigment of faeces.

Heme ⟶ Protoporphyrin IX ⟶ Bilirubin and Biliverdin ⟶ Stercobilin

Heme Pigment

Red colour of the blood is due to a pigment called heme. In the blood the heme is associated with the protein haemoglobin. Besides this, the pigment is also an essential component of many other important biomolecules such as cytochromes, peroxidase, myoglobin, etc.

Heme is structurally similar to chlorophyll and is made up of four pyrrole groups. In this case, there is an iron atom instead of magnesium in the centre of the molecule (Figure 11.44). The iron atom is associated with the protein part of the molecule. In haemoglobin, the pigment of the red blood cells, the iron atom is associated with histidine at positions 58 and 87 in the alpha-chain and positions 63 and 92 in the beta-chain of the globin (protein) part of the molecule.

The molecules containing heme as a component are usually associated with the transport of oxygen. In the blood, the heme part of the molecule is normally in the reduced form containing a water

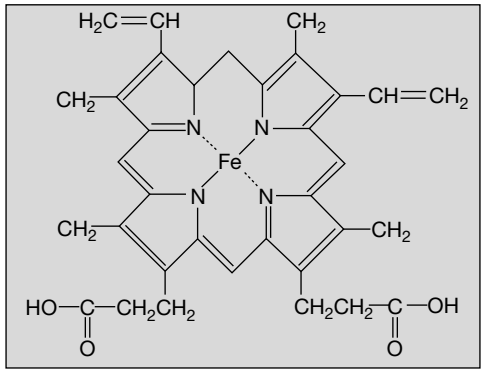


Figure 11.44 Structure of Haemoglobin

molecule covalently linked with the iron atom of the heme. In the presence of oxygen, this water molecule is replaced by an oxygen molecule forming oxyhemoglobin. The oxygen is carried to the various parts of the body in this form. Similarly, carbon monoxide can also bind with haemoglobin, replacing water molecule. This reaction is the biochemical basis of the toxicity of carbon monoxide, because CO-haemoglobin cannot carry oxygen.

The oxygen-carrying properties of haemoglobin are inhibited by certain chemicals such as nitrophenols, chlorates, aniline, etc. These chemicals do not bind with the iron atom but they change it from ferrous state to ferric state. The compound thus formed is brown in colour and is called methemoglobin. Methemoglobin cannot be oxygenated and therefore the oxygen-carrying properties of the haemoglobin is lost.

11.10 METABOLIC DISORDER OF AMINO ACIDS

Table 11.1 shows various metabolic disorder of amino acids.

Table 11.1 Metabolic Disorder of Amino Acids

Defective process	Defective enzyme	Symptoms and effects
Argininosuccinic academia (name of the disease)		
Urea synthesis	Argininosuccinase	Vomiting and convulsions
Argininemia (name of the disease)		
Urea synthesis	Arginase	Mental retardation
Albinism (name of the disease)		
Melanin synthesis from tyrosine	Tyrosine 3-monooxygenase (tyrosinase)	Lack of pigmentation: white hair, pink skin
Alkaptonuria (name of the disease)		
Tyrosine degradation	Homogentisate 1,2-dioxygenase	Dark pigment in urine and late developing arthritis

Defective process	Defective enzyme	Symptoms and effects
Carbamoyl phosphate synthetase I deficiency		
(Name of the Disease)		
Urea synthesis	Carbamoyl phosphate synthetase I	Lethargy, convulsions and early death
Homocystinuria (name of the disease)		
Methionine degradation	Cystathionine β-synthase	Faulty bone development and mental retardation
Maple Syrup urine disease (name of the disease)		
(branched chain ketoacidurea)		
Isoleucine, leucine and valine degradation	Branched-chain α-keto acid dehydrogenase complex	Vomiting, convulsions, mental retardation and early death
Methylmalonic academia (name of the disease)		
Conversion of propionyl-CoA to succinyl-CoA	Methylmalonyl-CoA mutase	Vomiting, convulsions, mental retardation and early death
Phenylketonuria (name of the disease)		
Conversion of phenyl-alanine tyrosine	Phenylalanine hydroxylase	Neonatal vomiting and mental retardation

EXERCISES

1. Write the pathway of phenylalanine and tyrosine from chorismate in plants and bacteria.
2. Write the biosynthesis of isoleucine from threonine, which is one of the precursors of isoleucine.
3. Describe the glucose–alanine cycle with the help of a diagram.
4. Write the deficiencies that occur due to any of the enzymes used in urea cycle.
5. Heme is structurally similar to chlorophyll. Write their importance.
6. Write the synthesis of serotonin from tryptophan.
7. Write the catabolism of threonine into acetaldehyde and glycine.
8. What is the importance of one-carbon pool in amino acid catabolism?
9. What is urea? Describe its formation and excretion in human body.
10. Explain the chemical reactions involved in formation of urea.
11. List the excretory products of nitrogen metabolism. How are they formed in the body?

MULTIPLE CHOICE QUESTIONS

1. Ammonia production by the kidney is depressed in
   (a) Acidosis
   (b) Alkalosis
   (c) Both (a) and (b)
   (d) None of these
   Ans. b
2. In the biosynthesis of porphyrins, the activation of glycine needs the coenzyme
   (a) NAD^+ (b) B_6–Po_4
   (c) FAD^+ (d) ATP
   Ans. b
3. Heme is synthesized by the incorporation of ferrous ion (Fe^{++}) into protoporphyrin III being catalysed by the enzyme
   (a) Ferroxidase
   (b) Ferroreductase
   (c) Ferrochelatase
   (d) None of these
   Ans. c
4. Haemoglobin takes up the number of molecules of oxygen
   (a) 1 (b) 2
   (c) 4 (d) 6
   Ans. c
5. Carboxyhemoglobin is formed by
   (a) CO (b) CO_2
   (c) HCO_3 (d) HCN
   Ans. a
6. The iron of heme is coordinated in β-chains at positions
   (a) 43 and 72 (b) 53 and 82
   (c) 63 and 92 (d) 73 and 102
   Ans. c
7. Amino acids provide the nitrogen for the synthesis of
   (a) The basis of the phospholipids
   (b) Uric acid
   (c) Glycolipids
   (d) None of these
   Ans. a
8. Sulphur-containing amino acids after catabolism produce a substance which is excreted
   (a) SO_2 (b) HNO_3
   (c) H_2SO_4 (d) H_3PO_4
   Ans. c
9. The transaminase activity needs the coenzyme
   (a) ATP (b) B_6–PO_4
   (c) FAD (d) NAD^+
   Ans. b
10. Synthesis of glutamine is accompanied by the hydrolysis of
    (a) ATP (b) ADP
    (c) TPP (d) None of these
    Ans. a
11. In brain, the major mechanism for removal of ammonia is the formation of
    (a) Glutamate (b) Glutamine
    (c) Aspartate (d) None of these
    Ans. b
12. Carbamoyl phosphate synthetase structure is markedly changed in the presence of
    (a) N-Acetyl glutamate
    (b) N-Acetyl aspartate
    (c) Oxalate
    (d) None of these
    Ans. a
13. The competitive inhibitor of arginine is
    (a) Citrulline (b) Malate
    (c) Lysine (d) Serine
    Ans. c
14. One molecule of urea is synthesized at the expense of the mols of ATP
    (a) 2 (b) 6
    (c) 4 (d) 3
    Ans. d
15. Uremia occurs in
    (a) Cirrhosis of liver (b) Nephritis
    (c) Diabetes mellitus (d) None of these
    Ans. b

16. Clinical symptom in urea cycle disorder is
    (a) Mental retardation
    (b) Nephritis
    (c) Diabetes mellitus
    (d) None of these

    Ans. a

17. The sparing action of methionine is
    (a) Tyrosine (b) Cystine
    (c) Arginine (d) None of these

    Ans. b

18. NH_4^+ aminates glutamate to form glutamine requiring ATP and
    (a) Mg^{++} (b) Ca^{++}
    (c) Na^+ (d) None of these

    Ans. a

19. Urea biosynthesis occurs mainly in the liver involving the number of amino acids
    (a) 3 (b) 4
    (c) 6 (d) 2

    Ans. c

20. Glutathione is a
    (a) Dipeptide (b) Tripeptide
    (c) Polypeptide (d) None of these

    Ans. b

12 Metabolism of Nucleic Acids

CHAPTER OBJECTIVES

12.1 Introduction to Nucleic Acids Metabolism

12.2 Biosynthesis of Purine Nucleotide

12.3 Regulation of Purine Nucleotide Biosynthesis by Feedback Inhibition

12.4 Biosynthesis of Pyrimidine Nucleotides

12.5 Regulation of Pyrimidine Biosynthesis by Feedback Inhibition

12.6 Catabolism of Purine Nucleotide

12.7 Catabolism of Pyrimidine Nucleotide

12.8 Salvage Pathway

12.9 Biosynthesis of Deoxyribonucleotides

12.10 Formation of Deoxythymidylic Acid

12.11 Regulation of Deoxyribonucleotide Biosynthesis

12.12 Biosynthesis of Nucleotide Coenzymes

12.13 Metabolic Disorder of Nucleic Acid

12.1 INTRODUCTION TO NUCLEIC ACIDS METABOLISM

The nucleic acid metabolism is concerned with the pathway by which nucleic acids and their components are anabolized (formed) and catabolized (broken down). The chemistry of nucleic acids and their components has been described in the chapter 5. This chapter deals with the fundamental mechanism for controlling macromolecular synthesis and activity in cells. This phase of the chapter deals in part with the fundamental mechanism for controlling macromolecular synthesis and activity in cells.

12.2 BIOSYNTHESIS OF PURINE NUCLEOTIDE

In the biosynthesis of purine, a nitrogenous heterocyclic base, for example, adenine, guanine, hypoxanthine and xanthine are involved. De novo synthesis involves a complex, energy-expensive pathway, that yields inosine 5′-monophosphate (IMP), a purine ribonucleotide. A separate pathway

is followed for the formation of AMP and GMP from IMP. Both DNA and RNA contain adenine and guanine. Hypoxanthine and xanthine are important intermediates in the synthesis and degradation of the purine nucleotides. Two types of pathways lead to the biosynthesis of purine nucleotide are de novo pathway and the salvage pathway. De novo synthesis of nucleotide begins with their metabolic precursors; amino acids, ribose 5-phosphate, CO_2 and NH_3. Salvage pathway recycles the free bases and nucleosides released from nucleic acid break down.

The mechanism of purine biosynthesis has been reviewed by John Buchanan. By studying purine synthesis in cell-free systems of pigeon liver, it has been established that purine biosynthesis is effective de novo from several small molecules. The various components, which contribute to the synthesis of purine ring, are derived from formate, CO_2, glutamine, aspartic acid and glycine as shown in Figure 12.1.

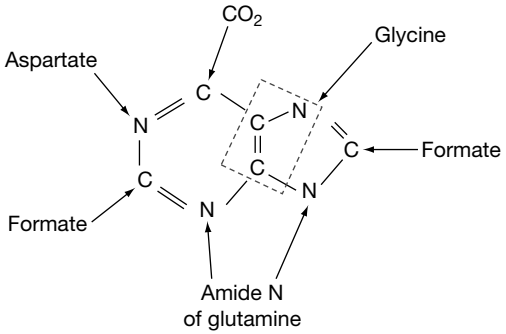


Figure 12.1 Sources of the purine ring. The sources of carbon and nitrogen are different

Purine biosynthesis is accompolished by de novo pathway: Nucleotide bases are assembled from simpler compounds. In de novo pathways, the nucleotide bases are made from simple starting materials and require ATP hydrolysis.

The two important main bases of purine nucleotide are adenine and guanine. De novo synthesis of purine nucleotide is completed in many steps. De novo pathway of purine biosynthesis leads to the complete synthesis of purine ring. Purine is a heterocyclic aromatic organic compound, consisting of a six-membered pyrimidine ring fused to five-membered of an imidazole ring. Steps of purine biosynthesis of de novo pathway are given hereunder (Figure 12.2).

Step 1: The synthesis of inosinic acid begins with D-ribose-5-phosphate, which is formed in the pentose cycle. D-Ribose-5-P is converted to 5-phosphoribosyl-1-pyrophosphate by the action of ATP. This reaction is catalysed by the kinase enzyme.

Step 2: 5-Phosphoribosyl-1-pyrophosphate reacts with glutamine to form 5-phosphoribosyl-1-amine. Glutamine donates amino group, which is attached at C-1 of PRPP, with the formation of 5-phosphoribosyl-1-amine. The enzyme that catalysed this reaction is glutamine-PRPP amidotransferase.

Step 3: In this step, 5-phosphoribosylamine reacts with glycine to form glycinamide ribonucleotide. GAR synthetase is the enzyme used to catalyse this reaction, and ATP is consumed to activate the glycine carboxyl group.

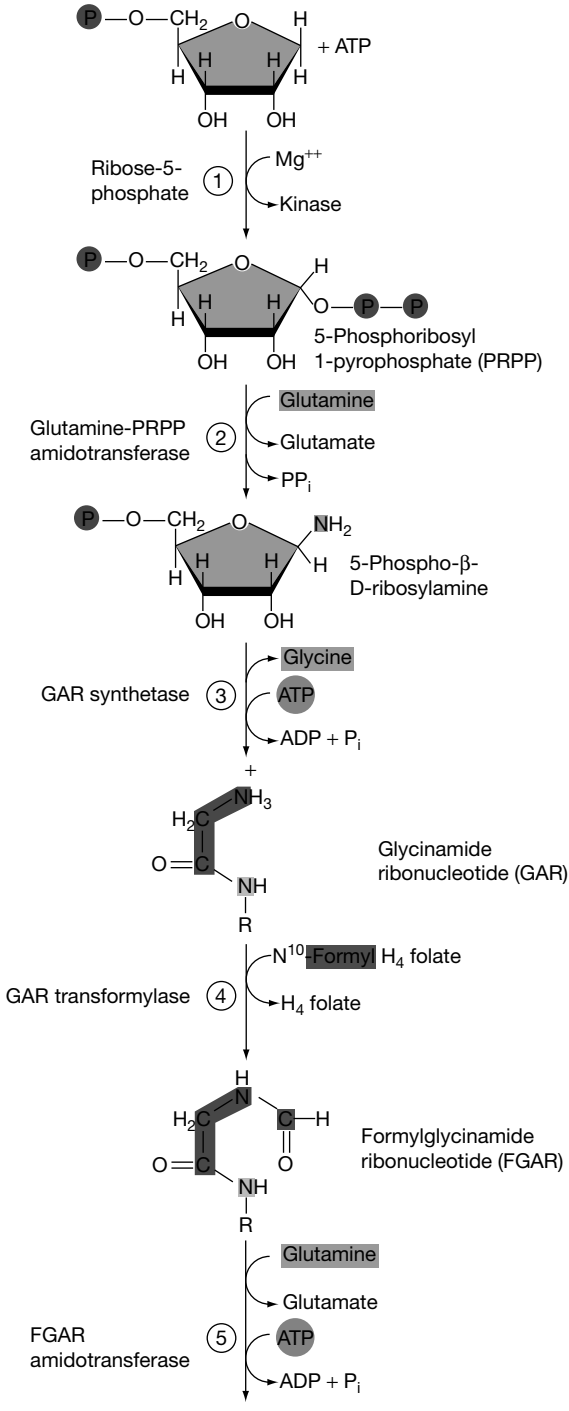


Figure 12.2 Continued

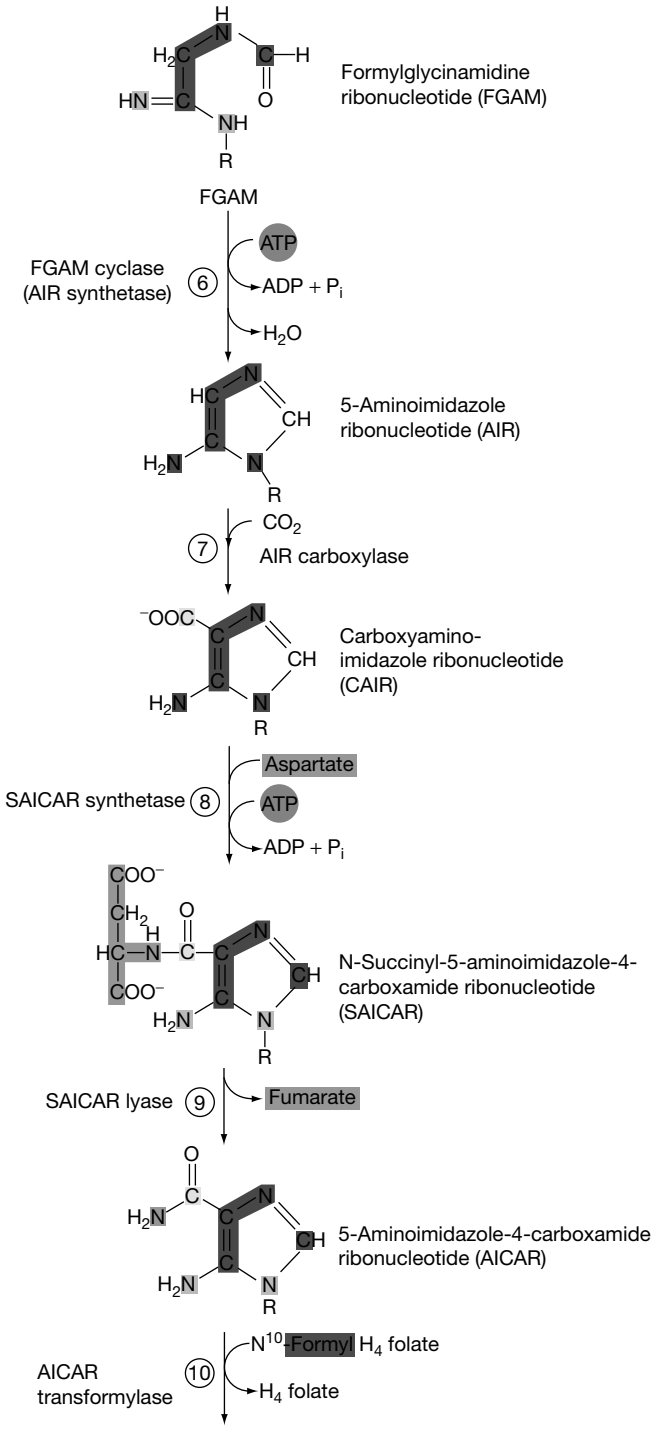


Figure 12.2 Continued

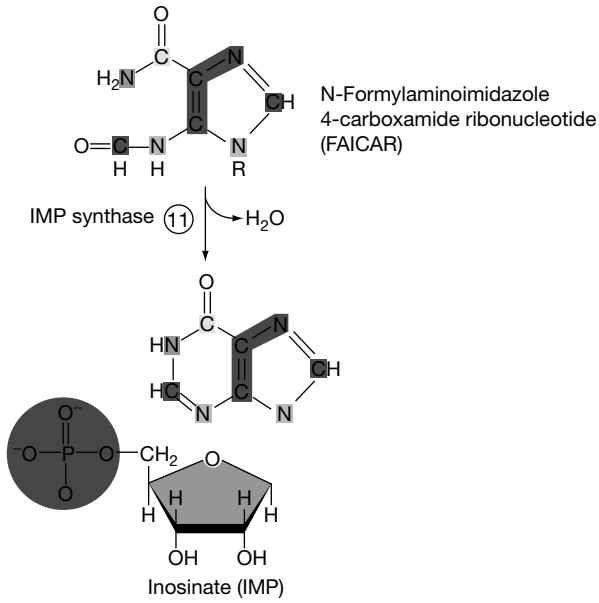


Figure 12.2 De novo synthesis of purine nucleotide. The biosynthesis of inosinic acid begins with D-ribose-5-phosphate leads to the construction of purine ring of inosinate (IMP) through many steps.

Step 4: Glycinamide ribonucleotide is converted to formylglycinamide ribonucleotide (FGAR) by N^{10}-anhydroformyltetrahydrofolic acid (N^{10}-anhydroformyl-FH_4), and glycinamide ribotide transformylase is the enzyme used to catalyse this reaction.

Step 5: Formylglycinamide ribonucleotide then reacts with glutamine and ATP to form formylglycinamidine ribonucleotide (FGAM). FGAR amidotransferase is the enzyme used to catalyse this reaction.

Step 6: In this step, formylglycinamidine ribonucleotide (FGAM) reacts with ATP in which dehydration occurs and the process of dehydration leads to ring closure to yield the five-membered imidazole ring of the purine nucleus, as 5-aminoimidazole ribonucleotide (AIR). The enzyme used to catalyse this reaction is FGAM cyclase.

Step 7: 5-Aminoimidazole ribonucleotide (AIR) is then carboxylated to carboxyaminoimidazole ribonucleotide by the enzyme aminoimidazole ribonucleotide carboxylase.

Step 8: The compound carboxyaminoimidazole ribonucleotide reacts with aspartic acid and ATP to form N-succinyl-5-aminoimidazol-4-carboxamide ribonucleotide. The enzyme used to catalyse this reaction is SAICAR synthetase.

Step 9: N-Succinyl-5-aminoimidazol-4-carboxamide ribonucleotide is then cleaved into fumaric acid by the enzyme SAICAR lyase to form 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR).

Step 10: 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) converted to N-formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR) by the enzyme AICAR transformylase.

Step 11: Inosinic acid is finally formed by the action of the enzyme IMP synthase upon N-formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR). The ring closure takes place to yield the second fused ring of purine nucleus.

12.2.1 Biosynthesis of AMP and GMP from IMP

The first intermediate of complete purine ring is inosinate (IMP). The conversion of inosinate to adenylate requires the insertion of amino group derived from aspartate. Formation of adenylate from inosinate is a two-step reaction; in the first step, inosinate reacts with aspartate and GTP is the source of high-energy phosphate in synthesizing adenylosuccinate. This reaction is very similar to the Steps 8 and 9 of purine biosynthesis in which aspartate is used to introduce N-1 of the purine ring. The only difference in this reaction is inspite of ATP, GTP is used as a source of energy. This reaction is catalysed by the enzyme adenylosuccinate synthetase. In the second step, adenylosuccinate is converted to adenylate (AMP) with the release of fumarate, catalysed by the enzyme adenylosuccinate lyase.

Guanylate (GMP) is formed by the NAD^+ requiring oxidation of inosinate (IMP) at C-2, followed by addition of an amino group derived from glutamine. This reaction is catalysed by the enzyme amidotransferase, and ATP is cleaved to AMP and PPi (Figure 12.3).

12.3 REGULATION OF PURINE NUCLEOTIDE BIOSYNTHESIS BY FEEDBACK INHIBITION

De novo purine nucleotide synthesis is regulated by feedback inhibition by various steps. The first mechanism is exerted on the first reaction, that is, transfer of a amino group to PRPP to form 5-phosphoribosylamine. This reaction is catalysed by the allosteric enzyme glutamine-PRPP amidotransferase. This enzyme is inhibited by the end product of IMP, AMP and GMP. AMP and GMP act synergistically in this concerted inhibition. Whenever either AMP or GMP accumulates to excess, the very step in its biosynthesis from PRPP is inhibited (Figure 12.4).

In the second control mechanism, which is exerted at a later stage, excess of GMP in the cell inhibits the formation of its own synthesis by inhibiting the formation of xanthylate from inosinate by inhibiting the enzyme IMP dehydrogenase without affecting the formation of AMP. In the same way, AMP inhibits is own synthesis by inhibiting the formation of adenylosuccinate from IMP by inhibiting the enzyme adenylosuccinate synthetase without affecting the synthesis of GMP.

The third mechanism balances the synthesis of the two nucleotides, GMP and AMP, by reciprocal arrangement. GTP is required in the conversion of IMP to AMP, whereas ATP is required for the conversion of IMP to GMP. Therefore, GTP regulates the synthesis of AMP and ATP regulates the synthesis of GMP.

Finally, the allosteric regulation of the enzyme ribose phosphate pyrophosphokinase PRPP synthetase inhibits the synthesis of PRPP. The inhibitors of the enzymes are ADP and GDP.

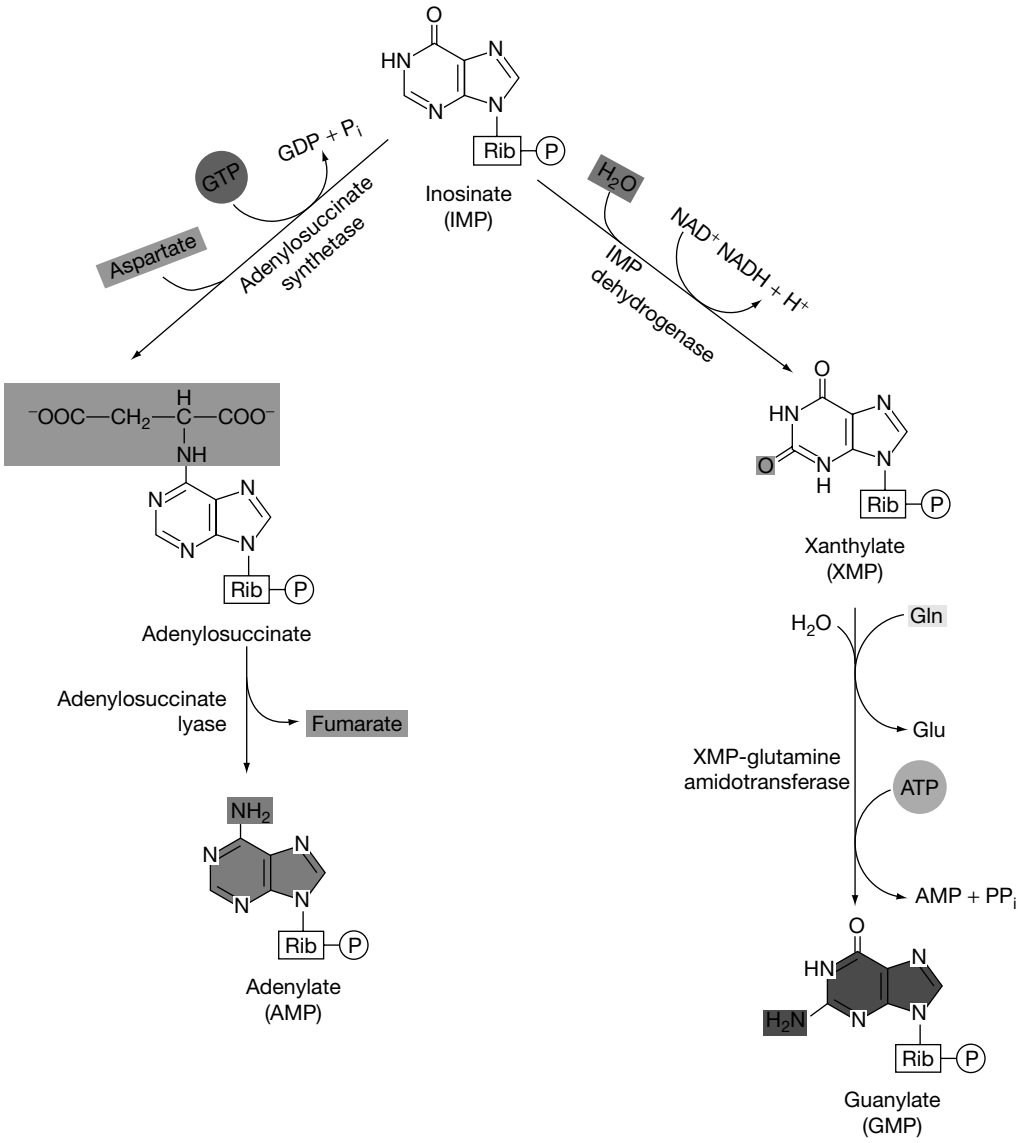


Figure 12.3 Biosynthesis of AMP and GMP from IMP

12.4 BIOSYNTHESIS OF PYRIMIDINE NUCLEOTIDES

The biosynthesis of pyrimidine nucleotides is reviewed by the scientist Reichard. The two major bases of pyrimidine are cytosine and thymine in DNA and cytosine and uracil in RNA. The pyrimidine, like the purines, is synthesized as the nucleotides. The discovery, in particular, aided in the study of pyrimidine synthesis was that orotic acid (6-carboxyuracil) could satisfy the pyrimidine of several bacteria. The biosynthesis of pyrimidine nucleotide is completed in many steps. The steps are as follows: (Figure 12.5)

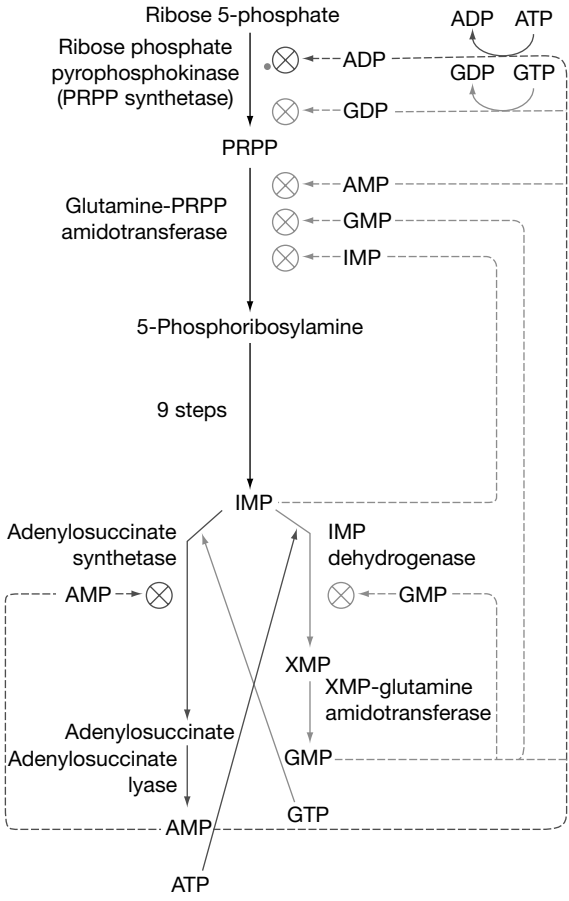


Figure 12.4 Regulatory mechanism by feedback inhibition in the biosynthesis of adenine and guanine nucleotides

Step 1: The first reaction in the synthesis of pyrimidine nucleotide is the reaction of carbamoyl phosphate with aspartic acid to form N-carbamoylaspartate. This reaction is catalysed by the enzyme aspartate transcarbamoylase (ATCase).

Step 2: The enzyme dihydroorotase act on N-carbamoylaspartate to give ring closure with the formation of dihydroorotate.

Step 3: Dihydroorotate is then oxidized to orotate by the enzyme dihydroorotate dehydrogenase which requires NAD^+.

Step 4: Orotate is converted into orotidylate by reacting with 5-phosphoribosyl 1-pyrophosphate. This reaction is catalysed by the enzyme orotate phosphoribosyl-transferase.

Step 5: Orotidylate is decarboxylated to uridylate (UMP) by the enzyme orotidylate decarboxylase.

Step 6: Uridylate (UMP) is converted into uridine 5′– triphosphate (UTP) through phosphorylation by ATP and kinases.

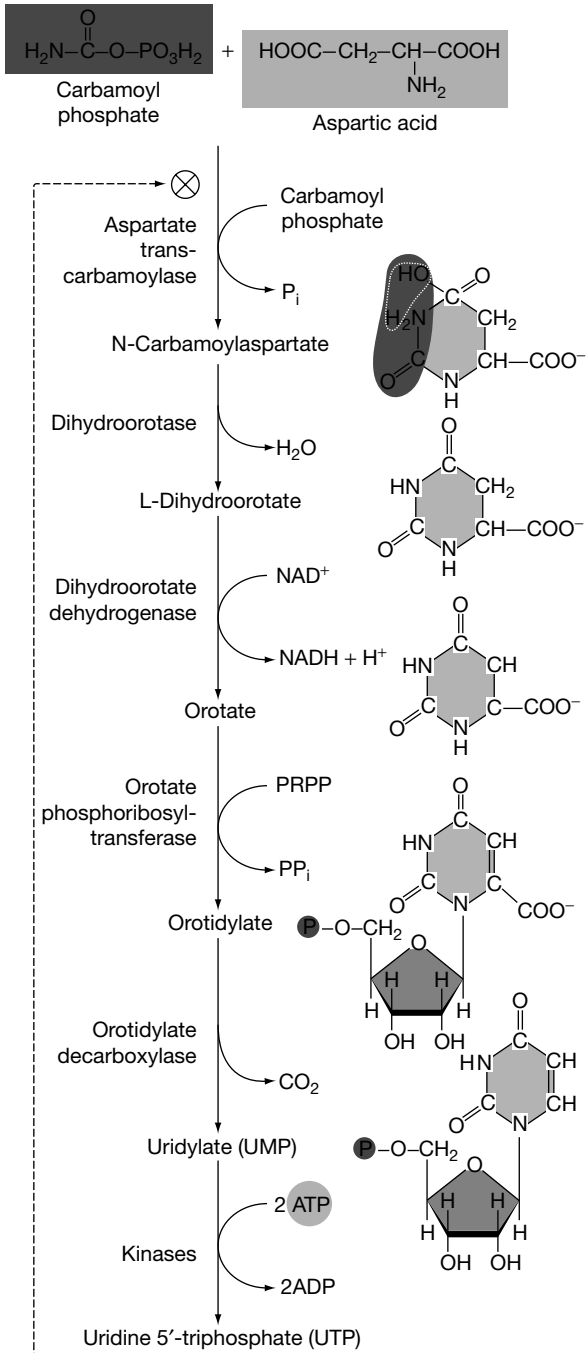


Figure 12.5 Continued

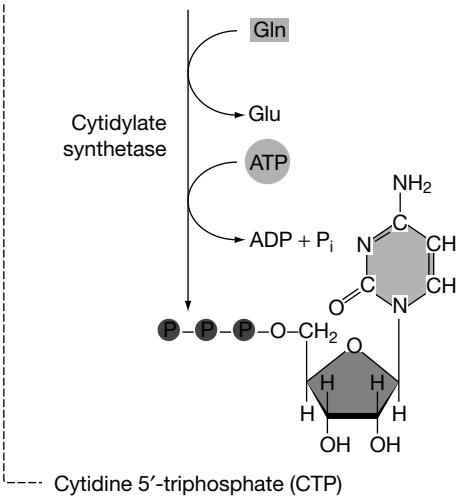


Figure 12.5 The biosynthesis of pyrimidine nucleotides. The biosynthesis of pyrimidine nucleotide starts with the reaction of carbamoyl phosphate with aspartic acid to form N-carbamoylaspartate. Carbamoyl phosphate shown in dark shade and aspartic acid in light shade. The N-carbamoylaspartate is shown in two shades indicating that dark shade is of carbamoyl phosphate and light shade is of aspartic acid

12.4.1 Synthesis of Cytidine 5′–Triphosphate (CTP)

The formation of cytidine 5′–triphosphate is through the amination of UTP with NH_3 to form CTP. Amino group is derived from glutamine. This reaction is catalysed by the enzyme cytidylate synthetase.

12.4.2 Synthesis of TTP Thymidine 5′–Triphosphate

The synthesis of thymine nucleotide involves both conversion of ribonucleotide to deoxyribonucleotide and methylation of pyrimidine ring and enzyme from *E. coli* converted deoxyuridylic acid to thymidylic acid in the presence of serine, tetrahydrofolic acid, ATP and Mg^{++}. It was found that N^{10}-hydroxymethyltetrahydrofolic acid could replace the serine and tetrahydrofolic acid requirement (Figure 12.6).

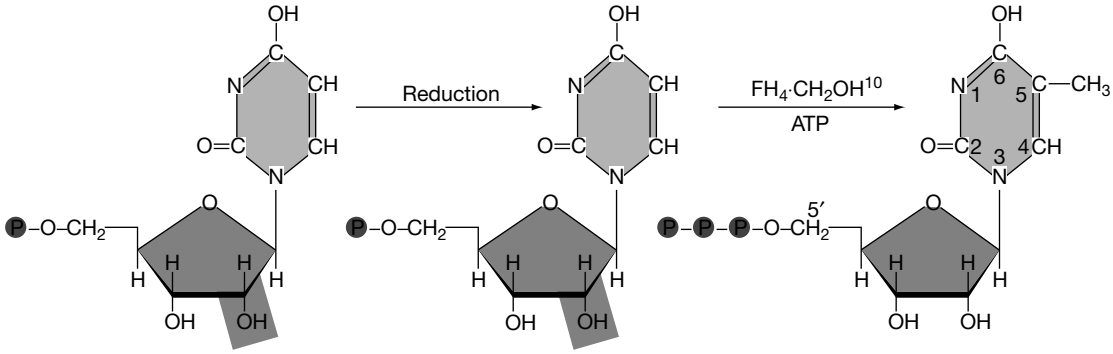


Figure 12.6 Synthesis of Thymidine Nucleotide

12.5 REGULATION OF PYRIMIDINE BIOSYNTHESIS BY FEEDBACK INHIBITION

The rate of pyrimidine nucleotide synthesis in bacteria is regulated through aspartate transcarbamoylase (ATCase). The first step of pyrimidine biosynthesis is the conversion of aspartate to carbamoylaspartate by the enzyme ATCase, this enzyme is inhibited by CTP, which is the end product of pyrimidine nucleotide biosynthesis (Figure 12.7). The bacterial ATCase enzyme consists of six catalytic subunits and six regulatory subunits. CTP is an allosteric inhibitor and binds to the allosteric subunits. The catalytic subunits bind to the substrate molecules. When CTP is bound to the regulatory subunits, it undergoes a conformation change. This change is transmitted to catalytic subunits, which also undergoes the conformational change and becomes inactive and do not allow the synthesis of pyrimidine nucleotide. When CTP is not bound to the regulatory subunits, the enzyme is active and leads to the synthesis of pyrimidine nucleotide. ATP is a positive modulator that prevents the changes induced by CTP (Figure 12.8).

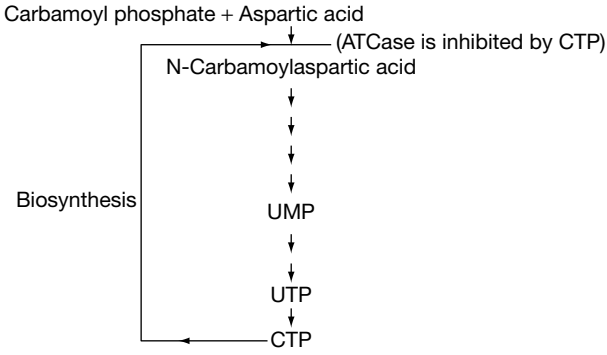


Figure 12.7 Regulation of pyrimidine biosynthesis

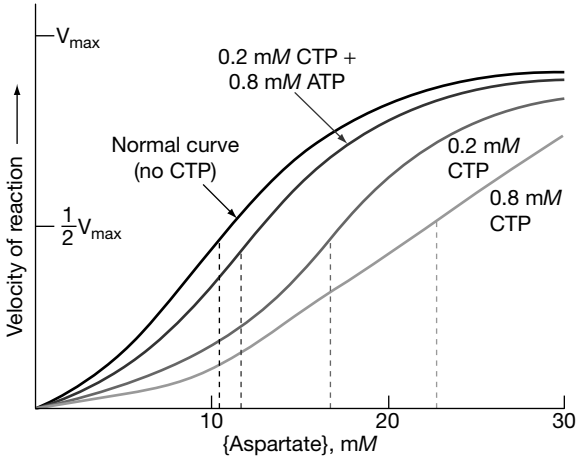


Figure 12.8 Aspartate transcarbomylase is allosterically regulated by CTP and ATP (as shown in the graph). The increased concentration of CTP increases the substrate concentration giving half- maximal velocity from about 10 mM to about 23 mM. This effect is reversed by ATP to almost normal

12.6 CATABOLISM OF PURINE NUCLEOTIDE

Degradation of adenylate to uric acid is through various steps. Adenylate yields adenosine, which is deaminated to inosine by the enzyme adenosine deaminase.

Inosine is hydrolysed to hypoxanthine, and hypoxanthine is oxidized to xanthine by the enzyme xanthine oxidase and then xanthine to uric acid. Uric acid is the excreted end product of purine catabolism in primates, birds and some other animals (Figure 12.9). In most mammals and vertebrates, uric acid is further degraded to allantoin by the action of enzyme urate oxidase (Figure 12.10).

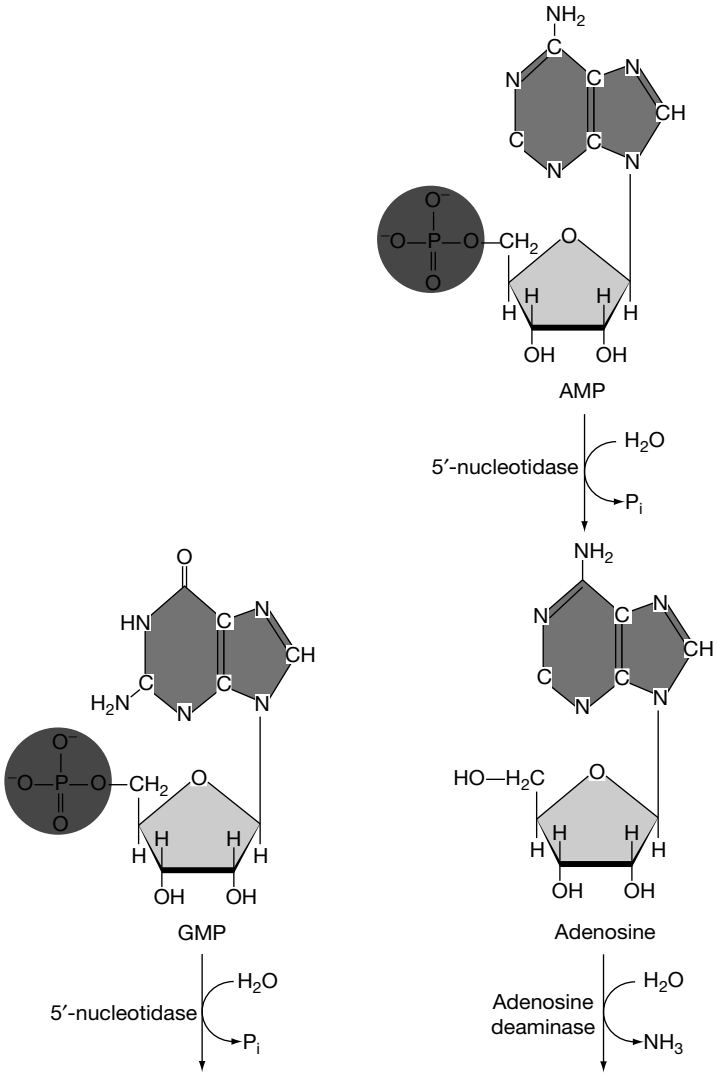


Figure 12.9 Continued

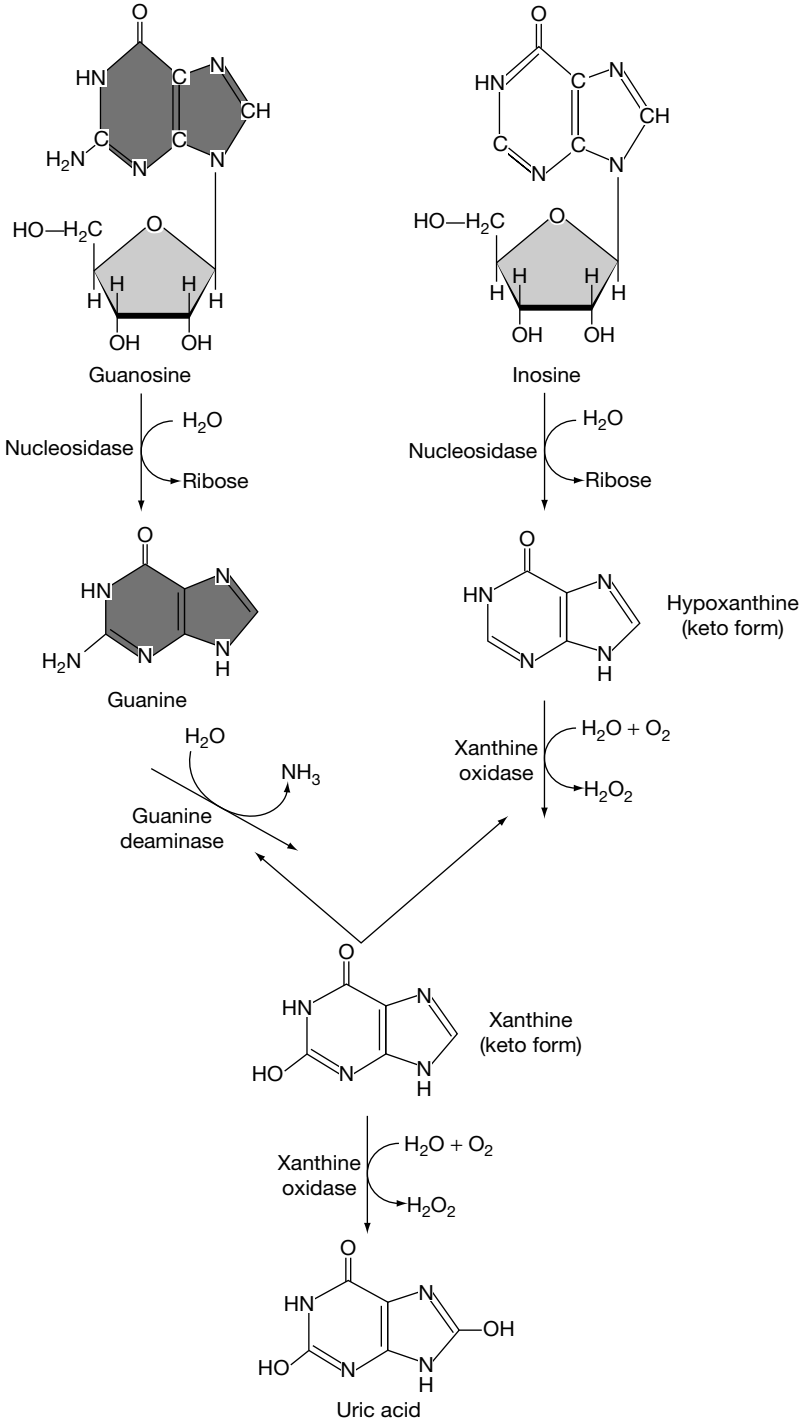


Figure 12.9 Catabolism of purine nucleotide

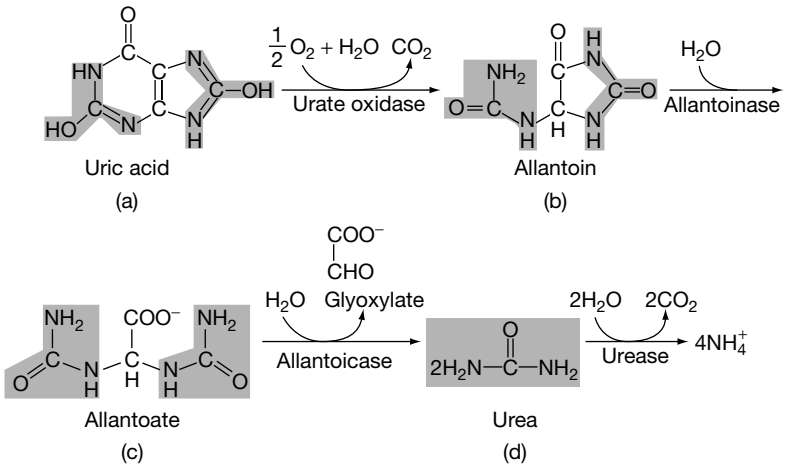


Figure 12.10 (a) Man and other primates, birds and reptiles convert purine to uric acids and excrete uric acid. (b) Mammals other than primates (except Dalmatian dog) carry the breakdown still farther excrete allantoin. (c) Bony fishes excrete allantoic acid (allantoate). (d) Most fishes, amphibians and cartilaginous fishes and marine invertebrates carry the breakdown still farther to urea and excrete ammonia

12.6.1 Catabolism of GMP

The end product of GMP is also uric acid. GMP is first hydrolysed to guanosine, which is then cleaved to form guanine by the action of the enzyme nucleosidase. Guanine undergoes hydrolytic removal of its amino group to yield xanthine which is converted to uric acid by the action of enzyme xanthine oxidase. Xanthine is formed from guanine (by hydrolytic removal of its amino group), and then, the enzyme xanthine oxidase converts xanthine to uric acid.

12.7 CATABOLISM OF PYRIMIDINE NUCLEOTIDE

Degradation of pyrimidine nucleotide leads to NH_4^+ production and ultimately leads to urea synthesis. Thymine nucleotide is first degraded to dihydrothymine by the enzyme dihydrouracil dehydrogenase, then to β-ureidoisobutyrate by the enzyme dihydropyrimidanase, then β-ureidoisobutyrate is degradated to β-aminoisobutyrate by the enzyme β-ureidopropionase, then β-aminoisobutyrate is further degraded to methylmalonyl-semialdehyde by the enzyme aminotransferase. The end product of thymine catabolism is methylmalonyl-semialdehyde. Methylmalonyl–semialdehyde is an intermediate product of valine catabolism. It is further degraded to propionyl-CoA and methylmalonyl-CoA to succinyl-CoA (Figure 12.11).

12.8 SALVAGE PATHWAY

Salvage pathway recycle already used bases by re-attaching them to a ribose. Purine and pyrimidine bases are recycled by salvage pathways. Free bases of both purine and pyrimidine nucleotide are released in cells during the degradation of nucleotide. Free purines are salvaged in large part.

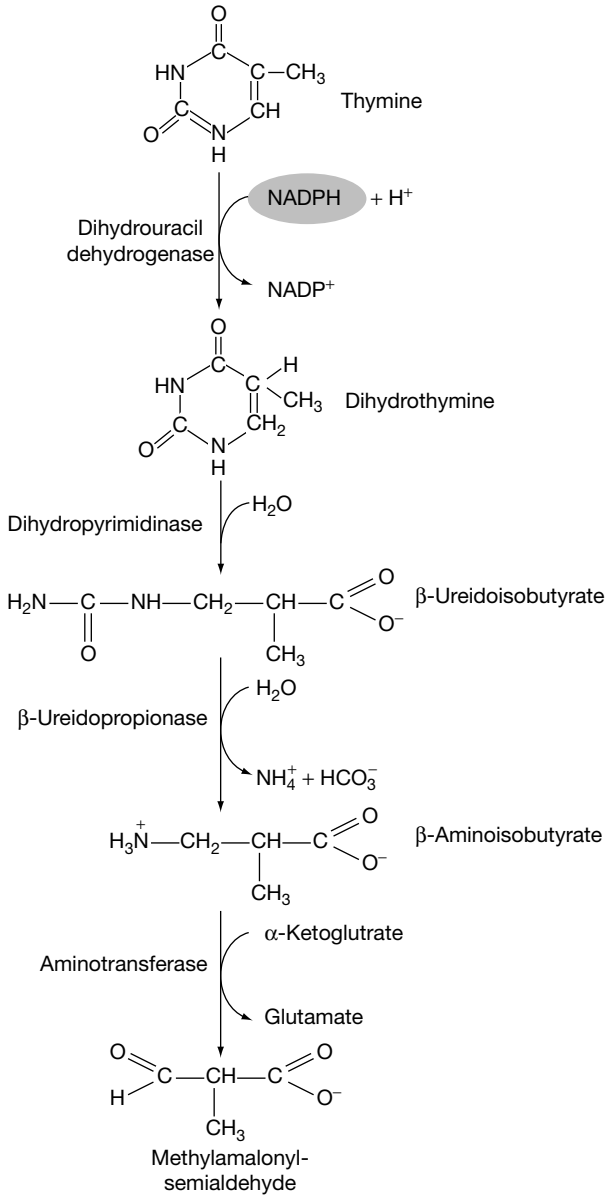


Figure 12.11 Degradation of pyrimidine nucleotide leads to NH_4^+ production and ultimately leads to urea synthesis. Thymine nucleotide is first degraded to dihydrothymine and the end product of thymine catabolism is methylmalonyl-semialdehyde

Salvage pathway for adenine is very simple as compared to de novo pathway of purine biosynthesis. Free adenine reacts with PRPP to form AMP and pyrophosphate by the enzyme adenosine phosphoribosyltransferase. Free guanine and hypoxanthine, which is the deamination product

of adenine, are salvaged in the same way as that of the adenine by the enzyme hypoxanthine–guanine phosphoribosyltransferase.

The salvage pathway for pyrimidine bases is similar as that of the purine bases.

12.9 BIOSYNTHESIS OF DEOXYRIBONUCLEOTIDES

In view of the fact that the deoxyribonucleotides differ from the ribonucleotides only in containing 2-deoxyribose instead of ribose as the pentose unit, it was understood that they would be formed by the pathway described above for the ribonucleotides, with the phosphorylated ribose precursor replaced by an analogous 2-deoxyribose derivative. Much early research, therefore, centred on the mechanism of biosynthesis of 2-deoxy-D-ribose. Although an enzymatic pathway for the formation of free 2-deoxyribose-5-phosphate from simpler precursors does in fact exist in some cells, it is known that the deoxyribonucleotides are not normally synthesized starting from deoxyribose as a building block but are formed by direct reduction of the 2-carbon atom of the corresponding ribonucleotides. An important experiment was carried out by I. A. Rose and B. S. Schweigert pointing to this conclusion with cytidine labelled with ^{14}C in both pyrimidine ring and the ribose. When presented to animal or bacterial cells, this compound was incorporated into the cytidylic acid residues of RNA and into the deoxycytidylic acid residues of DNA. The ratios of the isotopes in the pyrimidine and ribose (or deoxyribose) portions were identical in the cytidylic and deoxycytidylic acid residues. This finding indicated that the ribose moiety was not detached from the cytosine ring of the labelled cytidine prior to the formation of the labelled deoxycytidylic residues of DNA. Ribose had been detached first, and it would have become diluted by nonisotopic ribose or deoxyribose prior to formation of the deoxyribonucleotide, with a corresponding change in the ratio of ^{14}C in the pyrimidine and pentose residues. Direct isotopic tests on cell-free bacterial extracts subsequently confirmed that labelled ribonucleotides are in fact directly converted into the corresponding deoxyribonucleotides.

There appear to be two pathways for the direct reduction of ribonucleotides, depending on the species. P. Reichard and his colleagues have shown that in *E. coli* all four ribonucleoside diphosphates (ADP, GDP, UDP and CDP) are directly reduced to the corresponding deoxy analogues dADP, dGDP, dUDP and dCDP, respectively, by a multienzyme system. In the overall process, the reduction of the ribose moiety to 2-deoxyribose requires a pair of hydrogen atoms, which are ultimately donated by NADHP and H^+. However, the immediate electron donor is not NADHP, but the reduced form of a small heat-stable protein (108 amino acid residues) called thioeredoxin, which has two free-SH groups contributed by two cysteine residues. Thioredixin may be reversibly oxidized and reduced. It's oxidized, or disulphide form, which contains a residue of cystine, is reduced to the dithiol form by NADPH + H^+. However, the immediate electron donor is not NADPH but the reduced form of a small heat-stable protein (108 amino acid residues) called thioredoxin, which has two free-SH groups contributed by two cysteine residues. Thioredoxin may be reversibly oxidized and reduced.

It's oxidized, or disulphide, form, which contains a residue of cystine, is reduced to the dithiol form by NADPH + H^+ in a reaction catalysed by thioredoxin reductase:

$$\text{Thioredoxin (–S–S–)} + \text{NADPH} + H^+ \xrightleftharpoons{\text{Thioredoxin reductase}} \text{Thioredoxin (–SH)}_2 + \text{NADP}^+$$

Thioredoxin reductase is a flavoprotein of molecular weight 68,000; it contains two molecules of bound FAD. The reducing equivalents of the reduced thioredoxin formed are then

transferred to the ribonucleoside 5′-diphosphate acceptor (NDP) by ribonucleoside diphosphate reductase:

$$\text{Thioredoxin}(-\text{SH})_2 + \text{NDP} \xrightarrow{Mg^{2+}} \text{Thioredoxin}\ (-\text{s}-\text{s}-) + \text{dNDP} + \text{H}_2\text{O}$$

This reaction is rather complex and requires two proteins or subunits, as well as Mg^{2+}. The complete system can reduce any of the four ribonucleoside 5′-diphosphates to yield the corresponding 2-deoxy-D-ribose analogues.

In various other microorganisms, including some species of *Lactobacillus*, *Rhizobium*, *Euglena* and *Clostridium*; however, there is a different pathway, which in most cases requires the ribonucleoside 5′-triphosphates rather than the diphosphates. Moreover, it requires coenzyme B_{12} and can use either thioredoxin or dihydrolipoic acid as a reducing agent. Since coenzyme B_{12} is capable of acting as an intermediate carrier for the exchange of a hydrogen atom on one carbon atom for a substituent group on a vicinal carbon atom, the mechanism of reduction of the ribose moiety to 2-deoxyribose in *Lactobacillus* may possibly involve not only carbon atom 2 but also an adjacent carbon atom.

12.10 FORMATION OF DEOXYTHYMIDYLIC ACID

DNA contains thymine (5-methyluracil) instead of the uracil present in RNA. Deoxythymidylic acid (dTMP) is formed from deoxyuridylic acid (dUMP) by thymidylate synthetase, which catalyses the methylation of the uracil moiety in a reaction requiring the participation of a folic acid coenzyme, N^5, N^{10}-methylenetetrahydrofolate (Figure 12.12a) as a methyl donor. The formation

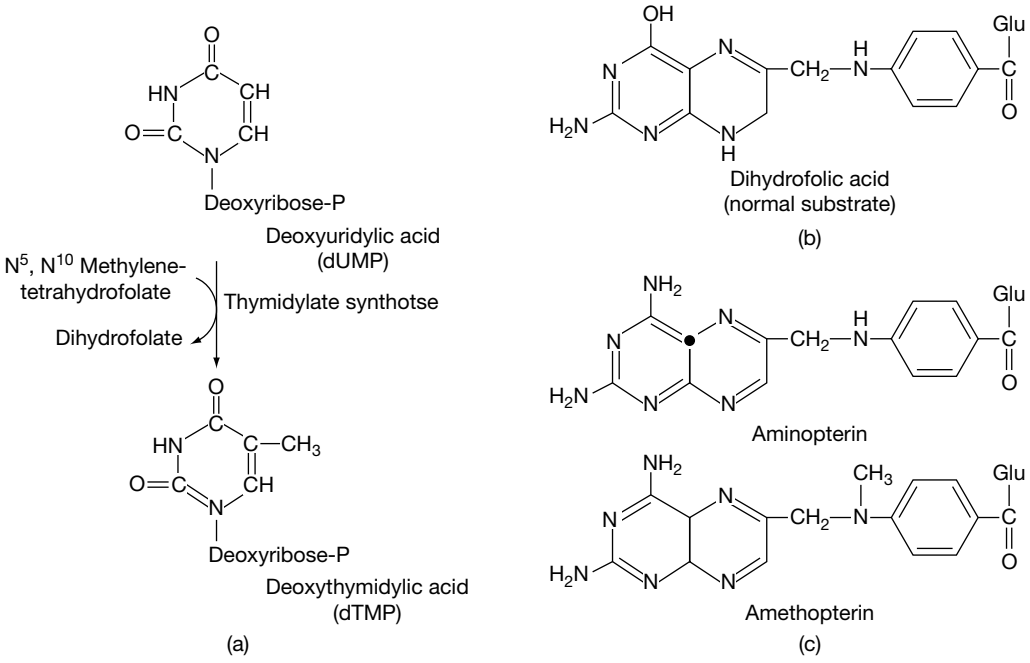


Figure 12.12 (a) dUMP is methylated to dTMP by thimidylate synthetase. (b) dihydrofolate (normal substrate) (c) Aminopterin and amethopterin have structural resemblance to dihydrofolate and they inhibit competitively the conversion of dihydrofolate into tetrahydrofolate by dihydrofolate reductase

of thymidylate and thus of DNA is greatly retarded by the drugs aminopterin and amethopterin (Figure 12.12c). These agents, which are called anti-folic drugs, slow the growth of some types of cancer, particularly leukaemia. Because of their structural resemblance to dihydrofolate, (Figure 12.12b) they inhibit competitively the conversion of dihydrofolate into tetrahydrofolate by dihydrofolate reductase.

$$NADHP + H^{+} + \text{Dihydrofolate} \rightarrow NADP^{+} + \text{Tetrahydrofolate}$$

The formation of thymidylate is particularly sensitive to depressed levels of tetrahydrofolate. In this way, DNA synthesis in the rapidly dividing malignant leukocytes is inhibited.

12.11 REGULATION OF DEOXYRIBONUCLEOTIDE BIOSYNTHESIS

In *E. coli*, one set of allosteric mechanisms for the regulation of deoxyribonucleotide biosynthesis involves the reaction between reduced thioredoxin and the NDPs and catalysed by the ribonucleoside-diphosphate reductase system (Figure 12.13). The reduction of CDP to dCDP and of UDP to dUDP by this enzyme is strongly accelerated by ATP, whereas the reduction of ADP to dADP and of GDP to dGDP is stimulated by dGTP and dTTP. On the other hand, dATP acts as a feedback inhibitor for the reduction of all the ribonucleoside 5′- diphosphates (Figure 12.13).

Since 5-phosphoribose-1-pyrophosphate is the ultimate source of the pentose units of both the ribonucleotides and deoxyribonucleotides, regulation of ribosephophate pyrophosphokinase by the allosteric inhibitors ADP and GDP is also important in the biosynthesis of deoxyribonucleotides.

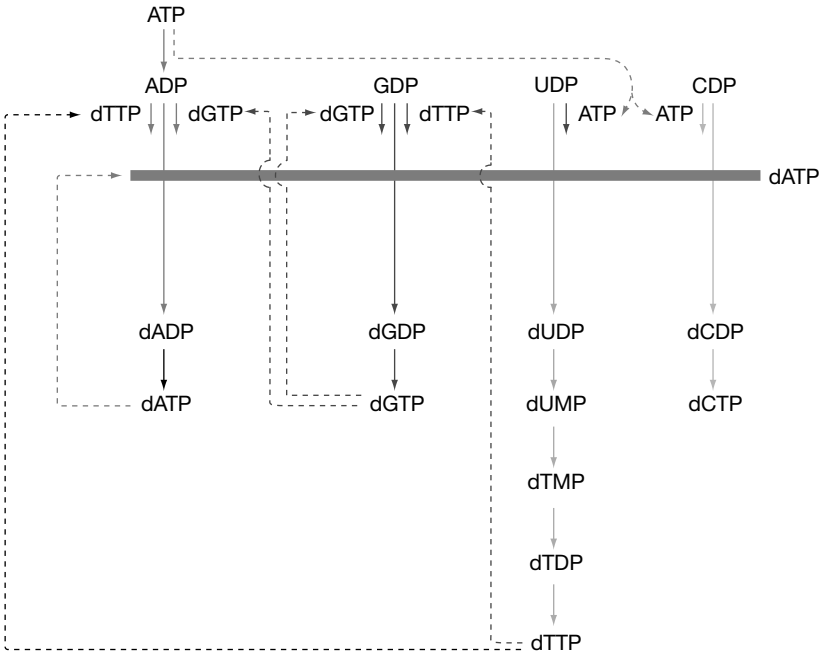


Figure 12.13 Deoxyribonucleotide biosynthesis is regulated by allosteric mechanisms. ATP, dGTP and dTTP are important stimulatory modulators and dATP is a general inhibitor

12.12 BIOSYNTHESIS OF NUCLEOTIDE COENZYMES

Flavin adenine dinucleotide, the pyridine nucleotides and coenzyme A are derivatives of adenylic acid. Flavin mononucleotide (FMN), which is not a true nucleotide and is more accurately called riboflavin phosphate, is synthesized by the enzyme riboflavin kinase from free riboflavin (vitamin B_2) and ATP in the reaction.

$$\text{Riboflavin} + \text{ATP} \xrightarrow{Mg^{++}} \text{FMN} + \text{ADP}$$

FAD is then formed from FMN by the action of FMN adenylyltransferase:

$$\text{FMN} + \text{ATP} \xrightarrow{Mg^{++}} \text{FAD} + \text{PP}$$

NAD is synthesized from free nicotinic acid in bacteria in the following series of reactions:

$$\text{Nicotinic acid} + \text{5-phospho-}\alpha\text{-D-ribose 1-pyrophosphate} \rightarrow \text{Nicotinic acid mononucleotide} + pp_i$$

$$\text{Nicotinic acid mononucleotide} + \text{ATP} \rightleftharpoons \text{desamido-NAD}^+ + pp_i$$

$$\text{Desamido-NAD}^+ + \text{glutamine} + \text{ATP} + H_2O \rightleftharpoons \text{NAD}^+ + \text{glutamic acid} + \text{ADP} + P_i$$

In bacteria, the amide group of NAD is inserted after construction of the nicotinic acid analogue of NAD, desamido-NAD. In mammals, however, free nicotinamide can be directly utilized via nicotinamide mononucleotide instead of free nicotinic acid and nicotinic acid mononucleotide. $NADP^+$ is formed from NAD^+ by the following reaction.

$$\text{NAD}^+ + \text{ATP} \rightleftharpoons \text{NADP}^+ + \text{ADP}$$

Coenzyme A (see the structure of CoA in Chapter 15) is assembled, starting from the free vitamin pantothenic acid, in the reaction sequence shown in Figure 12.14. In the last reaction, a phosphate group is introduced into the 3′-hydroxyl group of the adenosine portion of dephospho-CoA to yield the complete coenzyme A molecule.

$$\text{Pantothenic acid} + \text{ATP} \xrightarrow{\text{Pantothinate kinase}} \text{4′-phosphopantothenic acid}$$

$$\text{4′-phosphopantothenic acid} + \text{cysteine} \xrightarrow{\text{Phosphopantothenyl cystein synthetase}} \text{4′-phosphopantothenyl cysteine}$$

$$\text{4′-phosphopantothenyl cysteine} \xrightarrow{\text{Phosphopantothenyl cystein decarboxylase}} \text{4′-phosphopantotheine}$$

$$\text{4′-phosphopantotheine} + \text{ATP} \xrightarrow{\text{Pantetheinephosphate adenylytransferase}} \text{Dephospho coenzyme A} + \text{ppi}$$

$$\text{Dephospho coenzyme A} + \text{ATP} \xrightarrow{\text{Dephospho CoA kinase}} \text{Coenzyme A} + \text{ADP}$$

Figure 12.14 Biosynthesis of CoA

12.13 METABOLIC DISORDER OF NUCLEIC ACID

High uric acid overproduction results from the inability to recycle either hypoxanthine or guanine, which interrupts the inosinate cycle producing a HPRT deficiency, and lack of feedback control of synthesis results in rapid catabolism of hypoxanthine or guanine bases to uric acid.

12.13.1 Lesch–Nyhan Syndrome

Purines can be synthesized in two ways.

- De novo—'from scratch' and
- Salvage pathway—by recycling the nucleotides.

In patients with Lesch–Nyhan syndrome, HPRT, (hypoxanthine–guanine phosphoribosyltransferase) is absent which is the key enzyme in salvaging and recycling old purines from DNA and RNA degradation. The de novo pathway is controlled by the feedback inhibition provided by the nucleotides made by salvage pathway and lead to energy conservation. The salvage pathways are impaired due to the absence of the enzyme HPRT and lead to overproduction of de novo synthesis due to the absence of negative inhibitors. Xanthine oxidase breaks the excess purines to uric acid and leads to hyperuricemia. The children suffer from mental retardation and have poor coordination **in Lesch–Nyhan syndrome which is a genetic disorder.**

12.13.2 Symptoms of Hyperuricemia

- Formation of urate crystals
- Formation of kidney stones

Impaired kidney function leads to renal failure and gout-like arthritis.

12.13.3 Xanthinuria and Xanthine Lithiasis

Xanthinuria and xanthine lithiasis occurs due to excess excretion of hypoxanthine and xanthine, and this condition occurs due to the deficiency of the enzyme xanthine oxidase which converts hypoxanthine and xanthine to uric acid. This is a genetic disorder and leads to liver damage.

12.13.4 Gout

Various genetic defects in PRPP synthetase present clinically as gout. It results in overproduction and overexcretion of purine catabolites. The levels of urate increases in the serum, which crosses the gout solubility limit, leads to crystallization of sodium urate in soft tissues and joints and causes gouty arthritis.

12.13.5 Characteristics of Gout

Hallux (big toe) is the painful and swollen joint of arthritis. This disease is also known as the 'Disease of Kings' caused by rich foods and alcohol. In this disease, body does not process uric acid properly, and so blood has high levels of uric acid. Uric acid precipitates out in the joints, causing swelling. Certain foods are high in purines, which increase uric acid production.

12.13.6 Treatment of Gout

Gout is effectively treated by diet control, that is, without taking purine-rich food and alcohol. It is also treated by drugs such as allopurine, which is a xanthine oxidase inhibitor. Allopurine is a structural analogue of hypoxanthine and xanthine and has a higher affinity for xanthine oxidase. Allopurinol inhibits xanthine oxidase, the enzyme that catalyses the conversion of purines to uric acid. Xanthine and hypoxanthine are the excreted products of purine metabolism when the enzyme xanthine oxidase is inhibited as compared to uric acid both xanthine and hypoxanthine are more water-soluble and do not form crystalline deposits.

EXERCISES

1. How the biosynthesis of deoxyribonucleotide is regulated?
2. Differentiate between FMN and FAD with the help of their structure.
3. Explain how the formation of deoxythymidylic acid takes place.
4. Write in brief: biosynthesis of deoxyribonucleotides.
5. Write the biosynthesis of purine nucleotide.
6. How the biosynthesis of purine nucleotide is regulated by feedback inhibition?
7. Write the breakdown of purine nucleotide.
8. Write in detail
   (a) the degradation of pyrimidine nucleotide and
   (b) biosynthesis of CoA.
9. What is the difference between de novo pathway and salvage pathway of purine nucleotide?
10. How the pyrimidine biosynthesis is regulated by feedback inhibition?
11. Write short notes on
    (a) Lesch–Nyhan syndrome
    (b) Xanthinuria and Xanthine lithiasis
    (c) Gout
12. Write the biosynthesis of nucleotide coenzymes.
13. Write the biosynthesis of AMP and GMP from IMP.
14. How aminopterin and amethopterin inhibit competitively the conversion of dihydrofolate into tetrahydrofolate?

MULTIPLE CHOICE QUESTIONS

1. Lesch–Nyhan syndrome is due to the deficiency of the enzyme
   (a) Hypoxanthine–guanine phosphoribosyltransferase
   (b) Xanthine oxidase
   (c) PRPP synthetase
   (d) None of these

   Ans. a

2. Xanthinuria and xanthine lithiasis occurs due to the deficiency of the enzyme
   (a) Hypoxanthine–guanine phosphoribosyltransferase
   (b) Xanthine oxidase
   (c) PRPP synthetase
   (d) None of these

   Ans. b

3. Gout is effectively treated by without eating purine-rich food and alcohol. Drug treatment is also given, like drug allopurine is given which is
   (a) A xanthine oxidase inhibitor
   (b) A hypoxanthine-guanine phosphoribosyltransferase inhibitor
   (c) PRPP synthetase inhibitor
   (d) None of these

   Ans. a

4. Aminopterin and amethopterin have structural resemblance to
   (a) Dihydrofolate
   (b) Tetrahydrofolate
   (c) Folate
   (d) None of these

   Ans. a

5. Thioredoxin reductase is a flavoprotein of molecular weight 68,000; it contains two molecules of bound
   (a) FAD (b) FMN
   (c) $FADH_2$ (d) None of these

   Ans. a
6. Thymine nucleotide is first degraded to dihydrothymine by the enzyme
   (a) Dihydrouracil dehydrogenase
   (b) Dihydropyrimidanase
   (c) β-ureidopropionase
   (d) None of these

   Ans. a
7. Dihydrothymine then degraded to β-ureidoisobutyrate by the enzyme
   (a) Dihydrouracil dehydrogenase
   (b) Dihydropyrimidanase
   (c) β-ureidopropionase
   (d) None of these

   Ans. b
8. β-ureidoisobutyrate is degradated to β-aminoisobutyrate by the enzyme
   (a) Dihydrouracil dehydrogenase
   (b) Dihydropyrimidanase
   (c) β-ureidopropionase
   (d) None of these

   Ans. c
9. β-aminoisobutyrate by the enzyme β-ureidopropionase, β-aminoisobutyrate is further degraded to methylmalonyl-semialdehyde by the enzyme
   (a) Dihydrouracil dehydrogenase
   (b) Dihydropyrimidanase
   (c) β-ureidopropionase
   (d) Aminotransferase

   Ans. d
10. De novo purine nucleotide synthesis is regulated by feedback inhibition by various steps. The first mechanism is exerted on the first reaction that is transfer of an amino group to PRPP to form 5-phosphoribosylamine. This reaction is catalysed by the allosteric enzyme
    (a) Glutamine–PRPP amidotransferase
    (b) PRPP synthetase
    (c) Aminotransferase
    (d) None of these

    Ans. a
11. Man and other primates, birds, reptiles convert purine to
    (a) Uric acids (b) Allantoin
    (c) Urea (d) None of these

    Ans. a
12. Mammals other than primates (except Dalmatian dog) carry the breakdown still farther excrete
    (a) Uric acids (b) Allantoin
    (c) Urea (d) None of these

    Ans. b
13. Bony fishes excrete
    (a) Uric acids
    (b) Allantoin
    (c) Allantoic acid (allantoate)
    (d) Urea

    Ans. c
14. Most fishes, amphibians and cartilaginous fishes and marine invertebrates carry the breakdown still farther to urea and excrete
    (a) Uric acid
    (b) Allantoin
    (c) Allantoic acid (allantoate)
    (d) Ammonia

    Ans. d
15. Flavin mononucleotide (FMN), which is not a true nucleotide and is more accurately called riboflavin phosphate, is synthesized by the enzyme
    (a) Riboflavin kinase
    (b) FMN adenylyltransferase
    (c) PRPP synthetase
    (d) Aminotransferase

    Ans. a

13 Enzymes and Coenzymes

CHAPTER OBJECTIVES

13.1 Introduction and Classification of Enzymes
13.2 The Nature of Enzymes
13.3 Kinetics of Enzyme-Catalysed Reactions
13.4 Inhibition of Enzymes
13.5 Kinetic Aspects of Allosteric Enzymes
13.6 Cofactors
13.7 Isozymes
13.8 Enzyme Mechanisms
13.9 Activation of Enzymes

13.1 INTRODUCTION AND CLASSIFICATION OF ENZYMES

In analytical biochemistry, enzymes have an important place, and many investigations require the detection and quantitation of enzymes. Studies of the enzyme content of blood plasma are particularly useful in clinical biochemistry both in the monitoring of normal metabolic processes and in the detection of abnormal levels of enzyme production or release. Enzyme assays also provide convenient methods for assessing the quality of foodstuffs and checking the efficiency of sterilization and pasteurization processes.

Enzymes are valuable analytical tools and offer sensitive and specific methods of quantitation for many substances. The increasing availability of highly purified enzyme preparations, in both solution and immobilized forms, permits the development of a wide range of methods.

Because of the difficulties in measuring the amount of an enzyme in the conventional units of mass or molar concentration, the accepted unit of enzyme activity is defined in terms of reaction rate. The International Unit (U) is defined as the amount of enzyme which will result in the conversion of one mole of substrate to product in one minute under specified conditions, The SI unit of catalytic activity, which is becoming more acceptable, is the **katal** and is defined as the amount of enzyme which will result in the conversion of one mol of substrate to product in one second. A convenient subunit is the nanokatal, which is equal to 0.06 International Units.

The specific activity of an enzyme preparation is expressed as the catalytic activity per milligram of protein (units mg^{-1} protein) and is a convenient way of comparing the purity of enzyme preparations.

Enzymes are classified under six main headings depending on the nature of the chemical reaction involved, and each class being sub-divided on the basis of the substrate and the particular coenzyme or grouping involved in the reaction (Table 13.1).

Table 13.1 Classification and the System of Nomenclature of Enzyme

EC Numbering of Enzymes			
Group	**Reaction catalyzed**	**Typical reaction**	**Enzyme example (s) with trivial name**
EC 1 *Oxidoreductases*	To catalyze oxidation/ reduction reactions; transfer of H and O atoms or electrons from one substance to another	$AH + B \rightarrow A + BH$ (reduced) $A + O \rightarrow AO$ (oxidized)	Dehydrogenase, oxidase
EC 2 *Transferases*	Transfer of a functional group from one substance to another. The group may be methyl-, acyl-, amino- or phosphate group	$AB + C \rightarrow A + BC$	Transaminase, kinase
EC 3 *Hydrolases*	Formation of two products from a substrate by hydrolysis	$AB + H_2O \rightarrow AOH + BH$	Lipase, amylase, peptidase
EC 4 *Lyases*	Non-hydrolytic addition or removal of groups from substrates. C – C, C – N, C – O or C – S bonds may be cleaved	$RCOCOOH \rightarrow RCOH + CO_2$ or $[x-A-B-Y] \rightarrow [A=B+X-Y]$	Decarboxylase
EC 5 *Isomerases*	Intramolecule rearrangement, i.e. isomerization changes within a single molecule	$AB \rightarrow BA$	Isomerase, mutase
EC 6 *Ligases*	Join together two molecules by synthesis of new C – O, C – S, C – N or C – C bonds with simultaneous breakdown of ATP	$X + Y + ATP \rightarrow XY + ADP + Pi$	Synthetase

13.2 THE NATURE OF ENZYMES

Enzymes are proteins, although many are conjugated proteins and are associated with non-protein groups. Their catalytic activity is dependent on the maintenance of their native structure, and any slight variations may result in significant changes in this activity.

A common feature of enzymes is the presence of a cleft or depression in the structure which is lined with mainly hydrophobic amino acid residues and into which the substrate fits. Certain

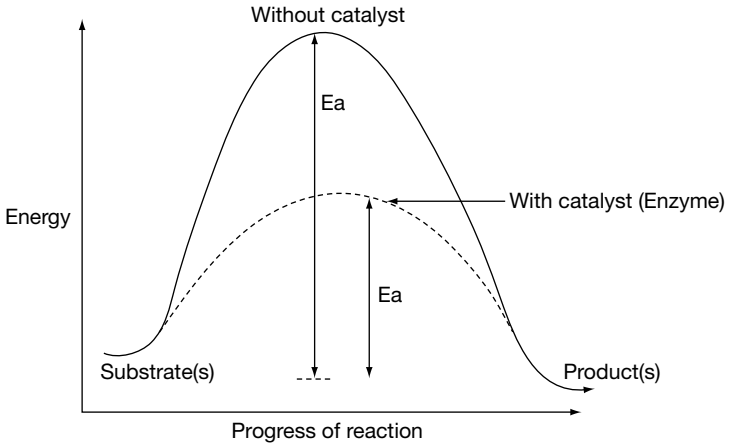


Figure 13.1 Energy diagram of enzyme-catalysed reaction. E_a is the free energy of activation

amino acid residues, which are concerned with either the orientation of the substrate, and hence the specificity of the enzyme, or are involved in the catalysis of the reaction, are located in this cleft. Those which are associated with the latter role form the active site of the enzyme and are often located towards the base of this cleft. In most cases, these amino acid residues are ionic or reactive and include histidine, lysine, cysteine and serine as well as glutamic and aspartic acids. In addition, the binding of ions from the solution, particularly cations, may also aid either the location of the substrate or the catalysis of the reaction (Figure 13.1).

13.2.1 Environmental Effects

Effect of Temperature

An increase in temperature increases the rate of denaturation of the enzyme with the loss of secondary and tertiary structure (Figure 13.2). Denaturation occurs more readily with pure

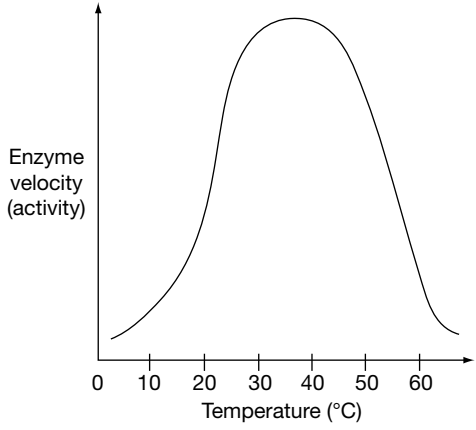


Figure 13.2 The rate of enzyme catalysis is altered by temperature. The velocity of enzymatic reactions is maximum at an optimum temperature after which the enzymes are denatured with loss of catalysis

solutions of enzymes than with impure and both are preserved best at low temperature with other protecting molecules present, storage at –20°C in bovine serum albumin or glycerol solutions being frequently used. A 50 per cent (v/v) solution of glycerol is particularly useful because it is fluid at –20°C and, being nonprotein, can subsequently be removed very easily.

It is sometimes suggested that enzymes show an optimum temperature but the most suitable temperature for a particular reaction is a compromise between maximal activity for a short period of time and a falling activity due to denaturation for a longer period of time. Many assays are performed at 37°C either because it is mistakenly assumed that the human body temperature is the optimum temperature for an enzyme or, more realistically, because above this temperature the rate of inactivation of the enzyme becomes far more significant. The International Union of Biochemistry originally recommended that 25°C should be regarded as a standard temperature but subsequently raised it to 30°C because of difficulties in keeping to the lower temperature in hot climates. It is possible to convert enzyme activities quoted for one temperature to equivalent activities at another temperature, by using predetermined conversion factors, but doubts have been raised about the validity of such methods. Currently, no standard temperature is specified, but it is recommended that the assay temperature should be quoted in all references to enzyme activities.

Effect of pH: The change in pH alters the enzymes' activity, and they work with a definite optimum pH. The effects of pH are due to the changes in the ionic state of the amino acid residues of the enzyme and the substrate molecules, causing varying efficiency in the binding of a substrate. Specific residues are implicated in the catalytic process and any alteration in their charge will significantly affect the rate of reaction. Extremes of acidity or alkalinity cause distortion of protein structure and lead to permanent denaturation, whereas narrow pH range does not lead to permanent denaturation and will be reversible (Figure 13.3). In the design of assays, it is important to appreciate that an enzyme may show different pH optima for different substrates, and it may not be true for every assay method.

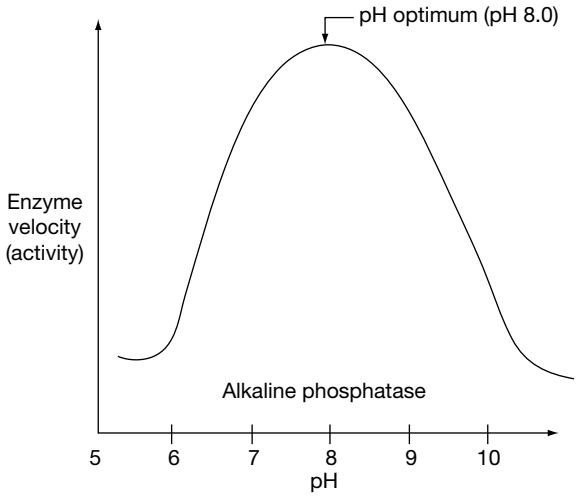


Figure 13.3 The rate of enzyme catalysis is dependent on the pH

13.3 KINETICS OF ENZYME-CATALYSED REACTIONS

The law of mass action states that the rate of a chemical reaction is proportional to the product of the concentrations of the reactants. This means that the rate of a reaction, which has a single component, will increase in direct relation to the increasing concentration but for a two-component reaction, it will increase in proportion to the square of the concentration. These relationships may be expressed by the following equations:

$$\text{Rate} = k_1 \text{ (concentration)(single reactant)}$$

$$\text{Rate} = k_2 \text{ (concentration)} \times \text{(concentration)(two reactants)}$$

where k_1 and k_2 are the reaction are velocity constants or rate constants for the reactions. The reactions are said to show first- and second-order kinetics, respectively. Occasionally, situations arise where increases in the concentration of a reactant do not result in an increase in the reaction rate. Such reactions are said to show zero-order kinetics.

It might be expected that enzyme-catalysed reactions would show extremely complex kinetics because two or more substrates are frequently involved. However, experimental studies of the effect of substrate concentration on the rate of reaction show consistent results (Figure 13.4). At high concentrations of substrate, the rate is almost constant (zero-order kinetics), but at low concentrations, it shows a direct relationship to concentration (first-order kinetics). The most satisfactory explanation for these results suggests that the rate of an enzyme-catalysed reaction is dependent upon the formation of the products by the breakdown of an enzyme–substrate complex as given in equation (13.1).

$$E + S \rightleftharpoons ES \rightleftharpoons E + P \tag{13.1}$$

The formation of the products involves only one component, the ES complex, and hence shows first-order kinetics. However, a high concentration of substrate will saturate the enzyme present giving a maximum concentration of the complex and hence a maximum reaction rate. Further increase in the substrate concentration will not increase the concentration of the complex, and the rate will remain constant (zero-order kinetics).

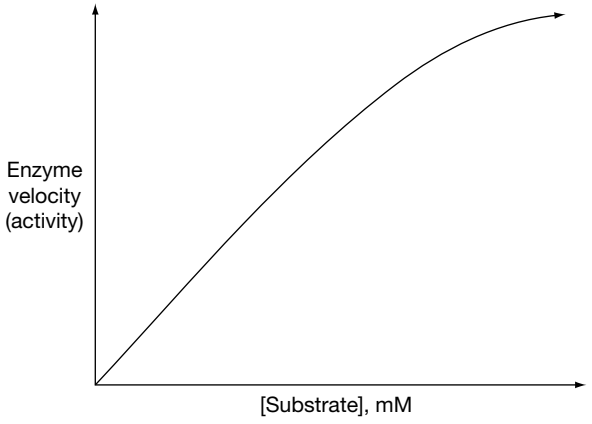


Figure 13.4 The effect of substrate concentration on the kinetics of enzyme catalysis

13.3.1 Michaelis–Menten Equation

The concept of an enzyme–substrate complex was fundamental to the appreciation of enzyme reactions and was initially developed in 1913 by Michaelis and Menten who derived an equation which was crucial to enzyme studies. Subsequent to Michaelis and Menten, several other workers approached the problem from different viewpoints, and although their work is particularly useful in advanced kinetic and mechanistic studies, they confirmed the basic concepts of Michaelis and Menten.

The ES complex is formed when the enzyme and substrate combine (reaction 13.1), but it may also be formed by the combination of the enzyme and product (reaction 13.4). The complex normally breaks down to form free enzyme and product (reaction 13.3), but it may also revert to the free enzyme and substrate (reaction 13.2). These reactions are summarized in equation (13.2):

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P \tag{13.2}$$

The dissociation constant of the complicated equilibrium involving the enzyme–substrate complex is known as the Michaelis constant, K_m, and involves the rate constants for each of the four reactions involving the ES complex.

$$K_m = k_2 + k_3/k_1 + k_4 \tag{13.3}$$

When concentration of the complex is constant, the rate of formation of the complex is balanced by the rate of disappearance of the complex. If measurements of the reaction rate for any substrate concentration are made before the product is present in appreciable amounts the reverse reaction will be negligible, and the rate constant k_4 can be ignored. Under these conditions

$$K_m = k_2 + k_3/k_1 \tag{13.4}$$

It is extremely difficult to determine the relative amounts of free and complexed enzymes, but it is possible to measure the total activity of the enzyme. The proportion of free enzyme can be represented as the difference between the total enzyme (E) and that complexed with substrate (ES).

$$\text{Rate of ES formation} = k_1[\text{E-ES}][\text{S}]$$

$$\text{Rate of ES removal} = k_2[\text{ES}] + k_3[\text{ES}]$$

where the square brackets indicate the concentration of the reactant.

Therefore

$$k_2 + k_3/k_1 = [\text{E-ES}][\text{S}]/[\text{ES}] = K_m$$

Rearrangement of the equation results in

$$K_m = [\text{E}][\text{S}]/[\text{ES}] - [\text{ES}][\text{S}]/[\text{ES}]$$

$$K_m = [\text{E}][\text{S}]/[\text{ES}] - [\text{S}]$$

$$[\text{ES}] = [\text{E}][\text{S}]/K_m + [\text{S}] \tag{13.5}$$

By definition, the observable rate of the product formation (v) is proportional to the concentration of the enzyme–substrate complex, (ES).

$$v = k_3[\text{ES}]$$

Therefore, substituting in equation (13.5)

$$v = k_3[E][S]/K_m + [S] \tag{13.6}$$

The true concentration of enzyme is difficult to measure especially in terms of molar concentration, but if the substrate concentration is large compared with that of the enzyme, all of the enzyme will be present as the ES complex, and the reaction will proceed at maximum velocity. Under these conditions of excess substrate and maximum velocity (V_{max})

$$V_{max} = k_3[E]$$

Substituting in equation (13.6) results in the common form of the Michaelis equation:

$$v = V_{max} \times [S]/K_m + [S] \tag{13.7}$$

The equation gives a measure of the Michaelis constant (K_m) in terms of measured velocity of the reaction (v) which results from a substrate concentration ([S]) and the maximum velocity (V_{max}) which can be achieved using very high concentrations of substrate.

The value for the maximum velocity is related to the amount of enzyme used, but the Michaelis constant is peculiar to the enzyme and is a measure of the activity of the enzyme. Enzymes with large values for K_m show a reluctance to dissociate from the substrate and hence are often less active than enzymes with low K_m values.

Determination of the Michaelis Constant

The reaction velocity produced by a fixed amount of enzyme with varying concentrations of substrate is determined, and a plot of the two variables shows a characteristic shape. The substrate concentration, which results in a velocity—which is half the maximum velocity—is numerically equal to the Michaelis constant.

$$V_{max}/2 = V_{max} \times [S]/K_m + [S]$$

$$[S] = K_m$$

While this method is extremely simple, it is also experimentally inaccurate. Because of the hyperbolic nature of the relationship, the curve approaches maximum velocity asymptotically making the deduction of a value for V_{max} difficult. Any error in assessing this value will be reflected in the value ascribed to K_m.

Lineweaver and Burk described a method for the determination of K_m which uses the reciprocal form of the Michaelis equation converting it to a linear relationship.

$$1/v = K_m/V_{max} \times 1/[S] + 1/V_{max} \tag{13.8}$$

A plot of the reciprocal of velocity against the reciprocal of the substrate concentration (Figure 13.5) gives a straight line graph with intercepts of

$$1/[S] = -1/K_m$$

and

$$1/v = 1/V_{max}$$

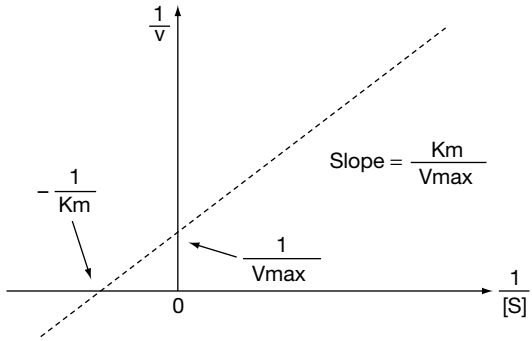


Figure 13.5 Double reciprocal plot analysis of enzyme-catalysed reaction. The double reciprocal plot is obtained by plotting inverse of substrate concentration (1/S) and inverse of velocity (1/V)

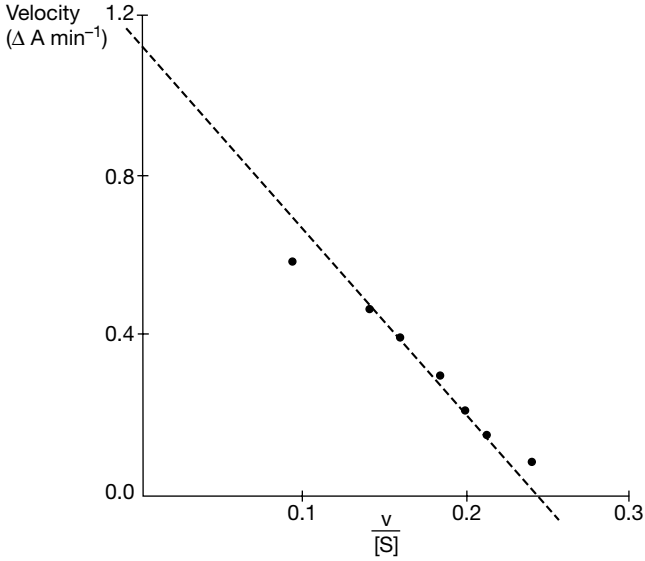


Figure 13.6 Hofstee plot for determining the Michaelis constant. The intercept of the *y*-axis gives the maximum velocity as 1.14 absorbance units per minute and the intercept on the *x*-axis (V_{max}/K_m) gives a Michaelis constant of 4.7 m mol l^{-1}

An alternative method known as the **Hofstee plot** (Figure 13.6) uses the Michaelis equation in the form

$$v = V_{max} - K_m v/[S]$$

In which v is plotted against v/[S] and gives intercepts at V_{max} and V_{max}/K_m.

Both of these methods are useful because the linear relationships permit not only a simplified graphical treatment but also the statistical and subsequent computer handling of the data. The Lineweaver–Burk method is more frequently used, although it does suffer from the disadvantages that the experimental values, which are the least precise (i.e., those involving very low substrate

concentrations and resulting in very low velocities), are the furthest from the origin and hence tend to exert the most influence on the graphical presentation. This feature may be compensated for by using weighted linear regression analysis. It is for this reason that the Hofstee plot is felt by some to be a more reliable method.

13.4 INHIBITION OF ENZYMES

A substance which decreases the rate of an enzyme-catalysed reaction is known as inhibitor and its effects may be permanent or transient. The inhibition of some reactions by substances which may be products of either that reaction or a subsequent reaction provides a control mechanism for cellular metabolism, while the selective inhibition of enzymes is the basis of many aspects of pharmacology and chemotherapy.

Some inhibitors structurally resemble the substrate and are bound by the enzymes but cannot be converted to the products. Because the formation of the complex between the enzyme and the inhibitor is a reversible reaction, the inhibitor can be displaced by high concentrations of the normal substrate. The inhibition causes an increase in the K_m value for the enzyme, but the maximum velocity for the reaction remains unaltered (Figure 13.7). Such substances are known as competitive inhibitors, and their effects can be reduced and even eliminated by high concentrations of substrate. The classical example of competitive inhibition is the action of the sulphonamide antibiotic drugs due to their structural similarity to the natural substrate p-aminobenzoic acid.

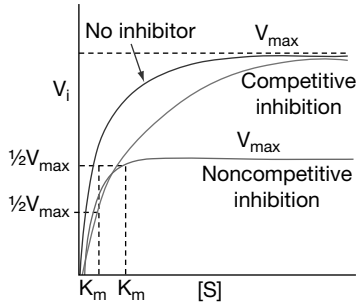


Figure 13.7 Plot of enzyme velocity in the presence and absence of an inhibitor. Note that in the presence of an inhibitor, the K_m increases

Another type of inhibitor combines with the enzyme irreversibly at a site which is often different from the substrate-binding site and as a result will inhibit the formation of the product by the breakdown of the normal enzyme–substrate complex. Such non-competitive inhibition is not reversed by the addition of excess substrate, and generally, the inhibitor shows no structural similarity to the substrate. Kinetic studies reveal a reduced value for the maximum activity of the enzyme, but an unaltered value for the Michaelis constant (Figure 13.7). There are many examples of non-competitive inhibitors many of which are regarded as poisons

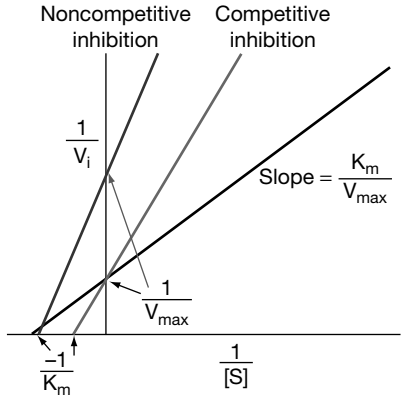


Figure 13.8 A double reciprocal plot analysis of velocity and its relationship with substrate concentration. The data are inverse plot of velocity (1/V) versus inverse of substrate (1/S) in the presence and absence of an inhibitor. The plot is used to calculate K_m and V_{max}

because of the crucial role of the inhibited enzyme. Cyanide ions, for instance, inhibit any enzyme in which either an iron or copper ion is part of the active site or prosthetic group, for example, cytochrome c oxidase. The organophosphorus compounds, of which the nerve gases are examples, inactivate enzymes that rely on the hydroxyl group of serine residues for their activity, for example cholinesterase.

Not all inhibitors fall into either of these two classes but some show much more complex effects. An uncompetitive inhibitor is defined as one which results in a parallel decrease in the maximum velocity and the K_m value (Figure 13.8). The basic mode of action of such an inhibitor is to bind only to the enzyme–substrate complex and not to the free enzyme thereby reducing the rate of formation of products. Alkaline phosphatase extracted from rat intestine is inhibited by L-phenylalanine in such a manner.

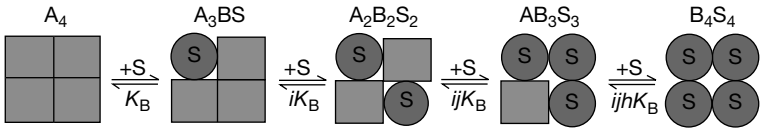
13.5 KINETIC ASPECTS OF ALLOSTERIC ENZYMES

Allosteric enzymes show various activation and inhibition effects, which are competitive in nature and related to conformational changes in the structure of the enzyme. Such allosteric enzymes are often crucial enzymes in metabolic pathways and exert control over the whole sequence of reactions. The name allosteric refers to the fact that inhibition of the enzyme is done by substances that are not similar in shape to the substrate.

The characteristic kinetic factor for most allosteric enzymes is the atypical relationship of activity and substrate concentration. So far, we have considered enzymes that possess independent substrate-binding sites, that is, the binding of one molecule of substrate has no effect on the intrinsic dissociation constants of the vacant sites. Such enzymes yield normal hyperbolic velocity curves. However, if the binding of one substrate (or effector) molecule induces structural or electronic changes that result in altered affinities for the vacant sites, the velocity curve will no longer follow Michaelis–Menten kinetics, and the enzyme will be classified as an 'allosteric' enzyme. In all likelihood, the multiple substrate (or effector)-binding sites of allosteric enzymes reside on different protein subunits. Generally, allosteric enzymes yield sigmoidal velocity curves. The binding of one substrate (or effector) molecule facilitates the binding of the next substrate (or effector) molecule by increasing the affinities of the vacant binding sites. The phenomenon has been called cooperative *binding*, or *positive* cooperativity with respect to substrate binding, or a *positive* homotropic *response.* A positive heterotropic response signifies that an effector other than the substrate is being bound at a specific regulatory site which increases the affinities of the vacant binding sites.

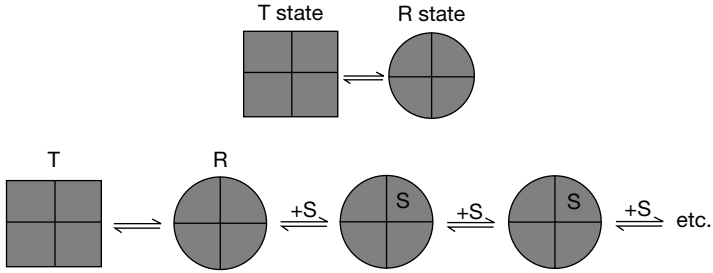
Two major models for cooperative binding have been proposed. These are the 'progressive', or 'sequential' interaction model, and the 'concerted' or 'symmetry' model. Both models are based on the observation that all allosteric enzymes are composed of subunits, that is, they are oligomers. The 'sequential' model of Koshland, Nemethy and Filmer (based on earlier suggestions of Adair and Pauling and on the 'induced fit' model of Koshland) assumes that the affinities of vacant sites for a given ligand change in a progressive manner as sites are filled (thus introducing the possibility of negative as well as positive homotropic responses). The 'sequential' model can be visualized as follows: A ligand (substrate or effector) binds an unoccupied site on one subunit of an oligomeric enzyme. As a result, the subunit undergoes an induced conformational change. New interactions between subunits are established, and this results in a change in the binding constants of the unoccupied sites. For example, if the binding constant for the first substrate

molecule is K_B, the binding constant for the second substrate molecule might be altered to iK_B. The second substrate molecule bound changes the binding constant of the vacant sites by another factor, j (to ijK_B) and so on. The sequential change in effective K_B requires that the subunits undergo the ligand-induced conformational change in a sequential manner.



The sequential model can be made very general and applicable to most allosteric enzymes by providing for restricted interactions between subunits, as dictated by the geometry of the oligomers.

The 'concerted-symmetry' model of Monod, Wyman and Changeux assumes that the oligomeric enzyme pre-exists as an equilibrium mixture of higher- and lower-affinity forms. When a substrate binds preferentially to the higher-affinity R ('relaxed') state, the equilibrium is displaced in favour of that state:



The transition between states is concerted, that is, all the subunits of the oligomers change conformation simultaneously. Because more sites are produced in the transition than were used up by the binding of ligand, the substrate saturation curve is sigmoidal. The concerted-symmetry model does not permit negative homotropic responses. Compounds that bind preferentially to the T ('tight' or 'taut') state act as inhibitors and compounds that bind preferentially to the R state act as activators (i.e. they mimic the substrate by promoting the appearance of more higher-affinity substrate sites).

A number of allosteric enzymes exhibit competitive-type kinetics since they involve changes in the apparent K_m of the substrate (i.e. $[S]_{0.5}$) but do not change the V_{max}. They are called K systems. Conversely, non-competitive systems are referred to as V systems because they involve changes in the V_{max} but not in the K_m.

The basic concept of allosteric enzymes initially proposed by Monod is an extension of Koshlund's induced fit theory. An allosteric enzyme in order to be catalytically active must be in a conformation which allows the binding of the catalytically active site must be in a conformation which allows the binding of the substrate. Its structure is normally flexible and may only be stabilized by the binding of other molecules. Hence the binding of an activator at the activator site locks the enzyme in a form in which the substrate-binding site is available. The binding of an inhibitor at a separate inhibitor site causes distortion of both the substrate-binding site and the activator site (Figure 13.9).

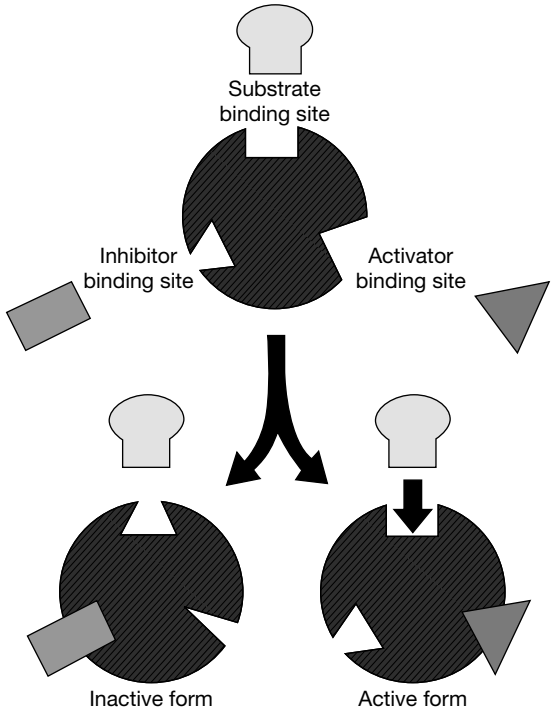


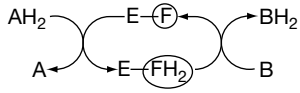
Figure 13.9 Allosteric enzymes. The binding of an activator stabilizes the enzyme in an active form, while the binding of an inhibitor distorts the active site causing a loss of activity

13.6 COFACTORS

A large number of enzymes require an additional component before the enzyme protein can carry out its catalytic functions (Table 13.2). The general term cofactor encompasses this component. Cofactors may be divided rather loosely into three groups which include (a) prosthetic groups, (b) coenzymes and (c) metal activators.

A prosthetic group is usually considered to be a cofactor firmly bound to the enzyme protein. Thus, for example, the porphyrin moiety of the haemoprotein peroxidase and the firmly associated flavin–adenine dinucleotide in succinic dehydrogenase are prosthetic groups.

This idea can be depicted as follows:

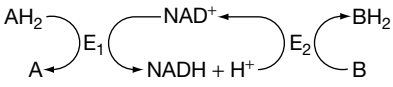


A coenzyme is a small, heat-stable, organic molecule which readily dissociates off an enzyme protein and in fact can be dialysed away from the protein. Thus, NAD^+, $NADP^+$, tetrahydrofolic acid and thiamin pyrophosphate are examples of coenzymes.

The function of a coenzyme, namely to interact with different enzymes, can be depicted as follows:

Table 13.2 Various Vitamins Function as Coenzymes

Coenzymes Derived From Vitamins	Coenzyme	Reaction type	Coenzyme class
B_1 (Thiamine)	TPP	Oxidative decarboxylation	Prosthetic group
B_2 (Riboflavin)	FAD	Oxidation/Reduction	Prosthetic group
B_3 (Pantothenate)	CoA – Coenzyme A	Acyl group transfer	Cosubstrate
B_6 (Pyridoxine)	PLP	Transfer of groups to and from amino acids	Prosthetic group
B_{12} (Cobalamin)	5-deoxyadenosyl cobalamin	Intramolecular rearrangements	Prosthetic group
Niacin	NAD^+	Oxidation/Reduction	Cosubstrate
Folic acid	Tetrahydrofolate	One carbon group transfer	Prosthetic group
Biotin	Biotin	Carboxylation	Prosthetic group



with NAD^+/NADH oscillating between E_1 and E_2.

The metal activator group is represented by the requirement of a large number of enzymes for metallic mono- or divalent cations such as K^+, Mn^{2+}, Mg^{2+}, Ca^{2+} or Zn^{2+}. These may be either loosely or firmly bound to an enzyme protein, presumably by chelation with phenolic, amino, phosphoryl or carboxyl groups. On the other hand, Fe^{2+} ion bound to a porphyrin moiety and Co^{2+} bound to the vitamin B_{12} complex would be included in the group in which porphyrin and vitamin B_{12} belong.

13.7 ISOZYMES

An enzyme, which has multiple molecular forms in the same organism, catalysing the same reaction is known as an isozyme. The most thoroughly studied isozyme is lactic dehydrogenase (LDH) which can occur in five possible forms in organs of most vertebrates, as observed by careful starch gel electrophoretic separation. Two basically different types of LDH occur. One type, which predominates in the heart, is called heart LDH. The other type, characteristic of many skeletal muscles, is called muscle LDH. The heart enzyme consists of four identical monomers which are called H subunits. The muscle enzyme consists of four identical M subunits, each subunit of which is enzymically inactive. The two types of subunits, H and M, have the same molecular weight (35,000) but different amino acid compositions and different immunological properties. There is genetic evidence that the two subunits are produced by two separate genes. Lactic dehydrogenase can be formed from H and M units to yield a pure H tetramer and a pure M tetramer. Combinations of H and M subunits will produce three additional types of hybrid enzymes. The possible combinations of the M and H subunits are therefore:

These various combinations have different kinetic properties, depending on the physiological roles which they perform. Isozymes are widespread in nature with over a hundred enzymes now known to occur in two or more molecular forms.

13.8 ENZYME MECHANISMS

The initial steps in enzyme-catalysed reactions involve the binding of the reactants to the enzyme surface, and one of the functions of the enzyme is to orientate these reactants relative to each other. This idea was suggested by Fischer as a 'lock and key' hypothesis, the enzyme being the lock and the substrate the key and although it explained the idea of the specificity of an enzyme, the idea of a rigid protein structure was difficult to accept. Koshlund proposed an alternative 'induced-fit' hypothesis which says that the binding of the substrate causes alteration in the geometry of the enzyme resulting in the correct orientation of the appropriate groups both in the substrate and in the enzyme.

Another hypothesis suggests that the binding of a substrate to an enzyme causes a strain or deformation of some of the bonds in the substrate molecule, which are subsequently broken. The effectiveness of this mechanism depends upon the strength of the binding force and does not necessarily involve any movement of the protein but suggests the idea of a flexible enzyme.

Many enzymatic reactions are a consequence of either nucleophilic or electrophilic attack on the substrate, the former being the most common. A nucleophile is a 'positive-centre-seeking' species and is capable of donating electrons. If such a species approaches another group, it will tend to push electrons away from the 'positive centre.' If the reaction to be catalysed is

$$A - X \rightarrow A^+ + X^-$$

then, an approach by a nucleophile (C^-, the catalyst) will make the reaction more feasible.

$$C^- \rightarrow A \text{ ---- } X \rightarrow C \text{ ----- } A + X^-$$

When a bond is broken, the nature of the leaving group is important in determining the energy of activation. The basicity of the group is related to its nucleophilicity and if the basic nature of the leaving group is diminished so also will be the tendency to re-form the broken bond:

$$H^+ + A \text{ ---- } X \rightarrow A \text{ ----- } X \text{ ---- } H^+$$

$$C^- \rightarrow A \text{ ---- } X \text{ --- } H^+ \rightarrow C \text{ ----- } A + H^+ X$$

This is an example of acid catalysis, and the effect is to pull electrons away from the leaving group. Often both acid catalysis and nucleophilic attack are involved in enzyme-catalysed reactions in what are known as 'push–pull' mechanisms.

13.9 ACTIVATION OF ENZYMES

Many enzymes require additional substances in order to function effectively. Conjugated enzymes require a prosthetic group before they are catalytically active, such groups being covalently or ionically linked to the protein molecule and remaining unaltered at the end of the reaction. Catalase, for instance, contains a heme group while ascorbate oxidase contains a copper atom.

The purification of some enzymes renders them inactive due to the removal of some substance essential for their activity but not classed as a prosthetic group. These are frequently inorganic ions and are not explicit participants in the reaction. Anionic activation seems to be nonspecific

and different anions are often effective. Amylase, for example, is activated by a variety of anions, notably chloride. Cationic activation is more specific, magnesium being particularly important in reactions involving ATP and ADP as substrates and it seems very likely that the cation binds initially to the substrate rather than to the enzyme.

In addition to these activations, there is a group of substances that are not simple activations but are often essential co-substrates for many different enzymes, and the most common examples are given in Table 13.3.

Table 13.3 Cofactors as Transporting Systems

Cofactor	Group transported	Type of reaction
NAD^+, $NADP^+$	Redox carrier	Oxidation
Flavins	Redox carrier	Oxidation
Cytochromes	Redox carrier	Oxidation
AMP, ADP	Phosphate	Energy transfer
Pyridoxal phosphate	Amino	Transamination
UDP	Glycosyl	Carbohydrate metabolism
Coenzyme A	Acyl	Lipid metabolism

EXERCISES

1. What is the mechanism of action of an enzyme? Discuss the factors which influence the enzyme action.
2. How are enzymes classified? Describe the general properties of enzyme and mention their mechanism of action.
3. Describe the mechanism of enzyme action and mention the role of coenzyme if required.
4. Write short notes on the following:
   (a) Mechanism of enzyme action
   (b) Specificity of enzyme action
   (c) Competitive inhibition of enzymes
   (d) Enzyme inhibitor
   (e) Isoenzymes
5. Write short note on the effects of the following factors on the activity of an enzyme.
   (a) Temperature
   (b) Substrate
   (c) Competitive inhibitors
6. Describe the mechanism of Michaelis–Menten equation.
7. Describe the Fischer's 'lock and key' hypothesis.
8. Describe the basic concept of allosteric enzymes. What are cofactors?
9. Distinguish between competitive, noncompetitive and un-competitive inhibition with the help of the graph plot.

10. Is the Hofstee plot superior than the Lineweaver–Burk method? If yes, then explain why.
11. Various vitamins function as coenzymes. Explain with the help of an example.
12. Koshlund proposed an alternative 'induced-fit' hypothesis. Explain.
13. Many enzymatic reactions are a consequence of either nucleophilic or electrophilic attack on the substrate. Explain with the help of an example.

MULTIPLE CHOICE QUESTIONS

1. Enzymes are usually named by adding the suffix 'ase' to the main part of the name of the substrate on which they act except
   (a) Erepsin
   (b) Maltase
   (c) Lactase
   (d) Sucase

   Ans. a
2. Some enzymes are named by their functions only, for example
   (a) Ptyalin
   (b) Pepsin
   (c) Reductases
   (d) Trypsin

   Ans. c
3. Some enzymes acting on the substrates are freely described by the adjectives, for example
   (a) Lactate dehydrogenase
   (b) Lipolytic
   (c) Cytochrome oxidase
   (d) Phosphatase

   Ans. b
4. The enzyme which uses oxygen as a hydrogen acceptor, for example, is
   (a) Tyrosinase
   (b) Succinate dehydrogenase
   (c) Aconitase
   (d) Carboxylase

   Ans. a
5. The enzyme which uses H_2O_2 as a substrate, for example, is
   (a) Catalase
   (b) Malate dehydrogenase
   (c) Ascorbic oxidase
   (d) Phosphorylase

   Ans. a
6. The enzyme which acts on single hydrogen donor with incorporation of oxygen, for example, is
   (a) Succinate thiokinase
   (b) Glycogen synthetase
   (c) Tryptophan oxygenase
   (d) Uricase

   Ans. c
7. The example of transglycosidase is
   (a) Hexokinase
   (b) Phosphorylase
   (c) GPT
   (d) Amino acid transacetylase

   Ans. b
8. Enzyme acting on peptide bonds, for example, is
   (a) Hexokinase
   (b) Chymotrypsin
   (c) GOT
   (d) Glucose-6-phosphatase

   Ans. b
9. Enzyme exergonic reaction means the system undergoes a loss of free energy eg,
   (a) Synthetase (b) Phosphatase
   (c) Hexokinase (d) Urease

   Ans. d
10. The group-transferring coenzyme is
    (a) CoA (b) NAD^+
    (c) $NADP^+$ (d) FAD^+

    Ans. a

11. The coenzyme containing an aromatic hetero ring in the structure is
    (a) Biotin
    (b) TPP
    (c) Sugar phosphate
    (d) Coenzyme Q

    Ans. b

12. Catalase contains a heme group while ascorbate oxidase contains a
    (a) Copper atom
    (b) Iron atom
    (c) Manganese atom
    (d) None of these

    Ans. a

13. The coenzyme derived from vitamin B_1 is
    (a) FMN (b) FAD
    (c) TPP (d) None of these

    Ans. c

14. The coenzyme derived from vitamin B_2 is
    (a) FMN (b) FAD
    (c) TPP (d) None of these

    Ans. b

15. The coenzyme derived from vitamin niacin is
    (a) NAD^+ (b) FAD
    (c) TPP (d) None of these

    Ans. a

14 Immunology

CHAPTER OBJECTIVES

14.1 Introduction
14.2 General Processes of the Immune Response
14.3 Antigenic Determinants on Immunoglobulins
14.4 Diseases Related to the Complement System
14.5 Complement Deficiencies
14.6 Antigen–Antibody Reactions
14.7 Complement
14.8 Transplantation
14.9 Interferons
14.10 Cytokines
14.11 Tissue Typing
14.12 Analytical Techniques—Precipitation Reactions
14.13 Analytical Techniques—Competitive Binding Immunoassays

14.1 INTRODUCTION

Antibodies are proteins which are produced by an animal by a process which is known as immune response, consequent to the introduction of a foreign substance into its tissues. A foreign substance that triggers the immune system is known as an antigen, and the antibodies, which are produced, are capable of binding with that antigen when allowed to react in an appropriate manner. Some substances, known as haptens, are not capable of initiating an immune response by themselves but when conjugated with a protein may result in the formation of antibodies.

Antigen–antibody reactions have provided the basis of very useful methods of qualitative and semi-quantitative analysis for many years particularly in microbiology. Antibodies have been used in the identification of bacteria, and often antibody preparations were given names which

described a demonstrable feature of their reaction with the antigen. Thus, lysins were antibodies which caused the disruption of cell membranes, opsunins rendered the antigen susceptible to phagocytosis, while agglutinins and precipitins caused the flocculation of cellular and soluble antigens, respectively. It was originally thought that the antibodies, which caused these different effects, were quite different from each other, but at the same time, it was not appreciated that the same antibody might have different effects depending on the environmental conditions and the presence of other substances.

14.2 GENERAL PROCESSES OF THE IMMUNE RESPONSE

The antibodies are produced by only one mechanism through which an animal may protect itself from substances or micro-organisms that are potentially harmful. A mechanical protection against infection is provided by the presence of an intact skin surface and membranes together with the secretion of mucus from many internal membrane surfaces. The acids secreted by the stomach and skin have a bactericidal effect similar to the presence of many body fluids of certain enzymes, particularly lysozyme.

Invasion of the tissues by an infective agent initiates an inflammatory response in the animal. This is nonspecific and is mediated primarily by substances released from tissues that are damaged as a result of either trauma or the toxic effects of the infective agent. The major mediator is the vasoactive amine histamine that causes an increased local blood flow and capillary permeability resulting in local oedema. A major aspect of the inflammatory response is the involvement of large numbers of phagocytic cells, particularly the polymorphonuclear leucocytes. These are chemotactically attracted to the inflamed tissues and are mainly responsible for the elimination of particulate material. This often results in the destruction of many of these cells and the formation of pus.

One very important group of cells known as the lymphocytes, which are widely distributed throughout the tissues, appear in increased numbers during an inflammatory response and are primarily responsible for the immune response. This is a specific response to the invading substance or agent by the animal and involves the production of cells and antibodies with the ability to recognize and bind the invading substance.

14.2.1 Cells Involved in the Immune Response

The lymphocytes are a very heterogeneous group of cells, almost identical when studied using light microscopy methods and only showing slight differences by electron microscopy techniques and yet the group contains cells with many different roles. Although large numbers of lymphocytes can be detected in the circulating blood and body fluids, the majority of lymphocytes are to be found in the group of tissues known collectively as the reticuloendothelial system. This includes the liver, spleen, bone marrow, thymus and lymph nodes, all of which are important in the immune response. Experiments involving the removal of various tissues from experimental animals have indicated that there are two different features to the immune response. The removal of the thymus, a small gland located behind the sternum, impairs the ability of a young animal to reject skin transplants but does not affect to the same extent its ability to produce antibodies. This aspect of the immune response is known as cell-mediated immunity and is due to a subpopulation of lymphocytes called T lymphocytes (thymus-derived). Experimental work with fowls has demonstrated that the removal of a lymphoid tissue nodule in the gut known as the Bursa of Fabricius

results in a reduced ability to produce antibodies but does not significantly alter the response to skin grafts. It was subsequently demonstrated that the production of antibodies, a feature known as **humoral immunity,** was associated with another subpopulation of lymphocytes known as B lymphocytes (bursa-derived). Although the equivalent of the Bursa of Fabricius in man has not been positively identified, it is most likely that the bone marrow acts as the source of B lymphocytes.

The detailed mechanisms of the immune response are not fully understood but certain major features of the process are generally accepted (Figure 14.1). The introduction of an antigen into the tissues of a susceptible animal results initially in increased proliferation of lymphocytes in the tissues of the reticuloendothelial system particularly the lymph nodes and the spleen. Subsequently, increased numbers of antibody-producing cells known as plasma cells can be seen. There is an initial lag period following the injection of the antigen after which antibody may be demonstrated in the serum, its concentration increasing up to a maximum level before declining. An animal will show different responses if the antigen is completely new to it (a primary response) or if the antigen has been encountered on a previous occasion (a secondary response)

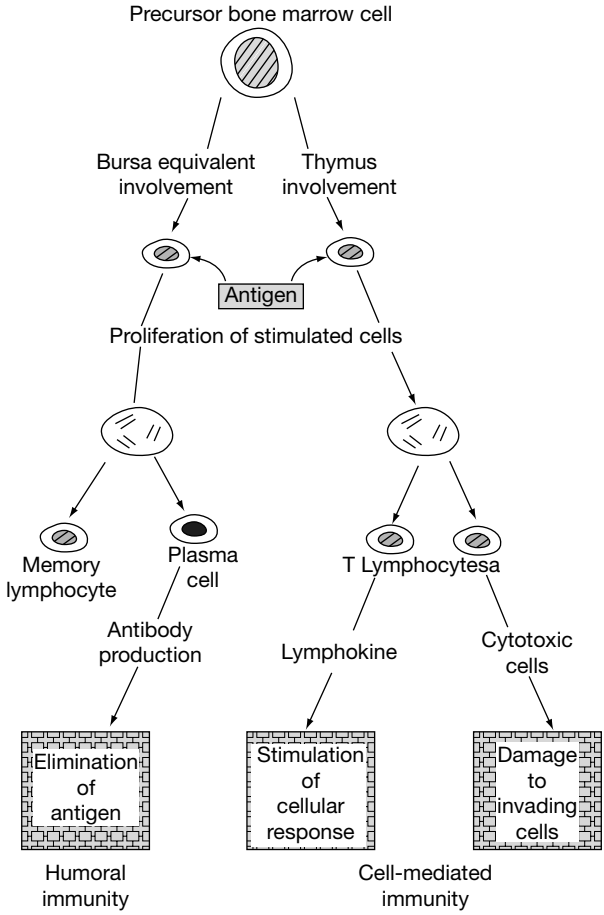


Figure 14.1 Cellular processes of the immune response. A simplified summary of the sequence of events in both the humoral and cell-mediated response to an antigen

(Figure 14.2). The secondary response shows a reduced lag period and a considerably increased rate of antibody synthesis compared with the primary response and the antibody persists for a longer period.

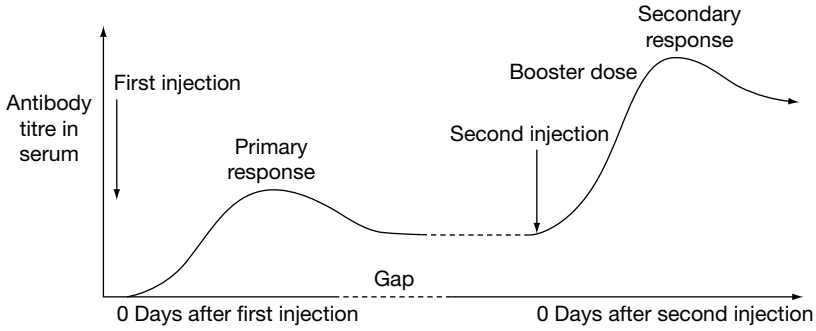


Figure 14.2 The kinetics of the immune response. The injection into an animal of a second dose or an antigen several weeks after the first injection will result in a response which is more rapid and more intense than the first

The kinetics of the response vary depending upon the antigen and the animal, but the relationship between the primary and secondary responses is characteristic.

An immunologically competent cell, whether it is a T or B lymphocyte, is thought to have specific membrane-bound receptors. The combination of these receptors with an appropriate antigen stimulates the proliferation of the cell resulting in a clone of identical cells, a process known as 'clonal selection.' The stimulation of a B lymphocyte results in the formation of a clone of plasma cells and hence the production of a specific antibody against the antigen. This antibody renders the antigen more susceptible to elimination by normal cellular processes and may cause the disruption of the cellular integrity of the antigen by a process which also involves a series of plasma proteins known collectively as complement. If the antigen is a toxic substance, its effect may be neutralized by combination with the antibody.

The stimulation of T lymphocytes results in two major classes of immunologically active cells, and one of which, the cytotoxic cell, is capable of binding to the antigen cell and cause irreversible lytic damage to the cell membrane. The other classes of T cells are capable of releasing soluble substances known as lymphokines that damage the invading cells and stimulate other aspects of the immune response.

An individual lymphocyte is capable of being stimulated by only a limited number of antigens (probably only one but at the most only two or three), and to provide a large number of different antibodies, it is essential that there are an equivalent number of different lymphocytes in the tissues.

14.2.2 Structure of Antibody (Immunoglobulins)

Antibodies are members of a group of proteins collectively known as immunoglobulins. The name is derived from the observation that during electrophoresis of blood plasma, the proteins associated with antibody activity migrate with the gamma globulin fraction. Immunoelectrophoretic studies have shown that the immunoglobulins can be divided into subclasses based on their antigenic nature and hence presumably on their overall structure and amino acid sequence.

Porter (1962) working with a purified rabbit immunoglobulin proposed the now characteristic four-chain structure involving two heavy chains and two light chains (Figure 14.3). Subsequently, information regarding the primary structure of the immunoglobulins was provided by comparison of the amino acid sequences of proteins obtained from patients suffering from the disease known as multiple myelomatosis. This disease involves the uncontrolled replication of an antibody-producing cell resulting in a clone of cells all producing the same immunoglobulin (Figure 14.4). Purification of this myeloma protein is relatively simple and provides a pure immunoglobulin.

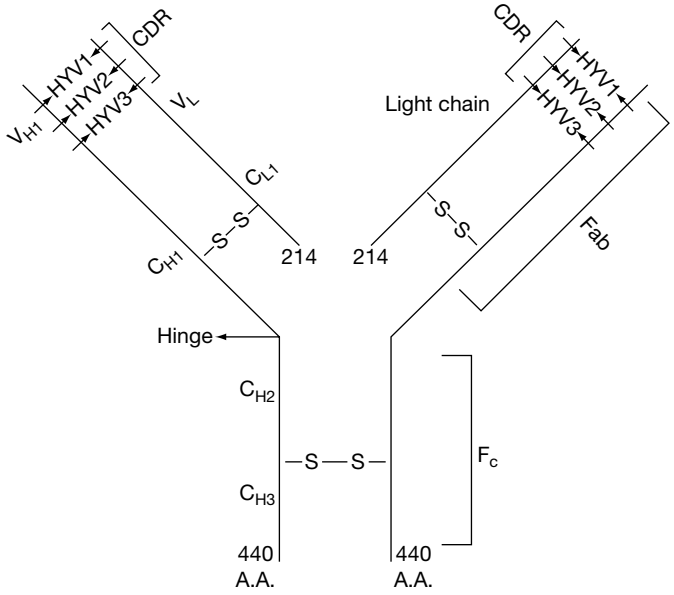


Figure 14.3 Basic structure of an IgG molecule. Two heavy chains (440 residues) and two light chains (214 residues) are joined by disulphide bonds and each shows a relatively constant amino acid sequence in one section (C-terminal end) and a variable sequence section (N-terminal end). The variable sections of both heavy and light chains are involved in the formation of the antigen-binding site

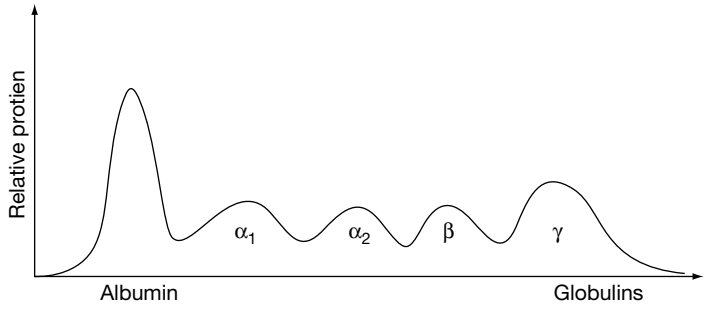


Figure 14.4 Myeloma proteins. Electrophoresis of serum from a patient suffering from multiple rnyelomatosis shows the presence of a large amount of a specific immunoglobulin. Although the immunoglobulin involved varies from one patient to another, it is usually homogeneous in any one individual

Comparison of the amino acid sequence of the heavy chains of a particular class of immunogobulin reveals that approximately three-quarters of each chain from the C-terminal end show very similar sequences (the constant section). The remaining quarter of the peptide chain (the variable section) shows considerable variation in the amino acid sequence and corresponds with that part of the chain associated with the antigen-binding site. Similar constant and variable regions are also demonstrable in the light chains, although in this case each involves approximately half of the peptide. The variation is particularly noticeable at three distinct sections (hypervariable sections) in the heavy chains, and it is suggested that these sections when associated with three similar sections in the light chains are responsible for the antibody activity and specificity of an immunoglobulin.

Five major subclasses of immunoglobulin (Table 14.1) have been recognized, and immunological studies have revealed that the differences between the classes lie in the heavy chains which, although of approximately the same size for all classes, vary considerably in the amino acid sequence. The light chains do not show such variation, and only two main types are demonstrable, known as the kappa (*k*) and the lambda (λ) chains. While all antibodies are immunoglobulins, there are some examples of immunoglobulins that do not show any antibody activity. Light chain production and heavy chain production are shown in Figures 14.5 and 14.6, respectively.

Table 14.1 Classes of Immunoglobulins

Immunoglobulin	RMM	Number of basic four chain units	Heavy chain	Antigenic valency	Levels in normal serum (in %)
IgA	*1.5×10^5	1 or 2	A	2	13
IgD	1.8×10^5	1	Δ	?	1
IgE	2.0×10^5	1	E	2	0.002
IgG	1.6×10^5	1	Γ	2	80
IgM	1.0×10^6	5	M	5 (10)	6

*Also exists as a dimer.

14.2.3 The Role of Antibodies

IgG comprises some 80 per cent of the total immunoglobulin in plasma, and because it is relatively small, it is capable of crossing membranes and diffusing into the extravascular body spaces. It can cross the placental membrane and provides the major immune defence during the first few weeks of life until the infant's own immune mechanism becomes effective.

IgM is a large molecule composed of five units, each one similar in structure to an IgG molecule. The tetramer contains an additional polypeptide, and the J chain (relative molecular mass 15,000) which appears to be important in the secretion of the molecule from the cell. It is an effective agglutinating and precipitating agent, and although potentially capable of binding 10 antigen molecules, it is usually only pentavalent. It does not cross membranes easily and is largely restricted to the blood stream.

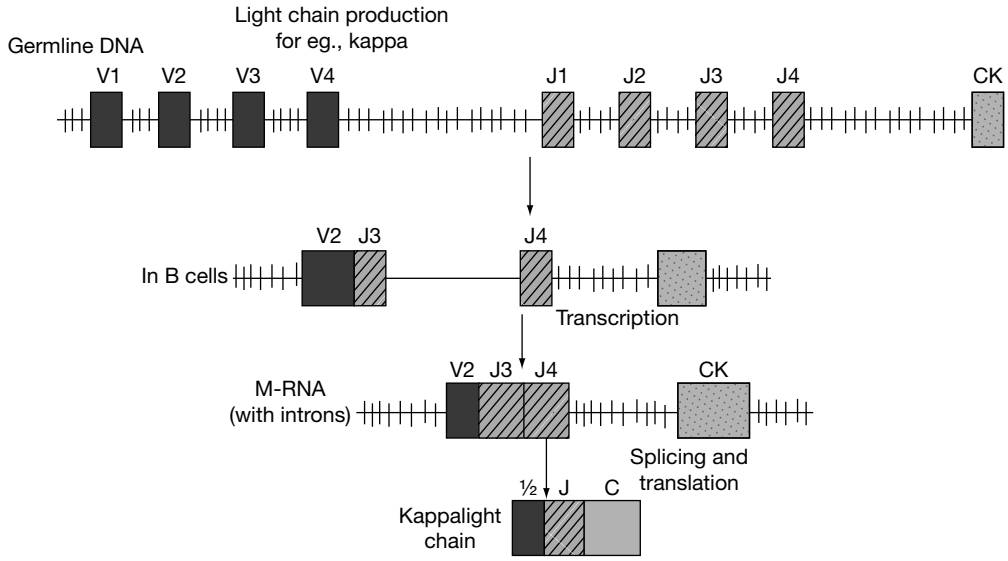


Figure 14.5 Light Chain Production

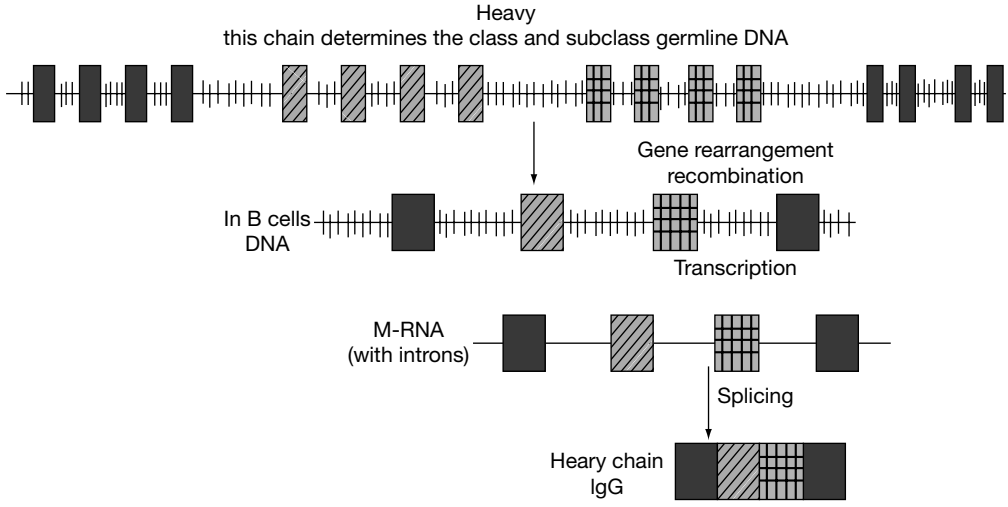


Figure 14.6 Heavy Chain Production

IgA is associated mainly with seromucus secretions such as saliva, tears, nasal fluids, etc., and is secreted as a dimer with both a J chain and a secretor piece (relative molecular mass 70,000), the latter apparently to prevent damage to the molecule by proteolytic enzymes. Its major role appears to be the protection of mucous membranes and its presence in blood, mainly as the monomer, may be as a result of absorption of the degraded dimer.

IgE is known as a cytophilic immunoglobulin because of its ability to bind to cells which may account for its low concentration in body fluids. When IgE reacts with an antigen, it causes degranulation of the mast cell to which it is bound with the release of vasoactive amines such as histamine. This process may well be helpful in initiating the inflammatory response, but in allergic individuals, the reaction is excessive and leads to a hypersensitive or over-reactive state.

14.3 ANTIGENIC DETERMINANTS ON IMMUNOGLOBULINS

Since antibodies (Abs) are glycoproteins, they can themselves function as potent immunogens to induce an Ab response. Such anti-Ig Abs are powerful tools for the study of B cell development and humoral immune responses. The antigenic determinants or episomes on Ig molecules fall in the following three major categories:

(i) Isotopic determinants
(ii) Allotypic determinants
(iii) Idiotypic determinants

14.3.1 Isotypic Determinants

They are constant region determinants that collectively define each heavy chain class and subclass and each light chain type and subtype within a species.

Each isotope encoded by a separate constant region gene and all members of a species carry the same constant region gene. Within a species, each normal individual will express all isotypes in their serum. Different species infect different constant region gene and express different isotype.

Anti-type Ab is routinely used for research purpose to determine the class on the subclass of serum Ab produced during immune response (or) to characterize the class of membrane-bound Ab present on B cell.

14.3.2 Allotypic Determinants

Although all members of a species inherit the same set of isotype genes, multiple alleles exist from some of the genes. These alleles encode solute amino acid differences called allotypic determinants, which is pure in some members of the species, but not in all members of the species. The r chain of all types are referred to as Gm markers.

14.3.3 Idiotypic Determinants

Idiotypes are the antigenic determinants created by the hypervariable regions of an antibody. The idiotypic determinants are generated by the conformation of heavy and light chain variable regions. Each individual antigenic determinant of the variable region is referred to as an idiotope.

14.4 DISEASES RELATED TO THE COMPLEMENT SYSTEM

The complement system is related to human disease in the following way:

1. Deficiencies in anyone of the protein components usually due to abnormalities in gene structure can have abnormal patterns or complement activation. If regulatory components are absent, too much complement activation may at the wrong time (or) wrong site, leading to excess inflammation and cell lysis. The absence of an integral component of the classical, alternative (or) terminal lytic pathways can result in too little complement activation and a lack of complement-medicated biologic function. Consequences in both cases are severe.

14.5 COMPLEMENT DEFICIENCIES

Deficiencies can be categorized on the basis of the functional type of protein that is lacking. Thus, there are deficiencies of the components of the classical alternative and terminal pathways and deficiencies in either soluble membrane regulatory proteins.

(i) Genetic deficiencies in classical and alternative pathway components, including Clq, Clr, C4, C2, C3 and factor D, have been identified. C2 deficiency is the most common one. The single most consistent clinical consequence of deficiencies in the early classical pathway proteins is the development of systemic lupus erythrocytes (SLE). More than 50 per cent of patients with C2 and C4 deficiencies, Sleigh suggested that the classical pathway is normally involved in regulating the immune system.

If normally generated immune complexes are not cleared from the limitation, they may deposit in blood vessel walls and tissues, where they activate the complement cascade and produce local inflammation. Such an inflammatory reaction may promote the breakdown of peripheral toluene to self-antigens leading to autoimmunity. Other reason includes genetic linkage of defective complement alleles in the MHC with others.

14.6 ANTIGEN–ANTIBODY REACTIONS

An antibody combines specifically with the corresponding antigen or hapten in a manner which is very similar to the binding of an enzyme to its substrate and involves hydrophobic and ionic interactions. The bonding between an antibody and antigen, however, involves no subsequent chemical reaction and its stability depends upon the complementary shape of the antigen and the binding site of the antibody. In analytical techniques, it is extremely desirable to use antibodies that have a high affinity for the antigen, a feature usually referred to as avidity.

Most antigens are large and may have many antigenic characteristics (determinants), and as a result, serum taken from an animal which has been immunized against that antigen will contain several, if not many, different antibodies against the different antigenic determinants. It is possible that another antigen may share some similar antigenic determinants with the original antigen with the result that some of the antibodies in an antiserum will bind to both antigens. Such an antiserum will show cross-reactivity between the two antigens and is said to lack specificity. Antisera used for analytical purposes should he specific, and it is essential that every antiserum should be thoroughly tested prior to its use.

14.6.1 Production of Antibodies

The raising of a specific antiserum by the immunization of an animal usually involves the parenteral injection of the pure antigen often introduced intramuscularly, although intravenous injection may be appropriate for particulate antigens. The feature of the primary and secondary responses to antigens suggests that a series of injections involving small amounts of antigen are likely to be more effective than a single large injection. Their precise sequence and timing can significantly affect the quality of the antiserum produced. An initial injection is normally followed by several booster doses given at 2-to-4-week interval. Too frequent injections, however, possibly giving a quicker response may result in an antiserum of reduced avidity.

The species of animal used should be as different as possible from the animal which is the source of the antigen. It should be relatively easy to handle and yet provide enough serum to make the process worthwhile. The animals most frequently used for the production of antibodies against human antigens are the guinea pig and rabbit, but for larger supplies of serum, goats and horses may be used.

Antigens vary considerably in their ability to initiate an immune response, and it is usual to incorporate an adjuvant into the sample prior to injection.

An adjuvant is a mixture of substances which stimulates an inflammatory response and prevents the rapid removal of the antigen from the tissues by the normal drainage mechanism.

Freund's adjuvant consists of an emulsion of dead mycobacteria in mineral oil, but simpler alternatives of aluminium phosphate or hydroxide have a similar effect. Of all the naturally occurring substances, proteins are generally the most immunogenic and need to have a relative molecular mass of at least 4000 and some structural rigidity to be effective. To raise antibodies against a nonimmunogenic molecule (**a hapten**), it is necessary to link it to a carrier protein which is capable of initiating a response. Bovine or human serum albumin is frequently used for this purpose as well as synthetic polypeptides such as poly-L-lysine. The hapten should be linked covalently with the carrier protein, a process usually achieved by a fairly simple reaction using a carbodiimide. This results in an amide bond between a carboxyl and an amine group on the protein and the hapten. Glutaraldehyde may be used giving a linkage between an amino group and a reactive hydrogen.

An antiserum will often contain unwanted antibodies because of other immunogenic substances in the injection. These must be removed if they are likely to reduce the specificity of the method. If a carrier protein was used to stimulate the immune response, the resulting antiserum will almost certainly contain antibodies against it. Such antibodies may be removed from the serum by adding to it some of the original carrier protein preferably insolubilized in some way. After allowing the constituents to react, the carrier protein can be removed together with the unwanted antibodies.

14.7 COMPLEMENT

The complement system is one of the major defence system of our body and consists of about 30 molecules constituting nearly 10 per cent of the total serum protein.

The functions of the system include controls of inflammatory reactions, clearance of immunity complexes, cellular activation and antimicrobial defence.

The system also plays a role in the development of the immune response and is a major epsilon system in autoimmune and hypersensitivity reactions. In plasma, these are present in functionally inactive form.

14.7.1 Complement Cascade

Complement is cultivated sequentially in a cascading manner, and each protein activating the protein that directly follows it in the sequence. Complement proteins are not antibodies, but the cascade is activated by an antibody.

Activation of the complement cascade has prevalent physiological and pathophysiological effects.

14.7.2 Complement System

The 11 proteins involved in antigen–antibody reaction are known as complement system. It was described by a Belgian, Jules Bordet in 1895. Complement works with antibodies to cause opsonization, chemotaxis and lysis of bacterial cells.

The pathway for activation of the complement set into motion by the interaction of antigen and antibody molecules is known as classical pathway.

Louis Pillemes (1955) proposed an alternate pathway or properdin system for the activation of complement system.

14.7.3 Alternative Pathway

The alternative pathway is spurred into action by molecules embedded in the surface membranes of invading microorganisms and does not require the presence of antibodies. It is thought to have evolved earlier than the classical pathway, which depends on the relatively recently evolved antibody molecule. Like the classical pathway, the alternative pathway produces both a C3 and a C5 convertase which leads to the production of C5b and then to the formation of the MAC.

C3, the complement component is spontaneously cleaved at low levels. This means that there are C3a and C3b fragments freely suspended in serum. The C3b component can attach to a number of different surfaces, both foreign and host cells alike. Sialic acid inactivates C3b, found on most mammalian cell surfaces. Microorganisms which lacks sialic acid, are steady sites for C3b deposition. C3b membrane-bound fragments are bound by Factor B. Factor D cleaves Factor B. The Ba fragment floats away, while Bb fragment stays associated with C3b. The consequential molecule C3bBb is the alternative pathways C3 convertase. The alternative pathways C3 convertase is, however, not stable.

So as to effectively split a pertinent number of C3 molecules, the C3 convertase requires the stabilization of another molecule, properdin (P), which binds to the C3bBb complex and extends the half-life of its activity.

The alternative pathway C3 convertase acts identical to the classical pathways enzyme of the same name and cleaves hundreds of C3 molecules into C3a and C3b.The C3b molecule remains attached to form the alternative pathway C5 convertase, C3bBb3b. This enzyme cleaves C5 into C5a and C5b. The C5 convertase, much like the C3 convertase before it, catalyzes the cleavage of hundreds to thousands of C5 complement component into C5a and C5b before it reverts to inactivity. C5a floats away and contributes to inflammation while the C5b fragment binds to the antigen surface. This binding of C5b is the initial step in the formation of the membrane attack complex (MAC).

The C5b membrane-bound complement component is bound by C6, the next complement molecule. The consequential bimolecular complex next binds C7 and then C8. The C5b-8 complex acts as a receptor for a variable number of membrane-disrupting C9 molecules. The resultant

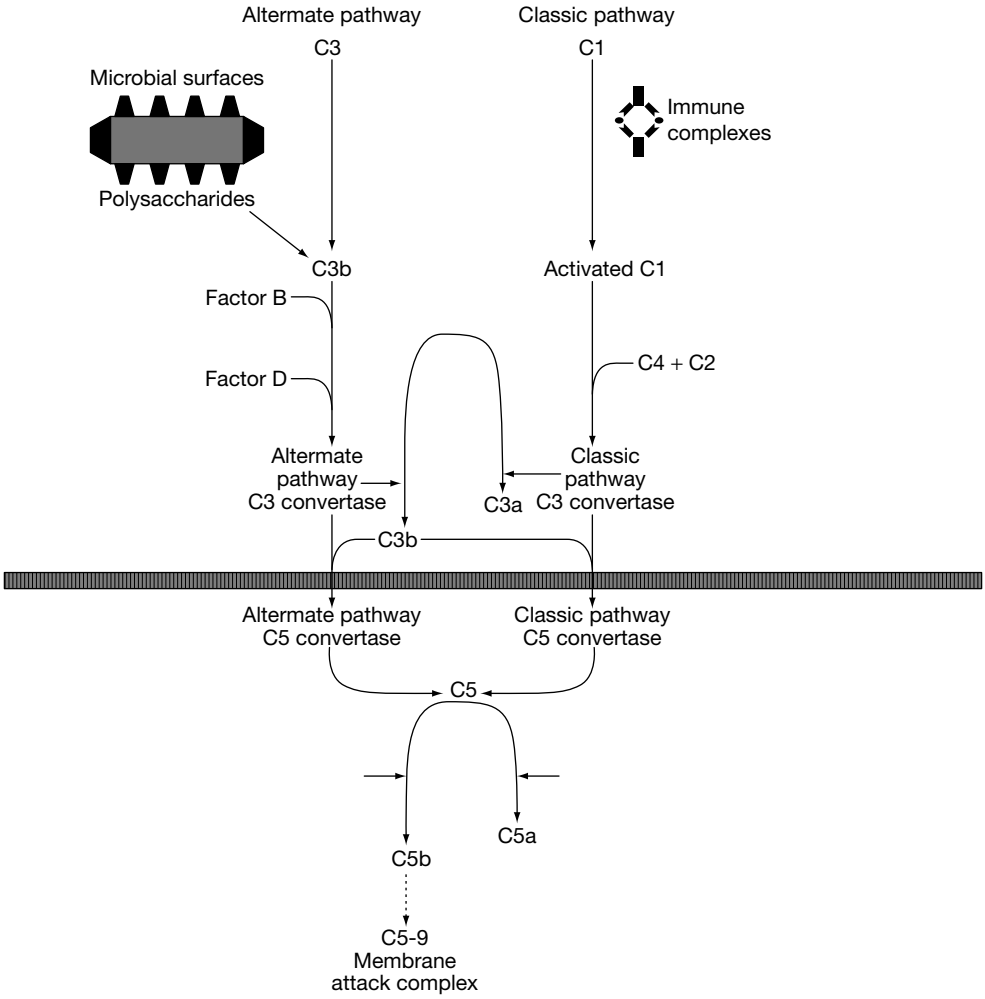


Figure 14.7 Complement Activation Pathways

C5b-8 complex and poly-C9 is given the name 'membrane attack complex.' The MAC creates a transmembrane pore leading to the lysis of the target cell (Figure 14.7).

14.7.4 Classical Pathway

The classical pathway of activation leading to the cleavage of C3 is initiated by the binding of two of more of the globular domains of the Clq component of CI, to its ligand. The C1-complex is consist of 1 molecule of C1q, 2 molecules of C1r and 2 molecules of C1s. Immune complex II containing 1g G and lg M and certain microorganisms and their products. This causes a conformational change in C1 complex that leads to auto activation of C1r complement. The enzyme C1r converts C1s into an active serine esterase that acts on thioester containing molecule C4 to

produce C4a and a reactive C4b which binds to microbial surface. C4b become activated to the surface. C4b then binds to C2 proteins which is cleaved by C1s, to C2a and C2b, forming the C4b2b complex (active in the presence of magnesium). C4b2b is an active C3 convertase cleaving C3 to C3a and C3b, which binds to the microbial surface or to the convertase itself. C3b later joins C4a2b to make C5 convertase.The activation of the classical pathway is regulated by a inhibitor and by a number of molecule that limit the production of C3 convertase.

Membrane attack complex are lipophilic in nature. It is attracted towards the lipid part of the cell and leads to its lysis. C3a, C5a acts as anaphylotoxins (Figure 14.7).

14.8 TRANSPLANTATION

Transplantation is the act of transferring cells, tissue or organs from one body site to another. The degree of immune response to a graft varies with type of graft. The following terms are used to denote different types of transplant.

14.8.1 Autograft

The self-tissue is transferred from one body site to another place in the same individual. These grafts are often carried out in the patients with burns by transferring healthy skin to a burnt area.

14.8.2 Isograft

If a tissue is transferred between genetically identical individuals, then it is called as isograft. In 100 strains of mice, an isograft can be performed between genetically identical tissues.

14.8.3 Allograft

In this case, the tissues are transferred between genetically different members of same species.

14.8.4 Xenograft

Here, the tissue is transferred between different species. For example, the graft of baboon heart into a human.

Auto- and isografts are usually accepted but the allograft and xenograft, which is genetically dissimilar to host is often recognized as a foreign graft, is rejected.

14.9 INTERFERONS

Interferons are proteins, and they are sensitive to typisin which destroys the activity of interferon. The molecular weight is about 25,000 daltons. Each interferon has its own specificity.

Interferons are biologically active substances, which are produced by the body of animals and also by the body of man. They are produced during the primary infection with virus.

Though they are produced during the first infection by the virus, they protect the organism from the virus.

Thus, interferon protects organism from the second infection by the virus as infected previously.

Interferons are produced not only against living viral particles, but also against heat-killed particles and inactivated viral particles.

Once interferons are produced in the body of an animal, the animal maintains the immunity against virus for protecting itself during second attack. Interferons are also produced against common cold, hepatitis and herpes virus.

Interferon also inhibits the rapid multiplication of some abnormal cells. Thus, interferons possess an anti-tumourogenic effect for controlling the multiplication of cells.

14.10 CYTOKINES

The development of an effective immune response involves lymphoid cells, inflammatory cells and haemopoietic cells. The complex interaction among these cells is mediated by a group by several lowmolecular weight proteins that are collectively designated as cytokines, to denote their molecule in cell-to-cell communication. Some cytokines posses direct effector functions of their own. So, cytokines serve as messengers of immune system. Unlike endocrine hormones that exert the effects over long distances, cytokines act locally.

In the body, cytokines are secreted from WBC and other cells in response to stimuli. Cytokines are regulatory proteins with low molecular weight.

14.11 TISSUE TYPING

Testing for histocompatability between an organ donor and the selected recipient is based on the presumption that tissue compatibility will promote graft acceptance and avoid immunorejection. Comparison of the tissue typing of a donor and the recipient has been carried out, usually some degree of Ag mismatching is observed, this is because of the extensive polymorphism of HLA Ag system. The basic approach is termed as ‘ HLA typing.’ It is carried out at the phenotypic level that is the types of protein are expressed on the cell surface and genotypic level that is the DNA sequence is present in the genes. Generally, HLA typing is carried out almost exclusively at the ‘phenotypic level’ using Abs to different HLA molecules. Since it is based on the use of serum-derived Abs, the approach is known as ‘serological typing.’ This serology remains the main technique for class I HLA molecules and uses DNA-based approaches.

14.11.1 Tissue Distribution of HLA Molecules

Class I molecules are expressed on the surface of virtually all cells except mature erythrocytes and thromboblast cells.

Class II molecules are only constitutively expressed on the surface of a small number of cell types, including macrophages, monocytes, follicular dendritic cells and B-lymphocytes. On some cells, such as macrophages, only DR molecules are expressed, whereas on other, such as lymphocytes, the three subclasses are expressed.

14.11.2 Structure of HLA Molecules

The class I molecules HLA-A, HLA-B and HLA-C are formed from polymorphic heavy chain glycoproteins that binds noncovalently to β2-microglobulin. The heavy or a chain of class I

molecules carries three distinct extra cellular regions or domains formed by disulphide binding. The polymorphic and membrane anchoring heavy chains contain both peptide-binding domains (al and a2) and Ig domain (a3) (Figure 14.8).

The class II molecules, HLA-DP and HLA-DQ and HLA-DR are formed from two glycoproteins, a and b chain class II molecules are heterodimers consisting of a non-covalently linked peptides (a and b) (Figure 14.8).

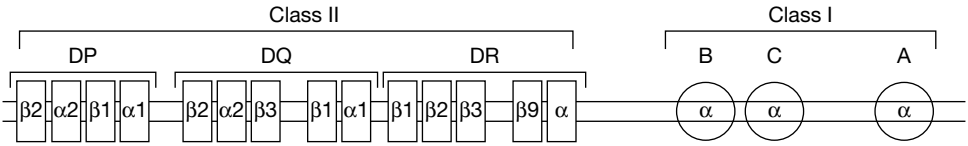


Figure 14.8 MHC of Humans on chromosome 6p21.3

14.11.3 HLA Polymorphism

Polymorphism is the phenomenon whereby numerous different alleles (allotypes) can occur at a single locus. Gene polymorphism in the HLA region is used in the HLA typing.

Polymorphism of HLA genes has two important consequences for the individual, that is, unselected individual has a diverse susceptibility to disease and also prompting rejection of organ transplants between each other.

14.11.4 Lymphocytotoxicity

When a fully HLA-compatiable donor is not available, transplantation may be successful. In this condition, mixed lymphocyte reaction can be used to assess the degree of class II MHC compatibility between a potential donor and a recipient.

Lymphocytes, from a potential donor, that have been X-ray irradiated or treated with mitomycin which serves as a stimulator cells, and lymphocytes from the recipient serves as responder cells.

14.12 ANALYTICAL TECHNIQUES—PRECIPITATION REACTIONS

Many soluble antigens may be detected and measured by precipitation using antibodies. The antigens need to be large with multiple antigenic determinants, while antibodies of the pentavalent IgG class are more effective than the divalent IgG class. It is noticeable that antisera produced in rabbits tend to be better precipitating agents than horse antisera but it is clear that immunoprecipitation reactions show considerable variation and that careful investigation and control of analytical methods which use antibodies are essential.

Because immunoglobulins are proteins, it is possible to raise specific antibodies against them. These antibodies may be used for the quantitation of immunoglobulins. It must be appreciated that such antibodies are directed against the protein or immunoglobulin nature of the antigen and not its antibody nature.

14.12.1 Immunoprecipitation in Solutions

The precipitation of a soluble antigen by an antibody produces an opalescent solution which can be measured either visually or photometrically. The degree of turbidity is proportional to

the amount of antigen for concentrations below the optimum. This relationship forms the basis of turbidometric or nephelometric methods of quantitation. The reaction is carried out in an aqueous solution in the presence of excess antibody, and a calibration curve is drawn using the turbidity measurements of a series of standard solutions of the antigen.

The degree of opalescence produced is only slight and considerable technical problems have to be overcome in order to achieve a reasonable degree of precision. Simple measurements of turbidity, using normal photometers in which the amount of incident light lost due to the scattering effect of the turbid solution, may be used. Such measurements simulate a Beer–Lambert relationship but generally show poor levels of precision and sensitivity.

The method can be considerably improved by the use of nephelometry rather than photometry. A nephelometer is similar in design to a fluorimeter, but it is designed to measure light scattered by the sample rather than light emitted by the sample. This means that the incident radiation is not required to excite the molecules and that the scattered radiation is of the same wavelength as the incident.

The extent of light scattering by a turbid solution increases as the wavelength of incident radiation decreases and for antigen–antibody complexes wavelengths in the near-ultraviolet (350–400 nm) are most frequently recommended.

The reasons for the sensitivity of nephelometry are similar to those described for fluorimetry, but reflections from cell and other surfaces will tend to reduce this sensitivity. Laser nephelometers have been developed in order to try to minimize interfering reflections and further improve sensitivity, although not yet in common use are gaining in popularity.

14.12.2 Immunoprecipitation in Gels

Gels are sometimes used in immunoprecipitation techniques to stabilize the precipitate, enabling both the position and the area of the precipitate to be measured. They also permit the formation of stable concentration gradients of one or both reactants. The point has already been made that maximum precipitation occurs when equivalent proportions of both antigen and antibody are available. Hence, if a high concentration of antigen is permitted to diffuse into a gel which contains a uniform concentration of antibody, at some point in the concentration gradient of antigen which is formed there will be optimal concentrations of both reactants and a precipitate will develop. The dimensions of the gradient will be related to the original concentration of the antigen, and hence, the distance between the precipitate and the original position will be proportional to the initial concentration. This principle forms the basis of single-radial immunodiffusion (SRID), a technique first described by Mancini (1965).

Single-radial immunodiffusion involves pipetting a measured volume of antigen into holes cut in a buffered agar gel which contains the antibody. The loaded gel is placed in a moist chamber at room temperature for at least 18 h to permit diffusion. Rings of precipitate will develop around each well due to a concentration gradient being formed by the radial diffusion of the antigen, the precipitate is most intense at the periphery and less intense towards the well. The area of precipitation is related to the concentration of antigen, and in practice, the diameter of each ring is measured to the nearest 0.1 mm, and a plot of diameter squared against concentration should give a straight line graph. It is usually advisable to take two measurements of the diameter at right angles to allow for any slight irregularities which often occur in the shape of the rings.

Single-radial immunodiffusion techniques provide very sensitive and specific methods for the quantitation of individual proteins. The lower limit of sensitivity is about 5 mg l^{-1}, although it is dependent upon the ability to detect and measure very small precipitation rings. Reducing the

amount of antibody in the gel will have the effect of increasing the average ring size but will also result in less total precipitate and a compromise has to be made between diameter and intensity of precipitate.

14.13 ANALYTICAL TECHNIQUES—COMPETITIVE BINDING IMMUNOASSAYS

The reversible nature of the antigen–antibody reaction has facilitated the development of competitive binding techniques with wide ranging applications and high levels of sensitivity and specificity. The basic concept of these techniques lies in the competition for a limited amount of antibody between the test antigen and a constant amount of reference antigen. The reference antigen, although identical immunologically, is distinguishable from the test antigen by the incorporation of an easily detectable group and is known as the label. The nature of the label used provides a means of classifying the various techniques, for example radio-immunoassay, enzyme immunoassay and fluoro-immunoassay.

When two types of antigen are present, labelled reference (Ag*) and unlabelled test (Ag), the basic reaction may be represented by the following equation:

$$Ag + Ag^* + Ab \rightleftharpoons Ag^*Ab + AgAb$$

Provided that only a limited amount of Ab is used, the equilibrium mixture will contain all of these components except free antibody and may be represented as follows:

$$\underset{\text{Free antigen}}{Ag + Ag^*} \rightleftharpoons \underset{\text{Bound antigen}}{Ag^*Ab + AgAb}$$

In an analytical method, the amounts of antibody and labelled antigen are held constant and the only variable in the system is the amount of test antigen. As the amount of this component varies, so the relative proportions of the other components in the equilibrium mixture will vary. An increase in the amount of the test antigen will result in an increased amount binding to the antibody (AgAb). This will force a parallel decrease in the amount of labelled antigen bound to antibody (Ag*Ab). Hence, the amount of labelled antigen bound to antibody in the resulting equilibrium mixture will be inversely proportional to the amount of test antigen introduced into the assay. In a comparable manner, the amount of free labelled antigen will be directly proportional to the amount of test antigen.

Hence

$$\text{test antigen} \propto \frac{1}{\text{Bound labelled antigen}} \text{ Free labelled antigen}$$

Such a relationship forms the quantitative basis of competitive binding immunoassays and also highlights the two major technical aspects of such techniques: first, the labelling of the reference antigen to make it readily distinguishable from the test antigen and yet not affect its antigenic nature and second, the ability to measure separately the amount of labelled antigen which is present in both the bound and the free forms. The different approaches to these two problems result in the various types of competitive binding immunoassay methods which are currently available.

14.13.1 Radioimmunoassay (RIA)

Radioimmunoassay was developed in the 1950s primarily as a result of monitoring the metabolism of insulin in patients with diabetes. It was originally used for the quantitation of peptide hormones, particularly insulin, but subsequently the method was extended to include the measurement of haptens such as drugs and non-peptide hormones especially the steroid hormones. The outstanding value of radioimmunoassay techniques compared with the chemical methods previously used lies in the vastly improved specificity and sensitivity of the analysis.

The technique involves the reaction of a limited amount of a specific antibody with a fixed amount of an isotopically labelled antigen and the test sample. When the reaction has reached equilibrium, the bound and free forms of the labelled antigen are separated from each other and the amount of radioactivity in one or both fractions is determined. Recently, techniques known as immunoradiometric methods have been developed in which the antibody rather than the antigen is labelled.

Labelled Antigen

It is essential that a pure sample of the antigen is available for labelling with an appropriate isotope and that once labelled the immunoreactivity of the antigen is not altered. Often, the only source of a hormone is the tissue in which it is synthesized and its extraction and purification prior to labelling may be difficult. Care must be exercised in the use of antigens extracted from different species and while, for instance, human and pig insulin are almost identical, there are considerable differences between human and pig adrenocorticotrophic hormone (ACTH).

The choice of isotope is important, and the most commonly used are carbon-14, tritium and the iodine isotopes 125 and 131. These are either beta or gamma emitters and while both types of emission can be conveniently measured, beta emitters require liquid scintillation counting techniques with the corresponding cost of consumable scintillation fluid. Gamma counters are expensive to purchase but are less costly to operate and are more amenable to automation. Carbon-14 and tritium are usually only used when they can be introduced during synthesis of the molecule and when a gamma emitter is not feasible. Some of the small peptide hormones, steroids and all of the drugs can be produced synthetically, and the isotope can be introduced during the synthesis. For most of the antigens extracted from tissues, the isotope has to be introduced into the molecule, and one of the iodine isotopes is frequently used.

The half-life of the isotope is extremely important, not only from the point of view of the shelf life of the reagent but also due to the effect on the basic qnantitative relationship. Because the sensitivity of the method depends upon the ability to detect as much of the labelled antigen as possible, isotopes with a short half-life will decay rapidly and so increase the proportion of reference antigen which will not be detectable. Another factor that influences the final purity of the labelled antigen is the purity of the original label. Iodine-125 may be obtained almost free from other isotopes of iodine while Iodine-131, although potentially more useful because of its high specific activity, contains appreciable amounts of the 127 and 129 isotopes.

Separation of Bound from free Antigen

The whole quantitative basis of radioimmunoassay depends upon the ability to separate the labelled antigen which is bound by the antibody from that which remains unbound (free). The

extremely low concentrations of both reactants mean that the antigen–antibody complex produced does not readily precipitate and a wide variety of techniques have been employed to achieve this separation.

Precipitation of the protein present in the reaction mixture results in the removal of the antibody and any antigen which is complexed with it. Various precipitating agents have been used such as solutions of ammonium sulphate, sodium bisulphite and polyethyleneglycol and certain organic solvents, for example ethanol and dioxane. However, such techniques are only valid for small antigens, when the unbound antigen is not precipitated. The efficiency of precipitation is extremely dependent upon the concentration of the precipitating agent and appreciably affected by such factors as temperature, pH and protein concentration. While such methods are economical, they are also nonspecific and tend to be unreliable.

Adsorption of the free antigen by a variety of adsorbent materials is useful particularly if the antigen is a small polar molecule such as a peptide or a steroid. The combination of antigen with an antibody generally inhibits its adsorption, although the presence of proteins in the sample tends to inhibit the adsorption of free antigen in any case. For this reason, it is often desirable to incorporate protein into the standard solutions.

Particulate charcoal shows very strong adsorptive properties tending to adsorb the antigen–antibody complex as well. It is also capable of disrupting the complex and 'stripping off' the antigen particularly if the avidity of the antiserum is not very great. In order to minimize these effects, charcoal is usually coated with a dextran which reduces its adsorptive properties preventing the large molecules and complexes gaining access to the surface of the particle by a molecular sieving effect. Dextran-coated charcoal can be bought ready for use or may be prepared by mixing a suspension of activated charcoal with a buffered solution of a dextran (average relative molecular mass of 80,000). The reaction time for adsorption to take place is critical. The charcoal is subsequently sedimented by centrifugation, and the supernatant fluid which contains the bound antigen is removed with the radioactivity in either fraction being measured.

Other adsorptive agents may be used, for example, silica, talc, etc., and as with charcoal, their adsorptive properties are modified by the presence of a protein in the reaction mixture. Adsorptive techniques have the advantage of being potentially very simple to use, but although satisfactory separation of the two fractions may be achieved, these methods require very careful control.

Immunoprecipitation, or the double antibody technique as it is often called, is an extremely popular and specific method of separating the bound antigen. It depends upon the precipitation of the antigen–antibody complex by the addition of an appropriate amount of a second antibody directed against the gamma globulins of the animal in which the first antibody was raised. The precipitating antibody obviously has to be raised in an animal of a different species from that used for the first antibody.

First antibody system		**Second antibody system**
(rabbit)		(goat)
$Ag + Ab$	$\rightleftharpoons$	$\underset{\text{precipitate}}{AgAb + \text{antirabbit } \gamma - \text{globulin}}$

Usually, the precipitating antibody is added after the initial reaction has reached equilibrium, and following a further reaction period, the mixture is centrifuged and the free antigen decanted with the supernatant fluid. The method may also be used with a preprecipitated double antibody system in which the two antibody preparations are combined in a single reagent and to which both the test and labelled antigen are added.

Although double antibody techniques tend to be more expensive, they are very specific and contamination between the fractions can be minimal. A major advantage lies in the fact that the technique can be applied to any antigen–antibody system.

Solid-phase systems have been developed in which the antibody is insolubilized in some way making the separation of the bound form from the free form a very simple process. Immunoglobulins may be covalently linked to the walls of plastic tubes without significantly affecting their ability to bind antigen. All the reactants are mixed in the tube, and after the reaction has reached equilibrium, the excess reagents, including the free antigen, may be removed by draining the tube leaving the bound antigen attached to the walls. A variety of fine particles, including cellulose, sephadex and even glass have been used to act as insoluble supports for the antibody, and a range of kits is commercially available which use many of these media.

Antibody Concentration

The properties of the antiserum used in radioimmunoassays are most important in attempting to produce valid analytical data. The avidity of an antiserum is a measure of its ability to bind firmly to the antigen while the concentration is expressed as the titre and measured as the greatest dilution of the antiserum which will still give a demonstrable reaction with the antigen. This information is extremely important in setting up any competitive binding immunoassay. The specificity of the antiserum is vital to the accuracy of the assay.

The concentration or titre at which an antiserum is used in an assay determines the analytical range and must be appropriate for the amount of labelled antigen used. The amount required is usually defined as that which in the absence of unlabelled antigen will bind 50 per cent of the available labelled antigen, Initially, a standard amount of labelled antigen must be selected, and in practice, the analytical range of a method is approximately one-half to five times the amount of labelled antigen used. Hence if the likely concentration range of test samples is known, a working amount of labelled antigen may be initially selected. The amount of antibody required is then determined by testing decreasing amounts of antiserum against the fixed amount of labelled antigen, a process known as titrating the antibody. For each antiserum dilution, the proportions of bound antigen are determined, the typical S-shaped titration curve from which the dilution of antiserum which will bind 50 per cent of the labelled antigen can be selected.

It is difficult to assess the avidity of an antiserum, primarily because of the lack of a precise definition of avidity but at least two methods have been suggested. The steepness of the antibody titration curve may give an indication of the avidity while a comparison of titration curves with and without the incorporation of a small amount of unlabelled antigen is also said to be useful.

14.13.2 Enzyme Immunoassay (EIA)

Enzyme immunoassays employ an enzyme as a label instead of a radioisotope and fall into two main categories. The heterogeneous assay involves an enzyme label simply as a means of detection and in which it is still necessary to separate the two forms of the antigen, bound and free, a process which is currently almost always achieved with the use of solid-phase systems. The homogeneous assay does not demand the physical separation of the two phases because the process of binding to the antibody modifies the activity of the enzyme label, enabling one of the two forms of the label to be detected and measured in the presence of the other.

Enzyme immunoassays lend themselves to a wide range of analytical applications and a very large number of specific methods have been described which include the quantitation of proteins,

hormones, drugs, viruses, antibiotics and microorganisms. They tend to be less sensitive with the corresponding radioimmunoassay method and while being prone to similar technical difficulties additional problems are introduced by the use of enzymes. However, they have the advantage of being relatively quick and easy to perform and the amplification effect of the enzyme enables a very wide range of sensitivity to be achieved. The precision of such techniques will obviously vary, but values of less than 10 per cent for the coefficient of variation are very feasible.

Heterogeneous Assays (ELISA)

The name ELISA is an acronym coined by the developers of the method and stands for enzyme-linked immunosorbent assay. It was developed primarily to ease the process by which the bound antigen is separated from the free and also to use a label other than a radioisotope.

The method involves the binding of an antibody to the walls of a plastic tube and subsequently adding the test and labelled antigens which compete for the available antibody. After a suitable reaction time, the tube is washed to remove all the reagents except those which are bound to the antibody. The amount of enzyme-labelled antigen which is bound to the tube wall can be measured by monitoring the enzymatic reaction after the addition of a suitable substrate. Under carefully controlled conditions, the amount of product formed is proportional to the amount of enzyme present and inversely proportional to the amount of test antigen (Figure 14.9).

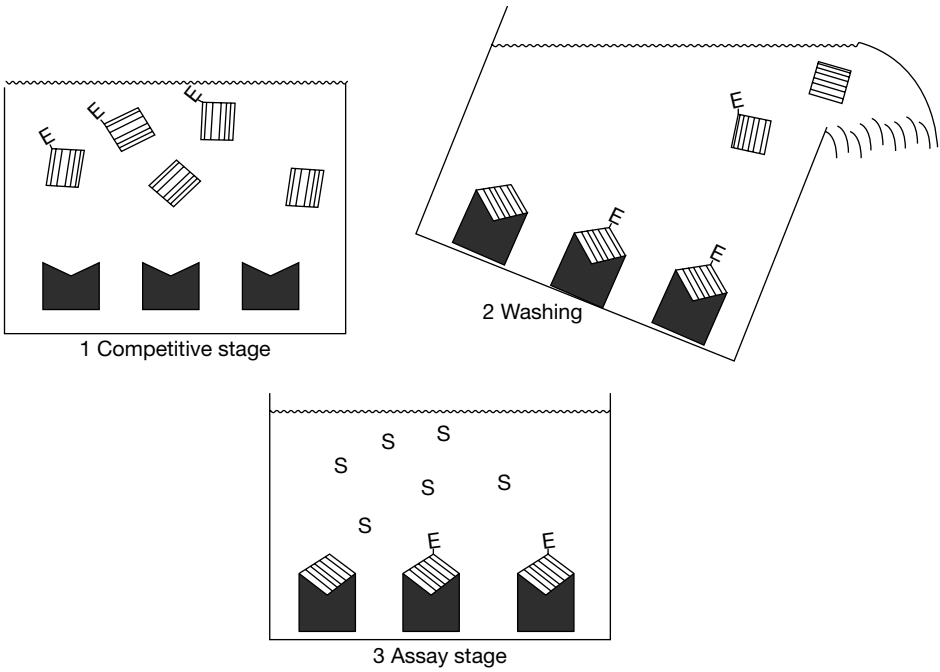


Figure 14.9 Enzyme-linked immunosorbent assay (ELISA). The enzyme-labelled antigen and the test antigen are introduced into a tube containing the immobilized antibody. After several hours, the unbound components are removed by washing and the substrate for the enzyme label is added. The amount of product formed (P_t) is proportional to the amount of labelled antigen which was bound. A control assay without test antigen gives the maximum amount of product (P_0) and the difference between the two values ($P_0 - P_t$) is proportional to the amount of unlabelled (test) antigen present

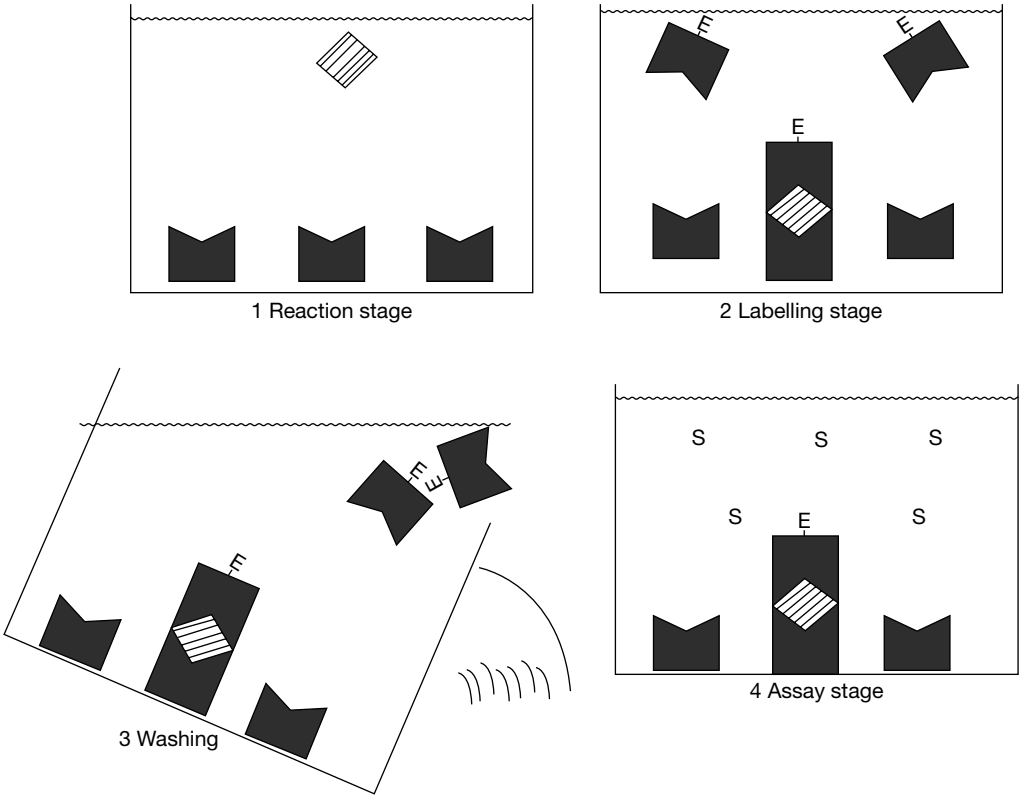


Figure 14.10 ELISA–Sandwich method. Unlabelled antigen is added to the immobilized antibody and allowed to react. The excess unbound antigen is removed by washing and enzyme-labelled antibody is added which binds to the antigen remaining. After removing the excess enzyme–antibody conjugate by washing, the enzyme label can be detected and measured by the addition of its substrate. The amount of product is proportional to the amount of antigen originally present and a standard curve can be produced in the usual way, providing the basis for a direct quantitative assay

The method is probably more useful in what is known as double antibody or sandwich form which uses enzyme-labelled antibody rather than antigen. In this form, the method is not competitive in nature and the quantitative aspects become more direct as a result. The specific antibody is bound to the walls of the vessel but instead of using a labelled antigen the test antigen is added and allowed to react. After washing the tube well to remove all the other components, an enzyme conjugate of the same specific antibody is added which binds to the antigen molecules already held by the solid-phase antibody producing the so-called sandwich (Figure 14.10). The excess enzyme–antibody conjugate is washed away, and a suitable substrate added and the amount of product formed in a given time is directly proportional to the amount of antigen originally present.

The enzymes commonly used as labels include alkaline phosphatase, peroxidase, β-galactosidase and glucose oxidase, and it is essential that the enzyme is capable of being covalently linked to the antigen or antibody without loss of its catalytic activity. Glutaraldehyde

is most frequently used as a linking agent while enzymes which are glycoproteins, such as peroxidase, may be linked via the carbohydrate group using periodic acid to form a reactive aldehyde group.

A major factor in the choice of an enzyme is the availability of a substrate which can be measured easily. The use of p-nitrophenyl phosphate and O-nitrophenyl-β-galactoside as substrates for alkaline phosphatase and β-galactosidase, respectively, is eminently suitable for such techniques because the absorption maxima of both products are in the visible region of the spectrum. Enzymes involving the use of two-stage reaction to a coloured product are sometimes employed, for example, glucose oxidase which requires the presence of peroxidase and chromogen in order to detect the hydrogen peroxide initially formed.

The ELISA technique can be used in variety of ways. In the manner described, it is suitable for the detection and quantitation of an antigen.

14.13.3 Fluoro-Immunoassay

Conjugates of antibodies and fluorescent groups have been used for several years in various microscopy techniques, but recently fluorescent groups have been used as labels in competitive binding immunoassays. Compared with radioimmunoassays and enzyme-immunoassays, fluoro-immunoassays have a considerable advantage in the stability of the reagent and the comparative ease with which measurements of fluorescence can be made. While fluorescence measurements are very sensitive, fluoro-immunoassay cannot usually equal the sensitivity of radioimmunoassay but in certain cases may be comparable to that offered by enzyme immunoassay.

In practice, fluoro-immunoassays are very similar to enzyme immunoassays and may be divided into two major types. A heterogeneous assay is one in which the labelled antigen is separated into the two forms, bound and free and the fluorescence associated with one of the forms is measured in a fluorimeter. Usually, solid-phase systems are used in which the antibody is bound to an insoluble support and the fluorescence of the bound labelled antigen is determined.

Homogeneous assays are those in which the separation is not necessary due to changes in the fluorescent properties occurring when the labelled antigen is bound by the antibody. The commonest system involves the quenching of fluorescence as in the case of the assay of gentamycin labelled with FITC (fluoroscein iso-thiocyanate) although in some cases the complexing with an antibody results in enhanced fluorescence. The binding by antibody of thyroxine labelled with FITC results in a fourfold increase in the fluorescence.

The fluorescent label must be stable and be easily detectable but must not interfere with the formation of the antigen—antibody complex.

EXERCISES

1. Discuss the structure of IgG. What are the regions in IgG that are responsible for specificity?
2. Classify immunoglobulins.
3. Write short notes on the following:
   (a) IgG (b) IGM
   (c) Antioxidant (d) Non-specific immunity

4. What is HLA? How is HLA responsible for tissue specificity?
5. What is the basis of electrophoresis? How is ELISA used for the quantification of immunoglobulins?
6. Describe the analytical techniques and the competitive binding immunoassays.
7. Write short notes on the following:
   (a) ELISA
   (b) EIA
   (c) RIA
8. How the testing for histocompatability is done? Explain.
9. What is transplantation? Distinguish between autograft, isograft, allograft and xenograft.
10. What is the complement system? Describe the alternate pathway of complement system.
11. What are adjuvant and Freund's adjuvants?
12. How the production of antibodies in the animal or human body takes place?
13. Explain the antigenic determinants on immunoglobulins. What are isotopic, allotypic and idiotypic determinants?
14. Describe the humoral and cell-mediated response to an antigen.
15. With the help of a figure, explain the kinetics of the immune response.

MULTIPLE CHOICE QUESTIONS

1. The B lymphocytes are responsible for which immunity?
   (a) Humoral (b) Cell mediated
   (c) Specific (d) Nonspecific
   Ans. a
2. How many chains are linked by disulphide bonds in the basic unit of all immunoglobulin molecules?
   (a) Four (b) Three
   (c) Two (d) One
   Ans. a
3. Immunoglobulins composed of more than one basic monomeric unit are termed as
   (a) Polymers
   (b) Monomers
   (c) Dimers
   (d) Trimers
   Ans. a
4. IgM are
   (a) Pentamers (b) Trimers
   (c) Monomers (d) Dimers
   Ans. a
5. The part of the antibody molecule which combines with antigens is formed by a few amino acids in the F region of H and
   (a) J chain (b) H chain
   (c) L chain (d) M chain
   Ans. c
6. There are two major types of L chains in man, the kappa and
   (a) Lamda chain
   (b) Alpha chain
   (c) Beta chain
   (d) Gamma chain
   Ans. a

7. IgG comprises 80 per cent of the gamma globulins and contains 2–4 per cent of
   (a) Carbohydrates
   (b) Protein
   (c) Nucleic acids
   (d) Lipids

   Ans. a

8. IgA has 5 to 10 per cent carbohydrate and does not cross the
   (a) Placenta (b) Uterus
   (c) Liver (d) Pancreas

   Ans. a

9. IgM is the major immunoglobulin expressed on the surface of _____ cells
   (a) B (b) T
   (c) C (d) D

   Ans. a

10. IgE is present in the serum at _____ concentrations
    (a) Low (b) High
    (c) Very High (d) Very Low

    Ans. a

11. No antibody activity is associated with
    (a) IgD (b) IgE
    (c) IgM (d) IgG

    Ans. a

12. IgA are
    (a) Pentamers (b) Trimers
    (c) Monomers (d) Dimers

    Ans. b

13. The transfer of self-tissue from one body site to another in the same individual is called
    (a) Autograft
    (b) Isograft
    (c) Allograft
    (d) None of these

    Ans. a

14. If a tissue is transferred between genetically identical individuals, then it is called
    (a) Autograft
    (b) Isograft
    (c) Allograft
    (d) None of these

    Ans. b

15. If a tissue is transferred between genetically different members of same species, then it is called
    (a) Autograft
    (b) Isograft
    (c) Allograft
    (d) Xenograft

    Ans. c

16. If a tissue is transferred between different species, for example, the graft of baboon heart into a human is called
    (a) Autograft
    (b) Isograft
    (c) Allograft
    (d) Xenograft

    Ans. d

15 Vitamins and Minerals

CHAPTER OBJECTIVES

15.1 Introduction to Vitamins
15.2 Fat Soluble Vitamin
15.3 Water-Soluble Vitamins
15.4 Minerals

15.1 INTRODUCTION TO VITAMINS

About 300 years ago, it was realized that some of the human disease such as night blindness, scurvy and beriberi were caused by the deficiency of some important nutrient in the diet. Later on, it was also realized that this nutrient was different from other nutrients such as the carbohydrates, fats and proteins. As early as 1720, the Austrian physician, Kramer recorded that scurvy could be cured by supplying orange or lime juice. In 1753, James Lind, an English physician, was able to cure scurvy with citrus fruits. During 1890–1897, Christian Eijkmann, a Dutch physician, was able to show that beriberi in human beings, could be cured when unmilled rice or a combination of milled rice and its brawn were substituted for milled rice in the diet. Apparently, the brawn of the rice contained some factor which could control beriberi. On the basis of these observations, Casimir Funk, a Polish biochemist, proposed that beriberi, pellagra, rickets and scurvy were diseases caused by nutritional insufficiency. In 1911, he could extract a chemical substance from rice polishing, which could control polyneuritis in pegion. It contained nitrogen in basic form and probably was amine, and he named this and similar other substances of vitamin. Later on, when it was found that not all such substances were amines, the name became vitamin.

The common feature about all the vitamins is that they are all organic substances, present in the food in small quantities. Further, they all are essential for growth and development and its absence in the diet causes specific deficiency disease. Vitamins differ from hormones in that they are supplied with the diet, while hormones are produced by the ductless glands in the body. Most vitamins and hormones, however, are involved in affecting the enzyme activity directly or indirectly. Several vitamins are incorporated as coenzymes or prosthetic groups of enzymes in both plants and animals.

Vitamins belong to several chemical groups. They are classified according to their solubility. Vitamins A, D, E and K are fat-soluble vitamins, whereas B group of vitamin and vitamin C are water soluble.

15.1.1 History of Vitamins

Many scientists have discovered fat-soluble and water-soluble vitamins which are listed in Tables 1 and 2, respectively.

Table 15.1 Fat-soluble vitamins

The discovery of fat soluble vitamins and their sources			
Vitamin	**Year of Discovery**	**Name of the Scientist**	**Food Source**
A	1915	McCollum	Butter fat egg yolk
D	1931	Angus and co-workers	Cod liver
E	1922	Bishop and Evans	Vegetable oils
K	1935	Almquist and Stakstad	Fish meal

Table 15.2 Water-soluble Vitamins

The discovery of water soluble vitamins and their sources			
Vitamin	**Year of Discovery**	**Name of the Scientist**	**Food Source**
B_1	1926	Jansen and Donath Warburg	Yeast, Liver
B_2	1932	Warburg and Christian	bottom yeast
B_3	1935	Warburg and Christian	Liver
B_5	1933	Williams and co-workers	Liver
B_6	1938	Many groups of Workers Keresztesy and Stevens, Stiller Harris and Folker Kuhn	Rice bran
B_7	1927	Boas	Liver, egg yolk
B_9	1941	Mitchell, Shell and Williams	Liver
B_{12}	1948	Rickes and co-workers	Liver
C	1932	Waugh and King	Lemon juice reducing heuromic acid.

15.1.2 Classification of Vitamins

Both fat- and water-soluble vitamins are used in multiple reactions and, therefore, most have multiple functions. See Tables 3 and 4 for a detailed description of these functions.

Table 15.3 Water soluble vitamin (all water soluble vitamin are soluble in hydrophilic environment)

Vitamin	Vitamin Common Name	Vitamin Chemical Name	Metabolic Function	Deficiency Disease	Upper Intake Level (UL/day)	Overdose Disease
B_1	Antiberiberi factor or Antineuritic factor	Thiamine	Thiamine pyrophosphate (TPP) as coenzyme in many decarboxylation reactions	Beriberi, Wernicke-Korsakoff syndrome	No tolerable upper level intake (UL) has been defined.	Drowsiness or muscle relaxation with large doses
B_2	"Yellow enzyme"	Riboflavin	Phototropic curvature of plant organs; FAD as a cofactor in respiration; Also in bioluminescence	Ariboflavinosis	No tolerable upper level intake (UL) has been defined.	No overdose disease
B_3	Pellagra Preventive (P-P) factor	Niacin, niacinamide	Participates in the formation of coenzyme A	Pellagra	35.0 mg	Liver damage (does > 2g/day) and skin rashes
B_5	Chick Antidermatitis factor or filtrate factor	Pantothenic acid	As a constituent in two pyrimidine nucleotide coenzymes, NAD and NADP	Paresthesia	No tolerable upper level intake (UL) has been defined.	Massive does yield mild intestinal distress and diarrhea at worst.
B_6	Rat Acrodynia factor or rat Antidermatitis factor	Pyridoxine, pyridoxamine, pyridoxal	Serve as growth factor to a number of bacteria; Also act as a carrier in active transport of amino acid across cell membrances	Anemia peripheral neuropathy.	100 mg	Impairment of proprioception, nerve damage (does > 100 mg/day)

(*Continued*)

Vitamin	Vitamin Common Name	Vitamin Chemical Name	Metabolic Function	Deficiency Disease	Upper Intake Level (UL/day)	Overdose Disease
B_7	Antiegg white injury factor or Coenzyme R	Biotin	As a prosthetic group for many enzymes which bring about carboxylation and synthesis of fatty acids	Dermatitis, enteritis	No tolerable upper level intake (UL) has been defined.	No overdoes disease
B_9	Liver Lactobacillus casei factor	Folic acid, folinic acid	Enzymatic synthesis of purines, pyrimidines and amino acids	Deficiency during pregnancy is associated with birth defects, such a neural tube defects	1,000 µg	May mask symptoms of vitamin B_{12} deficiency; other effects
B_{12}	Anti-pernicious anemia factor (APA factor)	Cyanocobalamin, hydroxycobalamin, methylcobalamin	Animals and micro-organisms only; Plants devoid of it	Megaloblastic anemia	No tolerable upper level intake (UL) has been defined	No ill effects on excessive ingestion
C	Antiscorbutic factor or Vitamin C	Ascorbic acid	Reducing agent; Biosyntheis of adrenal steroid hormones; Synthesis of collagen	Scurvy	2,000 mg	Vitamin C megadosage enhances Iron absorption lead to iron overload disorders such as haemochromatosis

Table 15.4 Fat soluble vitamin (all fat soluble vitamin are soluble in hydrophobic environment)

Vitamin	Vitamin Common Name	Vitamin Chemical Name	Metabolic Function	Deficiency Disease	Upper Intake Level (UL/day)	Overdoes Disease
A	Fat soluble A	Retinol, retinal, various retinoids, and four carotenoids	Utilized in vision and glyco protein synthesis	Night-blindness and Keratomalacia	300 μg	Hyper-vitaminosis A
D	Antirachitic factor	Ergocalciferol, cholecalciferol	Calcium and phosphate metabolism	Rickets and Osteomalacia	50 μg	Hyper-vitaminosis D
E	Antisterility vitamin and fertility vitamin	Tocopherols, tocotrienols	Antioxidant act as free radical scavenger	Deficiency is very rare; mild hemolytic anemia in newborn infants	1,000 mg	Increased congestive heart failure seen in one large randomized study
K	Anti hemorrhagic factor	Phylloquinone, menaquinones	Regulate synthesis of plasma clotting factors.	Bleeding diathesis	No tolerable upper level intake (UL) has been defined.	No known toxicity is associated with high doses of the phylloquinone (vit K_1) or menaquinones (vit K_2)

15.2 FAT SOLUBLE VITAMIN

15.2.1 Vitamin A

Introduction: Vitamin A occurs in animal tissues only, but many plant tissues contain substances that, when fed to rats suffering from xerophthalmia, for instance, will relieve the conditions. Thus, one must distinguish between vitamin A content and vitamin A activity. In other words, the animal can consume these pigments and brings about chemical alterations in them to produce vitamin A. Carrots and yellow corn contains no vitamin A, but since carotenes are present, they afford vitamin A activity to animals to eat them.

Structure and Properties of Vitamin A

β-carotene is a symmetrical molecule-containing two β-ionone ring connected by a carbon chain. It has the following structure shown in Figure 15.1. The two rings in β-carotene are marked as A and B. The formula of α-carotene can be indicated by showing the difference in the B ring of

this pigment compared with the B ring of β-carotene. The remainder of the molecule is the same. Since the A ring in these pigment is a β-ionone ring, these compounds are vitamin A precursors.

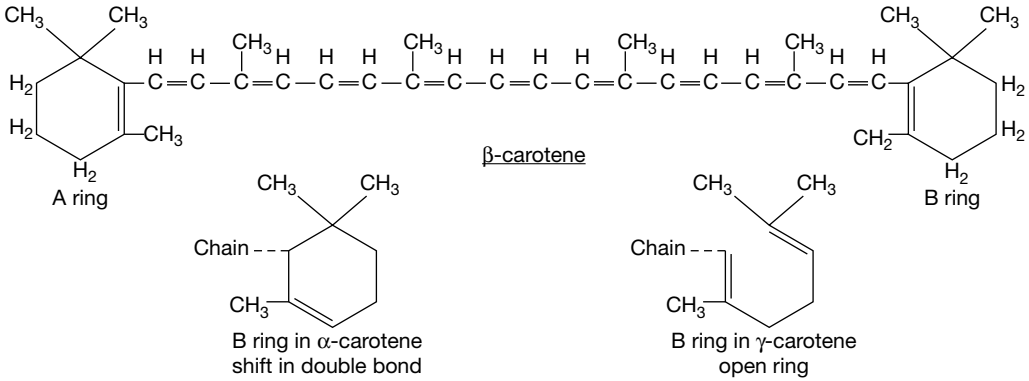


Figure 15.1 Structure of carotenes

The central double bond of β-carotene can be oxidized, and after scission at that point, it is theoretically possible that two molecules of vitamin A result. Other carotenes such as α- and γ-carotene (Figure 15.1) cannot yield more than one molecule of the vitamin on oxidative scission. This is because a α-ionone ring is an essential part of the vitamin A molecule (vitamin A_1) and β-carotene contains two such rings, α- and γ-carotene each have but one β-ionone ring. The second ring in these two pigments varies in structure, and there is no vitamin A activity associated with this part of the molecule after oxidation at the central double bond.

Vitamin A_1 (retinol$_1$), $C_{20}H_{29}OH$ and vitamin A_2 (retinol$_2$) $C_{20}H_{27}OH$, are alcohols with the accompanying structures. The structure of vitamin A_1 differs from the structure of vitamin A_2, and vitamin A_2 contains one more double bond in the ring.

Properties

Vitamin A is soluble in fat solvents and insoluble in water. It is viscous, colourless oil or pale yellowish substance. The vitamin is heat stable in the absence of air. Vitamin A can be destroyed on exposure to air or ultraviolet (UV) rays.

Coenzyme Activity: Vitamin A has no coenzyme activity.

Physiological Functions of Vitamin A and Carotenoids

Role of vitamin A in vision: Wald described in vitro synthesis of rhodopsin in a system containing (a) vitamin A, the precursor of retinene; (b) opsin, the protein of rhodopsin and (c) liver alcohol dehydrogenase, which bring about oxidation of vitamin A to retinene.

Both vitamin A and the retinenes exist in various cis-trans isomeric forms due to the conjugated double bonds. Only one isomer, the neo-b or 11-cis retinene, unites with opsin to form rhodopsin. Neo-b vitamin A was synthesized and converted into neo-b retinene. The aldehyde condenses with opsin to form rhodopsin, thereby establishing the physiological activity of the 11-cis isomer.

Rhodopsin has light-absorbing property due to polyene group of 11-cis retinal.

When light falls on rhodopsin splits into opsin and all trans-retinal. All trans-retinal may be isomerized to its 11-cis isomer in the presence of blue light, but in the eye, this isomerization is not significant. All the trans-retinol from the blood can be converted to all trans-retinal by retinene reductase by making use of NAD^+ and all trans-retinal then can be isomerized to its cis-isomer.

All trans-retinol from blood can be first isomerized to 11-cis retinol. All 11-cis retinol then can be converted to 11-cis retinal by retinol dehydrogenase in the presence of coenzyme $NADP^+$. Thus, the visual process involves continual removal of the active retinol from the blood into retinal (Figure 15.2).

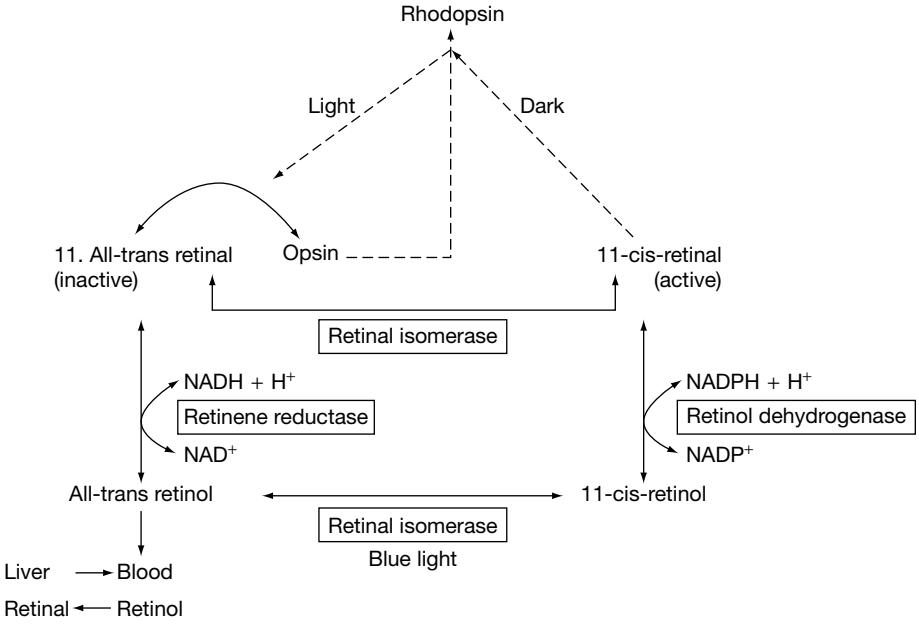


Figure 15.2 The Rhodopsin cycle

Cancer Prevention

Coupled with usability to aid immune system activity, vitamin A may be valuable in the fight against cancer.

Absorption of Vitamin A

The dietary intake of vitamin A in the form of vitamin A esters is hydrolysed in the lumen of the intestine by the enzyme lipase in the presence of bile salts and fats. The vitamin and the carotene are taken up by the intestinal mucosa where vitamin is esterified and carotene is converted first to retinal and then to retinol by retinol dehydrogenase (this enzyme is present in the liver serum). These esters, which are not the precursors of vitamin A, are absorbed and enter the intestinal lymphatics and finally to circulation in the form of chylomicrons. In the blood, the vitamin esters are attached to β-lipoprotein and are taken up by the liver. The vitamin is then released as retinol-binding protein for use anywhere required.

Storage: Ninety-five per cent of vitamin A in the form of esters is stored in the liver. Small amount of vitamin A is also present in other tissues, for example, lactating breast, adrenals, lungs and intestine.

Sources of Vitamin A

Plant sources: All leafy green vegetables supply provitamin A (carotene) in the diet. All pigmented vegetables and fruits, for example, carrots, papayas, tomatoes, sweet potatoes, pumpkins and apricots.

Animal sources: Many marine fish oils, especially the liver oils of soupfin shark, lingcod, haliput and sword fish.

Daily Requirement:

Adult	5000 IU
During pregnancy and lactation	6000–8000 IU
Children	2000–3000 IU
Infants	1500 IU
1 IU	= 0.3 μg of retinol
	= 0.6 μg of β-carotene

Normal concentration of vitamin A in blood:
24–60 IU/dl or 0.84–2.10 μmol/l.

Deficiency of Vitamin A

- **Eyes:** There are various eye conditions caused by the deficiency of vitamin A. Night blindness, or nyctalopia, is one of the early symptoms of vitamin A deficiency. Severe deficiency of vitamin A in children results in xerosis and keratomalacia. The eyelids stick together as a result of a purulent discharge. Small ulcers may appear on the cornea. Xerophthalmia with resulting blindness is common in poor children due to deficiency of vitamin A.
- **Bones and Teeth:** The bone growth is impaired due to deficiency of vitamin A. Teeth are derived from epithelial tissue, and so it is logical to expect a correlation between dietary vitamin A and tooth development.
- **Skin:** Deficiency of vitamin A has been associated with specific skin lesions. The deficiency leads to dryness and roughness of the skin.
- **Urolithiasis:** This is the condition in which urinary calculi are present is known as urolithiasis. The calculi were composed of calcium phosphate, and the deficiency of vitamin A allows keratinization of the genitourinary tract epithelium followed by bacterial invasion and alkalinuria.
- **Carbohydrate Metabolism:** Vitamin A has a specific function in the carbohydrate metabolism. Vitamin A plays a role in glucose synthesis from triose molecule.

Hypervitaminosis of Vitamin A

Excess vitamin A intake in humans leads to a number of symptoms such as headache, nausea, vomiting and drowsiness.

15.2.2 Vitamin D

Introduction: Vitamin D was discovered by Elmer McCollum in 1922. McCollum and co-workers demonstrated that cod liver oil contains a specific substance concerned with calcium deposition in rachitic rats. In 1924, Steenbock and co-workers and Hess reported that the irradiation of certain foods with UV light endowed them with antirachitic (vitamin D) activity. Angus and co-workers in 1931 isolated crystalline vitamin D. Vitamin D was obtained by UV irradiation of ergosterol, and this was named calciferol and was referred to as vitamin D_2.

Chemistry and Properties of Vitamin D

Ergosterol occurs in plants, and 7-dehydrocholesterol occurs in animals. Ergosterol is unsaturated and contains extra methyl group in its side chain than 7-dehydrocholesterol. Ultraviolet rays from sunlight cleave B ring of both the compounds. Ergocalciferiol (vitamin D_2) is formed in plants, and cholecalciferol (vitamin D_3) is formed upon exposure of skin to sunlight. Both vitamin D_2 and vitamin D_3 are of equal potency giving rise to D_2 calcitriol and D_3 calcitriol. As far as nutrition is concerned, vitamin D_2 and vitamin D_3 are important. The structure of ergocalciferiol (vitamin D_2) is the same as that of cholecalciferol (vitamin D_3) except that the side chain on position 17 is that of cholesterol. Vitamin D_2 has extra double bond and extra methyl group in the side chain as compared to vitamin D_3 (Figure 15.3). Ergosterol does not absorb

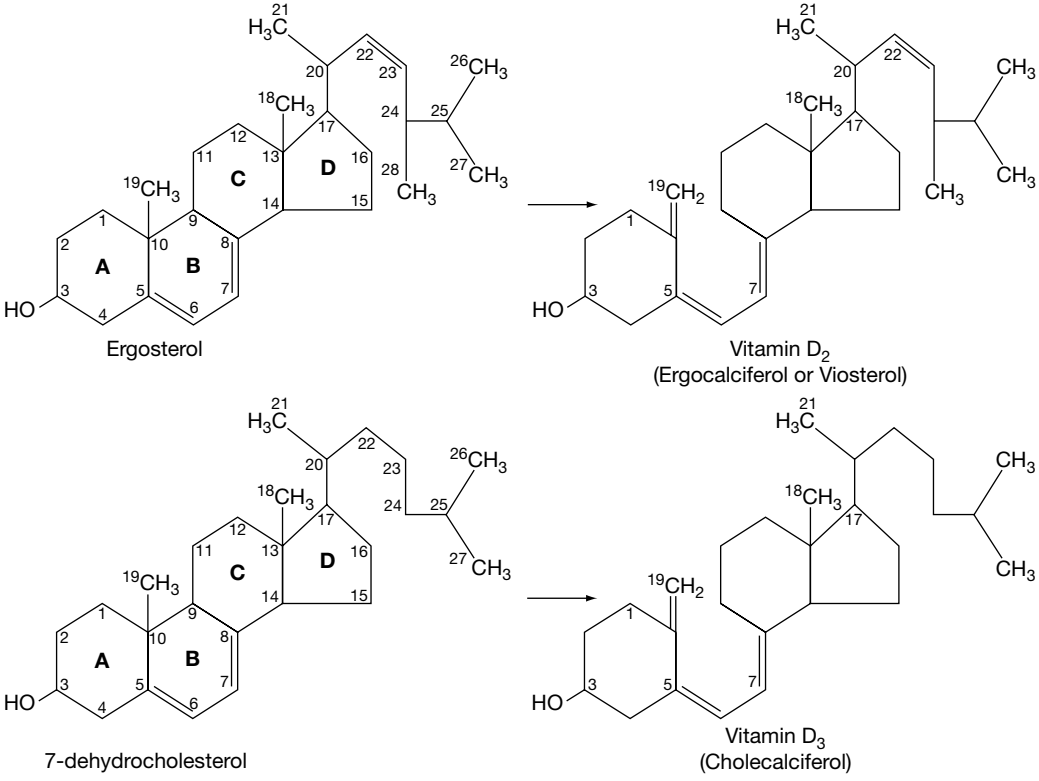


Figure 15.3 Activation of provitamins of the vitamin group

visible light but does absorb various wavelengths in the UV spectrum. The absorption maxima are at 260, 270, 282 and 293.5 mμ.

When ergosterol dissolved in alcohol, benzene, ether, etc., is irradiated with UV light, a series of photochemical reactions takes place.

Provitamin D_3 is synthesized in the body of man and other mammals. This is then activated in the skin by sunlight or UV rays and carried to various organs in the body for utilization or storage in the liver.

Properties of Vitamin D: Soluble in fat solvents but insoluble in water, heat stable, white crystalline material, ordinary boiling does not destroy it.

Physiological and Biochemical Functions

Vitamin D_3 when given to rachitic animals increases the permeability of the intestinal mucosa cells to calcium ion, apparently by changing the character of the plasma membrane to calcium permeation. It has been shown that vitamin D_3 induces the appearance of a specific calcium-binding protein (CaBP) in the intestinal mucosa of a number of animals. This protein has been isolated and purified; it has a molecular weight of 24,000 dalton and binds one atom of calcium per molecule of protein.

Vitamin D behaves more like a hormone than as the cofactor of an enzyme. That is, its effect is in controlling the production of a specific calcium-binding protein rather than influencing directly the activity of a specific enzyme.

Coenzyme Activity: Vitamin D has no coenzyme activity.

Absorption

- Vitamin D is readily absorbed in the small intestine.
- Since they are fat soluble, their absorption is enhanced by factors which favour fat absorption such as sufficient quantity of bile salt.

Storage: Vitamin D is stored largely in liver, kidney, intestine, adrenals and bones. A small amount of vitamin D is also excreted in bile but is partly reabsorbed in the intestine.

Sources: Liver of fish is a rich source of vitamin D. Vitamin D is present in egg, butter and milk. The cheapest source is sunlight which forms vitamin D_3 from 7-dehydrocholesterol in the skin.

Daily requirement

Infants and children	–	400 IU
Adult	–	200 IU
Women during pregnancy and lactation	–	400 IU

Normal Concentration in Blood of Vitamin D in Blood

Adult	–	700–3100 IU/l
Children	–	860–2100 IU/l

Deficiency of Vitamin D

Bow legs and knock knees are due to deficiency of vitamin D. The ankle, knees, wrists and elbow are swollen due to swelling of epiphyseal cartilages. Without adequate calcium and phosphorous

deposition during synthesis, bones weaken and bow under pressure, this disease is known as rickets in children, and osteomalacia in adults (soft bones) is due to inefficient calcium absorption in the intestine or pure conservation of calcium by the kidney.

Hypervitaminosis of Vitamin D

Vitamin D brings about increased absorption of Ca (calcium) and P (phosphorous) from the intestine and increased blood levels of these minerals. Hypervitaminosis of vitamin D leads to anorexia, thirst, constipation and polyuria.

15.2.3 Vitamin E

Introduction: Mattill and Conklin reported that natural foods contain nutrients specifically concerned with reproduction. They indicated that rats fed on a milk diet supplemented with yeast (B vitamins) and iron were unable to bear young ones. In 1922, Bishop and Evans announced the existence of a factor X in certain foods and indicated the necessity of the substance in the diet for normal rat reproduction.

Structure, Properties and Different Forms of Tocopherol

As early as 1927, it was recognized that vitamin E remained in the non-saponifiable fraction of the lipids of foods containing it. It was not until 1936 that the crystalline derivatives of the material with vitamin activity were isolated. Evans and co-workers prepared three derivatives of compounds from non-saponifiable fraction of wheat germ oil and showed that the original alcohol obtained by hydrolysis, of which one of them had high vitamin E activity. These workers named active alcohol as 'α-tocopherol.' Fernholz worked out the structure of 'α-tocopherol' in 1938, and it was synthesized by Karrer and co-workers in the same year.

There are eight naturally occurring tocopherol derivatives. Six are toco derivatives and two are tocotrienol. The toco and tocotrienol molecules are same except that tocotrienol has three double bonds in the side chain.

The pure tocopherols are oils and fat soluble, and a number of esters have been obtained in pure crystalline form. The tocopherols are extraordinarily stable to heat in the absence of oxygen, and they withstand acids at elevated temperatures. The tocopherols are excellent antioxidants.

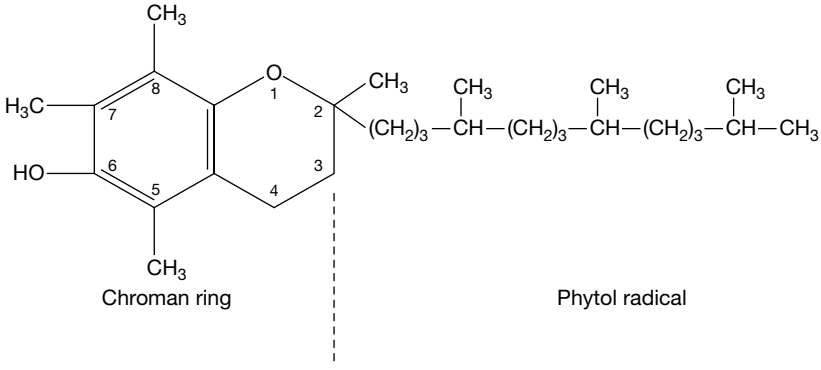


Figure15.4 α-tocopherol form of vitamin E

Physiological and Biochemical Functions

Characteristic symptoms of a vitaminosis E vary with the animal species. In mature female rats, reproductive failure occurs. They may be pregnant, but the foetuses die during the pregnancy and are absorbed from the uterus. In the male rat, germinal tissue degenerates. In rabbits and guinea pigs, acute muscular dystrophy results; in chickens, vascular abnormalities occur. In humans, well-defined syndrome of vitamin E deficiency has not been detected.

The most prominent effect that tocopherol has in in vitro systems is a strong antioxidant activity. It has been suggested that the biochemical activity of tocopherol is its capacity to protect sensitive mitochondrial systems from irreversible inhibition by lipid peroxidase. Thus, in mitochondria, prepared from tocopherol-deficient animals, there is a profound deterioration of mitochondrial activity because of haematin-catalysed peroxidation of highly unsaturated fatty acids normally present in these particles.

Absorption and Storage

(i) It is absorbed in the intestine in the presence of bile salts.
(ii) It is stored in the liver (mitochondria; microsomes) and fatty tissues.
(iii) It is present in high concentration in the adrenals, the pituitary, the uterus and the testes.

Sources: The most potent natural source of tocopherols is the vegetable oil; of these, wheat germ oil has the highest concentration. Corn oil, cottonseed oil and sunflower oil contain considerable amounts. Fish oil is a poor source. Lettuce and alfalfa are good sources.

Daily Requirement: Recommended daily amount (RDA) for a 25-year-old male for vitamin E is 15 mg/day. The dietary reference intake (DRI) for vitamin E is based on the α-Tocopherol form because it is the most active form as originally tested.

Hypervitaminosis of Vitamin E

Few adverse effects of vitamin E have been reported. If 80 times of the recommended intake has given, then it leads to increased congestive heart failure.

15.2.4 Vitamin K

Introduction: Vitamin K was discovered by the scientist Dam in 1929. He reported that chicks developed a haemorrhagic condition and prolonged blood clotting time. Dam in 1935 proposed the term vitamin K (Koagulations-vitamin) for a factor in certain food which protected chicks against this haemorrhagic syndrome. Dam reported that vitamin K activity was very high in hog liver as compared to many other foods. The factor was found to be fat soluble, even large amounts of the other fat-soluble vitamins had no beneficial effect on the course of the syndrome. Thus, vitamin K was differentiated from the other fat-soluble vitamins A, D and E.

In 1935, Almguist and Stakstav reported that fish meal was an excellent source of antihaemorrhagic factor or vitamin K. They also showed that in alfalfa the factor was localized in the unsaponifiable fraction of the ether extract.

Dam, Karrer and the co-workers isolated the vitamin from alfalfa in 1939. In 1939, Doisy and associates isolated the factor from both alfalfa and fish meal. The vitamin K was chemically different from the product obtained from fish meal. The vitamin K isolated from alfalfa

is designated as vitamin $K_1$and vitamin K, isolated from fish meal is designated as vitamin K_2. Vitamin K_1 is also known as phylloquinone or phytomenadione or phytomadione. Vitamin K_2 is also known as menaquinone or menatetrenone.

Structure and Properties of Vitamin K: Phthiocol is the first pure chemical shown to have vitamin K activity. It is not, however, a naturally occurring vitamin, but its structure was known, and thus, this helped in the elucidation of the quinoid structure of the vitamin. Vitamin K_1 is 2-methyl-3-phytyl-1, 4-nathoquinone. The vitamin K_2 contain different length side chains.

The original K_2 contains two farnesyl units in the side chain at position 3. This is equivalent to 6 isoprene units or 30 carbon atoms. Other K_2 molecules contain 7 isoprene units and 9 isoprene units in the side chain.

A number of vitamins of both the K_1 and K_2 series have been synthesized. A simple molecule, 2-methyl-1,4-naphthoquinone, is known as vitamin K_3 or menadione and is readily obtainable in pure form.

Some of the water-soluble derivatives are important. Since the natural products as well as the synthetic menadione are fairly insoluble in water, their parenteral administration is impractical. Diphosphates, diacetates, disulphates and bisulphate addition compound of menadione have been used for parenteral and oral therapy.

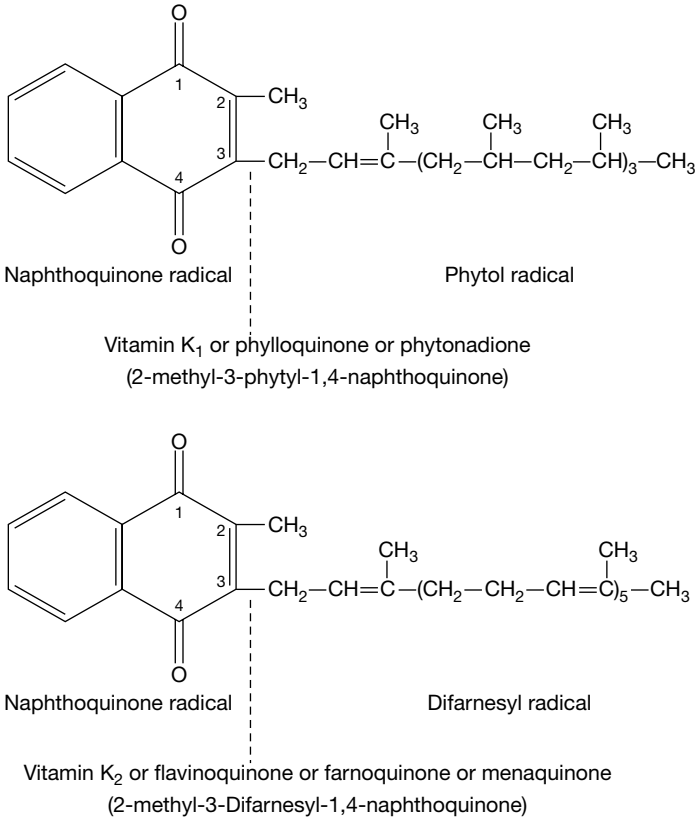


Figure 15.5 Structure of Vitamin K_1 and K_2

Coenzyme Activity: Vitamin K has no coenzyme activity.

Physiological and Biochemical Function: Vitamin K is involved in the carboxylation of certain glutamate residues in proteins to form gamma-carboxyglutamate residues (abbreviated Gla-residues).

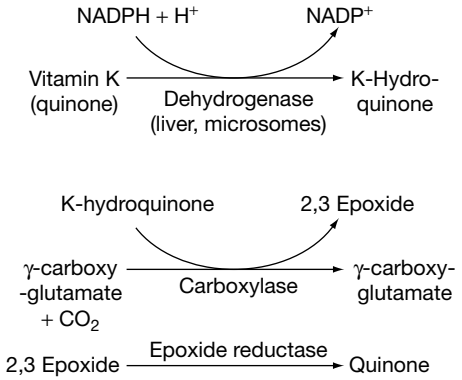


Figure 15.6 Vitamin K acts as a cofactor of the carboxylase that forms γ-carboxyglutamate residues in precursor proteins

The fundamental importance of vitamin K in the blood clotting process is well established. The process, which is highly complexed, is effected in that a deficiency of vitamin K results in a decreased level of prothrombin in the blood. The vitamin may also influence the overall process at the level of another factor proconvertin.

Prothrombin, a plasma proenzyme or zymogen, is converted into thrombin, a proteolytic enzyme, by the combined action of several factors. Thrombin in turn converts fibrinogen into fibrin, the protein from which clots are made.

Fibrinogen is a dimer with MW = 330,000 consisting of three polypeptide chains designated as alpha, beta and gamma. When thrombin acts on fibrinogen, a total of four peptide bonds are cleaved, and two small polypeptides with MW = 9000 are released. This modified fibrinogen molecule is now known as fibrin and is transferred from the form of a 'soft' clot to that known as 'hard' clot by $Ca2^+$ ions and still another protein.

'Cascade' phenomenon is a process in which an active factor is produced from an inactive form to produce an active one. The process is represented as follows:

The process of blood clotting is as shown below:

Absorption

Bile salts help in the absorption of vitamin K. The absorption is interrupted leading to haemorrhage in jaundice and other liver diseases when the bile secretion is very less.

Sources: The sources of vitamin K_1 are leafy green vegetables, such as spinach, cabbage, cauliflower, broccoli and kiwi fruit.

Daily Requirement: The daily requirement of vitamin K for a male is 120 micrograms/day, for adult women is 90 micrograms/day, for infants is 10–20 micrograms/day and for children 15–100 micrograms/day.

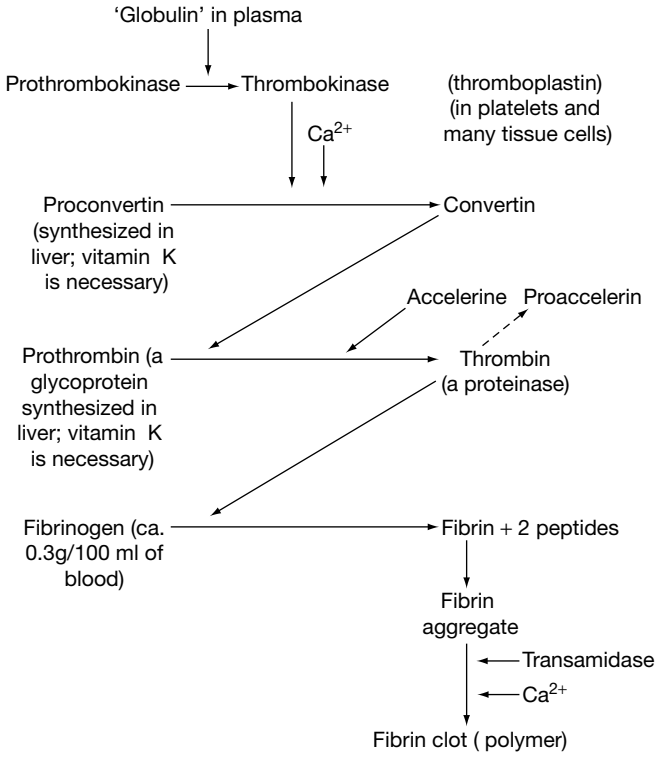


Figure 15.7 The process of blood clotting

Deficiency: Deficiency of vitamin K leads to haemorrhagic conditions due to low levels of prothrombin which leads to increased blood clotting time.

The deficiency of vitamin K leads to osteoporosis and coronary heart disease.

Hypervitaminosis of Vitamin K

In infants, large doses of vitamin K that is consumption of 30 mg/per day for 3 days leads to hyperbilirubinaemia in some cases.

15.3 WATER-SOLUBLE VITAMINS

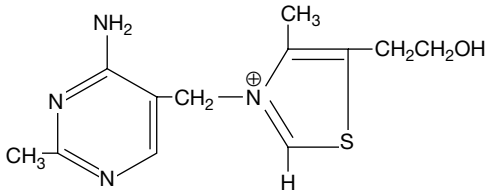
15.3.1 Thiamine (Vitamin B1)

Introduction: Thiamine deficiency in man leads to the condition known as beriberi and in animals the syndrome is referred to as polynuritis. Reference has been made to the studies by Takaki, who demonstrated that through proper diet re-forms the incidence of beriberi in Japanese Navy could be practically eliminated. Also, the important discovery of Eijkman that chickens develop symptoms resembling beriberi when fed a diet of polished rice symptoms disappear is a good case in point in this regard.

In 1926, the anti-beriberi vitamin was isolated from rice polishing by Jansen and Donath. The synthesis of thiamine was accomplished in 1936 by Williams and co-workers.

Structure and Properties of Thiamine

Soon after the purification and crystallization of thiamine, it was evident that a pyrimidine nucleus was a part of the molecule and that there also was present a substituted thiazole ring. In the synthesis of Williams and co-workers, the substituted pyrimidine (2-methyl-5-bromomethyl-6-aminopyrimidine hydrobromide) was reacted with 4 methyl-5-β-hydroxyethylthiazole to yield the bromide/hydrobromide of the vitamin. The naturally occurring thiamine is a chloride/hydrochloride.



Thiamine free base

Figure 15.8 Structure of thiamine free base

Structure of thiamine consists of two rings: the pyrimidine ring and a thiazole ring, and both these rings are connected by a methylene bridge. It is stable at acidic pH, and it is destroyed at elevated temperatures unless the pH is low. In alkaline solutions, complete destruction of thiamine results from boiling for short periods. At a pH of 3.5, boiling results in little destruction. In yeast, autoclaving at 120°C for short periods does not destroy the vitamin, but after two or three hours, nearly complete destruction is achieved.

The structure of thiamine as a free base is shown in Figure 15.7. The naturally occurring molecule and the synthetic vitamin contain a hydrochloride on the amino group and a chloride ion neutralizing the positive charge on the nitrogen atom of the thiazole ring.

Coenzyme activity of thiamine: An important derivative of thiamine is the pyrophosphate. This molecule is known as cocarboxylase and is the coenzyme or prosthetic group of the enzyme decarboxylase, which is involved in the decarboxylation of α-keto acids in the body. The structure of thiamine pyrophosphate is shown in Figure 15.9.

Thiaminokinase has been prepared from rat liver and from yeast. This enzyme in the presence of ATP and Mg^{++} synthesizes cocarboxylase from thiamine by transfer of pyrophosphate from the ATP to thiamine (Figure 15.9).

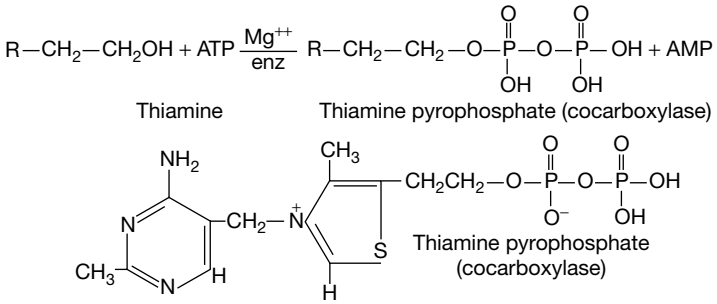


Figure 15.9 Structure of cocarboxylase

Green and others showed that various animal tissues, including rabbit muscle and pig heart, contain a thiamine-containing enzyme capable of catalysing the decarboxylation not only of pyruvate but also of α-ketoglutarate and α-ketobutyrate.

The non-oxidative decarboxylation of pyruvic acid (in plants and microorganisms) by the thiamine pyrophosphate (TPP) enzyme from yeast produces acetaldehyde. A proposed mechanism for this reaction is shown in Figure 15.10.

Lipoic acid (6,8-dithiooctanoic acid, or 6,8-thioctic acid) functions as a coenzyme in α-keto acid decarboxylation. Lipoic acid, also called pyruvate oxidation factor, protogen, and acetate replacement factor, is a growth substance for certain protozoa; hence, the name protogen. It can also replace acetate for growth in *Lactobacillus casei,* and so the name acetate replacement factor. The factor is required for the normal oxidation of pyruvate by *Streptococcus faecalis.* The term lipoic acid was used because the substance is fat soluble, and the term thioctic acid has been applied because it is an 8-carbon acid containing sulphur groups.

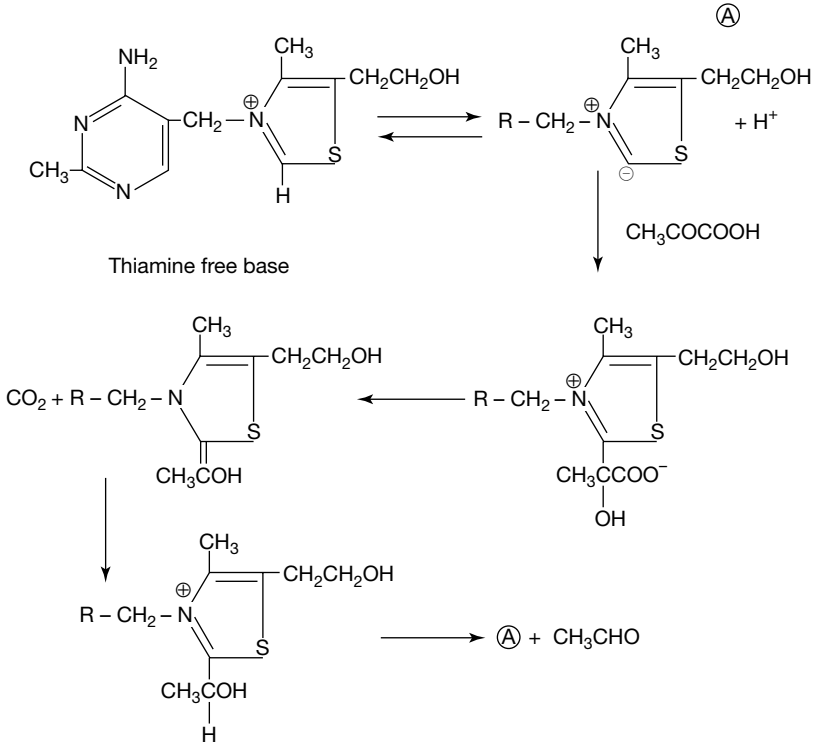


Figure 15.10 Mechanism for non-oxidative decarboxylation of pyruvic acid

In mammalian metabolism, the decarboxylation of pyruvic acid is an oxidative process (Figure 15.11) and involves the removal of CO_2 with the formation of the transient intermediate, α-hydroxyethyl thiamine pyrophosphate. This was referred to as 'active acetaldehyde.' 'Active acetaldehyde' appears to be α-hydroxyethyl thiamine pyrophosphate. The active acetaldehyde is then transferred to the oxidized form of lipoic acid forming acetyl lipoic acid (now reduced lipoic). As the acetyl is transferred to coenzyme A, the reduced lipoic acid is oxidized to the original form and

acetyl-CoA may enter one of its various metabolic reactions. Acetaldehyde in the free form is not a product of pyruvic acid decarboxylation in mammalian tissues. The oxidative decarboxylation of α-ketobutyric acid follows a similar scheme. The succinyl semi-aldehyde formed exists transiently on the coenzyme and then is converted into succinyl lipoic acid and then into succinyl CoA.

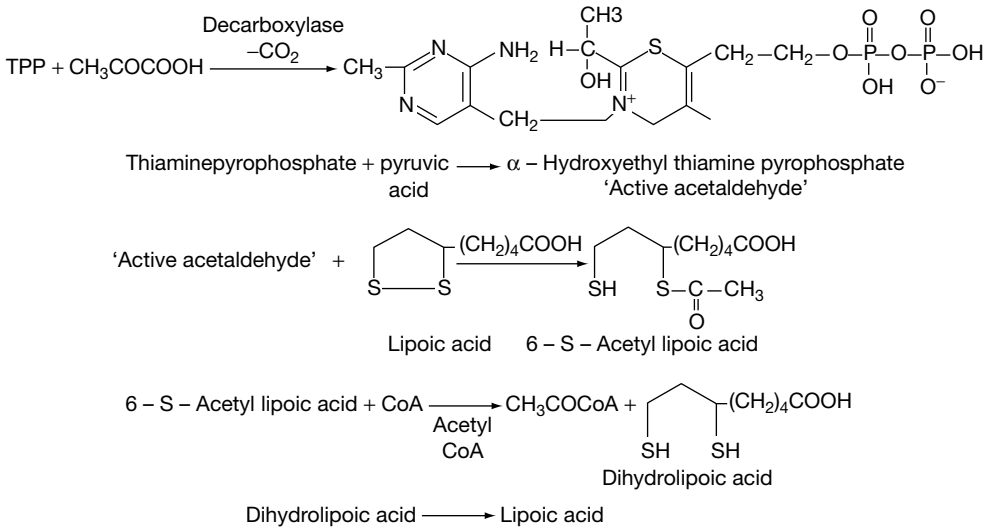


Figure 15.11 Oxidative decarboxylation of pyruvic acid

TPP is also involved as a coenzyme in a phosphoroclastic cleavage of α-keto acids. In such a reaction, the acyl of the acid is converted through acyl-CoA to acylphosphate, and the carboxyl group may become formate.

$$CH_3COCOO^- + Pi \xrightarrow{-CO_2} CH_3COCOOP + HCOO^-$$

Pyruvate + Inorganic phosphate ⟶ Acetylphosphate + Formate

Sources: The sources of thiamine are oatmeal, sunflower seeds, brown rice, potatoes, oranges, cauliflower and eggs.

Daily Requirement: It is difficult to fix a single requirement of vitamin B_1. The requirement is increased when metabolism is elevated as in fever, hyperthyroidism, increased muscular activity, pregnancy and lactation. Fat and protein reduces while carbohydrate increases the daily requirement of the vitamin. Some of the thiamine is synthesized by the bacteria in the intestine. Deficiencies of the vitamin occur not only by poor dietary intake but also in persons suffering from organic diseases.

Infants	0.3–0.5 mg
Children	0.7–1.2 mg
Adults (males)	1.2–1.5 mg
Adult (female)	1.0–1.1 mg
Pregnant women	1.3–1.5 mg
Lactating women	1.3–1.5 mg

Storage: Excess thiamine administered is not stored in the tissues. A part of the excess thiamine is excreted in urine and some of it is destroyed.

Deficiency: The disease caused due to the deficiency of thiamine is beriberi, and the disease is also common with chronic alcoholism. Beriberi is a neurological and cardiovascular disorder.

High Doses

Thiamine RDI is 1.4 mg/day. Thiamine daily doses about 50 mg leads to an increase in mental acuity. For thiamine, no upper tolerable intake levels (ULs) are defined.

15.3.2 Riboflavin (Vitamin B_2 or Vitamin G)

Introduction: The older terms nicotinic acid and nicotinic acid amide are replaced by the terms niacin and niacinamide, respectively. Funk had isolated nicotinic acid from rice polishing in 1914, but he did not realize that it was a vitamin. In 1935, Warburg Christian showed that nicotinic acid amide is an essential constituent of a coenzyme concerned in hydrogen transport. Now, two enzyme prosthetic groups containing niacinamide, coenzyme l and coenzyme ll are known. Niacinamide was isolated from liver concentrate by Elvehjem and co-workers and demonstrated the use of niacin in the treatment of pellagra.

Structure and Properties of Riboflavin

For the number of oxidative enzymes, the coenzymes are FMN and FAD and during the oxidation/reduction reactions these coenzymes remain attached to the enzymes. These coenzymes are oxidizing agents. Isoalloxazine ring reduction yields the flavoproteins in the reduced forms.

D is the chemical name of riboflavin indicates that the ribityl group is related to the D series of sugars. The one prime (1′) indicates attachment of the ribityl group at the first carbon atom and the 9 that this attachment is to position 9 of the isoalloxazine ring system (Figure 15.12). A number of syntheses are available for the dimethyl compound.

Riboflavin is sparingly soluble in water. The soluble form of riboflavin is riboflavin-5′-phosphate and it is costly.

Riboflavin is not destroyed when heated. Riboflavin is unstable in alkaline conditions. In milk, riboflavin is degraded slowly in dark.

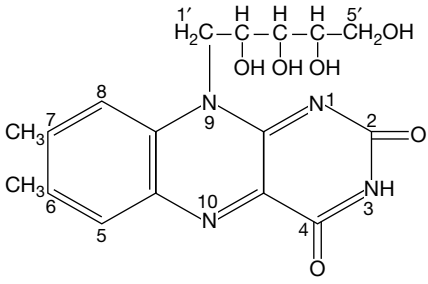


Figure 15.12 Riboflavin 6,7,-dimethyl-9-(D-1′-ribityl)-isoalloxazine

Coenzyme activity: The enzymes containing riboflavin are called flavoproteins. Two coenzymes are known, riboflavin phosphate or flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The first flavoprotein isolated was Warburg's yellow enzyme and it is composed of FMN and apoenzyme (specific protein). The enzyme can be separated into protein and FMN by dialysing against dilute HCl. No enzyme activity resides in the individual constituents, but upon recombining them activity reappears. Also, FAD has been isolated from yeast and animal tissues and various plants. Both the FAD and FMN have been synthesized.

FAD and FMN combine with different apoenzymes to form a large number of oxidation/reduction enzymes. FAD, for example, is found in xanthine oxidase, D-amino acid oxidase, aldehyde oxidase and fumaric dehydrogenase. FMN is associated with Warburg's yellow enzyme, cytochrome C reductase, L-amino acid oxidase and others. For the structure of FMN and FAD, see Figure 15.13.

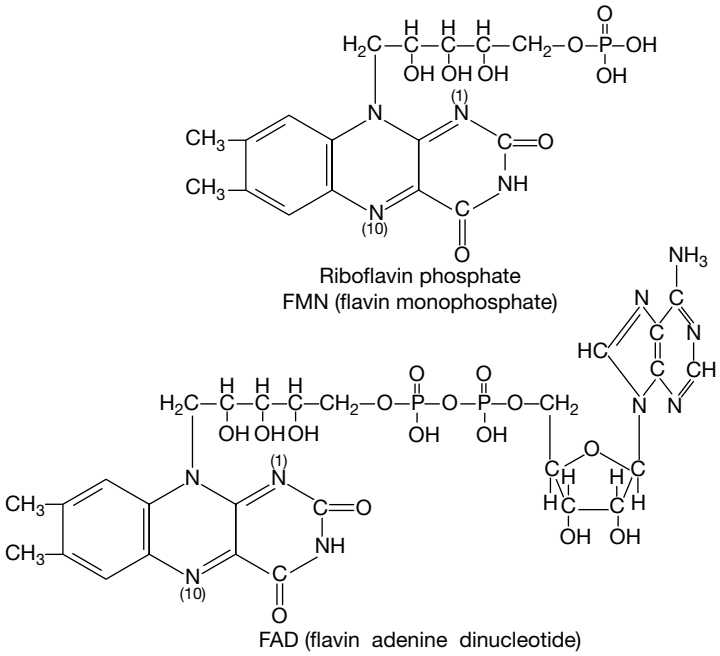


Figure 15.13 Structure of FMN and FAD

The mononucleotide (riboflavin-5′-phosphate) is synthesized from the vitamin and ATP under the influence of a flavokinase enzyme.

$$\text{Riboflavin} + \text{ATP} \xrightarrow{Mg^{++}} \text{FMN} + \text{ADP}$$

ATP reacts with the mononucleotide to form FAD. The enzyme mediating this reaction has been called flavin nucleotide pyrophosphorylase of FAD synthetase.

$$\text{FMN} + \text{ATP} \xrightarrow{Mg^{++}} \text{FAD} + \text{PP}$$

It was shown by Tong and others that FMN has a stimulating action in the conversion of iodide into iodoprotein by mitochondrial microsomal preparation of sheep thyroid glands.

Sources: Riboflavin is found in milk, meat, wheat bran, liver, kidney and cheese.

Daily Requirement: Riboflavin requirement for men is 1.3 mg/day and for women is 1.1 mg/day. For infants, the RDA is 0.3–0.4 mg/day, and for children, it is 0.6–0.9 mg/day.

Absorption and Storage: This vitamin is phosphorylated in the intestinal mucosa during absorption. It is absorbed from the small intestine through the portal vein and is passed to all tissues being stored in the body. The major part is excreted in urine, and a small part is metabolized in the body.

Deficiency of Riboflavin

Due to the deficiency of riboflavin, human beings suffer from splintered and red lips, swelling of the mouth lining and tongue, ulcers in the mouth and infection of eyes.

15.3.3 Niacin (Vitamin B_3 or Vitamin PP)

Structure and Properties of Niacin

Niacin or nicotinic acid is pyridine 3-carboxylic acid (Figure 15.14). Niacinamide or nicotinic acid amide is the acid amide. Niacin is readily prepared by oxidation of nicotine with strong oxidizing agents, such as permanganate or fuming nitric acid. Many other compounds, such as 3-ethylpyridine, can be oxidized to nicotinic acid. A synthesis from pyridine involves sulphonation of pyridine, distillation of the sodium salt of the 3-pyridine sulphonic acid with KCN to give the nitrile, and saponification of nicotinonitrile to yield nicotinic acid. Bromination of pyridine previous to sulphonation markedly increased the yield.

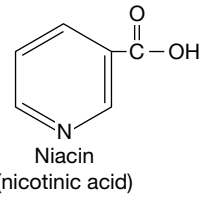


Figure 15.14 Structure of Niacin

Coenzyme activity: Two well-defined coenzymes contain nicotinamide. Nicotinamide is the form in which the vitamin is found in its physiologically active combinations. The first coenzyme I (CoI) is nicotinamide adenine dinucleotide NAD. It contains nicotinamide–ribose–phosphate–phosphate–ribose–adenine. Preiss and Handler consider the following reactions involved in the biosynthesis of NAD by enzymes in yeast, erythrocytes or liver.

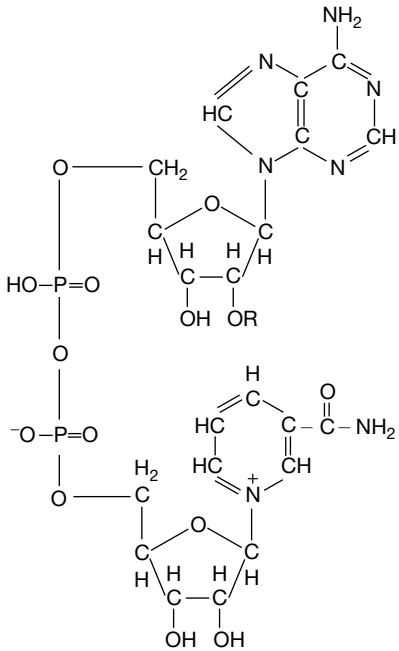
- Nicotinic acid + 5-phosphoribosylpyrophosphate → Nicotinic acid nucleotide + PP
- Nicotinic acid nucleotide + ATP ↔ desamido – NAD + PP
- Desamido – NAD + Glutamine + ATP + H_2O → NAD + Glutamate + AMP + PP.

The other coenzyme-containing niacin is called coenzyme II (CoII) or NADP for nicotinamide adenine dinucleotide phosphate. This is composed of nicotinamide–ribose–phosphate–phosphate–ribose–2′–phosphate–adenine. It differs from NAD only in the presence of a third phosphate group on carbon 2 of the ribose attached to adenine. It is synthesized from NAD and ATP. The NAD kinase enzyme mediating the reaction was isolated from pigeon liver by Wang and others.

$$NAD + ATP \xrightarrow{Mg^{++}} NADP + ADP$$

The primary action of the two coenzymes is to remove hydrogen from substrates as part of dehydrogenase enzymes and transfer hydrogen and/or electrons to the next coenzyme in the chain or to another substrate which then becomes reduced.

These enzymes are thus alternately oxidized and reduced. NADH represents the reduced form. Many metabolic processes utilize one or other of these coenzymes. The structure of NAD and NADP is shown in Figure 15.15.



Nicotinamide adenine dinucleotide, coenzyme I in which R = H (NAD)
Nicotinamide adenine dinucleotide phosphate, coenzyme II in which R = $PO(OH)_2$ (NADP)

Figure 15.15 Structure of NAD and NADP

Sources: The sources of vitamin niacin are peanuts, leafy vegetables, liver, meat, dairy products, egg, fish, cereal, legumes, mushrooms, nuts, dates, broccoli, tomatoes, carrots, sweet potatoes and asparagus.

Daily Requirement: The daily requirement of niacin for women is 14 mg/day, for men is 16 mg/day and for children is 212 mg/day.

Deficiency: Deficiency of niacin results in certain disorders as discussed hereunder.

- In humans, pellagra. However, this is not caused by the deficiency of niacin alone.
- Dermatitis accompanied by pigmentation and erythema of the tongue, which often becomes smooth and atropic.
- Nervous lesions.
- Gastrointestinal lesions.

Toxicity of Vitamin: The dosage of niacin about 1.5 to 6 g per day has certain side effects, such as skin flushing and itching, dry skin, skin rashes, including acanthosis nigricans, gastrointestinal problems and liver toxicity.

15.3.4 Pantothenic Acid (Vitamin B_5)

Introduction: Pantothenic acid is the substance that acted as a growth factor for yeast and other microorganisms, and the widespread distribution of this substance was demonstrated in 1933 by Williams and co-workers. This substance was given the name pantothenic acid by Williams and co-workers.

Concentrates of plant and animal tissues which showed various physiological activity were studied by other investigators. After few years the so-called chick antidermatitis factor isolated from liver, also called the filtrate factor, was identical to the substance given the name pantothenic acid by Woolley, Waisman and Elvehjem and also by Jukes. Williams and co-workers showed that the antidermatitis factor, like pantothenic acid, is alkali- and heat labile and is a hydroxy acid derivative of β-alanine. They were able to split β-alanine from the active compound and reactivate by coupling the remaining part of the molecule with synthetic β-alanine.

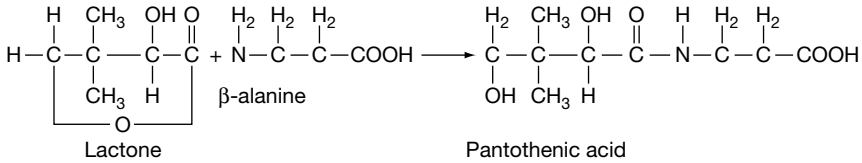
Pantothenic acid was crystallized in pure form in 1940. Its structure and synthesis was accomplished by investigators of the Merck Laboratories.

Structure and Properties of Pantothenic Acid

Pantothenic acid chemically is α, γ-dihydroxy-β-β-dimethylbutyryl-β′-alanide. The molecule is perhaps more easily comprehended if it is thought of as a dihydroxydimethylbutyric acid in peptide bond formation with β-alanine. The β′ indicates that the amino group is on the beta carbon atom rather than on the α- carbon, as found in ordinary alanine (Figure 15.16).

Many ways of synthesis of pantothenic acid have been developed. The direct condensation of β-alanine with the lactone of the substituted butyric acid gives excellent yields and the desired product is obtained directly.

The synthesis of calcium pantothenate was reported by Kagan and co-workers in high yield and in a high state of purity:



$((CH_2OHC(CH_3)_2CHOHCONHCH_2CH_2CONHCH_2CH_2S)_2$

$CH_3CHOHC(CH_3)_2CHOHCONHCH_2CH_2COOH$

pantothine

ω-methylpantothenic acid, N-(α,γ-dihydroxy-β, β-dimethylvaleryl)-β-alanine

$(CH_2CHC(CH_3)_2CHOHCONHCH_2CH_2S)_2$

bis-(β-pantoylaminoethyl)

disulphide

Figure 15.16 Pantothenic acid and related structures

In 1951, Lynen and co-workers characterized that pantothenic acid is a part of coenzyme A. The CoA molecule is a nucleotide of the following composition: adenine-phosphorylated ribose (C-3)-phosphate–phosphate–pantothenic acid–β-mercaptoethylamine (Figure 15.16).

The sulphydryl group (–SH) is readily acylated to form a great variety of acyl derivatives for the purpose of transferring these groups in synthesizing the reaction, that is, acetyl-CoA + choline → acetylcholine. Also, acyl groups are accepted by CoA in the metabolism of substrate molecules, that is, pyruvate → acetyl CoA + CO_2. CoA enters into metabolic pathways involving most classes of compounds.

Brown and Reynolds and Cheldelin and Baich reviewed the biosynthesis of CoA. Brown presented the scheme shown herewith. All of the enzymes required can be found in microorganism and in rat liver and kidney.

Pantothenic acid + ATP → 4′-phosphopantothenic acid

4′-phosphopantothenic acid + Cysteine → 4′-phosphopantothenylcysteine

4′-phosphopantothenylcysteine → 4′-phosphopantotheine

4′-phosphopantotheine + ATP → Dephospho coenzyme A + ppi

dephospho coenzyme A + ATP → Coenzyme A + ADP

A partial alternate route involves the reaction of 4′-phosphopantothenic acid with β-mercaptoethylamine to form 4′-phosphopantotheine.

The dephospho CoA lacks a phosphate group on carbon 3 of the ribose residue. Pantotheine is the part of the coenzyme A molecule composed of pantothenic acid in combination with β-mercaptoethylamine. This structure is also called *Lactobacillus bulgaricus* factor (LBF) because of its relation to growth of this organism. Pantothine is the disulphide compound and bears the same relation to pantotheine that cystine does to cysteine. The structure and synthesis were reported it 1950. LBF is a growth factor for various microorganisms and can replace pantothenic acid in the diet of various animals. It is widely distributed in nature and constitutes a second bound form of the vitamin.

Sources: The good sources of pantothenic acid are meat, whole grains, (pantothenic acid rich in outer cover of the grains) wheat bran, vegetables, like broccoli and avocados, rice, alfalfa and peanut meal.

Daily Requirement:

Age group	Age	Requirements
Infants	0–6 months	1.7 mg
Infants	7–12 months	1.8 mg
Children	1–3 years	2 mg
Children	4–8 years	3 mg
Children	9-13 years	4 mg
Adults men and women	14+ years	5 mg
Pregnant women	(vs. 5)	6 mg
Breastfeeding women	(vs. 5)	7 mg

vs. The Mean in the Age of Average.

Deficiency: The deficiency of this vitamin results in certain disorders as discussed hereunder.

Retarded growth in young animals is seen. Reproduction is seriously affected. Greying of hair in black rats. Adrenal cortical necrosis results from continued deprivation of the vitamin.

Hpervitaminosis: Toxicity of pantothenic acid is not known.

15.3.5 Pyridoxine (Vitamin B_6)

Introduction: The discovery of pyridoxine revealed the complex nature of the vitamin. Vitamin B_6 was given other names like rat acrodynia factor, rat antidermatitis factor and vitamin H.

Gyorgy showed that an eluate of a charcoal adsorbate of yeast extract was able to cure a type of dermatitis in rats that developed on synthetic rations containing B_1 (thiamine) and B_2 (riboflavin). This condition differed from that found in the absence of the pellagra-preventive factor (then thought to be B_2) and was characterized by denuding around the paws, nose and mouth. Thus, at least two antidermatitis factors for the rat became known, and the dual nature of the so-called B_2 preparations was established.

In 1938, Kerpasztesy and Stevens isolated pure substance from rice polishings and studied certain properties of the material. The structure of vitamin B_6 was elucidated by Stiller and others and by Harris and co-workers. Details of one method of synthesis were described by Harris and Folkers in 1939. The synthesis was also accomplished at about the same time by a group of German workers headed by Kuhn. The name of the compound was pyridoxine given at this time.

There are three compounds, interconvertible in the animal body, which possess vitamin B_6 activity. In general, pyridoxine is a general term indicating vitamin B_{6-} active material. Specifically this is not correct, since pyridoxine is a definite compound, as are pyridoxal and pyridoxamine and all three have B_6 activity. The phosphorylated compounds likewise have vitamin activity in animals.

Structure and Properties of Pyridoxine

Pyridoxine is a basic substance. The colourless crystals melt at 160°C. The compounds are soluble in alcohol and water, but only slightly soluble in ether or chloroform. The vitamin is generally used as the hydrochloride salt which melts at 260°C to 280°C with some decomposition. The salt is highly soluble in water at pH about 3, less soluble in alcohol and insoluble in ether. The vitamin shows marked absorption in the UV range.

The structure of pyridoxine and some related compounds are shown in Figure 15.17.

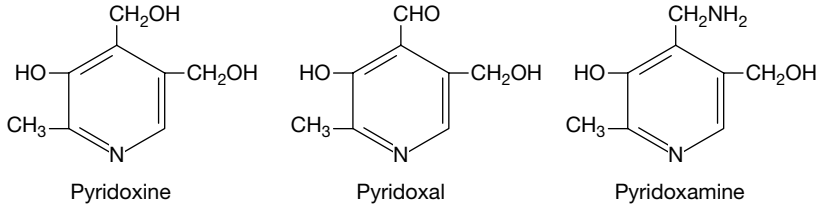


Figure 15.17 Pyridoxine

Coenzyme activity: Coenzyme pyridoxal phosphate is the active form of pyridoxine. The synthesis of the coenzyme from pyridoxal and ATP was demonstrated by Hurwitz.

$$\text{Pyridoxal} + \text{ATP} \rightarrow \text{Pyridoxal phosphate} + \text{ADP}$$

The phosphorylating enzyme, pyridoxal kinase, is found in human brain. All three forms of the vitamin are phosphorylated by the phosphorylating enzyme pyridoxal kinase and Zn^{++} plays an important role in activating them.

For the decarboxylation of amino acids, the coenzyme is required which is named as codecarboxylase, so as to differentiate it from cocarboxylase, (thiamine pyrophosphate) which is involved in decarboxylation of α-keto acid.

A numeral amino acid derivatives not originate from proteins are also decarboxylated by enzymes, needed pyridoxal phosphate, such as the functional role of the coenzyme in the decarboxylation of 5-hydroxytryptophan with the production of 5-hydroxytryptamine (serotonin) plus CO_2.

The next step in metabolism of tryptophan involves the enzyme kynureninase, which converts kynurenine to anthranilic acid, is pyridoxal phosphate–dependent.

Sources: The sources of this vitamin are milk or meat products. Dragon fruit is a good source of pyridoxine.

Daily Requirement: The recommended intakes of vitamin B_6 are up to 200 mg per day in adults.

Absorption and Storage: It is readily absorbed from the small intestine. The excess amount if ingested is not stored in the body but is excreted in urine.

Deficiency: The early descriptions of a pyridoxine deficiency in rats were one of multiple deficiency; however, other unknown factors were absent from the synthetic diets. At present, there is recognized specific syndrome representing pyridoxine requirement in rats. This includes acrodynia or a typical dermatitis which is generally symmetrical and affects the paws and various parts of the head. Seborrheic lesions are frequent. Oedema of the connective tissue layer of the skin is thought to be a characteristic feature. Loss of muscle tonus was observed after long-continued deprivation in rats.

Hypervitaminosis (Toxicity): The toxicity of vitamin B_6 is known only if taken in high doses that is more than 200 mg/per day for long period of time as it then can cause neurological disorders.

15.3.6 Biotin (Vitamin B_7 or Vitamin H)

Introduction: In 1916, the toxicity of diet high in egg white was observed. Later, Boas in 1927 described egg white injury in the rats that were fed diets containing raw egg white as the source of protein. She described muscle incoordination, dermatitis, loss of hair and nervous manifestations as symptoms of this syndrome. All these symptoms were prevented by giving diet with egg yolk. The factor was called antiegg white injury factor. She observed that cooked egg white was not toxic and that liver, yeast and certain other food apparently contained a substance that protected rats against the toxicity of the raw egg protein. The protective substance was called vitamin H by Gyorgy. Lease and Parsons showed that chicks were prone to egg white injury. Williams and co-workers some six years later demonstrated that egg white injury in rats and in

chicks was actually due to an antivitamin in egg white. This substance, known as avidin, was a basic protein and its ability to inactivate biotin was confirmed in 1941.

Previous to this, a potent growth stimulant for yeast had been isolated from dried egg yolk by Kogl and Tonnis and had been named as biotin. Coenzyme R was the name given to another growth factor isolated in 1933. In 1940, Gyorgy, Du Vigneaud and their co-workers announced the identity of vitamin H (antiegg white injury factor), coenzyme R and biotin. Du Vigneaud and co-workers characterized biotin and published its structure in 1942. Harris and others announced the synthesis of d-biotin in 1943.

Structure and Properties of Biotin

Biotin (d or natural isomer) is a monocarboxylic acid, only slightly soluble in water (0.03 to 0.04 g per 100 ml at 25°C and 1 g per 100 ml at 100°C) and alcohol (0.06 g per 100 ml at 25°C). Salts of the acid are quite soluble; the sodium salt can be prepared in 20 per cent aqueous solution. The free acid is practically insoluble in acetone and ether. The colourless crystalline needles melt from 231°C to 232°C. Water solutions (pH 4 to 9) are stable at 100°C, and the dry material is both thermostable and photostable. The vitamin is destroyed by acids and alkalies only on rigorous treatment and by oxidizing agents such as peroxide and permanganate.

The specific rotation, [α] 26/D, is 91.0° in 0.1 N NaOH and it shows maximum absorption in the UV at 234 nm.

Biocytin is a term designating a bound form of biotin first isolated from yeast by Wright and co-workers. It was identified as ε-N-biotinyl-lysine and later synthesized. It occurs in plant and animal tissues also. The synthetic material reacts in the same way as naturally occurring biocytin with respect to microbiological activity, combination with avidin hydrolysis rates, infrared absorption and other criteria. In biocytin, the ε-amino group of lysine and the carboxyl of biotin are combined in a manner similar to that in a peptide bond. Another bound form of the vitamin soluble bound biotin contained in peptic digests of hog liver and other tissues were found to be convertible to free biotin by an enzyme from liver named biotinidase. See the structure of biotin in Figure 15.18.

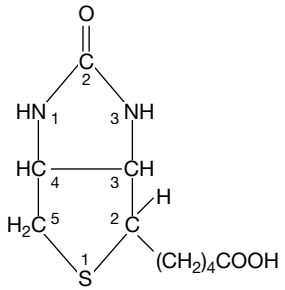


Figure 15.18 Structure of biotin

Biochemistry and coenzyme activity of biotin: In a few specific reactions, like carboxylations and decarboxylations, biotin is required. In aspartic acid and biotin-deficient medium, the growth of *L. arabinosus* is promoted by oxaloacetate. Coenzymes of various carboxylating enzymes are constituted by biotin. The formation of methyl malonyl CoA from the propionic acid upon reaction of propionyl CoA carboxylase with CO_2 and ATP, and then, propionyl CoA carboxylase in

this form incorporates CO_2 into propionic acid. The formation of a biotin–CO_2 combination (an active CO_2 molecule) takes place upon reaction of CO_2 with biotin (Figure 15.19). According to Lynen and co-workers, the CO_2 combines with the nitrogen atom. Another carboxylation reaction results in malonic acid from acetic acid. The carboxylation reaction is visualized as a two-step mechanism:

$$CO_2 + \text{Biotin-enzyme} + ATP \longrightarrow CO_2\text{–biotin-enzyme} + ATP + Pi$$

$$CO_2\text{–biotin-enzyme} + \text{Acetyl-CoA} \longrightarrow \text{Malonyl CoA} + \text{Biotin-enzyme}$$

Figure 15.19 The formation of an Active CO_2 Molecule

In oxalacetic transcarboxylase enzyme, the point of CO_2 attachment is on the nitrogen of biotin. According to Wood and others, in oxalacetic transcarboxylase enzyme the point of CO_2 attachment is on the nitrogen of biotin. Upon the urea production in the body, a reaction takes place between carbamyl phosphate and ornithine to form citrulline.

The biotin role in purine biosynthesis is well known in microorganisms. Biotin deficiency decreases fatty acid synthesis in animals.

Sources of Biotin: The sources of biotin are some vegetables, liver and egg yolk.

Daily Requirement: The daily requirement of biotin is 20–40 μg in children, 10–15 μg in infants and 50–60 μg in adults.

Deficiency: Biotin deficiency can be caused by the too much eating of raw egg whites, which contains the protein avidin in large amount, which strongly binds to biotin. Avidin is destroyed during cooking, whereas upon cooking biotin remains intact. Some symptoms of biotin deficiencies are conjunctivitis, alopecia, dermatitis and neurological symptoms.

15.3.7 Folic Acid (Vitamin B_9 or Vitamin M)

Introduction: The development of our present knowledge of folic acid, called petroglutamic acid, resulted from many studies of nutritional needs of animals, on the one hand, and of bacterial requirements, on the other. It is no wonder then that this compound has been assigned a wide variety of designation, since so many test animals and different bacteria have been employed under diverse experimental conditions in the elucidation of its nature. Also, a variety of symptoms were used as deficiency criteria in animals. Further complications surely resulted from the fact that the vitamin occurs in several chemical forms. A few of the names previously applied to this vitamin include vitamin M (a haematopoietic factor for monkey), vitamin B_c (chick growth factor), factor R (bacterial growth), norite eluate factor (*L. casei*), *L. casei* factor, vitamin$_{10}$ and vitamin$_{11}$.

In 1941, folic acid was assigned to a principle required by *S. lactis* R by Mitchell, Snell and Williams. The term folacin is now commonly used as a synonym for folic acid.

About this time, investigators recognized a difference between fermentation *L. casei* factor and liver *L. casei* factor, although the products of chemical degradation were the same. It developed that the fermentation factor contains three molecules of glutamic acid, one molecule of p-aminobenzoic acid and a substituted pteridine, while the liver factor is chemically similar save for the fact that only one molecule of glutamic acid is present per molecule of vitamin. In 1946, the isolation proof of structure and synthesis of folic acid were described by a group of workers.

Structure and Properties of Folic Acid: Folic acid is a yellow crystalline material soluble in water to about 0.1 per cent. It is soluble in dilute alcohol and can be precipitated from solution as the barium or lead salts or with basic precipitants, such as phosphotungstic acid. The structure of folic acid is shown in Figure 15.20.

The identity of vitamin M (folic acid), factor U, R and S, vitamin B_c, norite eluate factor, and liver *L. casei* factor with pterolglutamic acid is established. The fermentation *L. casei* factor differs in that three glutamic acid residues are present per molecule, and vitamin B_c conjugate contains seven glutamic acid residues.

The naturally occurring enzyme, vitamin B_c conjugase, hydrolyses folic acid–like compounds with several glutamic acid residues to pteroyglutamic acid and glutamic acid. This enzyme is widely distributed in animal tissues and may be of importance in converting pteroylglutamates to PGA, although pteroylglutamic acid, pteroyltriglutamic acid and pteroylheptaglutamic acid are active as haematopoietic agents for man.

Two diglutamic acid derivatives have been synthesized. Pteroyl-α-glutamylglutamic acid (diopterin) is inactive for *L. casei and S. faecalis* R (rhizopterin) but active in the types of human blood dyscrasias. Folic acid is water soluble and it is stable at neutral pH. Its activity is not lost if heated at 120ºC for 30 min at neutral pH.

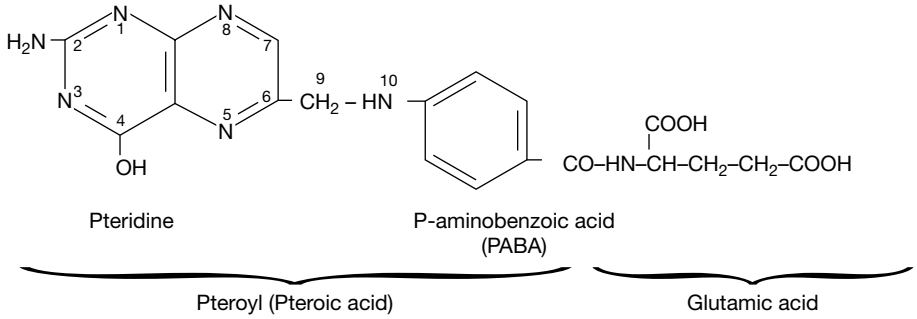
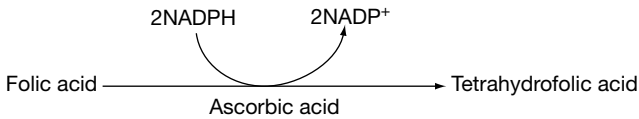


Figure 15.20 Pteroglutamic acid (PGA): Folic acid

Coenzyme activity: Folinic acid, citrivorum factor (CF) and leucoborin are names for a naturally occurring derivative of folic acid. Unlike folic acid, this molecule supports the rapid growth of *Leuconostoc citrivorum.* It has been synthesized and is one of the active coenzyme forms of the vitamin involved in one carbon (C_1) transfer mechanism. The reduction products of folic acid act as coenzymes. Folic acid is reduced to dihydrofolic acid (DHFA or FH_2) by folic reductase, and then, dihydrofolic acid is further reduced to 5,6,7,8-tetrahydrofolic acid (THFA or FH_4) by dihydrofolic reductase.



The formation of FH_4 from FA is associated with the oxidation of NADPH or NADH and requires the presence of ascorbic acid.

Structure of tetrahydrofolic acid, THFA or FH_4 is shown in Figure 15.21.

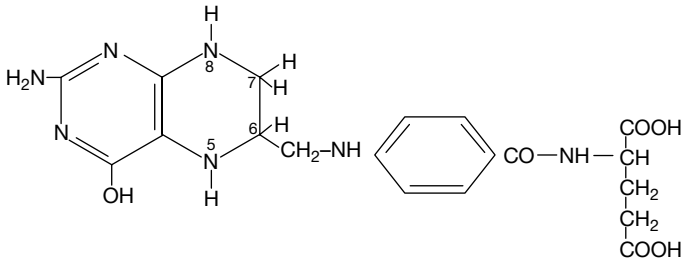


Figure 15.21 Tetrahydrofolic acid, THFA or FH4 (5,6,7,8-tetrahydropteroylglutamic acid)

The vitamins of B_9 group are involved in one-carbon metabolism in a way similar to the two-carbon metabolism in which CoA is involved.

Citrivorum factor (CF) is 5-formyl derivative of tetrahydrofolic acid and is so named because it supports the growth of *L. citrivorum*.

Sources: Leafy vegetables such as spinach, asparagus, turnip greens, lettuces, sunflower seeds, beans and peas, beer, grapefruit juice, banana, raspberry, grapefruit, strawberry and some vegetables like beets, corn and tomato juice.

Daily Requirement: The daily requirement details are shown in the table given hereunder.

Women		Pregnant Women	Men
RDA	400 μg DFE	600 μg DFE	400 μg DFE
UL	100 μg DFE	1000 μg DFE	1000 μg

Deficiency: Folic acid deficiency leads to certain disease like defect in human reproduction, heart disease, stroke, cancer, obesity and depression.

15.3.8 Vitamin B_{12} (Cobalamin)

Introduction: The antipernicious anaemia factor of liver extract was isolated in 1948 by Rickes and co-workers. Various workers had been studying the same or a similar substance found in other material. The 'animal proteins factor', which promotes the growth of animals on diets containing vegetable proteins and is found in such materials as fish solubles and cow manure, is similar to the factor isolated from liver extract. The name B_{12} was given to the vitamin.

Properties of Vitamin B_{12}

Cyanocobalamin crystals are tasteless and odourless. One gram dissolves in about 80 ml of water at room temperature, forming a neutral solution. The pure material is quite soluble in alcohol and insoluble in ether and acetone. In aqueous solution, crystalline cyanocobalamin has three absorption maxima at 278 nm, 361 nm and 550 nm, with extinction coefficients of 115, 107 and 64, respectively. It is remarkable that cobalamin contains about 4.35 per cent of cobalt. The molecular weight is 1355.

Cyanocobalamin has a net charge of one. The cobalt has a coordination number of six. It has one coordinate linked cyanide group, one coordinate pyrrole nitrogen and a coordinate link to a nitrogen of the 5,6-dimethylbenzimidazole moiety (Figure 15.22). Other B_{12}-active compounds are known in which the cyanide radical is replaced by various groups forming other cobalamins, such as hydroxycobalamin, chlorocobalamin, nitrocobalamin and thiocyanatocobalamin. Treatment with cyanide converts these molecules into cyanocobalamin.

The similarity of the cyanocobalamin molecule and the porphyrins is of interest. In B_{12}, two pyrrole rings are jointed directly rather than through the methene (–CH=) bridge as in other porphyrins.

Coenzyme activity, physiological action of vitamin B_{12}: Many microorganisms require B_{12} for growth. Lactic acid bacteria are highly sensitive to deficiency of this vitamin. The B_{12} requirement of some bacteria is met by methionine, or one of the pseudocobalamins. Other microorganisms synthesize quantities of B_{12} above their requirement.

Animal protein factor (APF) was known to be an essential nutrient for growth in experimental animals and after the discovery of B_{12}. It was established that the vitamin possessed high APF activity in the chick. The vitamin is known to be essential for the growth of other laboratory animals and nonruminant farm animals.

It is likewise essential in human nutrition. More specifically, has been B_{12}implicated in several metabolic reactions.

One coenzyme form of vitamin B_{12}, 5,6-dimethylbenzimidazole carbamide coenzyme, is identical with the vitamin except that in the place of –CN radical that is attached to cobalt, an

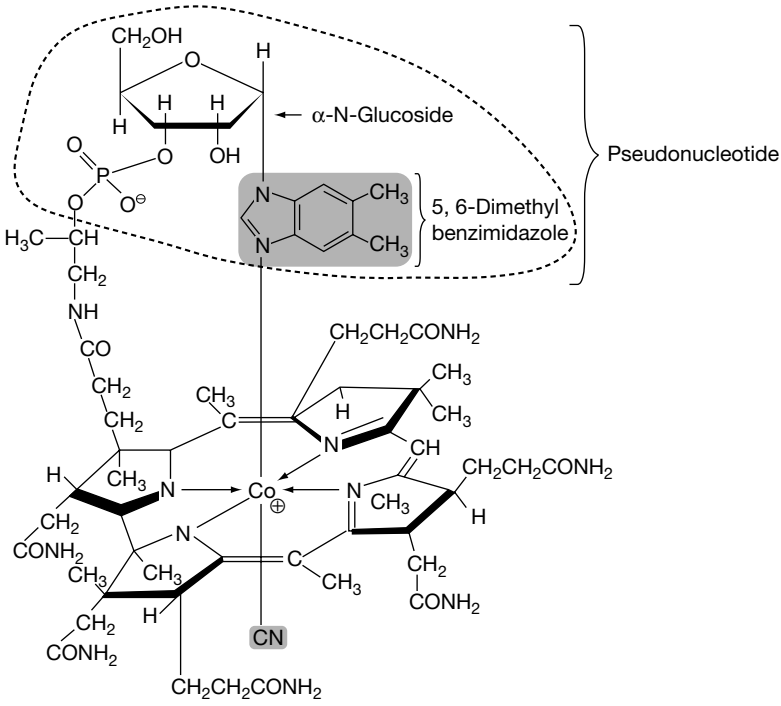


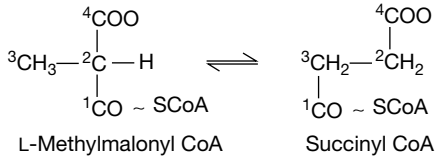
Figure 15.22 Vitamin B_{12}, cyanocobalamin

adenine nucleoside, in which the sugar is 5′-deoxyribose Vitamin C and the attachment of cobalt is to the 5′ carbon atom. The coenzyme acts in the enzyme system which catalyses the reversible isomerization of methylmalonyl CoA to succinyl CoA in mammalian and microbial metabolism and in the isomerization of methylaspartate to glutamate in microorganisms.

Vitamin B_{12} coenzyme reactions can be grouped into four general systems. Specific examples of these four general reactions are as follows:

1. **General:** Carbon–carbon bond cleavage

   Specific: L-Methylmalonyl CoA mutase, which uses 5′-deoxy-adenosyl cobalamin as coenzyme



2. Another coenzyme of B_{12} has a methyl group attached directly to cobalt in place of the deoxyadenosine nucleoside. This coenzyme participates in the formation of methionine from homocysteine in the presence of N^5-methyl FH_4 and other factors.

   Methyl activation:

$$\text{Homocystein} + N^5 - CH_3 - \text{THF} \xrightarrow[\substack{N_5-CH_3-\text{THF} \\ \text{Homocysteine transmethylase}}]{Mg^{++}} \text{Methionine} + \text{THF}$$

   The methyl B_{12} coenzyme is an intermediate in the transfer of the methyl group. This coenzyme also acts in the methylation of the purine ring in thymine synthesis. B_{12} has been implicated in cholesterol metabolism, porphyrin biosynthesis and other phases of metabolism.

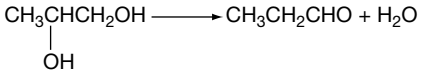
3. Another coenzyme function of cobamide coenzymes involves the metabolism of 1, 2-glycols. Abeles and others showed that cell-free extracts of *Aerobacter aerogenese* treated with charcoal lose their ability to mediate the conversion of ethyleneglycol to acetaldehyde and propanediol to propionaldehyde. Full activity was restored to the treated extract after addition of cobamide coenzyme. Further studies by these investigators suggested that the enzymatic reaction involves a transfer of hydrogen from C-1 to C-2 of the diol and a loss of the elements of water:

$$\underset{(2)}{CH_2OH} - \underset{(1)}{CH_2OH} \rightarrow CH_3CHO + H_2O$$

   Further purification studies of the enzyme called dioldehydrase showed that hydroxocobalamin and cyanocobalamin are inhibitors of the reaction. Certain interactions of enzyme and coenzyme were postulated and thought to be related to catalytic activity.

   General: Carbon–oxygen bond cleavage

Specific: (a) diol dehydrase. This type of reaction occurs in bacteria. The enzymatic mechanism is very complicated:



4. Another coenzyme function of cobamide coenzymes involves the carbon–nitrogen bond cleavage:

   General: Carbon–nitrogen bond cleavage

   Specific: D-α-lysine mutase:

Vitamin B_{12} Deficiency

Vitamin B_{12} deficiency has the following pathomorphology and symptoms:

The main syndrome of vitamin B_{12} deficiency is pernicious anaemia. The symptoms of the disease are as follows:

Megaloblastic anaemia, gastrointestinal symptoms and neurological symptoms.

Sources: The sources of vitamin B_{12} are fish and shellfish, meat (mainly liver), poultry, egg, beef, milk and milk products.

Daily Requirement: The daily requirement of vitamin B_{12} in an adult is 2 to 3 μg per day, in pregnant women is 2.6 μg per day and in lactating woman is 2.8 μg per day.

Absorption: Castle and others some years ago suggested that in pernicious anaemia, there is a deficiency of an intrinsic factor (stomach factor) and an extrinsic factor (food factor). The two factors were thought to react to form something required for the maturation of red blood cells. The extrinsic factor (EF) of Castle is now established to be vitamin B_{12}. The intrinsic factor, a low molecular weight mucoprotein, normally occurs in gastric juice; and pernicious anaemia is due to lack of this substance, since B_{12} is not absorbed in its absence. The mechanism by which intrinsic factor brings about absorption is still not clear.

However, it has been proposed that IF removes the vitamin from natural protein complexes with animal proteins. It also brings about absorption of the vitamin into the mucosal cells with the aid of an intestinal juice factor called releasing factor. Herbert studied the source of this factor in rats and found it to be in the proximal end of the small intestine. It is known that IF has a high degree of specificity. Fortunately, hog and human IF have similar actions in humans. In very large doses, B_{12} is absorbed in humans without IF, but not with doses found in ordinary diets. Small doses parenterally are highly effective in deficiency states.

After absorption into the blood, B_{12} is bound to plasma proteins and may circulate to the sites of activity. That portion converted into coenzymes is stored principally in the liver. Small amounts of B_{12} occur in blood of normal individuals. The variations are wide, but around 100 mg

to several hundred mg per ml of blood have been found and one-tenth or less of these amounts in pernicious anaemia patients. The stools of pernicious anaemia patients contain large amounts of the vitamin after oral administration if no intrinsic factor is given.

15.3.9 Vitamin C or L-Ascorbic Acid

Introduction: The earliest accurate description of the scurvy disease and its control was published by Lind in 1757 in his *Treatise on Scurvy*. Scurvy in man has been known for centuries. For centuries, the use of fresh vegetables and especially of the juice of lemons and of limes was established as specific cures for the disease scurvy. Years later, limes became a required item in the diet of the English Navy. Vitamin C deficiency not only was prevalent on long sea voyages because fresh foods were unavailable, but also was epidemic over parts of the world during times of famine and war. During the wars of our own times, outbreaks of scurvy in parts of the world have been common.

In 1907, Holst and Frolich initiated progress in the development of specific knowledge concerning vitamin C. These investigators showed that a scorbutic state could be produced in guinea pigs by restricting the diet of these animals to oats and bran. This development paved the way for intensive research into the properties of a protective substance in certain foods as well as into the biochemical defects associated with the scorbutic state.

The important information on the concentration of active anti-scorbutic material from lemon juice and on certain important chemical properties of the active substance was provided by Zilva and associates. They showed, for instance, that following oxidative destruction of the principle, the activity could be restored by reduction and proposed a relation between anti-scorbutic activity and reducing power of his preparations. A strong reducing substance was isolated from adrenal glands of rats and from citrus juices in 1928 by Szent Gyorgi. The name hexuronic acid was given to this material.

The hexuronic acid and reducing substance of Zilva were shown to be vitamin C, the anti-scorbutic vitamin. In 1932, Waugh and King reported that the vitamin C isolated by them from lemon juice and the reducing heuronic acid studied by others were identical because of the similarity in many chemical and physical properties as well as in biological potency in protecting guinea pigs against scurvy.

The true configuration of the molecule was established in 1933 by Reichstein and co-workers and Norman Haworth.

Chemistry of Ascorbic Acid

Vitamin C is sour in taste. It is odourless and white crystalline substance. It melts at 190°C–192°. It is insoluble in most organic solvents, although a 2 per cent solution can be made in alcohol. In water, the vitamin is soluble to the extent of 1 g in 3 ml.

Ascorbic acid forms salts of several metals. Ascorbic acid can be reduced by hydrogenation, by taking up iodine at the double bond. Dehydroascorbic acid is formed by oxidation of ascorbic acid. This is a freely reversible reaction. However, H_2S may be used for the reduction of the oxidized form. The dehydro form undergoes hydrolysis at the lactone ring with the formation of diketogulonic acid and oxalic acid (see Figure 15.23). The reverse of this reaction does not proceed in the body but can be brought about in the laboratory.

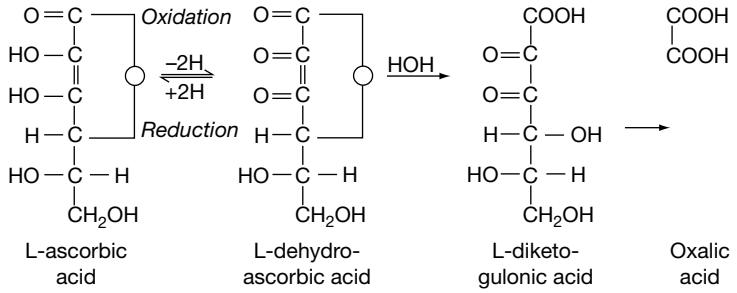


Figure 15.23 Oxidation of ascorbic acid yields dehydroascorbic acid, and this is a reversible reaction. The dehydro form undergoes hydrolysis at the lactone ring with the formation of diketo-gulonic acid

The greater stability of ascorbic acid solution depends on the decreased tendency towards hydrolysis of the lactone ring with decreasing pH. In alkaline solution, the hydrolysis is fairly rapid and such solutions lose vitamin activity in a short period. In vitro, the ascorbic acid undergoes oxidation and catalysed by the copper ion and the plant ascorbic acid oxidase. With increasing pH, rate of destructive oxidation is greater. Molecular oxygen, plays an important role in this type of oxidation, in processing various vitamin C-containing foods. Low-temperature storage of vegetables before processing, though usually impractical, and a quick preheating (blanching) just previous to canning or freezing also aid in decreasing ascorbic acid destruction.

Several synthetic compounds have some vitamin C activity. Thus, 6-desoxy-L-ascorbic acid has about one-third, and L-rhamnoascorbic has about one-fifth, D-araboascorbic about one-twentieth, and L-glucoascorbic around one-fortieth the activity of L-ascorbic acid. The activity of D-ascorbic acid is nil.

Sources: The sources of vitamin C are fruits like kakadu, rose hip, sea buckthorn, Indian gooseberry, acerola, papaya, strawberry, jujube, orange, lemon, melon and the camu camu fruit.

Daily Requirement: The daily requirement of vitamin C in infants is 35 mg per day, in children 40 mg, in adults 45 mg, in pregnant and lactating women is 60 and 80 mg per day, respectively.

Absorption and Storage: Ascorbic acid is readily absorbed from the small intestine peritoneum and subcutaneous tissues. It passes through the portal vein to the general circulation and to all tissues. It is supplied to the placental barrier readily. The placenta is also able to concentrate this vitamin. It is not stored in any particular organ and is distributed throughout the body. Each organ or tissue has an optimal saturation level of ascorbic acid. Excessive intake of ascorbic acid does not increase the saturation level, but the excess is excreted in the urine.

Deficiency: The disease due to deficiency of vitamin C is scurvy. The symptoms of the disease are the formation of spots on the skin, mainly on thighs and legs, spongy gums and bleeding through mucous membranes.

Vitamin C Hypervitaminosis: Vitamin C when taken in large doses leads to headache, diarrhoea, nausea, vomiting and lack of sound sleep.

15.4 MINERALS

Minerals are essential for various body functions such as for the contraction of muscle and nerve conduction. They are also important for cellular respiration systems. Minerals are important to maintain the body's acid–base balance and enzyme function. Minerals should be replaced every day as they are lost through sweating, urine, faeces and tears. Daily requirements of minerals are different for men, women, children and pregnant women.

Mineral are classified into the following two types:

1. Macrominerals
2. Microminerals

15.4.1 Sources of Minerals

Macrominerals

Sources:

- **Calcium:** Milk, egg, leafy green vegetable, fish, meat soya beans, etc.
- **Phosphorus:** Milk, peas, soya beans, meat, fish, eggs, almonds, wheat germ, etc.
- **Magnesium:** Cereals, vegetables, nuts and beans are rich sources of magnesium.
- **Sodium:** Table salt, eggs, meat, milk, cheese, butter, margarine, etc.
- **Potassium:** Vegetables like spinach, beans, peas; fruits like oranges; dairy products like milk, meat, nuts and butter.

Microminerals

Sources:

- **Iron:** Liver, eggs, meat, green vegetables, potatoes, soya beans, black beans, spinach, etc.
- **Zinc:** Most of the foods contain zinc.
- **Iodine:** Seafoods such as fish and fish oil. Vegetables like spinach, fruits and cereals.
- **Chromium:** Chromium is found in whole grains, egg yolks, brewer's yeast, liver, meats and nuts.
- **Cobalt:** Liver and red meat.
- **Copper:** Most of the foods contain copper.
- **Manganese:** Vegetables and most other foods.
- **Molybdenum:** Most of the foods contain molybdenum.
- **Selenium:** Foods containing selenium include meat, poultry, grains and seafood.
- **Fluorine:** Water, milk, etc.

15.4.2 Macro Minerals

Calcium: Quantitatively, this is present in the largest amount on account of its being the main constituent of bone and teeth. There is a total of about one kilogram calcium in man 98 per cent

of which is present in bone in the form of hydroxyapatite crystals only about 1 per cent in our teeth, and the rest in the other tissues and the circulation.

Blood and body fluids also contain calcium in small quantities and exert a regulatory function on neuromuscular irritability and blood coagulation and on the permeability of cell membranes and capillaries.

It is required for regulating a large number of cellular activities, nerve and muscle function, hormonal action, blood coagulation and cell motility. These actions are mediated by an intracellular protein—calmodulin—which is present in all nucleated cells and acts as a receptor molecule for Ca^{++}. Phenothiazine drugs act as potent inhibitors of calmodulin action.

Unlike potassium, which is present in all sources of food, calcium is present only in milk and milk products, egg yolk, legumes, nuts and green leafy vegetables. Adult man requires about 400–500 mg per day. The metabolism of this mineral is closely interrelated with phosphorus metabolism and regulated by vitamin D and parathormone.

Absorption

Unlike sodium, which is readily absorbed from the intestines, the absorption of calcium is incomplete. A high-protein diet favours its absorption, whereas a high-cereal diet will diminish it. On a high-protein diet, 15 per cent of dietary calcium is absorbed. If the protein content is low, only 5 per cent might be absorbed. The cereal contains phytic acid that forms an insoluble salt with calcium. Oxalates, present in vegetables like cabbage and spinach, also prevent absorption. An acidic pH of the intestine favours absorption. A ratio of food calcium to phosphorus not more than 2:1 and not less than 1:2 is necessary for the optimal absorption of calcium. Calcitriol promotes its absorption. The presence of large amounts of unabsorbed fatty acids, as in sprue syndrome, will cause its excretion as calcium soap.

Hypoparathyroidism: In this condition, the serum calcium levels are reduced to 7 mg/100 ml or and below the serum phosphate levels increase due to a decreased excretion in urine. A condition called 'tetany' occurs characterized by increased neuromuscular irritability, spasms and convulsions.

Rickets: It is another manifestation in children. It is mainly due to deficiency of vitamin D. Calcium metabolism is also affected.

Phosphorus

Phosphorus has many biological roles since its presence in the body is in the form of inorganic phosphate or phosphate esters. The absorption of phosphorus is regulated by vitamin D. Phosphorous is important for many functions in our body, like carbohydrate metabolism, transport processes, muscle function, structure of the cell membrane, nucleic acids and energy storage. Adenosine triphosphate (ATP) is an energy storage molecule and is used as a source of energy for several biological processes. Phosphorus is an important constituent of bones and teeth. The phosphate buffer system also maintains the pH range in our body.

Sources of phosphorus: Rich sources of phosphorous are black beans, milk, peas, soya beans, meat, fish, eggs, cottage cheese, almonds, wheat germ, etc.

The RDA of 700 mg of phosphorus is required for males and females. This is the RDA value of phosphorous.

The high doses of phosphorous may cause high blood pressure, nausea, diarrhoea, muscle paralysis, cramps, mental confusion and abnormal heart rhythms.

Paratharmone decreases re-absorption of phosphorous by renal tubule and decreases serum phosphorous levels.

Renal failure increases serum phosphorous due to diminished excretion by tubules.

Magnesium

Magnesium works in combination with several enzymes that are concerned in energy metabolism, synthesis of protein and nucleic acid. People suffering from magnesium deficiency are given supplements of magnesium such as many salts, chloride, gluconate, lactate, sulphate and oxide.

Magnesium is necessary for the activity of several enzymes in carbohydrate metabolism, for example, hexokinase, phosphofructokinase, enolase, pyruvate kinase, adenyl cyclase, glucose-6-phosphate dehydrogenase, etc.

Large doses of magnesium lead to low blood pressure and impairment of the central nervous system. Migraine headache is due to deficiency of magnesium ions.

Magnesium sources are cereals, vegetables, nuts and beans. Magnesium is excreted through urine and faeces.

Sodium

Sodium is present in both bone and soft tissues. But it is the constituent of the body fluids, including blood, which is of greatest importance metabolically. Whole blood contains 160 mg/100 ml and plasma contains 330 mg/ml. Adrenal cortical hormones are required for the normal sodium metabolism. In their deficiency, there is an increase in the renal excretion of sodium leading to a fall in blood sodium levels. In chronic renal failure and other conditions, such as cirrhosis of liver, there is retention of sodium and increased blood sodium and increased amounts of body fluids.

Normal diet contains 5 to 15 g added in food during preparation and 95 per cent of it is excreted in urine and sweat. About five 5 g of sodium chloride per day is adequate for normal healthy adult.

The sources of sodium are table salt, butter, eggs, cheese, meat, milk, etc.

High doses of sodium may cause hypertension.

Potassium

Potassium referred to as one of the key electrolytes in our body. Potassium, along with sodium, plays a vital role in regulating the fluid levels in our body. The normal diets contain about 4 g/day. It is excreted through urine. Plasma contains 5 m. eq/l. Along with sodium and calcium, it regulates neuromuscular irritability.

Hypofunction of adrenal cortex (Addison's disease) leads to increased serum K^+ level. Hyperfunction of adrenal cortex (Cushing's syndrome) lowers serum K^+ level. Certain diuretics like acetazolamide increase urinary excretion of potassium and lower its level in serum.

Sources of potassium are butter, milk, beans, spinach, peas, meat, fruits nuts, oranges and vegetables.

15.4.3 Micronutrients or Trace Minerals

Essential trace elements range from metals to nonmetals and their importance is due to their changeable oxidation state. In our body, trace elements are essential parts of oxidative–reduction enzymes. These trace elements play an important role in proteins transport, cofactors and detoxification.

Iron

Iron is a component of haemoglobin, myoglobin and cytochromes.

Absorption of iron is usually about 1 mg of food. Unabsorbed iron is excreted in faeces. Absorption occurs mainly in the stomach and duodenum. Gastric HCl converts organic iron to inorganic iron. Ascorbic acid and other reducing substances present in food convert ferric iron to ferrous iron.

Absorption into the intestinal epithelial cell occurs in the ferrous form. In the cell, it is reoxidized to ferric form for storage. When it has to leave the cell, it is reduced to ferrous form. It is transported in blood by a protein called 'apotransferrin' which is converted to transferrin when combined with iron. It is stored in tissues in combination with 'apoferritin' which is converted to 'ferritin' when combined with iron. 'Erythropoetin' facilitates transfer of mucosal iron to transferrin.

If the iron load is excessive, ferritin becomes denatured and breaks into subunits which aggregate to a particular matter called 'haemosiderin.'

Normal plasma has only 90–120 micrograms in men and 120–140 micrograms in women per 100 ml plasma.

Daily requirement for an adult male is 10 mg, for pregnant women, 30 mg and for lactating women, 15 mg.

Sources of iron are meat, liver, egg yolk, green leafy vegetables, etc.

Iron deficiency produces microcystic hypocromic anaemia.

Zinc

Zinc has some biological roles in growth, in the development of the testicles, healing of wound and in the regulation of immune system. Zinc is used as a cofactor for several enzymes, e.g., zinc is a component of alcohol dehydrogenase and few other enzymes. It is also associated with insulin molecule. Around 10–15 mg of zinc is present in our diet. Pancreas, liver, kidney and spleen have high turnover of zinc. Zinc-dependent enzymes take part in the proteins, carbohydrates, nucleic acids and lipids metabolism.

The daily requirement of zinc for men is around 15 mg, for women, 12 mg, for pregnant women, 15 mg and for lactating women, 16–19 mg.

A zinc deficiency in soil can lead to the deficiency in the usual food sources which ultimately lead to zinc deficiency in our diets. Zinc deficiency leads to delayed wound healing as well as impairment of acuity of taste. Zinc deficiency also occur in alcoholic cirrhosis.

Toxicity of zinc may cause flu-like symptoms, fever, fatigue, vomiting, etc.

Iodine

Iodine is well absorbed in the system. Iodine is circulated as both in free and bound form in the biological system. Iodine is stored in the thyroid gland and required for the synthesis of thyroid hormones, that is T3 and T4. These hormones are essential in controlling the basal metabolic rate of elders, and it controls the growth and development in children. Minimum of 25 micrograms of iodine is required daily. Recommended quantity is around 100–200 micrograms. Seawater contains high iodine, and therefore, vegetables grown in seawater are rich in iodine.

Food iodine is first converted to iodide and then absorbed from the intestine. Iodide can also be absorbed from lungs and skin. Iodine is mainly excreted through kidney, and small amount is excreted through saliva, skin, lung and intestine.

Deficiency

Deficiency of iodine leads to an increase in the size of thyroid gland and forms a large nodule that protrudes from the neck is called 'goiter.' Deficiency of iodine leads to a decreased BMR, sluggishness and obesity.

Toxicity

Hypersensitive to iodine can lead to skin rashes, fever, mucous membrane ulcers and swelling in the neck area.

Chromium

Chromium is a vital trace element that is required for nucleic acid, carbohydrate and fat metabolism.

Daily recommended quantity of chromium is 50 to 200 micrograms. The best sources of chromium are whole grains, egg yolks, brewer's yeast, liver, meat and nuts.

Deficiency of chromium is generally seen in people eating highly refined foods. Deficiency of chromium leads to high blood sugar levels. Abnormalities of nerve stimulation are also due to the deficiency of chromium.

Toxicity of chromium may lead to failure of liver, kidney, anaemia, etc.

Cobalt

Cobalt is a constituent of vitamin B_{12} function. Meat is a rich source of cobalt. In some of the lower animals, cobalt stimulates erythropoiesis. It is also said to liberate 'erythropoietin' hormone which stimulates erythropoiesis.

High doses of cobalt can lead to increased red blood cells, bone marrow hyperplasia, etc. High doses of cobalt affect the absorption of iron.

Copper

Copper is essential for the activity of several enzymes. It is essential for the activity of cytochrome, catalase, tyrosinase, monoamine oxidase and ascorbic acid oxidase. Traces of copper are required for normal synthesis of haemoglobin. The erythrocytes contain a colourless copper-containing protein called 'erythrocuprein' in small amounts. Cerebrocuprein is a copper-containing protein present in the brain. Erythrocuprein, cerebrocuprein and another copper-containing protein, hepatocuprein, in liver are all now identified to be an enzyme cytosolic speroxide dismutase.

A copper deficiency produced in experimental animals caused a fatal hypochromic microcytic anaemia. Copper favours absorption of iron from intestines and also its transport from and to tissues.

High doses of copper can cause diarrhoea with bluish green stool. Two diseases are associated with abnormal copper metabolism, which leads to diseases like Wilson's disease, and it is caused due to high levels of copper mainly in the brain, liver, kidney and eye. In male infants, defective copper transport into the blood leads to Menke's kinky hair syndrome.

Manganese

Manganese is important for the activity of several enzymes, such as phosphatase, arginase, carboxylase, isocitric dehydrogenase and cholinesterase, and some of them are involved in lipid, protein and carbohydrate metabolism.

Manganese deficiency produces slipped tendon disease in the chicken. No symptoms are known to occur in human.

Toxicity

High doses of manganese may cause dementia, psychiatric disorders similar to schizophrenia. Iron and manganese affect the absorption of each other.

Daily recommended dose of manganese is not known.

Molybdenum

Molybdenum is part of many enzymes, and it is required for the activity of xanthine oxidase and liver aldehyde oxidase. Molybdenum deficiency leads to headache, tetchiness and laziness. An overload of molybdenum interferes in the absorption of copper and iron. Daily recommended dose of molybdenum is not known.

Selenium

It is a non-metallic element. The sources of selenium are meat, poultry, grains and seafood. Its requirement in human is not yet proved. According to some reports, selenium may protect against cancer. It is important for many animal species. Deficiency of selenium deficiency produces hepatic necrosis, muscular dystrophy, necrosis of the cardiac muscle and several other disorders in various experimental animals.

Selenium is a component of the enzyme which converts the reduced glutathione to its oxidized form. In the conversion, H_2O_2 is used up and its accumulation is prevented. It is also required in some of the immune mechanisms, the biosynthesis of ATP in mitochondria. The renal cortex, pancreas, pituitary and liver contain high amount of selenium. Vitamin E has a beneficial role in some of the selenium deficiency conditions and vice versa.

Fluoride

Actually, fluoride may not act as a necessary trace element, but it has favourable effects on skeletal and dental health. Fluoride assists in the prevention of tooth decay. Hence, tooth decay is prevented due to fluoride. It is important for the prevention of development of osteoporosis in adults, particularly in postmanopausal women. Fluoride is present in drinking water in varying concentration depending on the soil. Normal bones and teeth contain minimal amount of fluoride. It is absorbed from the intestine just like chloride. It remains in the ECF and is taken up by

bones and teeth. Excretion is mainly through urine. Fluoride concentration of 1 ppm in drinking water is optimal. This supplies 1–2 mg of fluoride daily, in such population dental caries are 60–70 per cent less in children as compared to those who live in a population where the fluoride concentration in water is less.

Toxicity of fluoride occurred by ingestion of high levels of fluoride for long time will result in a clinical condition called fluorosis, an early sign in mottling and discoloration of teeth enamel. It can also result in increased bone density and calcification occurs at the point of insertion of tendons into bone and bony exostosis develops. In such a population, people suffer from fluoride toxicity as fluoride concentration in water is as much as 10 to 45 ppm.

EXERCISES

1. Give the chemistry, functions and deficiency manifestations of vitamin A.
2. Mention the sources of vitamin A. Describe the function and effects of deficiency of vitamin A. What is the daily requirement?
3. Describe briefly the chemical nature, action in the body, dietary sources and nutritional importance of vitamin A.
4. Name the fat-soluble vitamins, their occurrence, daily adult requirements and their importance to the body.
5. Give a brief account of the chemistry, sources, daily requirement and deficiency states of thiamine. Describe the mechanism of the action.
6. Describe briefly the chemical nature, mode of action, dietary sources and nutritional importance of thiamine.
7. Describe the chemistry, sources, daily requirement and metabolic functions of (a) thiamine and (b) niacin. Mention the diseases caused by their deficiency in diet.
8. What is riboflavin? Discuss its metabolic role in the body and the deficiency symptoms.
9. What are vitamins? Describe the functions and properties of any two vitamins of B-complex group.
10. Mention the sources of vitamin B_{12} and the effect of its deficiency. Describe the metabolic functions of vitamin B_{12}.
11. Describe the sources, requirements, functions and deficiency manifestations of vitamin C.
12. Describe the metabolism of calcium in the body.
13. Describe the sources, requirements and physiological functions of calcium.
14. What part does calcium play in bodily functions? How is the level of calcium in the blood regulated?
15. Mention the sources of iron in our diet. Describe the mechanism of iron absorption. Mention how iron is transported and stored in our body.
16. State briefly the sources of iron in our diet. Mention the functions of iron in the body. Discuss the metabolism of iron.
17. Discuss the metabolism of sodium in the body.
18. State how copper is metabolized in the body.

19. Write notes on the following:
    (a) Function of manganese in the body
    (b) Iron and its significance
    (c) Function of zinc
    (d) Function of fluorine

MULTIPLE CHOICE QUESTIONS

1. Zinc is a constituent of
   (a) Carbonic anhydrase
   (b) Malate dehydrogenase
   (c) Aldolase
   (d) Amylase

   Ans. a

2. Haemoglobin formation needs both
   (a) Iron and zinc
   (b) Iron and calcium
   (c) Iron and copper
   (d) Iron and magnesium

   Ans. c

3. Calcium is required for the activation of the enzyme
   (a) Isocitrate dehydrogenase
   (b) Fumarase
   (c) Succinate thiokinase
   (d) ATPase

   Ans. d

4. The absorption of calcium is increased by the dietary higher levels of
   (a) Fats (b) Proteins
   (c) Cereals (d) Vitamin A

   Ans. b

5. Calcium absorption is interfered by
   (a) Fatty acids
   (b) Amino acids
   (c) Vitamin D
   (d) Vitamin B_{12}

   Ans. a

6. The percentage of calcium in milligram in non-ionized form is about
   (a) 3 (b) 4
   (c) 5 (d) 6

   Ans. c

7. Retinal is reduced to retinol by retinene reductase in the presence of the coenzyme
   (a) NAD^+
   (b) $NADP^+$
   (c) $NADH+ H^+$
   (d) $NADPH + H^+$

   Ans. c

8. Retinol exists as an ester with higher fatty acids in the
   (a) Liver (b) Kidney
   (c) Lung (d) All of these

   Ans. d

9. Retinol is transported to the blood as retinol is attached to
   (a) α_1-Globulin
   (b) α_2-Globulin
   (c) β-Globulin
   (d) γ-Globulin

   Ans. a

10. Carotenes are transported with
    (a) Proteins (b) Lipids
    (c) Lipoproteins (d) Minerals

    Ans. c

11. In the blood, the vitamin esters are attached to
    (a) α_1-Lipoproteins
    (b) α_2-Lipoproteins
    (c) β-Lipoproteins
    (d) γ-Lipoproteins

    Ans. c

12. The preformed vitamin A is supplied by foods such as
    (a) Butter (b) Eggs
    (c) Fish liver oil (d) All of these

    Ans. d

13. Lumirhodopsin is stable only at a temperature below
    (a) –35°C (b) –40°C
    (c) –45°C (d) –50°C
    Ans. d
14. The normal concentration of vitamin A in blood in IU/dl is
    (a) 20–55 (b) 24–60
    (c) 30–65 (d) 35–70
    Ans. b
15. The activity of tocopherols is destroyed by
    (a) Oxidation (b) Reduction
    (c) Conjugation (d) All of these
    Ans. a
16. Some tocopherols are
    (a) Terpenoid in structure
    (b) Dionol in structure
    (c) Isoprenoid in structure
    (d) Farnesyl in structure
    Ans. a
17. Vitamin E is stored in
    (a) Mitochondria
    (b) Microsomes
    (c) Both of (a) and (b)
    (d) None of these
    Ans. c
18. Vitamin E protects the polyunsaturated fatty acids from oxidation by molecular oxygen in the formation of
    (a) Superoxide (b) Peroxide
    (c) Trioxide (d) All of these
    Ans. b
19. Vitamin K_2 was originally isolated from
    (a) Soya bean (b) Oysters
    (c) Putrid fish meal (d) Alfalfa
    Ans. c
20. Vitamin K regulates the synthesis of blood clotting factors
    (a) VII (b) IX
    (c) X (d) All of these
    Ans. d
21. Vitamin C is required in the metabolism of
    (a) Phenylalanine
    (b) Tryptophan
    (c) Both (a) and (b)
    (d) None of these
    Ans. c
22. Thiamine is also said to be
    (a) Anti-beriberi substance
    (b) Anti-neuritic vitamin
    (c) Aneurine
    (d) All of these
    Ans. d
23. Lipoic acid is also termed as
    (a) Thioctic acid
    (b) Protogen
    (c) Acetate replacement factor
    (d) All of these
    Ans. d
24. Folic acid is also termed as
    (a) SLR factor
    (b) Pteroyl-glutamic acid
    (c) Liver *Lactobacillus casei* factor
    (d) All of these
    Ans. d
25. Thiamine is oxidized to thiochrome in alkaline solution by
    (a) Potassium permanganate
    (b) Potassium ferricyanide
    (c) Potassium dichromate
    (d) Potassium chlorate
    Ans. b
26. Riboflavin in alkaline solution when exposed to ultraviolet light is converted into lumiflavin which ultraviolet light has a
    (a) Greenish yellow fluorescence
    (b) Bluish yellow fluorescence
    (c) Reddish yellow fluorescence
    (d) Light yellow fluorescence
    Ans. a
27. FMN is a constituent of the
    (a) Warburg yellow enzyme
    (b) Cytochrome C reductase
    (c) L-amino acid dehydrogenase
    (d) All of these
    Ans. d

28. Niacin is present in the maize in the form of
    (a) Niatin (b) Niacytin
    (c) Nicotin (d) Nicyn

    Ans. b

29. Nicotinic acid is essential for the normal functioning of
    (a) Skin
    (b) Intestinal tract
    (c) Nervous system
    (d) All of these

    Ans. d

30. Pyridoxine produces a coloured compound with
    (a) 2:6 dichloroquinone chlorimide
    (b) 2:6 dichloroquinone
    (c) 2:4 nitroquinone
    (d) All of these

    Ans. a

31. Pyridoxal phosphate is involved in the desulphuration of
    (a) Cysteine
    (b) Homocysteine
    (c) Both (a) and (b)
    (d) None of these

    Ans. c

32. Pentothenic acid deficiency causes
    (a) Nausea (b) Irritability
    (c) Anaemia (d) All of these

    Ans. d

33. Pantothenic acid exists in the tissues as
    (a) β-mercaptoethylamine
    (b) Coenzyme A
    (c) Pantoic acid
    (d) β-alanine

    Ans. b

34. Folic acid coenzymes take part in the synthesis of
    (a) Purines
    (b) Thymine
    (c) Both (a) and (b)
    (d) None of these

    Ans. c

16 Photosynthesis

CHAPTER OBJECTIVES

16.1 Introduction
16.2 Early Studies on Photosynthesis
16.3 Photosynthetic Apparatus
16. 4 Absorption of Light by Chlorophyll
16.5 The Supramolecular Complexes
16.6 Energy Conservation Process or Photosynthetic Electron Transport Chain
16.7 The CO_2 Reduction (Calvin) Cycle
16.8 The C_4-Pathway
16.9 Photorespiration
16.10 Crassulacean Acid Metabolism

16.1 INTRODUCTION

Photosynthesis is essential for living organisms on earth, since almost all life on the planet depends on it for a food source. Photosynthesis is a process by which CO_2 is converted into organic compounds that are found not only in photosynthetic organisms but also in all living cells. The conversion of CO_2 and H_2O to glucose, as of the same amount of energy that is released when glucose is oxidized to CO_2 and H_2O

$$6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6 + 6O_2$$

$$\Delta G' = +686{,}000 \text{ cal}$$

As the term photosynthesis implies, the energy for this process is provided by light.

The evolution of photosynthesis followed after the evolution of glycolysis and pentose phosphate metabolism. However, it occurs only in the presence of pigments such as chlorophyll. These pigments possessed the ability to absorb solar radiation and convert a portion of solar energy into chemical energy in the form of adenosine triphosphate (ATP), and thus, the process of photophosphorylation evolved.

When significant quantities of CO_2 had accumulated, it became the substrate for photosynthesis as reactions of photophosphorylation and pentose phosphate metabolism combined to yield a light-dependent reduction of CO_2.

The electrons for the reduction of CO_2 were supplied by H_2S and H_2, components present in the primordial atmosphere and certain photosynthetic bacteria remain today as evidence of those early forms of photosynthesis. As photosynthetic organisms continued to evolve, they acquired the capacity to use H_2O as a source of electrons. When this occurred, O_2 was produced and evolving forms were then provided with a new oxidant for a form of respiration, aerobic respiration, previously unknown.

Photosynthesis is the most efficient energy-conserving process operating against a thermodynamic gradient. The light energy is used to raise the chromophoric reaction centre to higher electronic level (to more negative potential) followed by the charge separation and generation of a 'hole' (acquiring a positive potential) and a reductant.

16.2 EARLY STUDIES ON PHOTOSYNTHESIS

16.2.1 Light and Dark Reactions

From the studies performed in 1905, Blackman showed that photosynthesis consists of the following two processes: light-dependent process and light-independent process that is limited in its rate by light-independent or dark reactions. The light-dependent processes exhibit the usual independence of temperature characteristic of photochemical reactions, while the dark reactions are sensitive to different temperatures. Today, the light-dependent processes are recognized as those in which light energy is converted into chemical energy, actually ATP and NADPH. The dark reactions, on the other hand, refer to the enzymatic reactions in which CO_2 is incorporated into reduced carbon compounds previously encountered in carbohydrate metabolism.

Evidence was first provided by Robert Emerson in the 1930s to show that the light-dependent phase of photosynthesis consisted of at least two light reactions. When Emerson measured the amount of photosynthesis carried out by the green algae, *Scenedesmus*, as a function of the wavelength of light, he observed that photosynthesis did not proceed at wavelengths greater than 700 nm. This was surprising, since light of this far-red wavelength was still being absorbed by the algal cells. Emerson subsequently showed that this decrease in the far-red region—the so-called 'far-red drop'—could be reversed to varying amounts if he supplemented the light at 700 nm with a second source of light having a wavelength of 650 nm. **This enhancement of the amount of photosynthesis caused Emerson to** postulate that, in the case of *Scenedesmus*, the assimilation of CO_2 in photosynthesis required light of two different wavelengths. Today, these requirements are met by postulating that these and many other photosynthetic organisms have two photosystems (PS I and PS II) that are activated by light of far-red wavelengths (680–700 nm) and shorter wavelengths (650 nm), respectively.

16.2.2 The Hill Reaction

In 1937, Robin Hill of Cambridge University initiated cell-free studies on photosynthesis by working with isolated chloroplasts rather than intact plants. He reasoned that more information might be obtained if grana or chloroplasts, which contain the chlorophylls, were studied separately from the cell. It would have been ideal if the chloroplasts could have carried out

both the oxidation of H_2O and the reduction of CO_2 to organic carbon compounds. This was not accomplished at that time. Nevertheless, chloroplasts were able to produce O_2 photochemically in the presence of a suitable oxidizing agent, e.g., potassium ferric oxalate. In this reaction, the ferric ion substitutes for CO_2 as an oxidizing agent during the photooxidation of H_2O

$$4Fe^{3+} + 2H_2O \text{ Chloroplasts} \xrightarrow{hv} 4Fe^{2+} + 4H^+ + O_2$$

Molecular oxygen is evolved in an amount stoichiometrically equivalent to the oxidizing agent added. This observation was of fundamental importance, for it permitted the study of the role of H_2O as a reducing agent in photosynthesis. The reaction is known as the Hill reaction and potassium ferric oxalate is known as Hill's reagent. Other compounds were subsequently shown to serve as Hill's reagents in studies on isolated chloroplasts; e.g., Warburg showed that benzoquinone could function as such.

16.2.3 Photophosphorylation

In 1952, it was known that both NADPH and ATP were needed in the conversion of CO_2 to carbohydrates in photosynthesis. Having obtained the NADPH via a Hill reaction, it was thought that re-oxidation of the reduced nicotinamide nucleotide by oxygen through the cytochrome electron transport system of plant mitochondria would produce ATP. In the intact plant cell, containing chloroplasts and mitochondria, both of these organelles would be involved in the production of the two coenzymes NADPH and ATP needed to drive the photosynthetic carbon reduction cycle (Figure 16.5). In 1954, Arnon and his associates questioned whether ATP so produced when they discovered that chloroplasts alone, when isolated by special techniques, could convert CO_2 to carbohydrates in the light reaction. Further studies in Arnon's laboratory showed that chloroplasts, in the absence of mitochondria, could synthesize ATP in two types of light-dependent phosphorylation reactions.

The first type, **cyclic photophosphorylation,** yields only ATP and produces no net change in any external electron donor or acceptor.

$$ADP + H_3PO_4 \xrightarrow[\text{Chloroplast}]{hv} ATP + H_2O$$

The second type, **non-cyclic photophosphorylation**, involves a process in which ATP formation is coupled with a light-driven transfer of electrons from water to a terminal electron acceptor such as $NADP^+$ with the resultant evolution of oxygen:

$$2NADP^+ + 2H_2O + 2ADP + 2H_3PO_4 \longrightarrow 2NADPH + 2H^+ + O_2 + 2ATP + 2H_2O$$

16.3 PHOTOSYNTHETIC APPARATUS

Photosynthesis is carried out by both prokaryotic and eukaryotic cells. The prokaryotes include the blue-green, and the purple and green bacteria; in these bacteria, the light-trapping process takes place in small structures called chromatophores. In eukaryotic organisms, photosynthetic process takes place in chloroplasts that contain the pigment 'chlorophyll.'

The chloroplast, whose structure and compositions are described in Chapter 3, contains the photosynthetic pigments, which are chlorophyll a and b in higher green plants together with certain carotenes, one of which is beta-carotene.

Chlorophylls: Chlorophylls are green pigment of plants, which absorb light for the process of photosynthesis. The most important type of chlorophyll is chlorophyll a, which is present in almost all green plants including some bacteria. Chlorophyll b is present in higher plants and green algae, chlorophyll c in diatoms and brown algae and chlorophyll d in some red algae. Purple bacteria contain another type of chlorophyll—the bacteriochlorophyll, which resembles chlorophyll a.

Chlorophylls in higher plants are specific to the chloroplasts. The chloroplasts are disc like or oval bodies and their average diameter is about 4 to 6 μ. The chloroplast is surrounded by a two-layered membrane, and the pigment is located in particulate structures called grana (singular – granum). The grana are embedded in the hydrophillic matrix of the chloroplast (see Chapter 3 for the structure of chloroplast). The size of the granum ranges from species to species, but the average range is from 0.3 to 0.7 μ. A granum is made up of about 40–50 disc-like sacs—the thylakoids. Adjacent grana are connected by tubular structures called lamellae. The nongranal portion of the chloroplast matrix is called stroma. Grana are lacking in algal cells, but free grana or chromatophores are found in bacteria.

The chlorophylls have a porphyrin structure or a tetrapyrrole nucleus with an Mg atom in the centre of the nucleus. A phytyl group and a methyl group are invariably attached to the nucleus. Various chlorophylls differ in their side chain (Figure 16.1). The structure of chlorophyll a was established by Hans Fischer in 1940. Later on, the structure was confirmed by R. B. Woodward, who could carry on the total synthesis of chlorophyll in 1960.

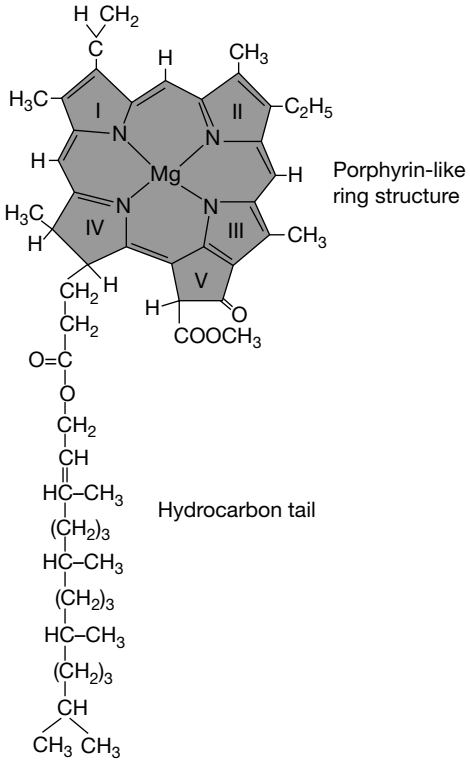


Figure 16.1 Structure of chlorophyll pigment

Chlorophylls absorb visible light efficiently because of their many conjugated double bonds. The absorption of light by chlorophylls is the most important biophysical event as the light energy trapped by the pigments is converted into chemical energy. In higher plants, both chlorophyll a and chlorophyll b work in tandem for the conversion of light into chemical energy.

16.4 ABSORPTION OF LIGHT BY CHLOROPHYLL

Chlorophyll a absorbs light with equal efficiency in both the blue and the red region of the visible spectrum. Since the energy content of blue light (Table 16.1) is about 50 per cent greater than the red light, one might expect that the former would be more effective in photosynthesis. This is not so as explained by the energy levels reached during excitation of the chlorophyll a molecule.

Blue light is sufficiently energetic to excite the second singlet state (60 kcal) of the chlorophyll a molecule (Figure 16.2). The lifetime of this state is estimated as only 10^{-11} s, far too short to be of use in photosynthesis.

Table 16.1 Energy Contents of Light of Different Wavelengths

Colour of light	Wavelength (nm)	Energy (cal/Einstein)
Far-red	750	38,000
Red	650	43,000
Yellow	590	48,000
Blue	490	58,000
Ultravoilet	395	72,000

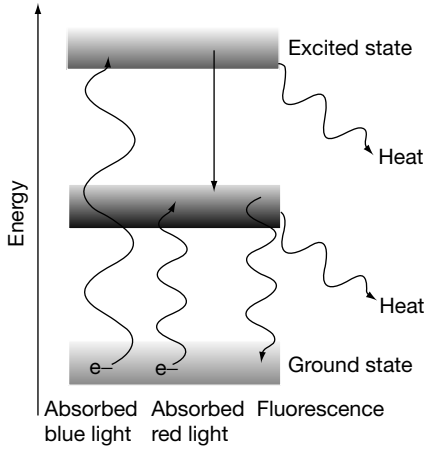


Figure 16.2 Energy states of the chlorophyll molecule. Each horizontal line represents a different energy state, and those above the ground states are unstable and lose energy by heat dissipation, fluorescence, phosphorescence or transfer to the energy trap of photosynthetic organisms

Therefore, heat is lost to reach the first singlet state, which, with an energy level of 40 kcal, can also be attained by the absorption of a red light. Again, the lifetime of this state is very short (10^{-9} s)

and loss of heat occurs to form the metastable triplet state. The lifetime of this transitional stage is sufficiently long (10^{-2} s) to permit the excited chlorophyll a molecule to transfer some of its energy to another molecule. The act of doing so is the first step in the conversion of light energy into chemical energy in photosynthesis. Otherwise, the triplet state decays by the loss of heat or phosphorescence to the ground state and no energy could be trapped.

16.5 THE SUPRAMOLECULAR COMPLEXES

Two photosystems, each consisting of light capturing antenna chl bound to proteins, they absorb and transfer the exciton to 'specialized' reaction centre chl a molecules (bound to proteins). The two photosystems are connected in series through electron transport carriers. Photosystem II (PS II) with its reaction centre P680 oxidizes water, while PS I with its reaction centre P700 reduces $NADP^+$.

Two photosystems made up of supramolecular chl/protein complexes and a light harvesting chl***a/b*** protein complex are involved in light capture, charge separation, electron transfer and energy conservation. These complexes along with cyt b_6-f complex and a coupling factor (the ATP synthase) are localized within the internal membranes called thylakoids. The thylakoids within chloroplast are suspended in the stroma. The thylakoids when stacked together form grana membranes.

Two photosystems are laterally as well as asymmetrically distributed. However, the PS II complex is primarily located in the appressed membranes, while the PS I is primarily located in the stromal lamellae and at end margins of appressed membranes.

The light-capturing and redox components involved in the electron transfer and energy conservation in the chloroplasts form four supramolecular entities that can be isolated using ionic/non-ionic detergents under carefully controlled salt and pH conditions. The four complexes that can be isolated are as follows:

1. The PS I
2. The PS II
3. The cytochrome b_6-f
4. CFo/CF1

The subunit composition and the functional, catalytic and structural role played by the subunits in each complex has been an area of intense research work.

16.5.1 The Photosystem I Complex

Photosystem I complex is primarily localized in the unappressed thylakoids. The native PS I complex consists of a core PS I and a peripheral antenna—LHC I. The native PS I complex is characterized by a chl***a/b*** ratio of 7–9 and contains approximately 200 chl/P700 ('PS I-200'). The LHC I binds about 100 chl and has a chl ***a/b*** ratio of 3.5.

Photosystem I core complex can be isolated by removing peripheral LHC I of native PS I complex (Mullet *et al*., 1980). The core complex binds 100 chl***a*** and is composed of PSI-A, PSI-B, PSI-C and eight other polypeptides. Chl***a*** molecules are bound to PSI-A and PSI-B only and which together bind P700 and all the electron acceptors A_O, A_1, F_X, F_B and F_A.

Photosystem I reaction centre complex is composed of PSI-A and PSI-B which together bind P700, A_O, A_1, F_X and 40 to 100 chl***a*** molecules (depending on the method of isolation). The reaction centre complex lacks PSI-C. The PSI-C binds F_A/F_B.

Photosystem I preparation from higher plant chloroplasts contains eight polypeptides. PS I complex from a wide variety of plant species have been shown to be composed of two large polypeptides (60 to 70 kDa on SDS-PAGE) and at least nine smaller polypeptides with apparent molecular masses between 1.5 and 22 kDa. The amino acid sequence of these polypeptides from various organisms show high homology with each other and suggest conversation of their structure. The gene for PS I polypeptide is denoted as ***psa,*** for example, genes for PSI-A, PSI-B and PSI-C are donated ***psaA***, ***psaB*** and ***psaC***, respectively.

Subunits of PS I reaction centre complex PSI-A, B and C are coded by the chloroplast genome, while PSI-D, E, F, G and H are nuclear coded. However, PSI-I, J and K with low molecular masses (< 7 kDa) are tightly bound to core complex and PSI-I and J are coded by the chloroplast genome.

16.5.2 The Photosystem II Complex

Photosystem II is a supramolecular complex made up of at least 25 proteins (Figures 16.2 and 16.3). A number of proteins bind chl, forming light-harvesting antenna. The smallest reaction centre complex, which binds the reaction centre pigment P680, the primary acceptor pheophytin as well as the secondary acceptors QA and AB, consists of D1/D2 and 9 and 4 kDa subunits of cyt$\boldsymbol{b_{559}}$. The heterodimeric D1-D2 proteins together are apoproteins of the PSII reaction centre. Further, PS II acts as a water-plastoquinone oxidoreductase.

Photosystem II core complex is composed of two major chl***a***-binding antenna proteins CP47 (CAP47) and CP43 (CP43) (Figure 16.3) which are coded by the chloroplast ***psbB*** and ***psaC*** genes, respectively. CP29 (CAP29) and the 22 kDa intrinsic protein shown to be a chl***a***-binding protein forms minor antenna.

At least seven proteins are involved in the process of light harvesting. The light-harvesting chl***a/b*** protein (LHC II/CABP II) is the major component of the thylakoids binding up to half of chl and protein mass. Careful analysis of LHC II suggests a chl***a/b*** ratio of 1.3/1.4 for LHC II. Further, LHC II is considered to contain 5–7 chl***a***, 4–6 chl***b*** and 2–3 xanthophy11 molecules per molecule of LHC II. The two apopolypeptides which together bind 13 chl ***a***, having apparent molecular masses of 25 and 27 kD.

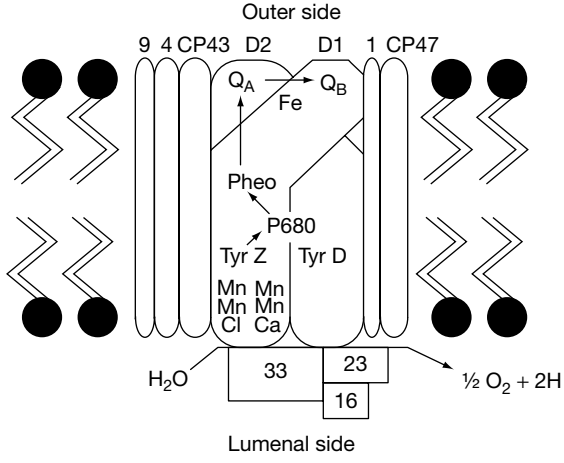


Figure 16.3 Structure of photosystem II

Each of the apopolypeptides of LHC II forms three transmembrance helices. The electron microscopic studies revealed that LHC II is arranged as trimeric unit which during mild SDS-PAGE often appears in a trimeric state. The phosphorylation of LHC II polypeptides controls the distribution of exciton between two photosystems. The phosphorylation of small mobile outer pool representing 27 and 25 kDa polypeptides of LHC II is now suggested to be controlled by the redox state of cyt***b***$_6$***-f*** complex.

Three additional chl***a/b*** proteins CP29 (CAP29), CP27 (CAP27 and CP24 (CAP24) together account for 5 to 10 per cent of the total chl associated with PS II. The isolation of inverted inside-out thylakoid vesicles made it possible to release and identify the proteins which are involved in water oxidation process on the luminal side of thylakoid membrane. Using this experimental approach, three proteins, viz., 33, 23 and 16 kDa were identified. All the three proteins are hydrophilic and water soluble, and none of them bind Mn.

16.6 ENERGY CONSERVATION PROCESS OR PHOTOSYNTHETIC ELECTRON TRANSPORT CHAIN

In higher plants, chl***a*** and chl***b*** are the light-absorbing species that are noncovalently complexed with proteins forming chl–protein complexes. Due to complexation with proteins, various spectroscopic forms of chl***a*** and chl***b*** exits in vivo resulting in the increase in the absorption cross-section by chloroplast. The light absorbed by the antenna chl is successively transferred to longer wavelength absorbing forms until the excitation energy is trapped by the reaction centre chl.

The frequency of 680 nm light is 4.41×10^{14} cycles s^{-1}. Thus, the act of absorption of a photon by P680 (the reaction centre of PS II) and therefore the time of excitation should be 2.5 fs (i.e., one cycle of frequency = 4.41×10^{14} cycles s^{-1}). The excitation energy transfer time between antenna chl should also be in the fs range.

The reaction centres of PS II and PS I are named P680 and P700, respectively, on the basis of their absorption maxima. P700 is generally considered to be a special dimer of chl a on the basis of dimer-like CD and absorption spectra and on the basis of low temperature resonance Raman spectrum.

A current model of Z-scheme of photosynthetic electron transport is shown in Figure 16.4. The linear photosynthetic electron transfer pathway in plants: the Z scheme. The energy from two separate photosystems (I and II) connected in series is used to oxidize water and reduce $NADP^+$. Photosystem (PS) II oxidizes two H_2O molecules into $O_2 + 4H^+ + 4e$ and reduces plastoquinone (PQ) to PQH_2 in a $2e^- / 2H^+$ reaction. Photosystem (PS) I oxidizes the electron-carrying molecules plastocyanin (PC) and reduces $NADP^+$ to NADPH. The PS II utilizes manganese (Mn), calcium delivers the electrons to Z, which is a tyrosine (try^- 161) residue on the D1 polypeptide. The other PS II reaction centre components are similar in function to those of the purple bacteria but involve chlorophyll, pheophytin and plastoquinone instead of their bacterial analogues. One or two molecules of bicarbonate (HCO_3) also appear to bind to Fe at or near the quinines and facilitate electron/proton transfer from Q_A to Q_B. PS I has a pair of primary donor chl molecules whose long wavelength absorption maximum is at 700 nm (P700). The light generated excited state (P700*) donates an electron to another chl molecule (A_0), and the resulting cation ($P700^+$) is re-reduced by plastocyanin. From A_0, the electron continues to move in an energetically downhill process to a phylloquinone (A_1, a vitamin K_1 centres (FeS_x, FeS_A, FeS_B and Fd or ferredoxin).

At the end of the chain, a complex known as the ferredoxin–$NADP^+$ oxidoreductase (FNR) transfers two electrons and a proton to convert $NADP^+$ to NADPH. A proton gradient created between outside of the membrane (the stroma), and lumen is dissipated through the coupling factor complex (CF_0–CF_1) to produce ATP.

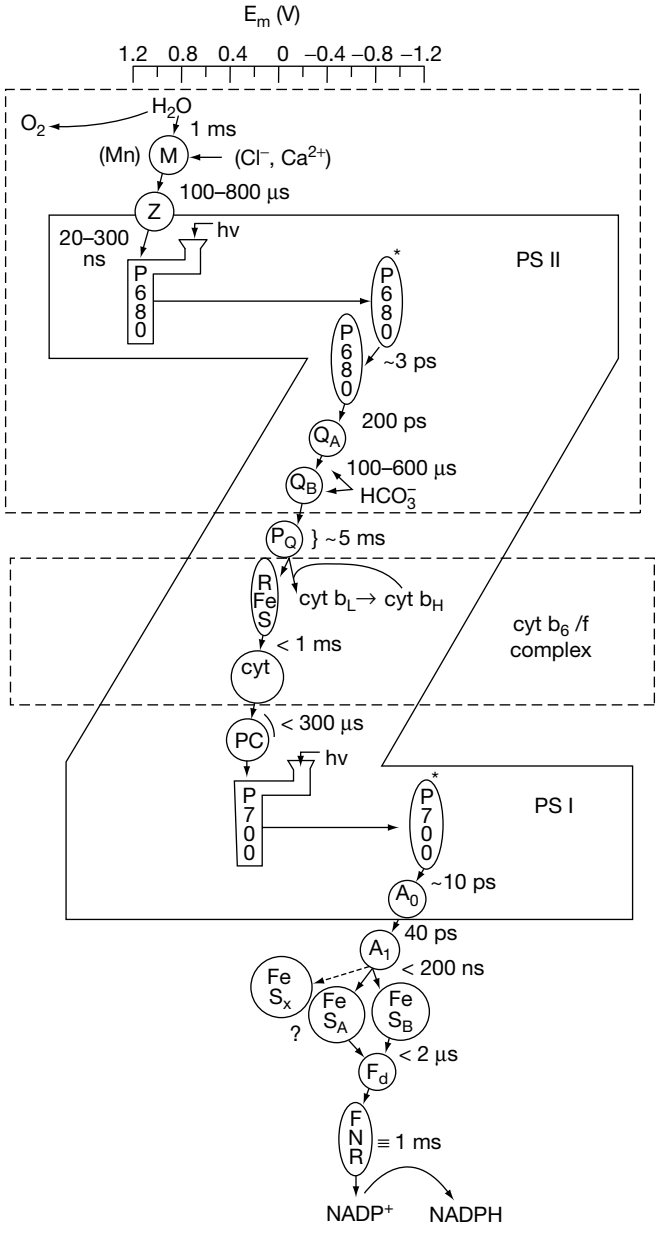


Figure 16.4 A model of Z-scheme of photosynthetic electron transport

16.7 THE CO_2 REDUCTION (CALVIN) CYCLE

Calvin postulated the operation of a carbon dioxide reduction cycle during photosynthesis (this cycle involves essentially only one new reaction, the carboxylation of ribulose-1,5-diphosphate, discussed below; the remainder of the reactions are identical or similar to reactions encountered previously in glycolysis and pentose phosphate metabolism) (Figure 16.5). All of the enzymes required to catalyse the reactions postulated in the Calvin cycle are known to occur in chloroplasts.

16.7.1 Carboxylation Phase

This rather confusing scheme can be better understood when the reactions are grouped into three phases. The first of these, the carboxylation phase, involves the single reaction catalysed by ribulose-1,5-diphosphate carboxylase (also called carboxydismutase). This key reaction involves the carboxylation not of a two-carbon compound, but of a five-carbon compound, ribulose-1,5-diphosphate, to yield two moles of 3-phosphoglyceric acid.

D-Ribulose-1,5-diphosphate $\xleftarrow{CO_2}$ Enediol $\longrightarrow$ β-keto acid intermediate $\xrightarrow{H_2O}$ Two molecules of 3-phosphoglyceric acid.

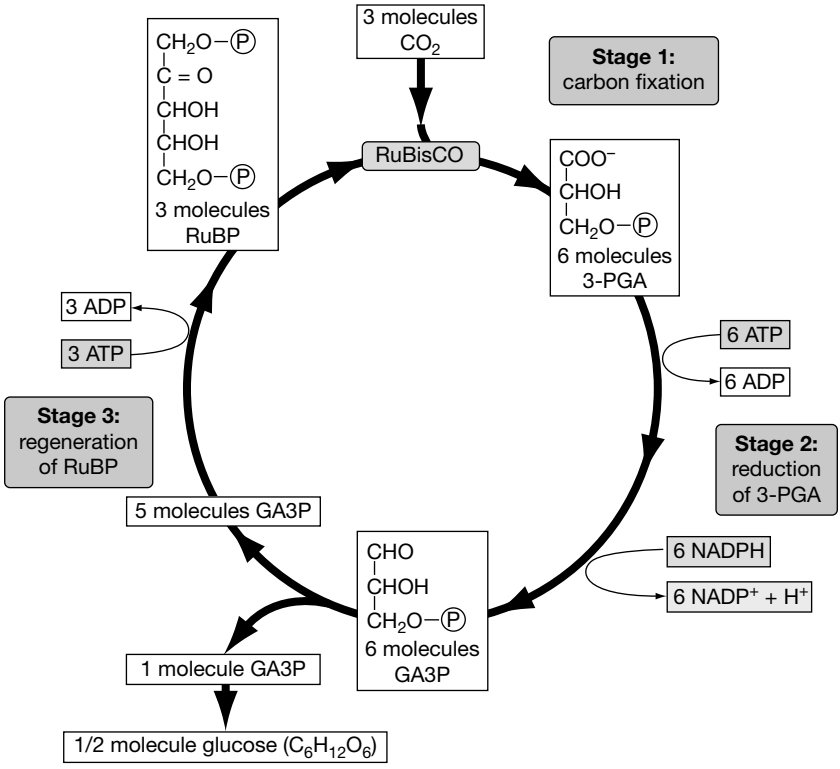


Figure 16.5 Calvin cycle showing three stages of the cycle: Stage 1: Carbon fixation, Stage 2: Reduction of 3-PGA and Stage 3: Regeneration of RuBP

In the presence of the enzyme ribulose-1,5-diphosphate carboxylase, CO_2 adds to the enediol form of ribulose diphosphate to form an unstable beta-keto acid which undergoes hydrolytic cleavage to form two molecules of phosphoglyceric acid. The equilibrium of the reaction is far to the right. The carboxylase was first purified as a homogenous protein by Horecker from spinach leaves, where it constitutes 5–10 per cent of the soluble protein. It has a molecular weight of 550,000 and is an oligomer composed of eight small monomers (12–16,000 MW) and eight large units (4–60,000 MW).

16.7.2 Reduction Phase

A second phase of the carbon reduction cycle, termed the reduction phase, consists of two reactions previously encountered in glycolysis. In these reactions, ATP and a reduced nicotinamide nucleotide are consumed. The first involves the phosphorylation of 3-phosphoglycerate by ATP to form 1, 3-diphosphoglycerate.

$$\text{3-Phosphoglyceric acid} + \text{ATP} \xleftrightarrow{\text{3-phosphoglyceric kinase}} \text{1, 3-Diphosphoglyceric Acid} + \text{ADP}$$

The second reaction involves reduction of the 1, 3-diphosphoglyceric acid by NADPH in the presence of an NADP-specific glyceralciehyde-3-phosphate dehydrogenase.

$$\text{1, 3-Diphosphoglyceric acid} + \text{NADPH} + H^+ \longleftrightarrow \text{Glyceraldehyde-3-phosphate} + NADP^+ + H_3PO_4$$

The chloroplast enzyme is activated by ATP and NADPH. It is in these two reactions that the NADPH and half of the ATP required to drive the carbon reduction cycle are utilized.

16.7.3 Regeneration Phase

The remainder of the reactions in the cycle compose the third, or regeneration phase, which accomplishes the regeneration of ribulose-1,5-diphosphate necessary to keep the cycle operating. Table 16.2 shows the reactions of this phase with the required stoichiometry. Note that a total of 36 carbon atoms present in 12 molecules of glyceraldehyde-3-phosphate at the end of the reduction phase are converted, by the reactions of the regeneration phase, into 1 molecule of fructose-6-phosphate (6 carbon atoms) and 6 molecules of ribulose-1,5-diphosphate (30 carbon atoms) at the end of the regeneration phase. The last reaction of the regeneration phase also requires ATP. The six molecules of ribulose-1,5-diphosphate produced by this phase are then available for the carboxylation process, and they can keep the cycle functioning.

Table 16.2 Stoichiometry of the Carbon Reduction Cycle

Carboxylation phase
$6\ \text{Ribulose.1, 5.diphosphate} + 6CO_2 + -6H_2O \longrightarrow \text{12, 3-Phosphoglycerate}$
Reduction phase
$\text{12, 3-Phosphoglycerate} + 12\text{ATP} \longrightarrow \text{12-1, 3-Diphosphoglycerate} + 12\text{ADP}$
$\text{12-1, 3-Diphosphoglycerate} + 12\text{NADPH} + 12H^+ \longrightarrow 12\ \text{Glyceraldehyde-3-phosphate} + 12NADP^+ + 12H_3PO_4$

Regeneration phase

$$5\ \text{Glyceraldehyde-3-phosphate} \longrightarrow 5\text{-Dihydroxy acetone phosphate}$$

$$3\ \text{Glyceraldehyde-3-phosphate} + 3\text{-Dihydroxy acetone phosphate} \longrightarrow 3\ \text{Fructose-1, 6-diphosphate}$$

$$3\ \text{Fructose-1, 6-diphosphate} + 3H_2O \longrightarrow 3\ \text{Fructose-6-phosphate} + 3H_3PO_4$$

$$2\ \text{Fructose-6-phosphate} + 2\ \text{Glyceraldehyde-3-phosphate} \longrightarrow 2\ \text{Xylulose-5-phosphate} + 2\ \text{Erythrose-4-phosphate}$$

$$2\ \text{Erythrose-4-phosphate} + 2\ \text{Dihydroxy acetone phosphate} \longrightarrow 2\ \text{Sedoheptulose-1, 7-diphosphate}$$

$$2\ \text{Sedoheptulose-1, 7-diphosphate} + 2H_2O \longrightarrow 2\ \text{Sedoheptulose-7-phosphate} + 2H_3PO_4$$

$$2\ \text{Sedoheptulose-7-phosphate} + 2\ \text{Glyceraldehyde-3-phosphate} \longrightarrow 2\ \text{Ribose-5-phosphate} + 2\ \text{Xylulose-5-phosphate}$$

$$2\ \text{Ribose-5-phosphate} \longrightarrow 2\ \text{Ribulose-5-phosphate}$$

$$4\ \text{Xylulose-5-phosphate} \longrightarrow 4\ \text{Ribulose-5-phosphate}$$

$$6\ \text{Ribulose-5-phosphate} + 6\text{ATP} \longrightarrow 6\ \text{Ribulose-1, 5-diphosphate} + 6\text{ADP}$$

SUM

$$6CO_2 + 18\text{ATP} + 12\text{NADPH} + 12H^+ + 11H_2O \longrightarrow \text{Fructose-6-phosphate} + 18\text{ADP} + 12NADP^+ + 17H_3PO_4$$

The overall stoichiometry of the carbon reduction cycle is given in the sum in Table 16.2. The fructose-6-phosphate produced can in turn be converted to glucose by reversal of the reactions encountered in the early stages of glycolysis.

$$\text{Fructose-6-phosphate} \longrightarrow \text{Glucose-6-phosphate} \xrightarrow{H_2O} \text{Glucose} + H_3PO_4$$

When this is done, the overall carbon reduction cycle becomes

$$6CO_2 + 18\text{ATP} + 12\text{NADPH} + 12H^+ + 12H_2O \longrightarrow \text{Glucose} + 18\text{ADP} + 18H_3PO_4 + 12NADP^+$$

Dividing this equation by six illustrates an important fact regarding the energetics of photosynthesis:

$$CO_2 + 3\text{ATP} + 2\text{NADPH} + 2H^+ + 2H_2O \longrightarrow C(H_2O) + 3\text{ADP} + 3H_3PO_4 + 2NADP^+$$

The above reaction shows that photosynthesis requires three moles of ATP and two moles of NADPH to convert one mole of CO_2 to the level of carbohydrate.

16.8 THE C_4 – PATHWAY

Many plants, including important crop plants of tropical origin—the tropical grasses sugar cane, corn and sorghum—possess an interesting variation for CO_2 assimilation. Early studies on these plants indicated that the CO_2 was incorporated initially into certain dicarboxylic acids, malate or aspartic acid rather than phosphoglyceric acid. The Australian team of M. D. Hatch and C. R. Slack initiated a series of studies in 1966 that stimulated work in many laboratories and resulted in our knowledge of the C_4 (or Hatch–Slack) pathway for CO_2 assimilation.

Plants that utilize the C_4 pathway also possess a common feature of leaf anatomy in which the vascular elements (phloem and xylem) are surrounded by a row of bundle sheath cells and then in turn by one or more layers of mesophyll cells. This characteristic anatomy (Kranz-type) has long been cited as a mechanism whereby plants living in dry or hot regions could minimize their loss of tissue water by transpiration, since the conducting elements were separated from the stomata on the surface of the leaf by one or more layer of mesophyll cells as well as the bundle sheath layer. This structural feature obviously also restricts the amount of CO_2 available for photosynthesis, and it is argued that the C_4-pathway represents the adaptation of tropical and desert plants to this stress. The C_4 pathway will be described in terms of the reactions occurring in the mesophyll and the bundle sheath cells.

16.8.1 Mesophyll Cells

CO_2 entering the leaf of a C_4-plant during stomatal opening will diffuse to the mesophyll where it serves as a substrate for phosphoenol pyruvic acid carboxylase.

$$\text{Phosphoenol pyruvic acid} + CO_2 + H_2O \longrightarrow \text{Oxalacetate} + H_3PO_4 \qquad (16.1)$$

This enzyme, which has a much higher affinity for CO_2 than does ribulose-1,5-diphosphate carboxylase, is localized in chloroplasts of the mesophyll cells. It therefore serves as a more efficient trap for the low levels of CO_2 that are encountered, and oxalacetate is produced.

The C_4 plants can be divided into those which have a high concentration of malic dehydrogenase in the mesophyll cells and those which have an active alanine/aspartic transaminase. In the former (reaction 16.2), oxalacetate is reduced to malate and in the latter (reaction 16.3), aspartic acid is formed. These two dicarboxylic acids then are believed to act as CO_2 carriers and enter the bundle sheath cells.

$$\text{Oxalacetate} + NADH + H^+ \xrightarrow{\text{Malic dehydrogenase}} \text{L-Malate} + NAD^+ \qquad (16.2)$$

$$\text{Oxalacetate} + \text{Alanine} \xleftrightarrow{\text{Transaminase}} \text{Aspartic acid} + \text{Pyruvate} \qquad (16.3)$$

The other unique reaction of the mesophyll is the one in which the CO_2 trapping agent, phosphoenol pyruvic acid is formed from pyruvate (that returns from the bundle sheath cells eventually). The enzyme that catalyses this reaction is pyruvate, phosphate dikinase (reaction 16.4).

$$\text{Pyruvate} + ATP + H_3PO_4 \longrightarrow \text{Phosphoenol pyruvate} + AMP + P\sim P \qquad (16.4)$$

This enzyme is also found in the mesophyll cells.

16.8.2 Bundle Sheath Cells

The plants that utilize malate as a carrier of CO_2 have a high level of the NADP-specific malic enzyme in the bundle sheath chloroplasts. This enzyme catalyses the formation (i.e. the release) of CO_2 from malate, which is then incorporated by means of the Calvin cycle. The enzymes of the Calvin cycle are found only in the bundle sheath chloroplasts, together with the NADP-malic enzyme. Pyruvate formed in this reaction returns to the mesophyll.

$$\text{L-Malate} + NADP^+ \longrightarrow \text{Pyruvate} + NADPH + H^+ + CO_2 \qquad (16.5)$$

The plants that utilize aspartic acid as a CO_2 carrier contain a transaminase in the bundle sheath cells that convert the aspartic acid back to oxalacetic acid.

$$\text{Aspartic acid} \xrightarrow{\text{Transaminase}} \text{Oxalacetic acid}$$

The fate of the oxalacetate depends again on the particular plant. One major group of aspartic-formers contain a NAD^+-specific malic dehydrogenase and a NAD^+-specific malic enzyme. These two enzymes thereby convert the oxalacetic acid first to malic acid and then to CO_2 and pyruvic acid.

$$\text{Oxalacetate} + \text{NADH} + H^+ \longrightarrow \text{L-Malate} + NAD^+ \tag{16.6}$$

$$\text{L-Malate} + NAD^+ \longrightarrow \text{Pyruvate} + \text{NADH} + H + CO_2 \tag{16.7}$$

Another, smaller group of aspartic carriers apparently convert the oxalacetate to PEP and CO_2 due to their content of PEP carboxykinase.

$$\text{Oxalacetate} + \text{ATP} \longrightarrow \text{Phosphoenol pyruvate} + CO_2 + \text{ADP}$$

In both the malate- and aspartic-carrier plants, the CO_2 is released in the bundle sheath cells where it presumably is concentrated to serve eventually as the substrate for ribulose-1,5-diphosphate carboxylase of the Calvin cycle. In the aspartic-carrier plants, an additional step is called for, as the amino acid moves from mesophyll to bundle sheath, it carries not only the CO_2 but also an amino ($–NH_2$) group. In order to avoid a net transfer of amino nitrogen into the bundle sheath cells, it is believed that the pyruvate formed in the reaction undergoes transamination to alanine that then moves out to the mesophyll, thereby balancing the $–NH_2$ groups. In the mesophyll, the alanine can transaminate with the oxalacetate formed initially. These relationships are shown in Figure 16.6.

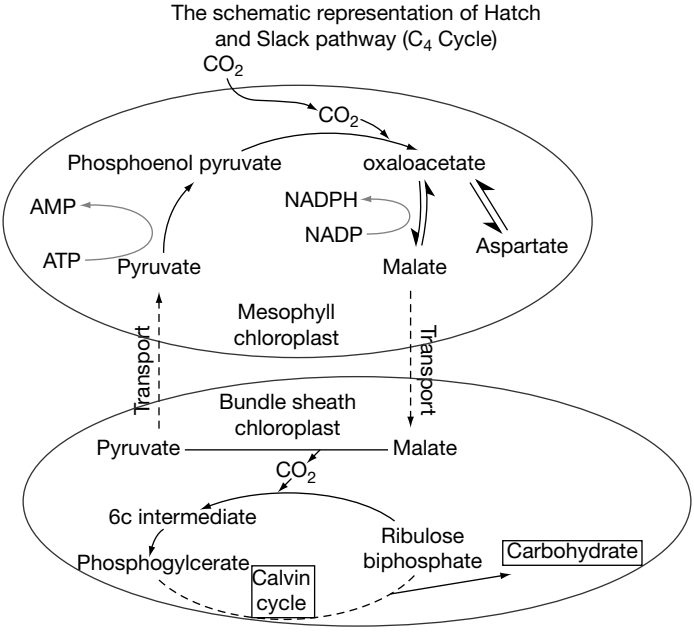


Figure 16.6 The role of malate or aspartate as CO_2 carriers in C_4 photosynthesis

In summary, the fixation of CO_2 into the C_4-dicarboxylic acids may be viewed as an efficient mechanism for trapping CO_2 and concentrating it in the bundle sheath for assimilation by means of the Calvin cycle. The C_4-pathway presumably has evolved in response to ecological situations characterized by the combination of high radiation, higher temperatures and a limited supply of H_2O. The capacity of C_4 species to survive and grow under these conditions is due to the ability of PEP-carboxylase to operate with low concentrations of CO_2.

16.9 PHOTORESPIRATION

Plants of course carry out the same general respiratory processes as animals and microorganisms in that they degrade carbohydrates by means of glycolysis and the Krebs cycle. They also exhibit β-oxidation and catalyse the general reactions of protein and amino acid catabolism. Moreover, these reactions occur in the same parts of the plant cell (mitochondria, cytoplasm, microsomes, etc.) as in animal cells. However, many plants also exhibit an additional metabolic activity termed photorespiration that occurs only when those plants are illuminated. Since photorespiration results in CO_2 evolution and O_2 consumption, it has the net effect of decreasing photosynthesis and therefore decreasing plant growth and crop yield. For this reason, the phenomenon has been increasingly studied.

Glycolic acid is a major photosynthetic product formed by certain plants under those experimental conditions—high O_2 and low CO_2—that favour photorespiration. Other evidence indicates the glycolic acid is the source of the CO_2 produced in photorespiration and it is proposed that the equivalent of two moles of glycolate are converted to one of CO_2 and one mole of 3-phosphoglyceric acid by enzymes occurring in the peroxisomes (microbodies that occur in green leaves) and mitochondria.

$$2\text{Glycolate} \longrightarrow \text{3-Phosphoglyceric acid} + CO_2$$

The key reaction in this process is the formation of glycolic acid (as phosphoglycolic acid); the enzyme involved is ribulose-1,5-diphosphate carboxylase, the carboxylation enzyme of the Calvin cycle. When this enzyme is exposed to high concentrations of O_2 (20 per cent and up) and no CO_2, ribulose-1,5-diphosphate is cleaved to 3-phosphoglyceric acid.

Phosphoglycolate so produced then enters a series of reactions that results in the release of 25 per cent of the carbon in the glycolic acid as CO_2 and the regeneration of 3-phosphoglycerate (Figure 16.7). The latter enters the Calvin cycle again to continue the process.

The list of plants that exhibit photorespiration includes the cereals, wheat and rice, many legumes and sugar beets, crops that are important from the standpoint of the world's food supply. In some of these, it has been estimated that the net assimilation of CO_2 by photosynthesis may be reduced by as much as 50 per cent by photorespiration. Some workers have proposed that it might be practical to increase the crop yield of such plants by finding a means to inhibit the photorespiration they exhibit. Other equally important food crops—corn, sorghum, sugar cane—do not exhibit the phenomenon of photorespiration. Since, however, the leaves of such plants do contain peroxisomes and presumably can still perform the individual reactions of photorespiration, some explanation is required. Some evidence suggests that these plants may rely on the C_4-fixation process described above, which, because of its greater efficiency of CO_2-fixation, can reutilize any CO_2 produced in photorespiration and effectively retain all of the CO_2.

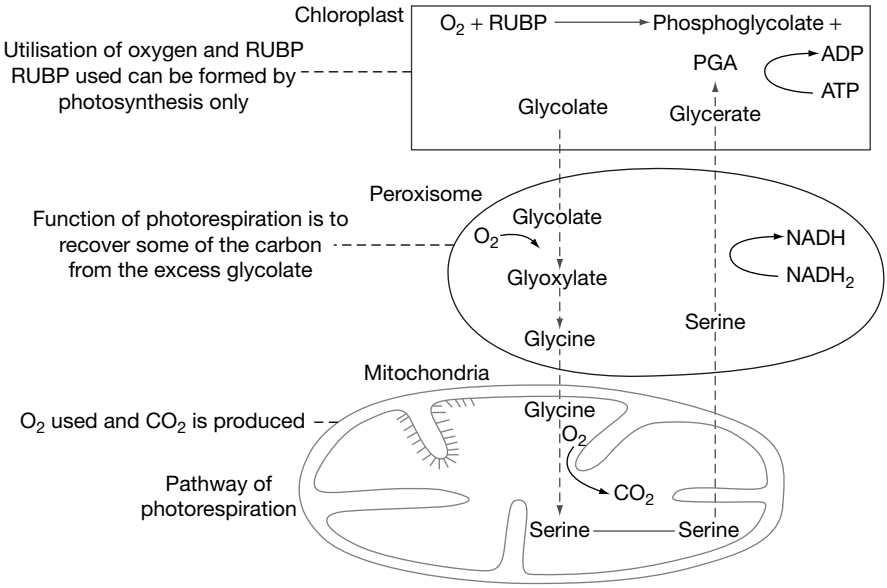


Figure 16.7 Glycolic acid metabolism and photorespiration, a process involving three organelles

16.10 CRASSULACEAN ACID METABOLISM

CAM during night: The plants undergoing crassulacean acid metabolism (CAM), during night time keep their stomata open, carbon dioxide enters the stomata and diffuses into the intracellular spaces of the mesophyll cells and is then fixed in the cytoplasm by phosphoenolpyruvate reaction. The prefixed CO_2 is stored in the vacuoles as malate for subsequent use, they are not instantly delivered to the Calvin cycle and used in the day time for photosynthesis (Figure 16.8).

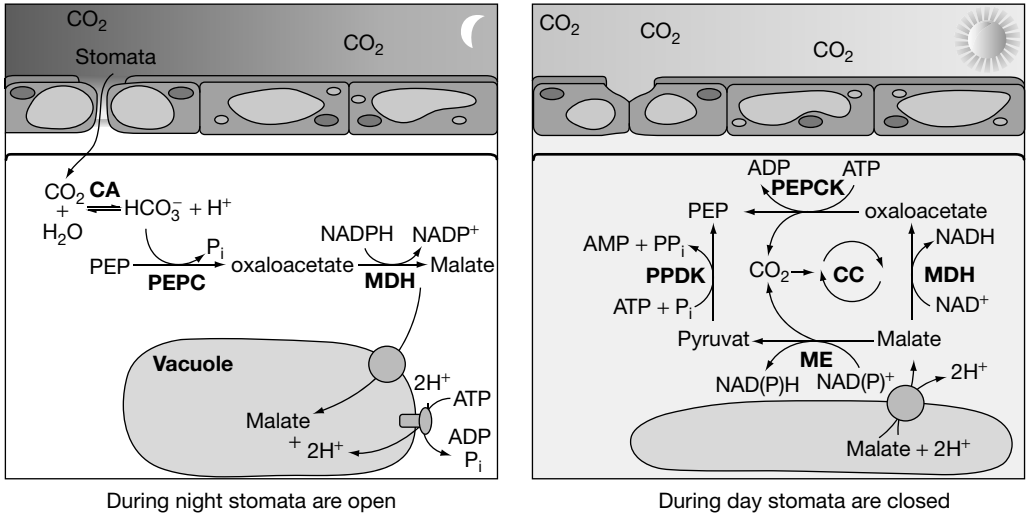


Figure 16.8 Crassulacean acid metabolism

CAM during day: The malate which is stored in the vacuoles is released into chloroplast, but during day time stomata remains closed. Malate is cleaved into pyruvate and carbon dioxide by either malic enzyme (ME) or phosphoenolpyruvate carboxykinase (PEPCK) depends on plant species. Carbon dioxide enters the Calvin cycle (Figure 16.8).

EXERCISES

1. Write in detail about the dark and light reactions in photosynthesis.
2. What is the difference between cyclic and noncyclic photophosphorylation?
3. Write about the photosynthetic apparatus of higher green plants.
4. What are the three stages of Calvin cycle? Explain with the help of a diagram.
5. Plants of course carry out the same general respiratory processes as animals and micro-organisms. Comment on the statement and explain with the help of a diagram.
6. What are C4 plants? Discuss the C4 pathway with the help of a diagram.
7. Write the stoichiometry of the carbon reduction cycle.
8. What are the four supramolecular complexes? Explain in detail.
9. Write the linear photosynthetic electron transfer pathway in plants, the Z scheme.
10. Explain the energy states of the chlorophyll molecule with the help of a diagram.
11. Write short notes on
    (a) Far-red drop
    (b) Emerson enhancement effect
    (c) Hill's reaction

MULTIPLE CHOICE QUESTIONS

1. Hatch–Slack pathway for CO_2 assimilation is also called as
   (a) C_3 pathway (b) C_4 pathway
   (c) Both (a) and (b) (d) None of these
   Ans. b
2. The native PS I complex consists of a core PS I and a peripheral antenna
   (a) LHC I (b) LHCII
   (c) Both (a) and (b) (d) None of these
   Ans. a
3. Emerson subsequently showed that this decrease in the far-red region—the so-called
   (a) Far-red drop
   (b) Far-green drop
   (c) Far-blue drop
   (d) None of these
   Ans. a
4. The plants undergoing crassulacean acid metabolism (CAM), during night time keep their stomata
   (a) Open (b) Closed
   (c) Both (a) and (b) (d) None of these
   Ans. a
5. Malate is cleaved into pyruvate and carbon dioxide either by malic enzyme (ME) or
   (a) Phosphoenolpyruvate carboxykinase (PEPCK)
   (b) PEP-carboxylase

(c) Both (a) and (b)
(d) None of these

Ans. a

6. Glycolic acid is a major photosynthetic product formed by certain plants under those experimental conditions—high O_2 and low CO_2—that favour
   (a) Photorespiration
   (b) Photophosphorylation
   (c) Photooxidation
   (d) None of these

   Ans. a

7. The energy from two separate photosystems (I and II) connected in series is used to oxidize water and reduce
   (a) $NADP^+$ (b) FAD^+
   (c) NAD^+ (d) None of these

   Ans. a

8. CO_2 entering the leaf of a C_4-plant during stomatal opening will diffuse to the mesophyll where it serves as a substrate for
   (a) Phosphoenol pyruvic acid carboxylase
   (b) Phosphoenolpyruvate carboxykinase
   (c) Both (a) and (b)
   (d) None of these

   Ans. a

9. All of the enzymes required to catalyse the reactions postulated in the Calvin cycle are known to occur in
   (a) Chloroplasts
   (b) Mitochondria
   (c) Ribosomes
   (d) Golgi apparatus

   Ans. a

10. The supra-molecular complexes along with cyt b_6–f complex and a coupling factor (the ATP synthase) are localized within the internal membranes called
    (a) Thylakoids
    (b) Stroma
    (c) Both (a) and (b)
    (d) None of these

    Ans. a

11. PS II complex is primarily located in the appressed membranes, while PS I is primarily located in the stromal lamellae and at
    (a) End margins of appressed membranes
    (b) End margins of nonappressed membranes
    (c) Both (a) and (b)
    (d) None of these

    Ans. a

12. Chlorophylls are green pigments of plants, which absorb light for the process of
    (a) Protein synthesis
    (b) Photosynthesis
    (c) Both (a) and (b)
    (d) None of these

    Ans. b

13. The nongranal portion of the chloroplast matrix is called
    (a) Grana (b) Stroma
    (c) Granum (d) None of these

    Ans. b

14. A granum is made up of about 40–50 disc-like sacs called
    (a) Grana (b) Stroma
    (c) Thylakoids (d) None of these

    Ans. c

15. The photosynthetic organisms have two photosystems (PS I and PS II) which are activated by light of far-red wavelength at 680–700 nm and shorter wavelength
    (a) 450 nm, respectively
    (b) 650 nm, respectively
    (c) 550 nm, respectively
    (d) 500 nm, respectively

    Ans. b

16. The prokaryotes include the blue-green, and the purple and green bacteria; in these bacteria the light-trapping process takes place in small structures called
    (a) Chromatophores (b) Chloroplast
    (c) Chlorophyll (d) None of these

    Ans. a

17. Photosystem I reaction centre complex is composed of PSI-A and PSI-B which together bind P700, A_O, A_1, F_X and
    (a) 400 to 500 chl***a*** molecules
    (b) 40 to 100 chl***a*** molecules
    (c) 200 to 300 chl***a*** molecules
    (d) None of these

    Ans. b

18. The light generated excited state (P700*) donates an electron to another chl molecule (A_0), and the resulting cation ($P700^+$) is re-reduced by
    (a) Plastocyanin
    (b) Phylloquinone
    (c) Both (a) and (b)
    (d) None of these

    Ans. a

19. From A_0, the electron continues to move in an energetically downhill process to
    (a) Plastocyanin
    (b) Phylloquinone
    (c) Both (a) and (b)
    (d) None of these

    Ans. b

20. The chlorophylls have a porphyrin structure or a tetrapyrrole nucleus with which atom in the centre of the nucleus is
    (a) Cobalt
    (b) Magnesium
    (c) Iron
    (d) None of these

    Ans. b

17 Hormones

CHAPTER OBJECTIVES

17.1 Introduction
17.2 Second Messengers
17.3 Classification of Hormones
17.4 Steroid Hormones
17.5 Peptide Hormones
17.6 Amino Acid Derivatives
17.7 Prostaglandins as Modulators of Hormone Action

17.1 INTRODUCTION

Hormones are regulatory biochemicals secreted by specific glands and transported to distant target organs through blood stream (circulatory system of the body) and exert control over various functions of the body. Although ductless glands are designated as the principal source of hormones, other tissues such as the gut are known to be associated with hormone production. In the field of plant biochemistry, hormone-like substances are elaborated by non-glandular tissues. Because the hormones act at sites distant to their origin, their function may be likened to that of chemical messengers. Since only minute amounts are usually secreted at any one time, the profound physiological changes elicited indicate the great potency of the hormones. Because of this high degree of activity, the hormones are considered to act catalytically in helping control various chemical reactions concerned with the maintenance and operation of the body.

Definition: Hormones are biochemicals produced by specific glands called as 'endocrine glands' and released into the blood stream through which they are carried to a remote tissue called the target organ.

In 1902, Bayliss and Starling first coined the name 'secretin' that is the active agent produced from the intestinal mucosa and subsequently coined the term 'hormone.' Like vitamins and enzymes, the hormones are effective in small amounts. As its name indicates, the hormones

are helpful in stimulating particular cells or organs ('hormaein' in Greek means 'to excite'), but not all of them are excitatory. Hormones differ from enzymes in terms of the following: (i) they are not protein in nature and (ii) as a rule do not act on the endocrine gland that has produced them. In fact, in many cases, the hormones could act by influencing the enzymes. This definition of hormones (endocrines) restricting their action to a distant target organ has a few exceptions. The hormonal secretions of one type of cell may regulate the activity of adjacent cells that have different characteristics and this direct action on contiguous cells is known as 'paracrine function' and the action on the same cell producing them known as 'autocrine function.'

The number of molecules of a hormone is quite small as compared to the number of similar non-hormone molecules like steroid hormones and cholesterol. But the cells in the target tissues are able to distinguish and pick up selectively the hormone molecules on account of the presence of receptors in the cells. The receptor not only binds the hormone but also sends a signal to speed up or slow down an intracellular function, for example, stimulation of cyclic AMP production. Alteration in the number of receptors in the cell is used by the organism to make the tissue more or less reactive to the hormone, for example, a decrease in the insulin receptors is said to be one of the causes for diabetes mellitus.

The hormone receptors are all proteins. The insulin receptor is a tetramer containing 2α and 2β monomers, and the α-subunit binds insulin and the β-subunit transmits the signal to the cell. The steroid hormone receptors have three domains in their polypeptide structure: a hormone-binding domain; a DNA-binding region and a specific region that guides the receptor about the precise region of the DNA. The binding of the hormone to the receptor can be modified by 'agonists' and 'antagonists.' This results in an altered response to the hormone. The agonists bind to the receptor and cause the hormone to exert maximal action. The antagonists, on binding to the receptor, markedly diminish the action of the hormone. Some forms of thyrotoxicosis and diabetes mellitus, respectively, are said to be due to agonistic and antagonistic action on the receptors.

Lipophilic hormones are transported in plasma by special proteins. They have relatively a long half-life. Their action is prolonged. They enter the cells of the target organ and bind to receptor proteins in the cytoplasm or the nucleus. They exert their function by altering the function of a portion of DNA through the hormone receptor elements (HRE) in the DNA. Steroid hormones of the adrenal cortex, testis and ovary belong to this group. Thyroid hormones, which are water soluble and easily transportable, also act in this manner.

Water-soluble hormones are easily transported in plasma in the free state. Their half-lives are very short and their actions are also limited to a very short period. They bind to receptors on the cell membrane and further action is mediated through a second messenger (the hormone itself being the first messenger). Most of the peptide hormones like insulin and glucagon, norepinephrine, epinephrine and hormones of the pituitary gland belong to this group.

Hypothalamus occupies a key place in the control of synthesis and release of most hormones. In response to specific neural messages from the peripheral tissues, it secretes minute quantities of hormones called 'releasing factors.' They are passed down the nerve fibres to the anterior pituitary gland (e.g., TRF, thyrotropin-releasing factor). Each releasing factor, in turn, will cause the release of a specific tropic hormone from the anterior pituitary gland (e.g., TRF causes the release of TH, thyrotropic hormone). The tropic hormone, in turn, is carried through systemic blood to the particular endocrine gland (in this case the thyroid) and stimulates the production of its characteristic hormones (the thyroid hormones). These hormones are released into peripheral circulation to reach the target tissue on which they act.

The release of some of the pituitary hormones is inhibited by certain inhibitory factors secreted by the hypothalamus (e.g., prolactin-inhibiting factor). In addition, the hypothalamus produces two hormones—oxytocin (OT) and vasopressin (ADH or antidiuretic hormone) which are bound to small proteins called 'neurophysins' and pass to the posterior pituitary and from that gland are released into systemic blood. All the hormones released by the hypothalamus are small polypeptides, and the structures of many of them have been elucidated. Some are even synthesized in the laboratory.

The liberation of calcitonin, insulin, glucagon and epinephrine is not controlled by hypothalamus. They are secreted by the respective endocrine glands directly in response to peripheral stimuli like changes in blood calcium and glucose levels.

Recently, the importance of thymus in the immune system of the body has come into light. Previously, the thymus was considered to be only a vestigial organ. Embryonic stem cells from the yolk sac and the liver of the foetus and the stem cells of the bone marrow of the adult migrate to the thymus and proliferate there. They may be called as the 'prethymocytes.' They acquire their immunological properties in the thymus. A small number of them (about 5 per cent) leave the thymus and re-enter circulation as the T-cells, the remaining 95 per cent are destroyed in the thymus itself. There are three types of T-cells in human beings. About 40 per cent of them have receptor sites for Igm and are called T_{μ} cells; another 10 per cent have receptors for IgG and are called the T_{γ} cells and the remaining 50 per cent do not have any receptors for any of the immunoglobulins. The T_{γ} cells are the killer cells and the T_{μ} cells are the helper cells.

In the aetiology of myasthenia gravis, thymus is now incriminated. It is said to be caused by the individual developing autoimmunity against muscle proteins. A deficiency of thymus-dependent, cell-mediated immunoresponse is said to be the cause of diseases like sarcoidosis and lepromatous reaction in leprosy.

The degeneration of thymus takes place with age. Its size is maximal at birth, begins to shrink at puberty and is generally not visible after middle age. This may be the reason for the inability of the elderly to develop resistance to infections to which they were not already exposed during childhood and to neoplasms.

Thymus is known to involve certain hormones which influence the development and maturation of certain types of lymphoid cells. Many hormones were identified such as thymosins, thymopoietins, thymic factors, lymphocyte-stimulating hormones, thymosterin, etc. They are all active mainly in stimulating immunological responses and also have minor side effects from which their names are derived. Calcitonin is also secreted by the thymus.

In experimental animals, it was observed that thymectomy soon after birth results in failure of the animals to grow, wasting and death in a few weeks or months. Lymphoid tissues and lymphoid cells of blood are deficient. Immunoglobulin response is poor. Inadequacy of thymus in early life in the human is associated with diminished humoral as well as cell-mediated immunological response and in some cases gammaglobulinaemia.

17.2 SECOND MESSENGERS

17.2.1 Cyclic AMP

The cell membrane on the outer surface has a regulatory site (R) where the hormone binds to it (Figure 17.1). The cell membrane on its inner surface has a 'catalytic site' 'C' adjoining the regulatory site, which contains the adenyl cyclase enzyme. At the catalytic site, interaction between

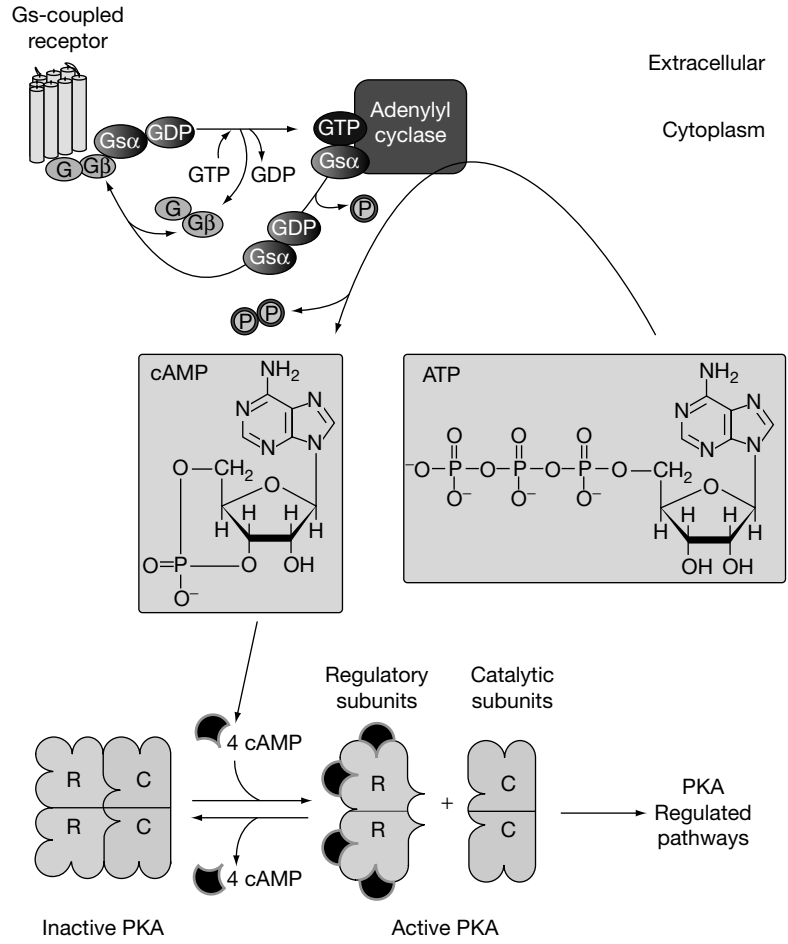


Figure 17.1 A model of adenylyl cyclase system. The figure shown below shows how cAMP interacts with intracellular protein kinases

the hormone and the receptor site will result in the stimulation of the adenyl cyclase; the adenyl cyclase will convert ATP to 3′ 5′-cyclic AMP (Figure 17.1) which enters the cytosol and in turn stimulates certain protein kinases which will phosphorylate certain enzyme molecules. Some enzymes, on phosphorylation, become active (e.g., phosphorylase), while some others become inactive (e.g., glycogen synthetase). Therefore, certain reactions are stimulated, while certain others are inhibited by the cyclic AMP. Cyclic AMP can be considered as the 'second messenger.' The hormone, therefore, need not enter the cell to produce its effects. Also, a small number of hormone molecules bound to the cell membrane can stimulate the production of a large number of cyclic AMP molecules in the cell.

The hormones calcitonin, chorionic, gonadotropins, corticotropin, epinephrine, FSH, glucagon, LH, MSH, norepinephrine, parathormone, TSH and vasopressin use cyclic AMP as the second messenger. Cholera toxin stimulates adenyl cyclase and causes production of excessive amounts of cyclic AMP in the mucosa cells of the intestinal epithelium.

The enzyme 'phosphodiesterase' converts cyclic AMP to ordinary AMP. Inhibitors of the enzyme phosphodiesterase like theophylline and caffeine thus prolong the action of the hormones which act through cyclic AMP. Insulin, on binding to the receptor on the target cell membrane, causes a decrease in cyclic AMP level, unlike epinephrine and glucagon; however, it acts on another nucleotide. GTP accelerates its conversion to cyclic GMP. The cyclic AMP and cyclic GMP seem to act in a reciprocal relationship, and this is known as the Yin-Yang hypothesis.

The regulation of the activity of adenyl cyclase system is mediated by two GTP-dependent regulatory proteins—Gs (stimulatory) and Gi (inhibitory). The G-proteins have three subunits—alpha, beta and gamma. It is the alpha-unit which shows alteration between the Gs and Gi states.

The G-protein also contains different receptor sites—Rs and Ri. Binding of the hormone to Rs causes production of Gs. Combination with Ri causes production of Gi. Cholera toxin causes irreversible formation of Gs. This, in turn, causes irreversible stimulation of adenyl cyclase. Pertussis toxin (whooping cough) causes irreversible formation of Gi.

In psuedohyperparathyroidism, parathyroid hormone is produced in normal amounts, but it has no effect on the target organ. This leads to hypocalcemia and hypophosphatemia. The defect appears to be a deficiency of the alpha-subunits of G-protein.

17.2.2 Cyclic GMP as Second Messenger

The enzyme guanylyl cyclase converts GTP to cyclic GMP. A number of peptides produced by cardiac muscle which can cause diuresis, increased urinary excretion of sodium and vasodilatation. They are said to act by stimulating guanylyl cyclase enzyme and formation of cyclic GMP which acts as a second messenger. Chemicals like nitroprusside, nitroglycerine, nitric oxide, sodium nitrite and sodium azide are powerful vasodilators and cause relaxation of smooth muscle. They seem to act by activating guanylyl cyclase and inhibiting cGMP phosphodiesterase, thereby increasing the levels of cyclic GMP and prolonging its action.

17.2.3 Phosphatidyl Inositol Biphosphate (PIP)

Upon hydrolysis, phosphoinositol biphosphate yields inositol triphosphate (IP_3) and diacylglycerol by the enzyme 'phospholipase-C.' When the hormone vasopressin binds to its specific receptor in the plasma membrane, changes occur in the G-protein similar to the changes in the beta-adrenergic receptor. The activated G-protein activates the phospholipase-C. This acts on PIP and releases IP_3 which causes a rapid release of Ca^{++} from the endoplasmic reticulum and sarcoplasmic reticulum (in smooth muscle) by opening up the calcium channels. The cytosolic calcium level is increased, and this triggers contraction of the smooth muscle, glycogen breakdown, etc. The specific phosphatases inactivate the IP_3 which convert it to inositol. Lithium inhibits this inactivation of IP_3 and is used therapeutically in the treatment of mental depression.

17.2.4 Diacetyl Glycerol

Proteinkinase C (PKC) is activated by diacetyl glycerol which, like PKA, phosphorylates serine and threonine residues in target proteins. PKC requires calcium for its activity. Diacetyl glycerol increases the affinity of PKC for Ca^{++} and thus activates it even at physiological levels of calcium. Phosphorylation inactivates diacetyl glycerol and forms phosphatidic acid or it may be hydrolysed to glycerol and fatty acids.

Cell division and cell proliferation is controlled by proteinkinase-C. Similar to diacetyl glycerol, certain substances called phorbol esters derived from croton oil activate proteinkinase C (PKC). But there are no mechanisms to breakdown these esters. Croton oil is hence carcinogenic and tumour promoter.

17.2.5 Calcium Ions

The calcium intracellular level is extremely low as compared to extracellular fluid levels. The intracellular level can be rapidly raised by opening up of calcium channels in the plasma membrane or the intracellular membranes. Calcium can rapidly bind to proteins and induce conformational changes in them. Calmodulin is a protein specially adapted to carry calcium and also bind to other proteins. Calmodulin contains two globular lobes joined by a long alpha-helix, each globe contains two hand-like structures called EF-hands. Each EF-hand can bind one Ca^{++} ion. The flexible region of the alpha-helix between the two lobes can give attachment to several proteins which may be enzymes, for example, calmodulin-dependent proteinkinase II (CaM kinase II) or pumps (Ca^{++} ATPase pump).

17.3 CLASSIFICATION OF HORMONES

Hormones can be classified into four groups as discussed hereunder.

1. **Steroid hormones:** The adrenal cortex secretes the steroid hormones (glucocorticoids and mineralocorticoids).

   Sex hormones: Oestrogens, androgens and progesterones, which are similar to hormones of testis (testosterone) and ovary (oestrogens and progesterone).
2. **Peptide hormones:** Peptide hormones are secreted by pancreas (insulin and glucagon).

   Parathyroid: Parathormone.

   Anterior pituitary: Growth hormone, prolactin, trophins: TSH, ACTH, FSH and LH.

   Posterior pituitary: Antidiuretic hormone (ADH), vasopressin and oxytocin.

   Gastrointestinal tract: Gastrin, secretin, cholecystokinin, vasoactive intestinal peptides and others.
3. **Amino acid derivatives: Thyroid:** Cacitonin, thyroxin (T4) and tri-iodothyronine (T3).

   Adrenal medulla: Epinephrine (adrenaline) and norepinephrine (noradrenaline)
4. **Miscellaneous hormones:** prostaglandins and cytokinins.

17.4 STEROID HORMONES

The adrenals: Adrenal glands in the human are situated close to the upper pole of the kidney and averages 45 by 26 by 6 mm in size and weigh about 10 g each. The adrenal gland consists of a medulla and a cortex, and each of them produces different hormones. In this category, the hormones produced by adrenal cortex are considered. It is a cyclic peptide with a molecular weight of 1640 and contains 14 amino acids.

17.4.1 The Adrenal Cortex

The source of adrenal cortex is the mesodermal tissue of the nephrotome portion of the embryo, and it is essential for life. It elaborates several hormones, all of which are steroid derivatives. Kendall and associates isolated and studied a number of hormones. They exert profound effects on (i) mineral metabolism, (ii) carbohydrate, fat and protein metabolisms (mainly carbohydrate) and (iii) sex hormone like actions. From adrenal cortex, nearly 50 distinct steroid compounds are isolated. They are classified according to their principal actions into one of the following three groups:

(a) Mineralocorticoids: (with 21 carbons).

(b) Glucocorticoids: (with 21 carbons).

(c) Sex hormones:

The sex hormones are oestrogens (18-carbon compounds), androgens (with 19 carbons) and progesterones (with 21 carbons). These hormones are similar to the hormones produced by testis (testosterone) and ovary (oestrogens and progesterones).

The mechanism of action of adrenocortical hormones is at the level of the cell nucleus. They bind to specific receptor proteins in the cytosol and the steroid/receptor complexes enter the nucleus where they bind to specific sites on the chromatin transiently and influence mRNA synthesis and through that protein (enzyme) synthesis. Inhibitors of RNA synthesis will thus prevent action of these hormones.

The 'pregnane' ring is the parent ring structure. The active hormones have a double bond between C-4 and C-5, and two keto groups—one at position 3 and another at position 20. Certain key groups exert profound influence on the biological activity of the compounds. A 'OH' at position 21 enhances sodium retention and is necessary for the effect on carbohydrate metabolism. A hydroxy or keto group at position 11 is also necessary for the effect on carbohydrate metabolism, but it diminishes the effect on mineral metabolism. A hydroxy group at position 17 enhances activity on carbohydrate metabolism.

Physiological Activity of the Corticoids

Mineral metabolism: Sodium and chloride reabsorption by the renal tubule is increased by corticoids, and on the other hand, corticoids decrease their excretion in sweat, saliva and gastrointestinal secretions. Aldosterone is the strongest hormone in this case. 11-Deoxycorticosterone and 11-deoxy, 17-hydroxycorticosterone (compound S) also have intense effects. They are produced in the zona glomerulosa. 11-Deoxycorticosterone acetate (DOCA) can be synthesized in the laboratory and can be absorbed by the buccal mucosa. Hence, it can be administered sublingually as a substitute for aldosterone; though, it is only 4 per cent as active as aldosterone. There is increased excretion of potassium and increased retention of sodium and water in the body.

The adrenocorticotropic hormone (ACTH) do not alter the secretion of aldosterone. Low sodium intake in food causes an increase in the production of aldosterone and vice versa. The regulatory mechanism seems to act through alteration of extracellular fluid volume, decrease of which stimulates aldosterone secretion which facilitates retention of sodium and an equivalent amount of water.

Aldosterone-stimulating hormone (ASH) is also active. Volume receptors said to be present in the walls of the renal afferent arterioles are stimulated by a decrease in the stretch of the arteriolar wall (fall in ECF volume). This causes the secretion of renin by the juxtaglomerular cells which in turn causes secretion of angiotensin II. The angiotensin II stimulates the relevant cells of the zona glomerulosa (of the adrenal cortex) to secrete aldosterone. Aldosterone secretion will lead to retention of sodium and water and expansion of ECF volume and thus causes stretching of the afferent arterioles of glomeruli and cuts down the secretion of renin. This is a 'feedback' mechanism of regulation.

Increased potassium concentration of plasma directly stimulates aldosterone production independent of ECF volume. The hormone seems to act at the nuclear level on the tubular epithelial cells.

In some diseases like liver cirrhosis, nephrosis and cardiac failure, there is an increased production of aldosterone which causes retention of fluid in the body (oedema and ascites). This is known as secondary aldosteronism. In primary aldosteronism, there is a hyperfunction of adrenal cortex to produce more of aldosterone. Certain drugs (e.g., spiranolactone or aldactone) block the action of aldosterone by binding to the aldosterone receptor and thus bring about diuresis and removal of excess water.

Licorice roots contain glycyrrhizinic acid which has mineralocorticoid activity. When it is used as a flavouring agent or in spices, there is a danger of causing hyperaldosteronism.

Effect on carbohydrate metabolisms: Glucocorticoids are the hormones with a 'O' or 'OH' at position 11 exercise their effects mainly on carbohydrate metabolism. Corticosterone (compound-B), 11-dehydrocorticosterone (compound-A) and the 17-hydroxy derivatives of these (compounds E and F) belong to this group. They are insulin antagonists in many respects. They cause increase in blood sugar level, decrease the utilization of carbohydrate, increase the synthesis of glycogen, and increase gluconeogenesis. Their stimulation of gluconeogenesis seems to be on account of increased synthesis of the enzyme 'phosphoenolpyruvate carboxykinase' (PEPCK), the rate-limiting enzyme in gluconeogenesis. Glucagon has an even greater effect in increasing the synthesis of this enzyme.

Effect on protein metabolism: Glucocorticoids stimulates gluconeogenesis from the metabolism of proteins. Large doses of these hormones can produce cessation of growth, negative nitrogen balance, muscle wasting and other reactions characteristic of protein breakdown.

Effect on fat metabolism: There do not appear to be direct effects of the corticoids on fat metabolism.

Anti-inflammatory effects: In addition to the metabolic effects, the cortical hormones exercise a profound anti-inflammatory effect and are invaluable in the treatment of collagen diseases like rheumatoid arthritis. They also decrease the antigen/antibody response and are useful in treating allergic conditions. They probably suppress synthesis of nucleic acid and protein in the lymphocytes. Cortisol also depresses immune response in organ transplantation procedures.

Biosynthesis of Steroid Hormones

The precursor for all steroid hormones is cholesterol. The enzyme desmolase cleaves the side chain of cholesterol between C_{20} and C_{22}. Different monooxygenases introduce OH at different

positions which require molecular oxygen and NADPH + H^+. Different enzymes exist for hydroxylation at different positions of the steroid ring—C_{11}, C_{17}, C_{21}, etc. Cytochrome P_{450} is required in the electron transport of this enzyme system. These various enzymes are present in the adrenal cortex, testis and ovary.

A lack of the enzyme 21-hydroxylase causes less production of glucocorticoids and mineralocorticoids.

Slight changes in the structure of the hormone molecule cause an emphasis of certain of the hormonal actions while suppressing most of the others (synthetic analogues of steroid hormones). Steroids having intense anti-inflammatory effect with little or no metabolic effects are thus produced (e.g., prednisone and prednisolone). Many of these compounds are used in therapy.

ACTH regulates the secretion of glucocorticoids. Lowered cortisone levels in blood stimulate ACTH production.

About 25 mg of cortisol and 2.5 mg of corticosterone and less than 0.1 mg of aldosterone are the principal hormones secreted by the adrenal cortex per day.

The transported hormones are bound to an alpha-globulin in the plasma (transcortin and corticosterone-binding globulin; CBG). In the liver, they are reduced to their tetrahydro derivatives and then conjugated with glucuronic acid and excreted through bile. Some are reabsorbed (entero-hepatic circulation) and a portion enters systemic circulation to be excreted by the kidney. Thus, liver is an important organ in inactivating the hormones. In hepatic failure, one cause for oedema and sodium retention may be on account of prolonged action of these hormones due to failure of the liver to inactivate them.

Hyperfunction of Adrenal Cortex

Hyperadrenocorticism is produced by the tumours of adrenal cortex. This manifests in the following: (1) negative nitrogen balance, (2) hypokalaemia, (3) hyperglycaemia and glycosuria, (4) retention of sodium and water resulting in oedema and hypertension and (5) hirsutism.

Hirsutism: Hirsutism is due to the hyperaction of the androgens of the adrenal cortex. Occurrence of hirsutism in childhood is called adrenogenital syndrome. The female assumes male secondary sex characters (growth of beard and moustache) and males show excessive masculinization.

Cushing's syndrome: Cushing's syndrome is mainly caused by the pituitary gland (basophilic adenoma of pituitary). The increased adrenocorticotropic hormone (ACTH) stimulation will lead to a hyperfunctioning of the adrenal cortex. It may also occur due to a primary adenoma of the adrenal cortex. The state is characterized by obesity involving the face, neck and trunk (such as the buffalo type), purpura, hirsutism, hypertension, hyperglycaemia and glycosuria.

Addison's disease: Generally, due to a tuberculous infection, a hypofunction of adrenal cortex, results in excessive loss of sodium and chloride in urine, low blood pressure, hypoglycaemia, general weakness, wasting and a brownish pigmentation of the skin. The pigmentation is due to excessive ACTH secretion by the pituitary, the ACTH having a melanocyte-stimulating effect.

17.4.2 Sex Hormones

These hormones are of two types: the male sex hormones and female sex hormones.

The Male Hormones

Testosterone is secreted or produced by the Leydig cells of the testis, and it is the principal male hormone. Pregnenolone is a precursor of testosterone, adrenal cortical hormones and progesterone, and it is a 19-carbon steroid. The testosterone is reduced to dihydrotestosterone (DHT) in the target tissues. DHT appears to be the active hormone. Only 400 μg is produced per day (compared with 5 mg of testosterone).

The less potent hormones are androstenedione and dehydroepiandrosterone (DHEA) and are produced in the testis and adrenal cortex (Figure 17.2). The hormones are transported by a specific plasma protein. Testosterone is metabolized to form androsterone and DHEA.

The growth of secondary sex organs—epididymis, vas deferens, prostate, seminal vesicles and penis— is promoted by testosterone. It also promotes muscular and skeletal growth and is protein anabolic. There is retention of calcium and phosphate also. It exerts protein anabolic effect by increasing the RNA and RNA polymerase of the cell nucleus and aminoacyl transferase of the ribosome. It also stimulates the mitochondrial activity. The protein anabolic effect is useful in the treatment of certain conditions of impaired growth or recovery from illness. Like adrenal cortical hormones, the androgens also first bind to cytosol receptor proteins, enter the cell nucleus and interact with nuclear chromatin to exert their actions.

Testosterone production by the Leydig cells is stimulated by LH. FSH stimulates synthesis of an androgen-binding protein (ABP) by the Sertoli cells. The ABP carries testosterone in high concentrations to the site of spermatogenesis, the seminiferous tubule.

Primary and secondary hypogonadism: It occurs before puberty and secondary sex characters do not develop. If it occurs at later stages, the secondary sex characters already developed regress. Primary hypogonadism is due to testicular dysfunction, and secondary hypogonadism is due to deficient secretion of LH and FSH.

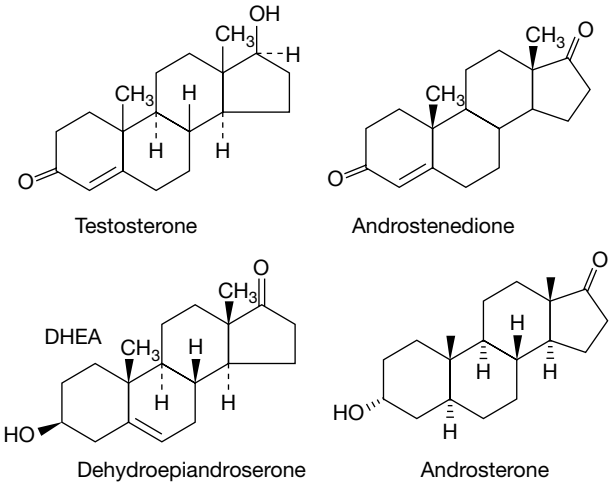


Figure 17.2 Structure of some male hormones

Synthetic analogues of androgens: If an additional methyl group is attached to C-17, then it is methyl testosterone, active on oral administration. Norsteroids are derivatives without a methyl group at C-10, for example, 19-nortestosterone and its 17-ethyl derivative, have potent protein anabolic effect and only a fraction of the androgenic effect. They are hence used as protein anabolic drugs. The hormones are excreted in bile and urine after conjugation in the liver with sulphuric acid or glucuronic acid. Their derivatives account for one-third of the urinary neutral 17-ketosteroids.

The Female Hormones

The female hormones are of two types as discussed hereunder.

(a) The follicular or oestrogenic hormones derived from the Graffian follicle.

(b) The progestational hormones are derived from the corpus luteum.

The follicular hormones: The production of this hormone takes place in ovary, placenta, adrenal cortex and testis in different amounts. They are C-18 steroids. Ring A is aromatic and methyl group at C_{10} is absent.

The main hormones are estrodiol and oestrone. In women, the hormones are responsible for the preparative phase of the menstrual cycle. They induce proliferation of endometrium, deepening of uterine glands, increased vascularity and changes in the fallopian tubules and vagina. The excretory product in urine is estriol (Figure 17.3). Oestrogens induce oestrus in the lower animals. A vaginal smear will show the altered histological appearance of oestrus. They suppress the secretion of FSH by pituitary. Oestrogens are necessary for the maintenance of female secondary sex characters.

Oestrogens exert protein anabolic effect locally on the target organ (uterus). The hormones are bound to a specific lipoprotein in that tissue. The RNA-polymerase activity of the tissue is increased. They also increase the phospholipid turnover rate in general and are hence lipotropic. They bring down the plasma lipids, if administered in conditions of hyperlipidaemia and coronary heart disease in men. Serum calcium and phosphate levels are increased on prolonged administration. Hypercalcification of bones may occur.

These are two synthetic substances (synthetic hormones) ethinyl estradiol, diethylstilbestrol which exert potent oestrogenic effect when administered orally.

Progestation hormones or luteal hormones. Pregnenolone is a precursor of progesterone and is a C-21 compound (Figure 17.3). The hormone is also formed by the placenta and also by adrenal cortex where it is the precursor of the several cortical hormones.

The action of the hormone is to cause endometrial development preparations for the reception of nutrition of the embryo. It suppresses ovulation, oestrus and the secretion of LH by pituitary. During pregnancy, progesterone production by corpus luteum continues through till near term.

The output of both oestrogen and progesterone suddenly falls on or about the 28th day of the menstrual cycle, menstrual flow starts and the uterine endometrium starts sloughing if pregnancy has not occurred. Progestin decreases peripheral blood flow and decreases heat loss from the skin. Hence, body temperature increases by about 0.5°C during the luteal phase of the menstrual cycle.

Three-fourth of the progesterone is eliminated through bile (as pregnanediol and pregnanetriol) and also through urine.

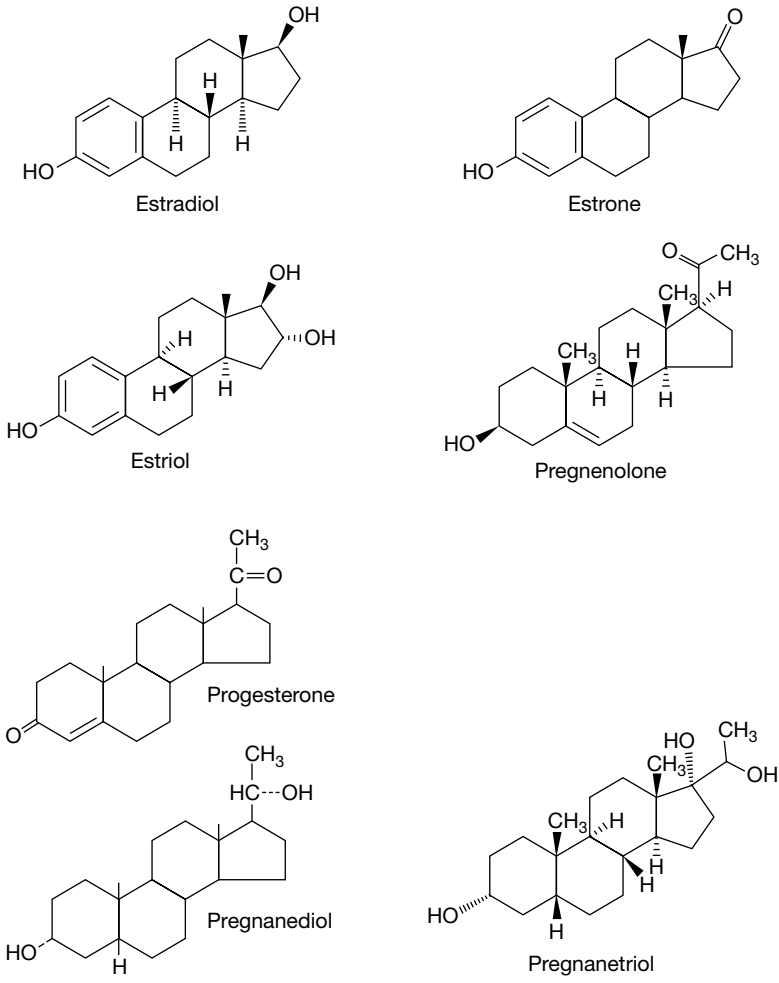


Figure 17.3 Structure of some female hormones

Progesterone: If progesterone is administered from 5th to 25th day of the menstrual cycle, it exerts an anti-ovulatory effect. It is, however, active only on parenteral administration. Synthetic progestins like 17-alpha-ethinyltestosterone and 17-alpha-ethinyl, 19-nortestosterone are active on oral administration, their activity being equal to or more potent than that of parenteral progesterone.

The another hormone from corpus luteum is relaxin. It is a polypetide hormone and is also produced by the placenta. It causes relaxation of symphisis pubis in animals.

The two synthetic substances are norethindrone and norethinodrel (synthetic hormones) which are active when taken by mouth. These synthetic oestrogens form the basis for oral contraceptive therapy. As described previously, they suppress the secretion of pituitary FSH and thus prevent ovulation from occurring and thus pregnancy is avoided. A constant search for orally active compounds that suppress FSH without exerting much hormonal effect is on.

Primary hypogonadism is caused by a disorder or failure of the ovaries, which may result from a chromosomal abnormality such as gonadal dysgenesis or Turner's syndrome, and the ovarian function may be defective and results in Stein–Leventhal syndrome or polycystic ovaries.

Secondary hypogonadism is caused by abnormal secretion of pituitary gland or hypothalamus, which leads to underproduction of the hormones that stimulate the ovaries to function. In either case, there is an imbalance between the male and female sex hormones, the former gain predominance, and hirsuitism, obesity, irregularties of menstrual cycle and infertility are caused. Hydatidiform mole and choriocarcinoma produce large amounts of human chorionic gonadotropins (hCG) that are excreted in urine in increased amounts. This can give a false pregnancy test when the urine is tested for pregnancy.

The menstrual cycle: Menstrual cycle occurs on an average every 28 days (varies from 25 to 35 days) in women. The menstrual cycle has the following three phases:

1. **Follicular phase:** Under the control of FSH, a single follicle in one of the ovaries begins to enlarge. This is accompanied by a rise in plasma oestrogen levels. It reaches peak levels and suddenly starts falling. The high peak reached or the sudden fall in oestrogen level causes release of lutenizing hormone (LH) mediated through pituitary regulation. These changes occur about 13th to 15th day after the last menstrual period. In 16 to 18 h thereafter, the ovum is released from the follicle (ovulation that indicates the end of the follicular phase).
2. **Luteal phase:** The granular cells of the ovary after ovulation, at the ruptured follicular site, undergo changes to form the corpus luteum. Corpus luteum produces progesterone and small quantities of estradiol. The estradiol secretion reaches a peak midway of the luteal phase (about 21st day of the cycle) and rapidly falls to quite low levels. Progesterone prepares the endometrium for the implantation of the blastocyst which will be formed if fertilization of the ovum by a spermatozoon has occurred. If implantation occurs (22 to 24 days of the menstrual cycle), chorionic gonadotropins take over the function of LH and stimulates the corpus luteum to produce progesterone. In the absence of implantation, the corpus luteum regresses and menstruation occurs.
3. **Menstruation:** Menstruation occurs at the end of the 28th day in the absence of fertilization of the ovum and implantation, and a new cycle starts.

Placental hormones: Implantation of the blastocyst in the endometrium forms the trophoblast which subsequently becomes the placenta. Placenta produces several hormones to support pregnancy and related changes in other internal organs.

1. **Human chorionic gonadotropin (HCG): HCG** has a close resemblance to LH structure and is a glycoprotein. The presence of these hormones can be seen in the urine a few days after implantation, even before the missing of the period is noticed. Their presence in urine forms the basis of pregnancy tests. Agglutination methods and ELISA techniques are available for early detection of pregnancy. The tests take only a few minutes to perform and have replaced the earlier time-consuming methods like the Aschheim–Zondek and Friedmann tests using rat or rabbit as experimental animals.

   Peak levels of HCG are reached in the middle of the first trimester. They start declining during the rest of pregnancy.
2. **Progestins:** During the first 6 to 8 weeks of pregnancy, corpus luteum supplies progestins. Placenta takes over the function subsequently.

3. **Oestrogens:** During pregnancy, oestrone and oestriol are produced continuously by the placenta. Their levels steadily rise.
4. **Placental lactogen:** This hormone is also known as somatomammotropin or placental growth hormone. Its actions are similar to those of prolactin and GH.

The onset of labour and the process of parturition are also under hormonal control. But the mechanisms are not clearly understood. Oestrogens, progestins, catecholamines and oxytocin play a major role.

Oestrogens and progestins promote the growth of mammary gland. Oestrogens promote growth of the duct system, and progestins promote growth of the alveolar system. Prolactin exerts an overall influence. Its concentration in serum increases from a mere 2 ng/dl to 200 ng/dl in late pregnancy. A sudden drop of progesterone levels soon after parturition triggers milk production by the mammary gland. Progesterone inhibits milk production.

After parturition, prolactin levels also fall. But bouts of secretion occur with each episode of suckling by the infant. If suckling does not take place, lactation gradually decreases. Suckling also releases oxytocin from pituitary, and this helps in expulsion of the milk from the mammary glands and in contraction of the uterus.

Menopause: The cessation of menstruation occurs around the age of 50 and women cease to have ovulation also. The oestrogens and progestins, which arise from the ovarian follicle during ovulation, are no longer produced. This stimulates the pituitary to secrete large amounts of FSH and LH, whose levels in blood are very much increased. Secondary sex organs of the female get atropic. There is severe osteoporosis on account of deficiency of oestrogens.

17.5 PEPTIDE HORMONES

17.5.1 Pancreatic Hormones

Islets of Langerhans produce four hormones as discussed hereunder**.**

(a) **Insulin**

(b) **Glucagon**

(c) **Somatostatin:** Somatostatin regulates the secretion of insulin and glucagon. It is also produced by the hypothalamus and inhibits GH secretion.

(d) **A pancreatic polypeptide:** Pancreatic polypeptide regulates gastrointestinal secretions.

Insulin

In 1922, Banting and Best extracted insulin from pancreas. It is a protein hormone and can be purified and separated in a crystalline form. Traces of zinc are invariably associated with insulin. Sanger and Smith elucidated its protein structure. Human insulin is made up of two polypeptide chains (A and B) linked together by two disulphide linkages and has a total of 51 amino acids. It has a minimum molecular weight of 5734. It is often present as an aggregate of two or more such simple molecules and shows a molecular weight from 12,000 to 48,000. Insulins from different sources (e.g., pig, cattle, sheep and horse) show minor differences in the amino acid composition and immunological activity. The closest resemblance of human insulin is with the pig's insulin. The biological action of the hormone can be prolonged by combining it with protamine or globin (protamine-zinc-insulin and globin-insulin) or by altering the size of the crystals (ultralente

insulin: large crystals and slow acting) so that it is slowly released from the site of injection and acts for several hours (12 to 24 h). Lente insulin is a 7:3 mixture of ultralente and regular insulins.

Insulin Biosynthesis

'Preproinsulin' is the primary translation product of the mRNA for insulin (Figure 17.4), and this large molecule migrates to the endoplasmic reticulum. The extra peptides are removed by the enzyme peptidase to release 'proinsulin' which is made up of the A and B chains linked together by another polypeptide containing 33 amino acids. The proinsulin in the pig has a molecular weight of 9082. It undergoes proteolytic cleavage and the insulin molecule separates out and forms aggregates of two to eight molecules. During the proinsulin stage, the beta-cell is filled with vesicles containing the prohormone. Fully formed insulin is associated with the granules of the beta-cell which forms within the vesicles. In islet cell tumours, proinsulin itself may be circulating in the plasma. The structures of preproinsulin, proinsulin and insulin are schematically presented in Figure 17.5.

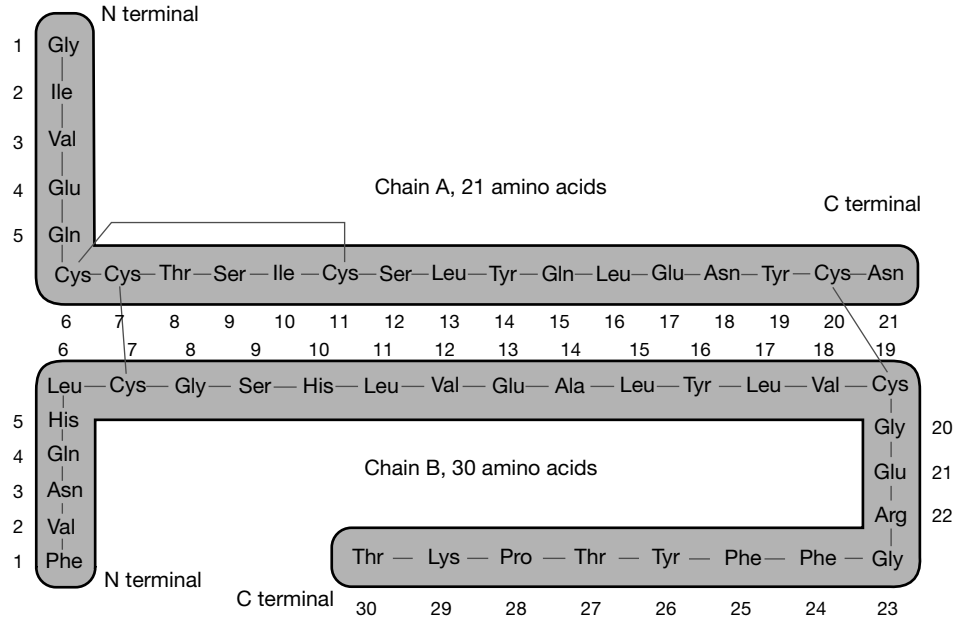


Figure 17.4 Structure of Insulin

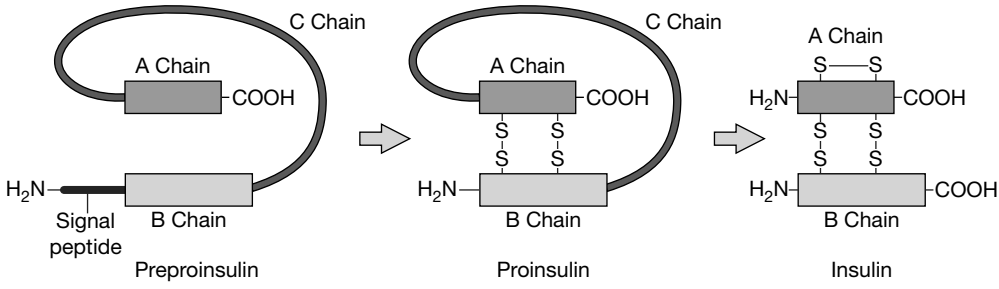


Figure 17.5 The structures of preproinsulin, proinsulin and insulin

Free insulin is released after the treatment of proinsulin with trypsin in vitro, and it will remove the connecting polypeptide. In the process, the 30th amino acid of the B chain, alanine, is also removed. It is called dalanated insulin (for dealaninated insulin) and has the same biological activity as insulin.

Monocomponent insulin, a highly purified insulin, is being marketed in recent years. It has minimum antigenicity and is particularly useful in cases of insulin resistance. About 250 units of insulin are present in human pancreas. About 50 units of insulin are required daily. It is likely that actual stimulus is provided not by the sugars themselves but by one of the products of their metabolism, probably arising out of the citric acid cycle. Cyclic AMP is also a stimulant for insulin secretion. Glucose stimulates calcium uptake and production of cyclic AMP by the beta-cell. Localization of calcium in certain areas of the beta-cell may be necessary for the secretion of insulin. Tolbutamide, one of the oral anti-diabetic drugs, also stimulates insulin release independent of alterations in glucose level and even when glucose metabolism is blocked by antimetabolites.

Amino acids leucine and arginine can also stimulate insulin secretion. The role of fatty acids in stimulating insulin production is not clear. Glucocorticoids and several of the hormones like growth hormone stimulate insulin secretion through their hyperglycaemic effect. Glucagon seems to stimulate insulin production not only by causing hyperglycaemia but also by increasing the cyclic AMP in the pancreas. The cyclic AMP stimulates glucose metabolism and thus supplies citric acid cycle intermediates which stimulate insulin secretion.

Insulin secretion is inhibited by epinephrine in spite of the hyperglycaemia. In vitro, insulin secretion is stimulated by calcium and potassium while inhibited by magnesium.

Intake of glucose by mouth is a better stimulus for insulin secretion than when taken by injection. This is attributed to the action of the intestinal hormones like pancreozymin, secretion **a**nd glucagon-like substances that are released when glucose is administered by mouth. They seem to stimulate insulin secretion. Vagal stimulation increases insulin secretion.

Insulin Metabolism

The enzyme glutathione-insulin transhydrogenase degrade insulin in the liver and kidney and which cleaves the -S-S linkages to -SH, thus separating the A and B chains. The hydrogen is derived from glutathione. The A and B chains undergo further hydrolysis by the enzyme 'insulinase.' The half-life of plasma insulin is only 7 to 15 min. Muscle, adipose tissue and liver are the major sites of its action. It is also active on the lens and leukocytes. It has little action on the metabolism of the renal tissue, erythrocytes and the gastrointestinal tract.

Receptors of Insulin

The receptor of insulin is dimeric in nature and consists of two alpha- and two beta-subunits. Both alpha- and beta-subunits are glycoproteins. The alpha-subunits project on the outer surface of the cell membrane. They bind insulin, probably at a cysteine-rich domain. The beta-units are transmembrane units and also project into the cytosol. They have tyrosine kinase activity and phosphorylation sites. Constant synthesis and degradation of the insulin receptors takes place (half life is 7-12 hours). When insulin binds to the receptor, conformational changes occur in the receptors. Neighbouring receptors aggregate together and move to the interior of the cell along with the insulin. This is one way of regulating insulin action by making less number of receptors available to insulin when insulin levels are high.

The receptors' excessive internalization may be one of the causes for insulin resistance in obesity and type II (noninsulin-dependent) diabetes (NIDDM). Insulin also promotes cell growth and differentiation just like insulin-like growth factors I and II (IGF-I and IGF-II). The action is by increasing translation by mRNA and by increasing gene expression.

Diabetes Mellitus

Diabetes mellitus is a metabolic disorder occurring as a result of lack of insulin or excess of insulin antagonists leading to a relative insulin lack. It is characterized by hyperglycaemia and glycosuria.

Three types of diabetes mellitus are discussed hereunder.

1. **Type 1:** Insulin-dependent diabetes mellitus (IDDM): By autoimmune mechanisms, beta-cells are damaged. T-lymphocytes accumulate in the islets and produce an inflammatory reaction (insulitis). There is a genetic basis—the beta-cells are treated as non-self or foreign. They are attacked by the immune mechanism of the body. The condition may he precipitated by infections, particularly viral infections.

   It occurs early in childhood and the onset is usually sudden. Polyurea, polydypsia and polyphagia (excess urine, thirst and hunger) are the main symptoms. Hyperglycaemia is often associated with ketoacidosis.

   Treatment is carried out by the administration of insulin. Occurrence of hypoglycaemia due to overdose of insulin is a risk, which has to be kept in mind.
2. **Type II:** Noninsulin-dependent diabetes mellitus (NIDDM): Generally, obese individuals develop this disease over a period in adult. It may be detected only during a routine medical check up. Beta-cell function is impaired, and also the effectiveness of insulin is decreased due to insulin resistance.

Conditions of Insulin Resistance

Obesity: Higher than normal levels of circulating insulin is found in many obese persons. In course of time, even the high levels of insulin are unable to overcome insulin resistance. Then, diabetes sets in. This is on account of beta-cell failure to meet the increased demands for insulin secretion to overcome the insulin resistance. Reduction of weight and decrease of obesity will decrease insulin resistance and may not require any other treatment. Adipose tissue produces certain regulatory substances (hormones)—leptin, resistin and adiponectin—which all contribute to insulin resistance. Increased levels of nonesterified fatty acids (NEFA) in plasma also contribute to insulin resistance.

Hyperglycaemia and hyperlipidaemia (particularly increased plasma triglycerides and VLDL) are observed.

Type II diabetes can be usually controlled by weight reduction, exercise and diet regulation. Oral hypoglycaemic agents are useful in treating this condition. Insulin is only rarely required.

3. **Secondary diabetes:** In this disease, there is hyperfunction of one or other of insulin antagonists leading to a relative insufficiency of insulin, for example, acromegaly (excess of growth hormone), Cushing's disease (excess of glucocorticoids), hyperthyroidism, etc.

Experimental diabetes can be produced by total pancreatectomy or selective destruction of the beta-cells by injecting alloxan, a pyrimidine derivative, which is highly and selectively toxic to the beta-cells. Injection of insulin antibodies can also cause diabetes. Phlorhizin causes

glycosuria by decreasing renal tubular reabsorption of glucose (renal glycosuria) and decreasing the renal threshold for glucose.

In the etiology of diabetes mellitus, glucagon is one of the contributory factors. Its blood levels are elevated in severe diabetes with ketoacidosis. The alpha-cells seem to be insensitive to the high blood glucose levels in diabetes and continue to secrete large amounts of glucagon. Somatostatin, a hypothalamic factor inhibiting the release of growth hormone, also inhibits the release of glucagon and is in experimental use as an adjunct to insulin in the control of severe diabetes mellitus. Somatostatin is also secreted by the D (delta) cells of the pancreas and the gastric mucosa. It is a small peptide containing 14 amino acids.

The patient suffering from diabetics undergoing treatment with insulin may develop, in course of time, antibodies to insulin. They bind to the insulin injected and make it ineffective, requiring an increase in the insulin dosage. A change in the brand of insulin may be effective in such cases. The development of resistance can be avoided by injecting highly purified preparations.

A second type of insulin resistance is due to the presence of antibodies against the insulin receptors. The antibodies bind to the insulin receptors, thus denying them access to insulin. Insulin fails to exert its normal action due to nonavailability of receptors.

A few cases of abnormal insulin production are reported. This is due to mutation occurring in the insulin gene which is located in the short arm of chromosome II. The abnormal insulin does not have normal physiological activity.

Glucagon

The alpha-cells of the pancreas secrete the hormone glucagon. In 1923, Kimball and Murlin noted hyperglycaemic effect of some pancreatic extracts due to the presence of this hormone. It is a polypeptide with a molecular weight of 3485. The polypeptide consists of 29 amino acid residues arranged in a straight chain. Unlike insulin, glucagon crystals are free from zinc. In addition to the alpha-cells of pancreas, the gastric and duodenal mucosa also elaborate glucagon-like substances. The structure of glucagon closely resembles that of secretin. Ingestion of carbohydrate in food stimulates the release of intestinal glucagon which in turn stimulates the pancreatic beta-cells to secrete insulin. Glucagon is transported in the blood in the free state and is destroyed in the liver by the enzyme glucagonase. The stimulus to glucagon production is lowering of blood glucose levels.

Hepatic adenyl cyclase enzyme is stimulated by glucagon, thereby leading to an increase in cyclic AMP level in the internal organs. This in turn stimulates the dephosphophosphorylase kinase enzyme resulting in the reactivation of phosphorylase. The final result of glucagon action is thus an increase in the active form of phosphorylase and increased breakdown of liver glycogen resulting in a blood sugar rise (hyperglycaemia). The effect is seen within minutes of glucagon administration and the hyperglycaemia lasts for an hour or more following a single injection.

The glucagon has no action on the muscle phosphorylase. Hence, the blood pyruvate and lactate do not show any change. Glucagon inhibits hepatic synthesis of protein from amino acids and synthesis of fatty acids and cholesterol from acetate. The amino acids are used for increased gluconeogenesis. There is also increased lipolysis and ketone body formation in the liver. Glucagon increases glomerular filtration rate (GFR) and augments the urinary excretion of sodium, chloride, potassium and phosphate.

Glucagon also stimulates lipolysis in adipose tissue. All these three actions are antagonistic to insulin action. Hence, diabetes produced by pancreatectomy (where insulin and glucagon are

both absent) is less severe than that produced by selective destruction of beta-cells by toxic substances like alloxan (where insulin is absent but glucagon is still present).

Somatotropin

This hormone is secreted by the D cells of the islets**.** It is secreted as a prohormone with a molecular weight of 11,500 and then broken down to the final size. It is a cyclic peptide, with a molecular weight of 1640 and contains 14 amino acids. It is also present in the hypothalamus and in many gastrointestinal tissues.

It inhibits the release of insulin and glucagon from the islets. The suppression of glucagon secretion has a beneficial effect in reducing ketosis in diabetes mellitus.

Pancreatic Polypeptide (PP)

It has 36 amino acids and a molecular weight of 4200. It is produced by the F cells of the pancreas.

17.5.2 The Parathyroids

In the thyroid, two pairs of glands embedded are parathyroids. They secrete a hormone called the parathyroid hormone or parathormone. It synthesizes a preprohormone which loses 25 amino acids to become the prohormone. The prohormone, in turn, loses six more amino acids to become the active parathormone. It has 84 amino acids, with a molecular weight of 8500. Its amino acid composition had been worked out. Parathormone activates adenyl cyclase in bone and kidney. Urinary cyclic AMP is increased. The hormone exerts its action chiefly on calcium and phosphorus metabolisms. Administration of the hormone (1) raises serum calcium, (2) lowers serum phosphorus and (3) increases urinal excretion of both calcium and phosphorus. Parathormone (PTH) is degraded by proteolytic enzymes (cathepsins) present in the parathyroids and in most tissues, more particularly in the Kupffer cells in the liver.

There is no storage of the hormone.

Bone and kidney on their cell membrane contain receptor proteins to the hormone. When the hormone combines with the receptor, there is an activation of the cascade system leading to the formation of cyclic AMP. PTH has an anabolic effect on bone at low concentrations. It stimulates osteoblastic activity and increases alkaline phosphatase. In higher concentrations, it exerts a catabolic effect and stimulates osteoblastic activity. Calcitriol synergizes PTH action on bone.

Synthesis of Calcitriol

Usually, 90 per cent of calcium in the glomerular filtrate is reabsorbed but due to the influence of PTH, the quantity reabsorbed increases to 98 per cent. Simultaneously, there is an inhibition of phosphate reabsorption. PTH has no direct effect on the absorption of calcium by the small intestine. But since calcitriol has such effect, by enhancing calcitriol formation, PTH indirectly increases calcium absorption by the small intestine.

Besides calcium loss, there is also a loss of mucopolysaccharide from bone matrix. Serum glycoprotein and sialic acid levels are increased following prolonged hormone action. The secretion of parathormone is regulated by the ionic calcium levels of plasma. Parathormone is continuously secreted by the gland on demand. There is very little storage—just enough for 1½ h

(compared with insulin stores in the pancreas which are adequate for several days and thyroxin stores in thyroid which are enough for several weeks).

Generally, hypoparathyroidism is caused by an accidental removal of the glands along with thyroid. Muscular weakness, low serum calcium and tetany are some of the manifestations.

Hyperparathyroidism is caused due to the tumours in the glands. Decalcification of bones, spontaneous fractures or bending of the bones, increased blood calcium level and decreased phosphate levels are some of the symptoms. The large amount of circulating calcium may be deposited in the form of calculi in the kidney and pancreas. Abnormal calcification of pericardium and other soft tissues may occur.

Calcitriol

Vitamin D, the 1, 25-dihydroxy derivative of cholecalciferol is now put into the category of a hormone and is known as 'calcitriol.' The principal action of the hormone is to stimulate intestinal absorption of both calcium and phosphate. The hydroxylation of cholecalciferol occurs in two stages. The -OH at position 25 is added in the liver by a 25-hydroxylase enzyme. The 25-hydroxylated cholecalciferol now is taken up by the kidney and another hydroxy group is added in position 1 by 1α-hydroxylase present in the epithelium of the proximal convoluted tubule. The α-hydroxylase of the kidney is stimulated by PTH. The enzyme is inhibited by high levels of calcitriol, a feedback mechanism.

Calcitriol is similar to the other steroid hormones. It enters the target cells (in the intestinal villi and the osteoblasts of bone and the distal convoluted tubule of the kidney) and binds to the nuclear chromatin. Special calcitriol receptor proteins are present in these cells. The binding of calcitriol causes increased production of calcium-binding protein (CBP) by those cells due to stimulation of the concerned genes. The net result is an increased calcium absorption by small intestine and increased tubular reabsorption of calcium by the kidney.

17.5.3 The Pituitary Gland

The pituitary gland is also known as hypophysis. Hypophysis is composed of two parts: neurohypophysis (a neural component) and adenohypophysis (a buccal component). Neurohypophysis consists of the posterior lobe and the infundibulum and the adenohypophysis consists of the anterior lobe and the intermediate or middle lobe.

Anterior Lobe Hormones

In the pituitary gland, three types of cells are present: (1) eosinophilic cells which take up acid dyes, (2) basophilic cells which take up basic dyes and (3) neutrophils (chromophobe cells) which do not take either acidic or basic stain. The individual hormones formed by these are not clearly identified.

Anterior pituitary produces hormones which can be classified into three groups as listed hereunder.

1. **Growth hormone:** prolactin and somatomammotropin group, are all proteins.
2. TSH, lutenizing hormone (LH), follicle-stimulating hormone (FSH) and chorionic gonadotropin (CG), all are glycoproteins.
3. ACTH, lipotropin (LPH) and MSH (melanocyte-stimulating hormone), all are peptides.

The hormones in each group have close structural resemblance.

Growth Hormone (Somatotropin)

The growth hormone (GH) is a polypeptide with a molecular weight of 21,500. It has 191 amino acids and two disulphide bridges.

The receptor of the growth hormones is a protein with a single-membrane spanning domain. When growth hormone binds to it, two neighbouring receptors form a dimer which can stimulate tyrosine kinases leading ultimately to the activation of several signal pathways for increased synthesis of protein by the concerned genes. IGF I and IGF II mediate the growth-related effects of GH. GH seems to act by stimulating protein synthesis and by enhancing the uptake of amino acids by cells. It causes a nitrogen retention (positive nitrogen balance) and also a phosphorus retention. The transport of amino acids across the cell membrane is increased. This transport is independent of its subsequent utilization for protein synthesis.

Liver glycogen is increased due to increased gluconeogenesis. The combined effect of the two is hyperglycaemia and a decreased glucose tolerance. The effect on protein metabolism is similar to insulin action but that on lipid and carbohydrate metabolisms is antagonistic to insulin action. Calcium, sodium, potassium and phosphate are retained under the influence of the hormone.

The production of 'somatomedins' is stimulated by GH from liver and kidney. They stimulate many of the anabolic effects of growth hormone and have also serum insulin like activity.

The release of not only growth hormone but also the release of insulin, glucagon, thyrotropin and FSH is inhibited by somatostatin. It is used along with insulin, experimentally, in the control of severe diabetes mellitus to suppress the secretion of glucagon in particular. The D cells of the pancreatic islets and the gastric mucosa also secrete somatostatin.

Immunoassay methods can measure the growth hormones in plasma. They are highest in children and steadily decrease with age. The levels are same in both sexes or sometimes higher in females. Exercise and stress cause a transient increase in its levels. Hypoglycaemia stimulates secretion of GH. Amino acids, particularly arginine, also cause an increased production of the hormone. Glucose and glucocorticoids inhibit the secretion of GH.

The regulation of GH seems to be a specific 'growth hormone–releasing factor' (GHRF) as well as a 'growth hormone release inhibiting factor' (GH-RIH) or 'somatostatin.'

'Gigantism' syndrome is due to hypersecretion of growth hormone, if it occurs during growing period and leads to 'acromegaly' if it occurs in the adult. Dwarfism is due to hyposecretion of growth hormone during growing periods.

Lactogenic Hormones (Prolactin, Mammotropin, Luteotropic Hormone (LTH))

The hormone stimulates the growth of the mammary gland and activates the corpus luteum and stimulates the production of progesterone by that structure. It also stimulates growth like the GH. It is also a protein hormone and has a molecular weight of 23,400. It is inhibited by a hypothalamic 'prolactin inhibiting factor.'

The structure and function of growth hormone receptors are similar to the prolactin receptors on the target cell membrane. Tumours of prolactin-secreting cells cause amenorrhoea (cessation of menses) and galactorrhoea (spontaneous discharge of milk from the breasts).

The tropic hormones are produced by anterior pituitary whose target organs are other endocrine glands in which the functions are stimulated by these hormones. The tropic hormones are produced inversely in relation to the plasma concentration of the hormones of the target

endocrines. Thus, high levels of thyroxine in plasma will inhibit the production of thyrotropic hormone (thyroid-stimulating hormone (TSH)) and vice versa.

Gonadotropins

(i) **Follicle-stimulating hormone (FSH):** This hormone promotes the growth of the Graafian follicle in the female and the testicular growth and early stages of spermatogenesis in the male. The blood levels of FSH are markedly increased during puberty and in the female at the time of ovulation. Its secretion is said to be regulated by the hypothalamus through a FSH-releasing factor. It has a molecular weight of 30,000. FSH binds to the Sertoli cells in the testis and induces the synthesis of an 'androgen-binding protein' in them. This protein helps in transporting testosterone to the seminiferous tubules and epididymis.

(ii) **Luteinizing hormone (LH) or interstitial cell stimulating hormone (ICSH):** This hormone in the female stimulates the final stages in the maturation of the Graafian follicle, ovulation and the development of corpus luteum. It stimulates production of oestrogen and progesterone. In males, it stimulates the interstitial cells to produce testosterone. LH activates phosphorylase leading to glycogenolysis. The HMP pathway is enhanced and produces the NADPH + H^+ required for the synthesis of the steroid hormones. The secretion of LH is also regulated by the hypothalamus which produces a hypothalamic-releasing factor (HRF).

The placenta of the pregnant female produces gonadotropic hormones similar to those of pituitary. They are called the chorionic gonadotropic hormones. The placenta is also said to elaborate a growth hormone and a thyroid-stimulating hormone.

The presence of the chorionic gonadotropic hormones in the urine of a pregnant woman forms the basis of several tests for pregnancy.

Thyrotropic Hormone (Thyroid-Stimulating Hormone (TSH))

It increases the uptake of iodine by the thyroid, and its incorporation into the thyroid hormones. One mechanism of its action may be by stimulating glucose metabolism in the thyroid. The secretion of TSH is regulated by a hypothalamic TSH-releasing factor (TRF). It is a mucoprotein with a molecular weight of 30,000.

Adrenocorticotropic Hormone (ACTH or Corticotropin)

In 1961, Hoffman and associates synthesized a portion containing the active part of a molecule. It stimulates the synthesis and release of adrenal cortical hormones. The stimulus on the hormone synthesis acts at a point before pregnenolone formation. Since pregnenolone is the precursor for mineralocorticoids, glucocorticoids, and also sex hormones, the synthesis of all the three is enhanced by ACTH. Aldosterone secretion is not much influenced by ACTH. It is a polypeptide hormone with a molecular weight of 4566.

Injection of ACTH therefore produces all the effects of administration of corticosteroids on the carbohydrate, protein, lipid and mineral metabolisms. The action of ACTH seems to be increased by phosphorylase activity and increased HMP pathway. The glucose metabolism of adrenal cortex is enhanced. Secretion of ACTH is reciprocally related to plasma levels of corticosteroids. Stress, fever, hypoglycaemia, epinephrine, oestrogens and vasopressin—all stimulate ACTH production. The hypothalamus is said to regulate ACTH secretion by producing a corticotrophin-releasing factor (CRF). Three distinct factors α_1, α_2 and β-are described.

Abnormalities: Hypersecretion and hyposecretion of these tropic hormones produce changes similar to hypersecretion or hyposecretion of the endocrine secretions which they influence. All the tropic hormones belonging to the glycoprotein group act through the cyclic AMP system.

Encephalins and Endorphins

Encephalins are the small peptides capable of exerting morphine-like effects and have been isolated from brain extracts. These encephalins bind to the same receptors where in the limbic system binding of drugs like morphine and other analgesics takes place and exert an analgesic effect. Two such peptides are isolated. Both are pentapeptides and differ only in the fifth C-terminal amino acid and are called 'encephalins.'

Leu-encephalin: Tyr-Gly-Gly-Phe-Leu
Met-encephalin: Tyr-Gly-Gly-Phe-Met

From the anterior pituitary, a beta-lipoprotein is isolated and has 91 amino acids and the sequence of the amino acids from 61 to 65 is same as that of Met-encephalin. Breakdown products of this beta-lipoprotein at this point have also analgesic properties. These small peptides are called 'endorphins.'

Hormones of the Middle or Intermediate Pituitary Lobe

The pars intermedia produces the melanocyte-stimulating hormone (MSH). MSH secretions are inhibited by cortical hormones, epinephrine and norepinephrine. In Addison's disease, where cortical hormones are low, MSH is secreted in increasing amounts leading to brownish pigmentation of the skin. MSH is said to exist in α, β and γ forms, each of which is a low-molecular-weight polypeptide.

Melatonin: It is produced by the pineal body. It is a derivative of serotonin and its action is opposed to that of MSH. Melatonin lightens the colour of the melanocytes of the skin.

Hormones of the Posterior Lobe of Pituitary

Two hormones are produced by the posterior lobe of pituitary: (1) vasopressin or pitressin and (2) oxytocin or pitocin. The hormones are elaborated by the supra optic neurons of the paraventricular nuclei of the hypothalamus and are only stored in the posterior pituitary in association with two proteins—neurophysin I and II, respectively. On release from the posterior pituitary, they are carried in association with plasma proteins to the kidney, mammary gland and liver.

Vasopressin or Antidiuretic Hormone (ADH)

In the cells of specialized neurons, this hormone is synthesized and combines with a large polypeptide called neurophysin and a small glycoprotein to form granules. The granules are discharged into circulation from the neurohypophysis in response to hypertonicity of the blood plasma or decrease in blood volume. They reach the distal convoluted tubules and bind to receptors on the tubular cells and cause an activation of the adenylate cydase and release of 3′, 5′-cyclic AMP. This, in turn, causes the luminal membrane to become water permeable and leads to reabsorption of water by the distal convoluted tubules.

The syndrome known as 'diabetes insipidus' is due to the deficiency of this hormone. Large volumes about 30 litres/day of urine of low specific gravity are passed.

Oxytocin: This hormone is a cyclic polypeptide of 8 amino acids and has a molecular weight about 1000. It causes contraction of the uterus and ejection of milk from the mammary gland.

17.5.4 Gastrointestinal Hormones

Gastrin: This hormone gastrin is secreted when hydrochloric acid of gastric juice or the substances present in or derived from food stimulate the pyloric mucosa. Gastrin is absorbed into blood and carried to the parietal cells of the gastric glands and stimulates them to secrete a HCl-rich gastric juice.

Gastrin I and gastrin II are the two hormones that have been identified. Both gastrin I and gastrin II are small polypeptides containing 17 amino acids. The C-terminal 4 amino acids seem to be most important for the function (-trp-met-asp-phe). A synthetic preparation—pentagastrin containing five amino acids (including the above four) is used clinically to stimulate gastric secretion.

Acetylcholine, vagal stimulation and intake of food, particularly if protein-rich, cause a release of gastrin. Glycine is a potent stimulus. Gastrin-producing pancreatic tumours, arising probably from the D cells, cause high gastrin production, increased HCl secretion and high incidence of peptic ulcers. It is described as Zollinger–Ellison syndrome.

Secretin: This hormone is formed by the duodenal and jejunal mucosa, and it is a polypeptide hormone containing 27 amino acids out of which 14 amino acids are similar to those found in glucagon. It has a glucagon-like action in increasing cardiac output and lipolysis. It is secreted on stimulation by ingested food and acid chyme from stomach. It stimulates the secretion of a bicarbonate-rich watery secretion by pancreas.

Cholecystokinin-pancreozymin: Once cholecystokinin and pancreozymin were considered to be two different hormones but now they are accepted to be one single hormone. This hormone is a polypeptide containing 33 amino acids, the C-terminal five amino acids being the same as that of gastrin. Much of the activity resides in the C-terminal 8 amino acids. It stimulates the secretion of the pancreatic enzymes and also causes contraction of the gall bladder. It has also gastrin-like and secretin-like actions and stimulates release of both insulin and glucagon from pancreatic islets. That is why oral glucose is more effective in stimulating insulin release than parenterally administered glucose. It is liberated from the duodenal and jejunal mucosa.

The gastrointestinal mucosa secretes several factors which are polypeptides having hormonal action and these are called ‘candidate hormones.’

Gastric inhibitory polypeptide (GIP) inhibits the gastric acid secretion and motility of the stomach. Vasoactive intestinal polypeptide (VIP) is secreted by the mucosa of the small intestine and colon. It also inhibits gastric acid secretion and inhibits the motility of the stomach and gall bladder. It stimulates pancreatic and intestinal secretions. Motilin stimulates gastric motility. Enteroglucagon stimulates glycogenolysis. Chymodenin stimulates chymotrypsin secretion by the pancreas.

Bulbogastrin is secreted by the duodenal bulb and inhibits gastric HCl secretion.

Urogastrone is extracted from normal urine, and when administered parenterally, inhibits secretion of HC1 by the gastric mucosa. The substance responsible for this action is named ‘urogastrone.’ Its exact nature and its relationship to other gastrointestinal hormones and its physiological role are not known.

17.5.5 Renin (Hormonal Function of the Kidney)

Goldblatt and others by their experiment have shown that occlusion of arterial blood supply to the kidney by any method leading to renal ischaemia will stimulate the renal cortex to liberate into the blood stream a substance called 'renin' which is a proteolytic enzyme. 'Angiotensinogen', an alpha-2 globulin, is present in the blood and produced in the liver. Renin acts on angiotensinogen and liberates from it a decapeptide (containing 10 amino acids) called angiotensin-I which is further acted upon by an enzyme present in blood and converted to angiotensin-Il which is an octapeptide containing only eight amino acids.

Angiotensin: It is about 200 times as active as norepinephrine. It increases the force of cardiac contraction, constricts the arterioles and causes contraction of smooth muscle. Angiotensin-I has similar, but much feebler, actions.

Antagonists to angiotensin II or its production or its action are used in the treatment of hypertension.

Saralasin is the substance in which the first and eighth amino acids of angiotensin are substituted by other amino acids. It acts as a competitive inhibitor by occupying the receptor sites. Propanolol, antagonizes the beta-adrenergic catecholamines and prevents the release of rennin. Teprotide blocks the conversion of angiotensin-I to angiotensin-II by combining with the converting enzyme.

The another proteolytic enzyme in kidney called 'angiotensinase' is capable of inactivating angiotensin by hydrolysing it.

It is not clear whether renal pressor system has any role in the normal person. But in diseases which cause a decrease in circulation through the kidney (e.g., glomerulonephritis, perinephritis), this system is responsible for the causation of a persistent hypertension. The kidney also produces 'kininogen', a substance having an anti-hypertensive effect. It also produces two hormones having the effect of stimulating red blood cell production by bone marrow—erythropoietin and erythrogenin.

It activates vitamin D by adding to the 25-hydroxycholecalciferol (the OH at C-25 is added by liver) one more —OH at C-1. It has also an enzyme system capable of hydroxylating at C-24 which will result in the formation of an inactive derivative of the vitamin D-24, 25-dihydroxycholecalciferol.

Few hormones like insulin, glucagon and aldosterone are destroyed by the kidney.

17.6 AMINO ACID DERIVATIVES

17.6.1 The Thyroid Gland

The iodine-containing hormones are secreted by the thyroid gland, which have a general stimulating effect on cell respiration and metabolism.

Biosynthesis and Chemistry of the Thyroid

Iodine is taken up by the thyroid from plasma against a concentration gradient and utilizes it for hormone synthesis. About one-third of the inorganic iodine of plasma derived from food is taken up by the gland, and the rest is excreted mainly by kidney. Small amounts are also excreted in saliva, milk and gastrointestinal secretions. The iodine concentration in thyroid is 10 to 100 times

that in plasma. The TSH (thyroid-stimulating hormone) of the pituitary stimulates the uptake of iodine by the gland. Thiocyanate and perchlorate competitively inhibit iodine uptake. Cyanide and dinitrophenol also inhibit the uptake by blocking cellular metabolism.

The enzyme peroxidase in the gland, oxidizes the inorganic iodide (I^-) to iodine (I^- or I^+) with the loss of one or two electrons. The active iodine is now taken up by the tyrosine moiety of the glycoprotein, thyroglobulin, which is the characteristic protein of the colloid of the thyroid and has a molecular weight of 660,000. It has 115 tyrosine residues in its molecule. The sequence of iodination of tyrosine and the condensation of iodotyrosines form the hormone.

The iodination of tyrosine and the subsequent coupling reaction between the iodotyrosines all occur while in the thyroglobulin molecule. The tri-iodo and tetra-iodothyronine (T_3 and T4) are then released by proteolytic enzymes present in lysosomes or vesicular membranes and enter the blood stream. Mono- and di-iodo tyrosine are also released but are deiodinated and the iodine is again used. Release of the hormone from the thyroglobulin is accentuated by TSH and by exposure to cold environment.

Blood plasma contains two proteins: TBG and TBPA. A thyroxine-binding globulin (TBG) which migrates between α_1 and α_2 globulins on electrophoresis, and a thyroxine-binding prealbumin fraction (TBPA), which take up thyroxine and transport it. A trace amount of the hormone is also in the free state and probably represents the active form of the hormone. In a normal individual, the plasma thyroxine levels (measured as 'proteinboundiodine' or PBI) vary from 4–8 μg/l00 ml. But the TBG has much reserve and can bind three times this amount.

Production of the TBG is increased by oestrogens (e.g., in pregnancy/and oral contraceptive pills) and is produced in the liver. It is decreased during treatment with androgens or glucocorticoids.

T_3 may be the major thyroid hormone. It disappears from the blood 20 times faster than T_4. About 80 per cent of the circulating T_4 is converted to T_3 in the tissues, particularly in the liver and kidneys. The deiodination in the tissues may produce 3, 5, 3^1 tri-iodothyronine as well as 3, 5^1, 3^1 tri-iodothyronine. The latter is called reverse T_3 and is less active than T_3.

The conversion of T_4 to T_3 is decreased by propylthiouracil and propranolol.

Thyroid contains several weeks store of T_4 and T_3. Hence, even a complete block of their biosynthesis by drugs or iodine deprivation elicits a response of increased production of TRH and TSH after several days.

TSH is required for the uptake of the plasma iodine by the gland, and its oxidation and incorporation into the thyroxine and also for the release of the hormone from the gland. The hypothalamus is said to elaborate a thyroglobulin-releasing factor (TRF). Plasma levels of the thyroxine regulate the hormone production by enhancing or decreasing TSH secretion of the pituitary. This is an example of feedback inhibition.

The Functions of Thyroid Hormones

1. **Calorigenic effect:** They increase the metabolism and oxygen consumption of all tissues.
2. **Protein metabolism:** These hormones cause a negative nitrogen balance because of higher amounts, and they cause excessive breakdown of protein due to an increased rate of metabolism. In physiological levels, they are protein anabolic and are necessary for normal growth. There is increased excretion of urinary creatine.

3. **Carbohydrate metabolism:** The increased rate of absorption of glucose from the intestines results in rapid hyperglycaemia during an oral glucose tolerance test. But since the rate of metabolism of glucose by tissues is also high, the level rapidly comes down to normal. There is also an increase in hepatic glucose-6-phosphatase activity and more rapid insulin destruction. Glycogenolysis is increased in the liver and muscle due to increased sensitiveness to epinephrine. Gluconeogenesis is also enhanced. All these effects lead to the development of a diabetic state. But the utilization of glucose by tissues through glycolysis as well as citric acid cycle is not impaired. On the other hand, it is increased. Hence, the diabetes is not of a severe type.
4. **Lipid metabolism:** The adipose tissue hormones favour lipolysis. This may be also an indirect effect through sensitization of the tissue to epinephrine action. The lipogenic effect of insulin is also enhanced. The important diagnostic effect is on plasma cholesterol levels. In hyperthyroidism, there is an increased hepatic synthesis of cholesterol, but a greater increase in its oxidation to bile acids and elimination. The result is a decrease in plasma cholesterol levels.

In hypothyroidism, the condition is reversed, a decrease in hepatic synthesis of cholesterol but a greater decrease in oxidation leading to a rise in plasma cholesterol.

Mechanism of Action of Thyroid Hormones

The thyroid hormones are slow in their action. They enter the target cell and are bound to a specific carrier molecule which is directly associated with nuclear chromatin. In moderate concentrations, they have an anabolic effect. RNA content, amino acid transport into the cell and protein synthesis in the cell increase. Higher concentrations of the hormones produce negative nitrogen balance. Protein synthesis is decreased; carbohydrate and lipid breakdown are increased; bone gets decalcified, mitochondrial swelling and uncoupling of oxidative phosphorylation occur.

Thyroid hormones increase the activity of Na^+, K^+-ATPase pump by increasing the number of pump units in all cells. This results in increased oxidative phosphorylation and O_2 consumption.

There is an interrelationship between GH and thyroid hormones. GH stimulates increased production of IGF-I by the liver which in turn enhances somatostatin levels. The somatostatin decreases TSH release and thyroid hormone synthesis and release. Children treated with GH for dwarfism occasionally develop hypothyroidism on this account.

Thyroid hormones and glucocorticoids enhance the transcription by the GH gene and hence cause increased production of GH. This may explain the anabolic effects of the thyroid hormones.

1. By a feedback mechanism the thyroid hormone itself suppresses its own secretion by the gland. This action is mediated through TSH.
2. Hyperplasia of thyroid (goiter) is caused due to the deficiency of iodine in food and drink. Excess of iodine causes hypofunction by decreasing TSH output and is used in the treatment of hyperthyroidism.
3. Uptake of iodine by the thyroid is inhibited by thiocyanate by blocking the concentrating mechanism.

4. Synthesis of the thyroid hormone is inhibited by thiourea, thiouracil and sulphonamides and produce goitre. The action may be by preventing oxidation of iodide directly by their action on iodide or through inhibition of oxidizing enzymes.

Hypothyroidism: Hypothyroidism manifests as cretinism in children and it produces myxedema in adults.

In myxedema, the skin is thick and puffy due to deposition of what is known as myxomatous material in subcutaneous layers, poor mental powers and there is hypersensitivity to cold.

Hyperthyroidism or exophthalmic goitre: Due to hyperfunction of this hormone the eye balls bulge out of the face (exophthalmos) and look large and protruding. Due to hyperfunction of this hormone wasting of the tissues and loss of weight are marked. There is hypersensitivity to hot climate. Body temperature, pulse and cardiac rates are increased. Plasma cholesterol level is decreased. Hyperglycaemia and glycosuria may occur. Plasma PBI levels are increased. Due to hyperfunction of this hormone, the BMR is increased from +20 to +80 per cent or more.

The thyroid activity can be evaluated by more recent techniques like determination of radio-iodine uptake by the thyroid gland, estimation of plasma T_3 and T_4, levels by ELISA method or by radioimmunoassay.

In hyperthyroidism, there is production of an abnormal protein 'thyroid-stimulating IgG' (TSI) which activates the TSH receptors. This results in excessive TSH-like stimulation of the thyroid gland, enlargement of thyroid and overproduction of T_3 and T_4. The production of TSI is not under feedback control.

17.6.2 Calcitonin

It was first extracted from parathyroid; afterwards, it was found that the thyroid contains even greater amounts of the hormone. It is produced by the C cells of the thyroid. It is a polypeptide with 32 amino acids and a molecular weight of 3600. Injection of the hormone causes a rapid fall of serum calcium and phosphorus. Calcium passes from the blood into bone. Urinary excretion of calcium and phosphate remain unaltered. It does not alter the absorption or excretion of calcium and phosphorus by the intestines. The hormone exerts a direct effect on the bone—acceleration of calcium deposition and inhibition of its resorption. The hormone thus favours calcium retention by bone. Its secretion is increased in hypercalcaemia. Calcitonin inhibits the synthesis of calcitriol in the kidney.

17.6.3 Adrenal Medulla

Adrenal medulla produces two hormones norepinephrine and epinephrine (Figure 17.6) (noradrenaline and adrenaline). The physiological action of epinephrine closely resembles that of sympathetic stimulation. Adrenal medulla, though it has several physiological functions, is not indispensable for life.

Chemistry of the Hormone

The formation of these hormones is from phenylalanine and tyrosine. Norepinephrine is formed first and is then converted to epinephrine by methylation of the side chain. Epinephrine is the main hormone produced by the medulla. Norepinephrine is found principally in the sympathetic nerves. The naturally occurring hormones are L-isomers and are about 15 times as active as the corresponding

D-isomers. Much of the hormones is oxidized to inactive compounds by the action of the enzyme monoamine oxidase (MAO) and others. The liver is the main site of this enzymatic inactivation.

They are also inactivated by methylation of the –OH in position 3.

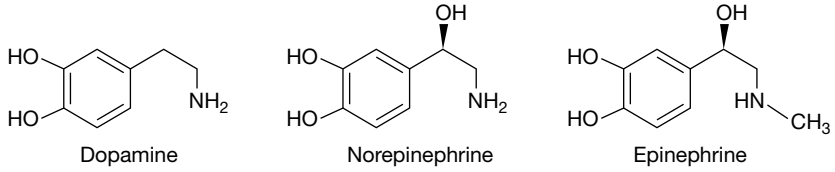


Figure 17.6 Structure of Dopamine, norepinephrine and epinephrine

Vanilmandelic acid (VMA): VMA, 4-hydroxy-3-methoxy-mandelic acid, is one of the principal products excreted in urine. This metabolite accounts for over 60 per cent of the catecholamine excretion in urine. The metabolites are conjugated with glucuronic acid or sulphate before excretion.

The hormones bound to the target tissues to exert physiological action. This binding is inhibited by drugs like reserpine, cocaine and chlorpromazine which thus block adrenergic action. Others which structurally resemble the hormones (e.g., β-hydroxytyramine and alpha-methyl-norepinephrine) compete for the binding sites in tissue and thus inhibit the hormonal action.

Catecholamines cannot cross the blood–brain barrier. L-Dopa can, however, penetrate the barrier and be converted to catecholamines in the brain. In Parkinson's disease, where there is a deficiency of catecholamines in the brain, administration of L-Dopa is found to relieve the symptoms.

Functions of Norepinephrine and Epinephrine

Norepinephrine acts as an overall vasoconstrictor without much effect on cardiac action and increases blood pressure. Epinephrine causes vasodilatation of arterioles of muscle and vasoconstriction of arterioles of skin and splanchnic area besides increasing rate and force of contraction of the heart. Overall effect is a rise in blood pressure. Epinephrine causes relaxation of smooth muscles of the stomach, intestine, bronchioles and urinary bladder and contraction of the sphincters of stomach and bladder. Its relaxant effect on bronchioles is used in the treatment of bronchial asthma. Epinephrine is by far more potent than norepinephrine in metabolic functions. Some of the metabolic functions are as follows:

(a) **Liver glycogenolysis:** Epinephrine has similar action as that of glucagon. By stimulation of the liver enzyme, adenyl cyclase, there is a final increase of phosphorylase activity and glycogenolysis, causing an elevation of blood sugar level.

(b) **Muscle glycogenolysis:** Epinephrine enhances the cyclic AMP level in muscle unlike of glucagon and causes glycogenolysis in it leading to increased blood pyruvate and lactate levels.

(c) Lipolysis is stimulated in adipose tissue leading to a rise in the NEFA levels of plasma.

(d) Glucose uptake by tissues is diminished.

(e) Insulin secretion by pancreas is decreased.

Nature of adrenergic receptors: The adrenergic receptors, which are stimulated by these hormones, are of two types, the alpha- and the beta-adrenergic receptors. The alpha-receptors

are mainly stimulated by norepinephrine and produce vasoconstriction. Epinephrine can stimulate both. Increased rate and force of contraction of heart, glycogenolysis and lipolysis are due to stimulation of the beta-receptors which results in increased cyclic AMP production. In the pancreas, epinephrine stimulates mainly the alpha-receptors leading to a depression of cyclic AMP level and a diminished insulin secretion.

For epinephrine, the beta-adrenergic receptor is present in the plasma membrane and has a molecular weight of 64,000. It has seven helices occupying the membrane and is a transmembrane protein. The amino terminal is linked to oligosaccharides and is on the external surface of the membrane. The C-terminal is on the cytosol side and contains serine and threonine residues which can be phosphorylated and dephosphorylated rapidly.

Regulation of secretion of adrenal medulla: The hormone secretion is stimulated by a number of factors like fear, anger and pain (emotional states), hypoglycaemia, muscular activity and hypotension. Hypotension mainly stimulates norepinephrine (noradrenaline) secretion, while hypoglycaemia promotes epinephrine (adrenaline) secretion. The 'alarm reaction' is probably first triggered off by adrenal medulla which pours out epinephrine into circulation which in turn stimulates ACTH secretion by the adenohypophysis. The latter may be an indirect effect mediated by a hypothalamic mechanism. Tumours of adrenal medulla (known as pheochromocytoma) produce intermittent hypertension, coronary insufficiency and cardiovascular failure. Estimation of catecholamines or vaniline mandelic acid (VMA) in urine helps in the diagnosis of the condition.

The enzymic synthesis of catecholamines from dopamine is inhibited by disulfiram. Reserpine and guanethidine increase the destruction of catecholamines. They act, therefore, as antihypertensives and tranquilizers.

Tyramine, methyldopa and alpha-methyltyrosine, when administered, produce substances resembling catecholamines, which compete with the hormone-binding sites, and increase the circulating levels of catecholamines.

Specific antagonist to norepinephirine is phentolamine (regitine). When injected intravenously, it causes a rapid fall of blood pressure in 2–5 min. This can be used as a clinical test for pheochromocytoma.

Several hormones like insulin, ACTH, vasoactive intestinal polypeptide (VIP), etc., are found in the brain tissue apart from the established role of epinephrine as a neurotransmitter. Special receptors for these hormones are found in the brain tissue. There appears to be a close relationship between the nervous system and the endocrine system.

Most endocrine glands are derived from epithelial cells. The neural crest is suggested as a possible embryological origin for many endocrine glands. This explains the close interplay between the nervous system and the endocrine system and the occurrence of endocrine cells in ectopic sites (e.g., production of parathormone and ACTH by malignant cells in lung cancer).

17.7 PROSTAGLANDINS AS MODULATORS OF HORMONE ACTION

Prostaglandins do not act as hormones themselves but rather modulate the action of hormones. Epinephrine, glucagon, ACTH and TSH stimulate lipolysis, whereas PGE_1 strongly inhibits the lipolytic effect of these hormones. These hormones prevent the rise of the intracellular cyclic AMP by inhibiting adenyl cyclase of adipose tissue cells.

EXERCISES

1. Define hormones and explain their mode of action. What are hormone receptors?
2. What are second messengers? Write a note on cyclic AMP and cyclic GMP as second messengers.
3. What are the physiological activities of the corticoids?
4. Write short notes on the following:
   (a) Hirsutism
   (b) Cushing's syndrome
   (c) Addison's disease
5. What do you understand by menstrual cycle that occurs in women? Write the three phases of menstrual cycle.
6. Which hormone is responsible for expulsion of the milk from the mammary glands? Write in detail about this hormone.
7. Write the insulin biosynthesis with a well-defined diagram.
8. Glucagon stimulates lipolysis in adipose tissue. Comment on the statement.
9. What are thyroid glands? Write the biosynthesis and chemistry of the thyroid hormones.
10. What are pituitary glands? Write the hormones secreted from the anterior lobe of the pituitary.
11. What are encephalins and endorphins?
12. Write the functions of norepinephrine and epinephrine.
13. What are gastrointestinal hormones? Write notes on gastrin and secretin.
14. State whether calcitonin is a parathyroid or the thyroid hormone. Write the functions of calcitonin.
15 Write short notes on the following:
   (a) Oxytocin
   (b) Cholecystokinin/pancreozymin
   (c) Vanilmandelic acid
   (d) Follicular or oestrogenic hormones
   (e) Vasopressin
   (f) Progesterone

MULTIPLE CHOICE QUESTIONS

1. Name of the scientists those in 1902 were the first to coin the term hormone for 'secretin' produced by intestinal mucosa.
   (a) Bayliss and Starling
   (b) Robert and Robert
   (c) Anderson and Bayliss
   (d) None of these

   Ans. a
2. The degeneration of thymus takes place with age. Its size is maximal at

birth, begins to shrink at puberty and is generally not visible
(a) After middle age
(b) Before middle age
(c) After old age
(d) Before old age

Ans. a

3. Which enzyme converts cyclic AMP to ordinary AMP?
(a) Phosphodiesterase
(b) Phosphoesterase
(c) Esterase
(d) None of these

Ans. a

4. Which enzyme converts GTP to cyclic GMP?
(a) Guanylyl cyclase
(b) Adenyl cyclase
(c) Both (a) and (b)
(d) None of these

Ans. a

5. Renin acts on angiotensinogen and liberates from it a decapeptide (containing 10 amino acids) called angiotensin-I which is further acted upon by an enzyme present in blood and converted to angiotensin-Il which is
(a) An octapeptide
(b) Pentapeptide
(c) Hexapeptide
(d) None of these

Ans. a

6. Two such peptides are isolated. Both are pentapeptides and differ only in the fifth c-terminal amino acid and are called
(a) Encephalins
(b) Endorphins
(c) Both (a) and (b)
(d) None of these

Ans. a

7. The substance in which the first and eighth amino acids of angiotensin are substituted by other amino acids. It acts as a competitive inhibitor by occupying the receptor sites is
(a) Saralasin
(b) Propanolol
(c) Catecholamines
(d) None of these

Ans. a

8. Which substance antagonizes the beta-adrenergic catecholamines and prevents the release of rennin?
(a) Saralasin
(b) Propanolol
(c) Catecholamines
(d) None of these

Ans. b

9. Which substance blocks the conversion of angiotensin-I to angiotensin-II by combining with the converting enzyme
(a) Saralasin
(b) Propanolol
(c) Teprotide
(d) None of these

Ans. c

10. The kidney also produces a substance having an anti-hypertensive effect.
(a) Kininogen
(b) Propanolol
(c) Teprotide
(d) None of these

Ans. a

11. Blood plasma contains two proteins, TBG and TBPA. A thyroxine-binding globulin (TBG) migrates between which of the following globulins on electrophoresis?
(a) α_1 and α_2
(b) γ_1 and γ_2
(c) β_1 and β_2
(d) None of these

Ans. a

12. It was first extracted from parathyroid; but afterward, it was found that the thyroid contains even greater amounts of the hormone. It is produced by the C cells of the thyroid. It is a polypeptide with

32 amino acids and a molecular weight of 3600. The substance is

(a) Calcitonin
(b) Calcitriol
(c) Catecholamines
(d) None of these

Ans. a

13. This hormone is formed by the duodenal and jejunal mucosa and it is a polypeptide hormone containing 27 amino acids out of which 14 amino acids are similar to those found in glucagon.

(a) Calcitonin
(b) Secretin
(c) Cholecystokinin
(d) Pancreozymin

Ans. b

14. It regulates the secretion of insulin and glucagon. It is also produced by the hypothalamus, and inhibits GH secretion. The substance is

(a) Somatostatin
(b) Secretin
(c) Cholecystokinin
(d) Pancreozymin

Ans. a

15. Which of the following regulates gastrointestinal secretions?

(a) Somatostatin
(b) Pancreatic polypeptide
(c) Calcitonin
(d) None of these

Ans. b

Appendix

NORMAL LABORATORY VALUES

Hematology

1	Bleeding time	1–7 minutes (Ivy method)
2	Clot retraction	Starts in 1–3 hours Completes in 6–24 hours No clot lysis in 24 hours
3	Prothrombin time	11–14.5 seconds
4	Partial thromboplastin time	Activated, 25–37seconds
5	Cellular measurement of red cells	Average diameter = 7.3µm Mean corpuscular volume (MCV):Men = 80–94fL, Women = 81–99fL Mean corpuscular hemoglobin (MCH) = 27–32 Pg Mean corpuscular hemoglobin concentration (MCHC) = 32–36%
6	Fragility of red cells	Starts at 0.45–0.38% Nacl Completes at 0.36–0.3% Nacl
7	Reticulocytes	0.2–2% of red cells
8	Hemoglobin (in blood)	Men = 14–18 gms/dL Women = 12–16 gms/dL
9	Hemoglobin (in serum)	2–3 mg/dL
10	Erythrocyte Sedimentation rate (ESR)	Less than 20 mm/h (Westergren method) 0–10mm/h(Wintrobe method)
11	Red blood count (RBC)	Men = 4.5–6.2 million/µL Women = 4.0–5.5 million/µL
12	Platelets	1,50,000–4,00,000/µL
13	White blood count (WBC)	5,000–10,000/µL

Differential Count of WBC		
1	Myelocytes	0%
2	Juvenile neutrophils	0%
3	Band neutrophils	0–5%
4	Segmented neutrophils	40–60%
5	Lymphocytes	20–40%
6	Eosinophils	1–3%
7	Basophils	0–1%
8	Monocytes	4–8%
Lymphocytes (Total) 1500–4000/µL		
1	B cell	5–25%
2	T cell	60–88%
3	Suppressor	10–43%
4	Helper	32–66%
5	H:S	>1

CLINICAL BIOCHEMISTRY

Physical Parameter

		Specific Gravity	pH
1	Blood	1.056 (varies with Hb and protein concentration)	7.35–7.45
2	Serum		
3	Plasma		
4	Urine		

Sugar

		Fasting	Post Periendial	Random
1	Blood			
2	Serum	70–100 mg%	100–140 mg%	70–110 mg%
3	Plasma			
4	Urine			

Lipids

		Total Lipids	Phospholipid	Inorganic Phosphorus (fasting)
1	Blood	–	–	–
2	Serum	450–1000 mg/dL	145–200 mg/dL	3–4.5 mg/dL
3	Plasma	–	–	–
4	Urine	–	–	–

		Triglycerides	Cholesterol	HDL Cholesterol	LDL Cholesterol	VLDL Cholesterol
1	Blood		–	–	–	–
2	Serum	<165mg/dL	150–220mg/dL	>50mg/dL.	<130mg/dL.	<40mg/dL
3	Plasma		150–220mg/dL	–	–	–
4	Urine		–	–	–	–

Proteins

		Total Protein	Albumin	Globulin	Fibrinogen
1	Blood				
2	Serum	6–8g/dL	3.5–5.5g/dL	2–3.6g/dL	
3	Plasma				0.2–0.6g/dL
4	Urine				

		Ferritin	Heptaglobin
1	Blood		
2	Serum	Men: 30–300ng/mL Women: 20–120ng/mL Children: 7–140ng/mL	40–170mg of Hb binding capacity
3	Plasma		
4	Urine		

Minerals

		Calcium	Calcium Ionized	Iron	Copper
1	Blood				
2	Serum	8.5–10.3mg/dL	4.25–5.25mg/dL	50–175µg/dL	100–200µg/dL
3	Plasma				100–200µg/dL
4	Urine				

		Potassium	Sodium	Zinc	Lead
1	Blood				
2	Serum	3.5–5meq/L	136–145meq/L	50–150µg/dL	
3	Plasma	3.5–5meq/L	136–145meq/L		
4	Urine				<80µg/24h

		Chloride	Bicarbonate	Magnesium
1	Blood			
2	Serum	96–106meq/L	24–28meq/L	1.8–3mg/dL
3	Plasma	96–106meq/L		1.8–3mg/dL
4	Urine			

Vitamins

		Ascorbic Acid	Folic Acid	Cyanocobalamin (B_{12})	Vitamin A
1	Blood				
2	Serum		2–20ng/mL	200Pg/mL	15–60µg/dL
3	Plasma	0.4–1.5mg/dL			
4	Urine				

		25-Hydroxycholecalciferol	1,25-dihydroxycholecalciferol	24-25-dihydroxy-cholecalciferol
1	**Blood**			
2	**Serum**	8–55ng/mL	26–65 Pg/mL	1–5ng/mL
3	**Plasma**			
4	**Urine**			

Enzymes

		Acid Phosphatase	Alkaline Phosphatase	Cholesteryl Esters
1	Blood			
2	Serum	1–5units	5–13 units	65–75% of total cholesterol
3	Plasma			
4	Urine			

		Amylase	α_1 Antitrypsin	Creatine Kinase (ck)
1	Blood			
2	Serum	80–180Units/dL	>180mg/dL	10–50I.U/L at 30°C
3	Plasma			
4	Urine			

		Lipase	Lactate Dehydrogenase	γ-glutamyl Transpeptidase
1	Blood			
2	Serum	<150units/L	55–140I.U/L at 30°C	<30 units/L at 30°C
3	Plasma			
4	Urine			

Hormones

		Thyroxine, Free (FT_4)	Thyroxine, Total (TT_4)	Thyroxine-binding
1	Blood			
2	Serum	0.8–2.4ng/dL	5–12µg/dL	12–28µgT_4/dL
3	Plasma			
4	Urine			

		Gastrin	Pepsinogen	Calcitonin
1	Blood			
2	Serum	Upto 100pg/mL	25–100ng/mL	<100Pg/mL
3	Plasma			
4	Urine			

Sr. No		RT_3U	Epinephrine	Norepinephrine
1	Blood			
2	Serum	25–36%		
3	Plasma		<0.1ng/mL	<0.5µg/L
4	Urine			

Sr. No		Dopamine	Aldosterone	Cortisol
1	Blood			
2	Serum			5–25µg/dL
3	Plasma	<135Pg/mL	2–9ng/dL	
4	Urine			

Sr. No		Deoxycortisol	Growth hormone	Thyroid Stimulating Hormone (TSH)
1	Blood			
2	Serum	>7µg/dL	Adults: 1-10ng/mL	<10µU/mL
3	Plasma			
4	Urine			

Sr. No		Follicle-stimulating Hormone (FSH)	Luteinizing Hormone (LH)	Adrenocorticotropic Hormone (ACTH)
1	Blood			
2	Serum	Men: 1–15mIU/mL Women: 1–30mIU/mL Castrate: 30–200mIU/mL	Men: 1–15mIU/mL Women: <1–30mIU/mL Castrate: >30mIU/mL	
3	Plasma			20–100Pg/mL
4	Urine			

Sr. No		Prolactin	Anti-diueretic Hormone (ADH, Vasopressin)	Insulin-like-growth Factor1(IGF–1)
1	Blood			
2	Serum	1–25ng/mL		
3	Plasma		0–2Pg/mL	400–2000Units/L
4	Urine			

		Testosterone, Free	Testosterone,total	Progesterone
1	Blood			
2	Serum	Men: 10–30ng/dL Women: 0.3–2ng/dL	Men: 300–1000ng/dL Women: 20–80ng/dL Luteal phase: upto 120ng/dL	Follicular phase: 0.2–1.5mg/mL Luteal phase: 6–32ng/mL Pregnancy: >24ng/mL Men: <1ng/mL (1ng/mL=3.2nmol/L)
3	Plasma			
4	Urine			

		Estradiol (E_2)	Renin Activity	Estriol (E_3)
1	Blood			
2	Serum	Men: 12–34Pg/mL Women: M.C.(1–10 days): 24–68Pg/mL M.C.(11–20days): 50–300Pg/mL M.C. (21–30 days): 73–149 Pg/mL (By Ria) (1Pg/mL=3.6Pmol/L)		Men and non-pregnant women: <0.2µg/dL (By Ria)
3	Plasma		Normal sodium intake Supine: 1–3ng/mL/h Standing: 3–6 ng/mL/h Sodium depleted Supine: 2–6 ng/mL/h Standing: 3–20ng/mL/h	
4	Urine			

		Chorionic Gonadotropin	Aldosterone	Catecholamines
1	Blood			
2	Serum	Men: <9mIU/mL Pregnant women: 10mIU/mL		
3	Plasma			
4	Urine		2–26µg/24h (varies with Na & K intake)	Total: <100µg/24h Epinephrine: <10 µg/24h Norepinephrine: <100µg/24h

		Cortisol, free	Serotonin	Triiodothyronine (T_3)
1	Blood		5–20µg/dL	
2	Serum			80–220ng/dL
3	Plasma			
4	Urine	20–100µg/24h		

		17-Ketosteroids	Metanephrine	Vanillylmandelic Acid (VMA)
1	Blood			
2	Serum			
3	Plasma			
4	Urine	Under 8 years: 0–2mg/24h Adolescents: 2–20mg/24h Men: 10–20mg/24h Women: 5–15mg/24h 1mg = 3.5 µm [Values vary with the method used.]	<1.3 mg/24h	Upto 7mg/24h (<35 µmol)

		Glucagon (Fasting)	Insulin
1	Blood		
2	Serum	20–100Pg/mL	4–25µu/mL
3	Plasma		
4	Urine		

Oxygen

		Capacity	Arterial Content	Arterial % Saturation	Arterial PO_2
1	Blood	16–24 vol % (vary with Hb conc)	15–23 vol% (vary with Hb conc)	94–100% of capacity	80–100mm Hg (vary with age)
2	Serum				
3	Plasma				
4	Urine				

Porphyrins

		delta-aminolevulinic acid	Coproporphyrin	Uroporphyrin	Porphobilinogen
1	Blood				
2	Serum				
3	Plasma				
4	Urine	1.5–7.5mg/24h.	<230µg/24h.	<50µg/24h.	<2mg/24h.

Nitrogenous Compound

		Urea Nitrogen	Ammonia	Uric Acid	Creatinine
1	Blood				
2	Serum	8–20mg/dL		Men: 3–9mg/dL Women: 2.5–7.5mg/dL	0.6–1.2mg/dL
3	Plasma	8–20mg/dL	10–80µg/dL	Men: 3–9mg/dL Women: 2.5–7.5mg/dL	0.6–1.2mg/dL
4	Urine				

Liver Function Test

		Bilirubin Total	Direct (Conjugated)	Indirect
1	Blood			
2	Serum	0.2–1.2mg/dL	0.1–0.4mg/dL	0.2–0.7mg/dL
3	Plasma			
4	Urine			

		SGOT (AST)	SGPT (ALT)	Alkaline Phosphatase
1	Blood			
2	Serum	0–41 IU/L at 37°C	0–45 IU/L at 37°C	5–13 units
3	Plasma			
4	Urine			

CEREBROSPINAL FLUID (CSF)

Appearance	Clear and colourless
Specific gravity	1.003–1.008
Cells	Infants: 0–20 mononuclears/μL.
	Adults: 0–5 mononuclears/μL
Pressure (reclining)	Newborns: 30–90 mm water
	Children: 50–100 mm water
	Adults: 70–200mm water
	[Average = 125mm water]
Glucose	50–85mg/dL(2.8–4.7 mmol/L)
Proteins	Total: 20–45 mg/dL (0.2–0.45 g/L) in lumbar cerebrospinal fluid
	IgG: 2–6mg/dL(0.02–0.06g/L)

RENAL FUNCTION TESTS

Specific gravity of urine	1.003–1.030
Maximal glucose reabsorptive capacity (Img)	Men: 300–450mg/min
	Women: 250–350mg/min
Inulin clearance (GFR)	Men: 110–150mL/min
	Women: 105–132mL/min
Creatinine clearance, endogenous (GFR)	Approximates inulin clearance
P–Aminohippurate (PAH) clearance (RPF)	Men: 560–830mL/min
	Women: 490–700mL/min
Maximal PAH excretory capacity (Tm PAH)	80–90mg/min
Filtration fraction (FF)	Men: 17–21%
	Women: 17–23%, [FF = GFR/RPF]
Fractional excretion of sodium	$FE\,Na^{+} = \frac{(U/P)Na^{+}}{(U/P)Cr} \times 100$
	[Adapted from current Medical Diagnosis and Treatment 1992]

Glossary

A

Acetyl CoA: Acetyl-coenzyme A, a high-energy ester of acetic acid that is important both in the tricarboxylic acid cycle and in fatty acid biosynthesis.

Acidosis: A metabolic condition in which the capacity of the body to buffer H^+ is diminished; usually accompanied by decreased blood pH.

Actin: A protein making up the thin filaments of muscle; also an important component of the cytoskeleton of many eukaryotic cells.

Active Site: The region of an enzyme molecule that contains the substrate binding site and the catalytic site for converting the substrate(s) into product(s).

Active Transport: Energy requiring transport of a solute across a membrane in the direction of increasing concentration.

Acyl Phosphate: Any molecule with the general chemical form $R–\underset{\underset{O}{\|}}{C}–OPO_3^{2-}$.

Adenine: A purine base found in DNA or RNA.

Adenosine Diphosphate (ADP): The nucleotide formed by adding a pyrophosphate group to the 5′-OH group of adenosine.

Adenosine Triphosphate (ATP): The nucleotide formed by adding yet another phosphate group to the pyrophosphate group on ADP.

Adenosine: A purine nucleoside found in DNA, RNA and many cofactors.

Adenylate Cyclase: The enzyme that catalyzes the formation of cyclic 3′, 5′ adenosine monophosphate (cAMP) from ATP.

Adipocyte: A specialized cell that functions as a storage for lipid.

Adipose Tissue: Connective tissue specialized for the storage of large amounts of triacylglycerols.

Aerobe: An organism that utilizes oxygen for growth.

Aerobic: Occurring in the presence of oxygen.

Alcohol: A molecule with a hydroxyl group attached to a carbon atom.

Aldehyde: A molecule containing a doubly bonded oxygen and a hydrogen attached to the same carbon atom.

Aldose: A simple sugar in which the carbonyl carbon atom is an aldehyde; that is, the carbonyl carbon is at one end of the carbon chain.

Aldosterone: A hormone produced by the cortex of the adrenal gland, instrumental in the regulation of sodium and potassium reabsorption by the cells of the tubular portion of the kidney.

Alkalosis: A metabolic condition in which the capacity of the body to buffer OH^- is diminished; usually accompanied by an increase in blood pH.

Alopecia Areata: Hair loss occurring in only one section of the scalp.

Allosteric Enzyme: An enzyme whose active site can be altered by the binding of a small molecule at a nonoverlapping site.

Allosteric Site: The specific site on the surface of an allosteric enzyme molecule to which the modulator or effect or molecule is bound.

Alzheimer's Disease: Also called SDAT (senile dementia Alzheimer's type). This disease is characterized by a general loss of intellectual ability and impairment of memory, judgment and abstract thinking, as well as changes in personality. Other symptoms include loss of speech, disorientation and apathy. Alzheimer's disease is the most common cause of dementia, rarely occurring before the age of 50. The disease takes from a few months to four or five years to progress to complete loss of intellectual function.

Amino Acids: α –Amino substituted carboxylic acids, the building blocks of proteins.

Aminoacyl-tRNA Synthetases: Enzymes that catalyze synthesis of an aminoacyl-tRNA at the expense of ATP energy.

Aminoacyl-tRNA: An aminoacyl ester of a tRNA.

Amino-terminal Residue: The only amino acid residue in a polypeptide chain with a free α-amino group; defines the amino terminus of the polypeptide.

Aminotransferases: Enzymes that catalyze the transfer of amino groups from α-amino to α-Keto acids; also called transaminases.

Amphibolic Pathway: A metabolic pathway used in both catabolism and anabolism.

Amphipathic: Containing both polar and non polar domains.

Ampholyte: A substance that can act as either a base or an acid.

Amphoteric: Capable of donating and accepting protons, thus able to serve as an acid or a base.

Anabolism: The phase intermediary metabolism concerned with the energy-requiring biosynthesis of cell components from smaller precursors.

Anaerobic: Occurring in the absence of air or oxygen.

Androgen: A steroid hormone, such as testosterone or androsterone, that promotes male characteristics.

Andropause: The male counterpart of menopause, when the production of testosterone decreases and there are accompanying mental symptoms.

Anemia: A condition marked by a deficiency of red blood cells or of hemoglobin in the blood, resulting in pallor and weakness.

Angstrom (Å): A unit of length equal to 10^{-10} m.

Anhydride: The product of the condensation of two carboxyl or phosphate groups in which the elements of water are eliminated to form a compound with the general structure.

R–X–O–X–R, Where X is either carbon or phosphorus.
‖ ‖
O O

Anion-exchange Resin: A polymeric resin with fixed cationic groups; used in the chromatographic separation of anions.

Anomers: The sugar isomers that differ in configuration about the carbonyl carbon atom. This carbon atom is called the anomeric carbon atom of the sugar.

Antibiotic: A natural product that inhibits bacterial growth (is bacteriostatic) and sometimes results in bacterial death (is bacteriocidal).

Antibody: A defense protein synthesized by the immune system of animals. See also immunoglobulin.

Anticodon: A specific sequence of three nucleotides in a tRNA, complementary to a codon for an amino acid in an mRNA.

Antigen: A molecule capable of eliciting the synthesis of a specific antibody in animals.

Antiparallel β-pleated Sheet (β-sheet): A hydrogen bonded secondary structure formed between two or more extended polypeptide chains.

Antiparallel: Describes two linear polymers that are opposite in polarity or orientation.

Antiport: Cotransport of two solutes across a membrane in opposite directions.

Apoenzyme: The protein portion of an enzyme, exclusive of any organic or inorganic cofactors or prosthetic groups that might be required for catalytic activity.

Apolipoprotein: The protein component of a lipoprotein.

Apoprotein: The protein portion of a protein, exclusive of any organic or inorganic cofactors or prosthetic groups that might be required for activity.

Apoptosis (app'-a-toe'-sis): Programmed cell death, in which a cell brings about its own death and lysis, signaled from outside or programmed in its genes, by systematically degrading its own macromolecules.

Aspartic Acid: Aspartic Acid, also known as aspartate, is an excitatory neurotransmitter in the brain stem and spinal cord. Aspartic acid is the excitatory counterpart to glycine, an inhibitory neurotransmitter. Low levels have been linked to feelings of fatigue and low mood, whereas high levels have been linked to seizures and anxiousness.

Asymmetric Carbon Atom: A carbon atom to which four different atoms or groups of atom are attached is said to be asymmetric carbon atom.

ATP (Adenosine Triphosphate): A ribonucleoside 5′-triphosphate functioning as a phosphate group donor in the cell energy cycle; carries chemical energy between metabolic pathways by serving as a shared intermediate coupling endergonic and exergonic reactions.

ATP Synthase: An enzyme complex that forms ATP from ADP and phosphate during oxidative phosphorylation in the inner mitochondrial membrane or the bacterial plasma membrane, and during photophosphorylation in chloroplasts.

ATPase: An enzyme that hydrolyzes ATP to yield ADP and phosphate; usually coupled to some process requiring energy.

Autophosphorylation: Strictly, the phosphorylation of an amino acid residue in a protein, catalyzed by the same molecule. Often extended to include phosphorylation of one subunit of a homodimer by the other subunit.

α Helix: A helical conformation of a polypeptide chain, usually right- handed, with maximal intrachain hydrogen bonding; one of the most common secondary structures in proteins.

5 Alpha Reductase: An enzyme that converts testosterone, the male sex hormone, into the more potent dihydrotestosterone (DHT).

B

Base Pair: Two nucleotides in nucleic acid chains that are paired by hydrogen bonding of their complementary bases; for example, A with Tor U, and G with C.

Base Stacking: The close packing of the planes of base pairs, commonly found in DNA and RNA structures.

Bidirectional Replication: Replication in both directions away from the origin, as opposed to replication in one direction only (unidirectional replication).

Bilayer: A double layer of lipid molecules with the hydrophilic ends oriented outward, in contact with water, and the hydrophobic parts oriented inward.

Bile Salts: Derivatives of cholesterol with detergent properties that aid in the solubilization of lipid molecules in the digestive tract.

Biochemical Pathway: A series of enzyme-catalyzed reactions that results in the conversion of a precursor molecule into a product molecule.

Biomolecule: An organic compound normally present as an essential component of living organisms.

Biotin: A vitamin; an enzymatic cofactor involved in carboxylation reactions.

Buffer: A conjugate acid-base pair that is capable of resisting changes in pH when acid or base is added to the system. This tendency will be maximal when the conjugate forms are present in equal amounts.

β Oxidation: Oxidative degradation of fatty acids into acetyl-CoA by successive oxidations at the β–carbon atom.

C

Calorie: The amount of heat required to raise the temperature of 1.0g of water from 14.5 to 15.5 °C. One calorie (cal) equals 4.18 joules (J).

Calvin Cycle: The cyclic pathway used by plants to fix carbon dioxide and produce triose phosphates.

cAMP: 3′, 5′ cyclic adenosine monophosphate. The cAMP molecule plays a key role in metabolic regulation.

Carbohydrate: A polyhydroxy aldehyde or ketone.

Carboxylic Acid: A molecule containing a carbon atom attached to a hydroxyl group and to an oxygen atom by a double bond.

Carboxyl-terminal Residue: The only amino acid residue in a polypeptide chain with a free α-carboxyl group; defines the carboxyl terminus of the polypeptide.

Carnitine Shuttle: Mechanism for moving fatty acids from the cytosol to the mitochondrial matrix as fatty esters of carnitine.

Carotenoids: It is a photosynthetic pigments made up of isoprene units.

Catabolism: That part of metabolism that is concerned with degradation reactions.

Catalyst: A compound that lowers the activation energy of a reaction without itself being consumed.

Catalytic site: The site of an enzyme involved in the catalytic process.

Cation-exchange Resin: An insoluble polymer with fixed negative charges; used in the chromatographic separation of cationic substances.

Cell: A usually microscopic structure containing nuclear and cytoplasmic material enclosed by a semi-permeable membrane; the basic structural unit of all organisms.

Cell Membrane: The interface between the cellular machinery inside the cell and the fluid outside. A semi-permeable lipid bilayer found in all cells containing biological molecules, primarily proteins and lipids.

Cell wall: A tough outer coating found in many plant, fungal, and bacterial cells that accounts for their ability to withstand mechanical stress or abrupt changes in osmotic pressure. Cell walls always contain a carbohydrate component and frequently also a peptide and a lipid component.

Cerebroside: Sphingolipid containing one sugar residue as a head group.

Chiral Compound: A compound that can exist in two forms that are non-superimposable images of one another.

Chlorophyll: A green photosynthetic pigment that is made of a magnesium dihydroporphyrin complex.

Chloroplast: A chlorophyll-containing photosynthetic organelle, found in eukaryotic cells, that can harness light energy.

Chromatin: The nucleoprotein fibers of eukaryotic chromosomes.

Chromatography: A procedure for separating chemically similar molecules. Segregation is usually carried out on paper or in glass or metal columns with the help of different solvents. The paper or glass columns contain porous solids with functional groups that have limited affinities for the molecules being separated.

Chromosome: A thread-like structure, visible in the cell nucleus during metaphase, that carries the hereditary information.

Chylomicron: A plasma lipoprotein consisting of a large droplet of triacylglycerols stabilized by a coat of protein and phospholipids; carries lipids from the intestine to the tissues.

Chymotrypsin: A well–studied protease that cleaves on the carbonyl side of amino acid residues with aromatic side chains.

Chymotrypsinogen: The zymogen precursor of chymotrypsin.

Cistron: A unit of DNA or RNA corresponding to one gene.

Citric Acid Cycle: A cycle system of enzymatic reactions for the oxidation of acetyl residues to carbon dioxide, in which formation of citrate is the first step; also known as the Krebs cycles or tricarboxylic acid cycle.

Codon: A sequence of three adjacent nucleotides in a nucleic acid that codes for a specific amino acid.

Coenzyme A: A pantothenic acid-containing coenzyme serving as an acyl group carrier in certain enzymatic reactions.

Coenzyme B_{12}: An enzymatic cofactor derived from the vitamin cobalamin, involved in certain types of carbon skeleton rearrangements.

Coenzyme: An organic molecule that associates with enzymes and affects their activity.

Cofactor: A small molecule required for enzyme activity. It could be organic in nature, like a coenzyme, or inorganic in nature, like a metallic cation.

Competitive Inhibition: A type of enzyme inhibition reversed by increasing the substrate concentration; a competitive inhibitor generally competes with the normal substrate or ligand for a protein's binding site.

Complementary Base Sequence: For a given sequence of nucleic acids, the nucleic acids that are related to them by the rules of base pairing.

Configuration: The spatial arrangement in which atoms are covalently linked in a molecule.

Conformation: The three-dimensional arrangement adopted by a molecule, usually a complex macromolecule. Molecules with the same configuration can have more than one conformation.

Conjugate Acid-Base Pair: A proton donor and its corresponding deprotonated species; for example, acetic acid (donor) and acetate (acceptor).

Corpus Luteum: A temporary endocrine gland formed from the ruptured ovarian follicle (which enclosed the egg) after an egg is released during ovulation. The corpus luteum produces the hormones progesterone and oestradiol (an oestrogen).

Corticosteroids: Hormones secreted from the cortex of the adrenal gland that include glucocorticoids (e.g. cortisol), mineralocorticoids (e.g. aldosterone) and androgens (e.g. testosterone).

Corticosterone: A glucocorticoid hormone produced by the adrenal glands. Cortisol is the main glucocorticoid in most animal species but corticosterone is higher in animals such as amphibians, rodents, reptiles and birds.

Corticotrophin-releasing Hormone: Corticotrophin-releasing hormone is the main element that drives the body's response to stress. It is also present in diseases which cause inflammation. Too much or too little corticotrophin-releasing hormone can have a range of negative effects.

Cortisol: Cortisol is a steroid hormone which regulates a wide range of processes throughout the body including metabolism and the immune response. It also has a very important role in helping the body respond to stress.

Covalent Bond: A chemical bond that involves sharing of electron pairs.

Cristae: Infoldings of the inner mitochondrial membrane.

C-reactive Protein (CRP): An inflammatory marker - a protein that the body releases in response to inflammation. Thus, elevated levels of CRP in the blood mean that there is inflammation somewhere in the body. CRP is not normally present in the blood of a healthy patient. CRP levels can increase by as much as 1000-times with inflammation. Conditions that commonly lead to marked changes in CRP include infection, trauma, surgery, burns, inflammatory conditions, and advanced cancer. Moderate changes occur after strenuous exercise, heatstroke, and childbirth. Psychological stress and some psychiatric illnesses can cause small changes in CRP levels. CRP is the only inflammatory marker that has been found to be an indicator of heart health. Therefore, doctors often carry out a CRP test at the same time as cholesterol and other lipid tests to help predict a patient's risk of heart attack.

Creatinine: Creatinine is a normalizing parameter used to calculate neurotransmitter levels. Creatinine is produced at a constant rate through the kidneys. Therefore, by using creatinine as the constant factor, spot urinary measurements can be performed with out having to factor in the patient's hydration state, possible renal disorders or diuretic substances that may have been used.

Cushing's Disease: is the collection of clinical symptoms and signs resulting from a pituitary tumour that causes excessive amounts of the hormone cortisol to be released by the adrenal glands.

Cushing's Syndrome: is the name given to the collection of signs and symptoms that occur when the body is exposed to too much of the hormone cortisol.

Cytidine: A pyrimidine nucleoside found in DNA and RNA.

Cytochromes: Heme-containing proteins that function as electron carriers in oxidative phosphorylation and photosynthesis.

Cytochrome bc Complex: A membrane bound electron transfer protein complex found in all anoxygenic photosynthetic organisms that oxidizes reduced quinone and reduces a c-type cytochrome. The complex contains a c-type cytochrome, two b-type cytochromes and an FeS center.

Cytochrome bf Complex: A membrane bound electron transfer protein complex found in all oxygenic photosynthetic organisms that oxidizes reduced plastoquinone and reduces plastocyanin (or cytochrome c). The complex contains a c-type cytochrome, two b-type cytochromes and an FeS center.

Cytoplasm: The contents enclosed by the plasma (or cytoplasmic) membrane, excluding the nucleus.

Cytosine: A pyrimidine base found in DNA and RNA.

Cytoskeleton: The filamentous skeleton, formed in the eukaryotic cytoplasm, that is largely responsible for controlling cell shape.

Cytosol: The liquid portion of the cytoplasm, including the macromolecules but not including the larger structures like subcellular organelles or cytoskeleton.

D

Dalton: A unit of mass equivalent to the mass of a hydrogen atom (1.66×10^{-24} g)

Dark Reactions: Reactions that can occur in the dark, in a process that is usually associated with light, such as the dark reactions of photosynthesis.

De Novo Pathway: A biochemical pathway that starts from elementary substrates and ends in the synthesis of a biochemical.

Deamination: The enzymatic removal of an amine group, as in the deamination of an amino acid to an alpha keto acid.

Deficiency: The amount lacked; a shortage: an estrogen deficiency or vitamin deficiency.

Degenerative: Of, relating to, involving, or tending to cause degeneration; to diminish in quality, esp. for a former state of coherence, balance, integrity, etc.

Dehydroepiandrosterone (DHEA): A steroid hormone naturally produced by the adrenal glands and sold in synthetic form as a nutritional supplement.

Dehydrogenase: An enzyme that catalyzes the removal of a pair of electrons (and usually one or two protons) from a substrate molecule.

Denaturation: The disruption of the native folded structure of a nucleic acid or protein molecule; may be due to heat, chemical treatment, or change in pH.

Depression: A condition of general emotional dejection and withdrawal; sadness greater and more prolonged than that warranted by any objective reason.

DHT (Dihydrotestosterone): A conversion of testosterone that is considered to be an aging-bio-marker. Among its affects are the appearance of body-hair, the loss of scalp hair and the onset of prostate gland problems.

Diabetes Insipidus: Diabetes insipidus (water diabetes) is caused by a reduction in, or failure to respond to, anti-diuretic hormone. This results in passing too much dilute urine and increased thir.

Diabetes Mellitus: A metabolic disease resulting from insulin deficiency; characterized by a failure in glucose transport from the blood into cells at normal glucose concentrations.

Diagnostic Test: Any kind of medical test performed to aid in the diagnosis or detection of disease or poor health.

Diffusion: The net movement of molecules in the direction of lower concentration.

Digestion: Enzymatic hydrolysis of major nutrients in the gastrointestinal system to yield their simpler components.

Disorder: A disturbance in physical or mental health or functions.

Disulfide Bridge: A covalent linkage formed by oxidation between two cysteine SH groups either in the same polypeptide chain or in different polypeptide chains. Reversible by adding reducing agents.

DNA Polymerase: An enzyme that catalyzes the formation of 3'-5' phosphodiester bonds from deoxyribonucleotide triphosphates.

DNA: Deoxyribonucleic acid. A polydeoxyribonucleotide in which the sugar is deoxyribose; the main repository of genetic information in all cells and most viruses.

Dopamine: Dopamine is an excitatory and inhibitory neurotransmitter, depending on the dopaminergic receptor it binds to. It is derived from the amino acid tyrosine. Dopamine is the precursor to norepinephrine and epinephrine, which are all catecholamines. The function of dopamine is diverse but plays a large role in the pleasure/reward pathway, memory, and motor control. Dopamine, like norepinephrine and epinephrine, is stored in vesicles in the axon terminal. Dopamine plays a significant role in the cardiovascular, renal, hormonal, and central nervous systems.

Dosage: The amount of medicine to be given.

Double Helix: A structure in which two helically-twisted polynucleotide strands are held together by hydrogen bonding and base stacking.

E

Eicosanoid: Any fatty acid with 20 carbons.

Electron Acceptor: A substance that receives electrons in an oxidation–reduction reaction.

Electron Carrier: A protein, such as a flavoprotein or a cytochrome, that can reversibly gain and lose electrons; functions in the transfer of electrons from organic nutrients to oxygen or some other terminal acceptor.

Electron Donor: A substance that donates electron in an oxidation–reduction reaction.

Electron Transfer: Movement of electrons from substrates to oxygen via the carriers of the respiratory (electron -transfer) chain.

Electrophoresis: The movement of particles in an electrical field. A commonly-used technique for analysis of mixtures of molecules in solution according to their electrophoretic mobilities.

Elongation Factors: (1) Proteins that function in the elongation phase of eukaryotic transcription. (2) Specific proteins required in the elongation of polypeptide chains by ribosomes.

Enantiomorphs: Isomers that are mirror images of one another.

Endergonic Reaction: A reaction with a positive standard free energy change.

Endonuclease: An enzyme that breaks a phosphodiester linkage at some point within a polynucleotide chain.

Endoplasmic Reticulum: A system of double membranes in the cytoplasm that is involved in the synthesis of transported proteins. The rough endoplasmic reticulum has ribosomes associated with it. The smooth endoplasmic reticulum does not.

End-product (feedback) Inhibition: The inhibition of the first enzyme in a pathway by the end product of that pathway.

Energy Charge: The fractional degree to which the AMP-ADP-ATP system is filled with high-energy phosphates (phosphoryl groups).

Enzyme: A molecule most often a protein, that contains a catalytic site for a biochemical reaction.

Epimers: Two stereoisomers with more than one chiral center that differ in configuration at one of their chiral centers.

Epinephrine: Epinephrine, also known as adrenaline, is an excitatory neurotransmitter and hormone essential for lipolysis, which is a process in which the body metabolizes fat. Epinephrine is derived from the amine norepinephrine. As a neurotransmitter, epinephrine regulates attentiveness and mental focus. Epinephrine is synthesized from norepinephrine. As a hormone, epinephrine is secreted along with norepinephrine principally by the medulla of the adrenal gland

Epithelial Cell: Any cell that forms part of the outer covering of an organism or organ.

Erythrocyte: A cell containing large amounts of hemoglobin and specialized for oxygen transport; a red blood cell.

Escherichia Coli (E. coli): A Gram negative bacterium commonly found in the vertebrate intestine. It is the bacterium most frequently used in the study of biochemistry and genetics.

Essential Amino Acids: Amino acids that cannot be synthesized by humans (and other vertebrates) and must be obtained from the diet.

Essential Fatty Acids: The group of polyunsaturated fatty acids produced by plants, but not by humans: required in the human diet.

Estradiol: The most powerful female hormone that occurs naturally. An estrogenic hormone produced by the ovaries and used in treating estrogen deficiency and certain menopausal and postmenopausal conditions.

Estriol: An estrogenic hormone occurring in urine during pregnancy; used in conditions involving estrogen deficiency.

Estrogen: Any of several steroid hormones produced chiefly by the ovaries and responsible for the development and maintenance of female secondary sex characteristics.

Estrogen Dominance: A condition in which a person has excess amounts of estrogen in the body.

Estrone: An estrogenic hormone produced by the ovarian follicles and found during pregnancy in urine and placental tissue; used in the treatment of estrogen deficiency and certain menopausal and postmenopausal conditions.

Ether: A molecule containing two carbons linked by an oxygen atom.

Eubacteria: One of the five kingdoms of living organisms. Eubacteria have a plasma membrane but no internal organellels or nucleus.

Eukaryote: A cell or organism that has a membrane-bound nucleus.

Exocytosis: The fusion of an intracellular vesicle with the plasma membrane, releasing the vesicle contents to the extracellular space.

F

Facilitated Diffusion: Diffusion of a polar substance across a biological membrane through a protein transporter: also called passive diffusion or passive transport.

Fatty Acid: A long-chain hydrocarbon containing a carboxyl group at one end. Saturated fatty acids have completely saturated hydrocarbon chains. Unsaturated fatty acids have one or more carbon-carbon double bonds in their hydrocarbon chains.

Feedback Inhibition: Allosteric enzyme Inhibition at the beginning of a metabolic sequence by the end of product of the sequence; also known as end-product inhibition.

Fermentation: The energy-generating breakdown of glucose or related molecules by a process that does not require molecular oxygen.

Fischer Projection Formulas: A method for representing molecules to show the configuration of groups around chiral centers; also known as Fischer projection formulas.

Flagellum: A cell appendage used in propulsion. Bacterial flagella have a much simpler structure than eukaryotic flagella, which are similar to cilia.

Flavin-linked Dehydrogenases: Dehydrogenases requiring one of the riboflavin coenzymes, FMN or FAD.

Flavin Nucleotides: riboflavin containing nucleotide coenzymes (FMN and FAD).

Flavoprotein: An enzyme containing a flavin nucleotide as a tightly bound prosthetic group.

Follicle Stimulating Hormone: Follicle stimulating hormone is produced by the pituitary gland. It regulates the functions of both the ovaries and testes. Lack or insufficiency of it can cause infertility or subfertility both in men and women.

Forgetfulness: Loss of remembrance or recollection; a ceasing to remember. Also called memory loss, foggy thinking, or foggy memory.

Free Radicals: Harmful molecules that cause damage in the body; leading factors in the development of blood vessel disease (atherosclerosis), cancer and other conditions. Byproducts of normal body processes, byproducts of the breakdown of certain medicines, found in pollutants.

Fluorescence: Emission of light by excited molecules as they revert to the ground state.

Furanose: A sugar that contains a five-membered ring as a result of intramolecular hemiacetal formation.

G

G proteins: A family of heterotrimeric GTP-binding proteins that act in intracellular signaling pathways. Commonly, ligand binding to a serpentine receptor induces the exchange of GTP for bound GDP, enabling the G protein to activate a downstream enzyme in a signaling pathway.

G proteins have intrinsic GTPase activity and therefore self-inactivate.

Galactorrhea: Secretion of milk from the breast of a non-lactating person.

Gamma-Aminobutyric Acid (GABA): A neurotransmitter of the central nervous system that inhibits excitatory responses.

Gangliosides: Sphingolipids containing complex oligosaccharides as head groups; especially common in nervous tissue.

Gel Fitration Chromatography: A technique that makes use of certain polymers that can form porous beads with varying pore sizes. In columns made from such beads, it is possible to separate molecules, which cannot penetrate beads of a given pore size, from small molecules that can. Also called gel-exclusion or molecular seive chromatography.

Genetic Information: The hereditary information contained in a sequence of nucleotide bases in chromosomal DNA or RNA.

Gigantism: Gigantism is a rare condition of accelerated growth and increased height as a result of too much growth hormone secretion during childhood or adolescence.

Globular Protein: A folded protein that adopts an approximately globular shape. May also be called soluble proteins.

Glucagon: Glucagon is secreted to maintain glucose levels in the bloodstream when fasting and to raise very low glucose levels.

Glucagon-Like Peptide 1: Glucagon-like peptide 1 is a hormone produced in the gut and released in response to food. It causes reduced appetite and the release of insulin.

Glucagonoma: A glucagonoma is a very rare tumour of the pancreas in which there is an increase in release of the hormone glucagon causing a characteristic skin rash and raised blood sugar levels.

Gluconeogenesis: The production of sugars from nonsugar precursors such as lactate or amino acids. Applies more specifically to the production of free glucose by vertebrate livers.

Glucose-Dependent Insulinotropic Peptide: Glucose-dependent insulinotropic peptide is a hormone produced by the small intestine in response to eating food. Its main action is to encourage the release of insulin into the bloodstream to control blood sugar levels.

Glutamate: Glutamate is the major excitatory neurotransmitter in the brain which is necessary for memory and learning. In fact, it is believed that 70% of the fast excitatory CNS synapses utilize glutamate as a transmitter. Excitatory neurotransmitters increase the activity of signal-receiving neurons and play a major role in controlling brain function.

Glycine: Glycine is a principal inhibitory amino acid in the brain stem and spinal cord that regulates excitatory neurotransmission in much the same way as GABA. Glycine, much like GABA and taurine, can become elevated to compensate for elevations in excitatory neurotransmitters, primarily, glutamate and aspartic acid.

Glycogen: A polymer of glucose residues in 1, 4 linkage, with 1,6 linkages at branch points.

Glycogenesis: The process of converting glucose to glycogen.

Glycogenic: Describing amino acids whose metabolism may lead to gluconeogenesis.

Glycolipid: A lipid containing a carbohydrate group.

Glycolysis: The catabolic conversion of glucose to pyruvate with the production of ATP.

Glycoprotein: A protein linked to an oligosaccharide or a polysaccharide. Glycosaminoglycans. Long, unbranched polysaccharide chains composed of repeating disaccharide subunits in which one of the two sugars is either N-acetylglucosamine or N-acetylgalactosamine.

Glycosaminoglycan: A heteropolysaccharide of two alternating units: one is either N- acetyglucosamine or N-acetylgalactosamine; the other is a uronic acid (usually glucuronic acid). Formerly called mucopolysaccharide.

Glycosidic Bond: The bond between a sugar and an alcohol. Also the bond that links two sugars in disaccharides, oligosaccharides, and polysaccharides.

Glyoxylate Cycle: A pathway that uses some of the enzymes of the TCA cycle and some enzymes whereby acetate can be converted into succinate and carbohydrates.

Glyoxysome: An organelle containing some enzymes of the glyoxylate cycle.

Goitre: Goitre is a general term for an enlarged thyroid gland. Depending on the type of swelling, location, how it affects gland function and how long it has been present, goitre has various effects and is treated in a variety of different ways.

Golgi Apparatus: A complex series of double-membrane structures that interact with the endoplasmic reticulum and that serve as a transfer point for proteins destined for other organelles, the plasma membrane, or extracellular transport.

Golgi Complex: A complex membranous organelle of eukaryotic cells; functions in the posttranslational modification of proteins and their secretion from the cell or incorporation into the plasma membrane or organellar membranes.

Gonadotrophin-Releasing Hormone: Gonadotrophin-releasing hormone is released from nerve cells in the brain. It controls the production of luteinising hormone and follicle stimulating hormone from the pituitary gland.

Gram Molecular Weight: The weight in grams of a compound that is numerically equal to its molecular weight: the weight of 1 mole.

Grana: Stacks of thylakoids, flattened membranous sacs or disks, in chloroplasts.

Growth Hormone (GH): A hormone secreted by the pituitary gland. GH stimulates growth and repair of the body as well as the activities of the immune system. With age, GH release diminishes (also known as HGH or human growth hormone).

Guanine: A purine base found in DNA or RNA.

Guanosine: A purine nucleoside found in DNA and RNA.

H

Hair Loss: The medical description of the loss of hair from the head or body, sometimes to the extent of baldness, that is involuntary and unwelcome. In some cases, it is an indication of an underlying medical concern, such as iron deficiency. Also called alopecia.

Hairpin Loop: A single-stranded complementary region of DNA or RNA that folds back on itself and base-pairs into a double helix.

Haworth Perspective Formulas: A method for representing cyclic chemical structures so as to define the configuration of each substituent group; the method commonly used for representing sugars.

Health: The general condition of the body or mind with reference to soundness and vigor.

Helix: A spiral structure with a repeating pattern.

Heme Protein: A protein containing a heme as a prosthetic group.

Heme: An iron-porphyrin complex found in hemoglobin and cytochromes.

Hemiacetal: The product formed by the condensation of an aldehyde with an alcohol; it contains one oxygen linked to a central carbon in a hydroxyl fashion and one oxygen linked to the same central carbon by an ether linkage.

Hemoglobin: A heme protein in erythrocytes; functions in oxygen transport.

Henderson-Hasselbalch Equation: An equation that relates the pKa, to the pH and the ratio of the proton acceptor (A^-) and the proton donor (HA) species of a conjugate acid base pair.

Heterochromatin: Highly condensed regions of chromosomes that are not usually transcriptionally active.

Heteroduplex: An annealed duplex structure between two DNA strands that do not show perfect complementarity. Can arise by mutation, recombination, or the annealing of complementary single-stranded DNAs.

Hexose Monophosphate Pathway: An oxidative pathway beginning with glucose 6-phosphate and leading, via 6- phosphogluconate, to pentose phosphates and yielding NADPH. Also called the pentose phosphate pathway and the hexose monophosphate pathway.

Hexose: A sugar with a six-carbon backbone.

High-Performance Liquid Chromatography (HPLC): Chromatographic procedure, often conducted at relatively high pressures, using automated equipment that permits refined and highly reproducible profiles.

Hirsutism: Hirsutism is the presence of excess hair growth in women and is commonly caused by an imbalance of hormones.

Histamine: Histamine is an excitatory neurotransmitter involved in the sleep/wake cycle and inflammatory response. Depending on the receptor histamine activates a wide array of biological actions can occur. For instance, one receptor helps regulate the sleep/wake cycle whereas another receptor helps regulates norepinephrine, serotonin, and acetylcholine release. There are also other receptors that may be activated to induce inflammatory response, which is commonly associated with the exposure to an allergen.

Histones: The family of basic proteins that is normally associated with DNA in most cells of eukaryotic organisms.

Homopolysaccharide: A polysaccharide made up of only one type of monosaccharide unit.

Hormone: A chemical substance made in one cell and secreted so as to influence the metabolic activity of a select group of cells located at other sites in the organism.

Hormone Pellet Therapy: A sustainable delivery method or treatment option for bioidentical hormone therapy. Every three to six months a pellet made of bioidentical hormones is inserted just under the patient's skin. The pellets, which contain customized levels of estradiol or testosterone, react to the needs of the body, so they actually dissolve releasing the bioidentical hormones as needed.

Hormone Receptor: A protein that is located on the cell membrane or inside the responsive cell and that interacts specifically with the hormone.

Hormone Therapy: The use of hormones in medical treatment. Also called hormonal therapy.

Host Cell: A cell used for growth and reproduction of a virus.

Hot Flash: A sudden feeling of feverish heat typically as a symptom of menopause. Also called a hot flash.

Hydrogen Bond: A weak, noncovalent, attractive force between one electronegative atom and a hydrogen atom that is covalently linked to a second electronegative atom.

Hydrolysis: The cleavage of a molecule by the addition of water.

Hydrophilic: Preferring to be in contact with water.

Hydrophobic: Preferring not to be in contact with water, as is the case with the hydrocarbon portion of a fatty acid or phospholipid chain.

Hyperthyroidism: Pathologically excessive production of the thyroid hormones.

Hypogonadism: Diminished hormonal or reproductive functioning in the testes or the ovaries.

Hypothalamus: An area of the brain that is believed to be the command center for instructions to the endocrine system.

Hypothyroidism: Deficient activity of the thyroid gland.

Hysterectomy: Surgical removal of part or all of the uterus. Also called surgical menopause.

I

Imine: A molecule containing a nitrogen atom attached to a carbon atom by a double bond. The nitrogen is also covalently linked to a hydrogen. Immunofluorescence. A cytological technique in which a specific fluorescent antibody is used to label an antigen. Frequently used to determine the location of an antigen in a tissue or a cell.

Immune Response: The capacity of a vertebrate to generate antibodies to an antigen, a macromolecule foreign to the organism.

Immunoglobulin: A protein made in a B plasma cell and usually secreted; it interacts specifically with a foreign agent. Synonymous with antibody. It is composed of two heavy and two light chains linked by disulfide bonds. Immunoglobulins can be divided into five classes (IgG, IgM, IgA, IgD, and IgE) based on their heavy-chain compon

In Vitro: Literally, "in glass," describing whatever happens in a test tube or other receptacle, as opposed to what happens in whole cells of the whole organism (*in vivo*).

Induced Fit: A change in the shape of an enzyme that results from the binding of substrate.

Inducers: Molecules that cause an increase in a protein activity when added to cells.

Inducible Proteins: Those which are synthesized in different amounts depending on cellular signals.

Initiation Factors: Those protein factors that are specifically required during the initiation phase of protein synthesis.

Insomnia: Inability to obtain sufficient sleep, especially when chronic; difficulty in falling or staying asleep. Also called sleeplessness.

Intercalating Agent: A chemical, usually containing aromatic rings, that can sandwich in-between adjacent base pairs in a DNA duplex. The intercalation leads to an adjustment in the DNA secondary structure, as adjacent base pairs are usually close-packed.

Interferon: One of a family of proteins that are liberated by special host cells in the mammal in response to viral infection. The interferons attach to an infected cell, where they stimulate antiviral protein synthesis.

Intermediary Metabolism: In cells, the enzyme-catalyzed reactions that extract chemical energy from nutrient molecules and utilize it to synthesize and assemble cell components.

Interstitial Cystitis: A urinary bladder disease of unknown cause characterized by urinary frequency, urgency, pressure and/or pain in the bladder and/or pelvis. Also known as painful bladder syndrome (PBS).

Iodine: An element required in small amounts for healthy growth and development. The thyroid gland requires iodine to synthesize thyroid hormones; a deficiency of the element leads to goiter.

Ion Channel: An integral protein that provides for the regulated transport of a specific ion, or ions, across a membrane.

Ion-Exchange Resin: A polymeric resinous substance, usually in bead form, that contains fixed groups with positive or negative charge. An anion exchange resin has positively-charged

groups and is therefore useful in exchanging the anionic groups in a test sample; a cation exchange resin is itself negatively charged, and has the opposite application. The resin is usually used in a column chromatographic procedure.

Irritable Male Syndrome: The term for a set of symptoms caused by a drop in testosterone levels in males. These symptoms are similar to those of the male menopause or andropause.

Isoelectric Point or pH: The pH at which a protein has no net charge.

Isomerase: An enzyme that catalyzes an intramolecular rearrangement.

Isomerization: Rearrangement of atomic groups within the same molecule without any loss or gain of atoms.

Isomers: Any two molecules with the same molecular formula but a different arrangement of molecular groups.

Isoprenoid: Any of a large number of natural products synthesized by enzymatic polymerization of two or more isoprene units: also called terpenoids.

Isotopes: Stable or radioactive forms of an element that differ in atomic weight but are otherwise chemically identical to the naturally abundant form of the element; used as tracers.

Isozymes: Multiple forms of an enzyme that catalyze the same reaction but differ from each other in their amino acid sequence, substrate affinity, $V_{max,}$ and/or regulatory properities; also called isoenzymes.

K

Keratins: Insoluble protective or structural proteins consisting of parallel polypeptide chains in α-helical or β conformations.

Ketogenic: Describing amino acids that are metabolized to acetoacetate and acetate.

Ketone Bodies: Refers to acetoacetate, acetone, and β-hydroxybutyrate made from acetyl-CoA in the liver and used for energy in nonhepatic tissue.

Ketone: A functional group of an organic compound in which a carbon atom is double-bonded to an oxygen. Neither of the other substituents attached to the carbon is a hydrogen. Otherwise the group would be called an aldehyde.

Ketosis: A condition in which the concentration of ketone bodies in the blood or urine is unusually high.

Kilobase: One thousand bases in a DNA molecule.

Kinase: An enzyme catalyzing phosphorylation of an acceptor molecule, usually with ATP serving as the phosphate (phosphoryl) donor.

Km: See Michaelis constant.

Krebs Cycle: A cycle system of enzymatic reactions for the oxidation of acetyl residues to carbon dioxide, in which formation of citrate is the first step; also known as the Krebs cycles or tricarboxylic acid cycle.

L

Lectins: Agglutinating proteins usually extracted from plants.

Leukocyte: White blood cell; involved in the immune response in mammals.

Leukotrienes: A family of molecules derived from arachidonate; muscle contractants that constrict air passages in the lungs and are involved in asthma.

Ligand: A (usually small) molecule that binds to another, such as oxygen when it binds to myoglobin.

Ligase: An enzyme that catalyzes the joining of two molecules together. In DNA it joins 5′-OH to 3′ phosphates.

Light Harvesting Complex: A protein complex that harvests light energy and converts it

to exciton energy that can migrate to a reaction center. The light is absorbed by pigment molecules (*e.g.*, chlorophyll, bacteriochlorophyll, carotenoids, phycobilin) that are attached to the protein.

Lipases: Enzymes that catalyze the hydrolysis of triacylglycerols.

Lipid Bilayer: Model for the structure of the cell membrane based on the interaction between the hydrophobic regions of phospholipids.

Lipid: A biological molecule that is soluble in organic solvents. Lipids include steroids, fatty acids, prostaglandins, terpenes and waxes.

Lipoate (Lipoic Acid): A vitamin for some microorganisms; an intermediate carrier of hydrogen atoms and acyl groups in α-keto acid dehydrogenases.

Lipopolysaccharide: Usually refers to a unique glycolipid found in Gram negative bacteria.

Lipoprotein: A lipid protein aggregate that serves to carry water–insoluble lipids in the blood. The protein component alone is an apolipoprotein.

Liposome: A small, spherical vesicle composed of a phospholipid bilayer, which forms spontaneously when phospholipids are suspended in an aqueous buffer.

Luteinizing Hormone: A hormone produced by the anterior lobe of the pituitary gland that stimulates ovulation and the development of the corpus luteum in the female and the production of testosterone by the interstitial cells of the testes in the male.

Lyase: An enzyme that catalyzes the removal of a group to form a double bond, or the reverse reaction.

Lymphocytes: A subclass of leukocytes involved in the immune response. B lymphocytes synthesize and secrete antibodies; T lymphocytes either play a regulatory role in immunity or kill foreign and virus-infected cells.

Lysosome: An organelle that contains hydrolytic enzymes designed to break down proteins that are targeted to that organelle.

M

Matrix: The aqueous contents of a cell or organelle (the mitochondrion, for example) with dissolved solutes.

Meiosis: A type of cell division in which diploid cells give rise to haploid cells destined to become gametes.

Melatonin: A hormone derived from serotonin and secreted by the pineal gland in inverse proportion to the amount of light received by the retina. Darkness perceived by the retina (or sleep) induces greater production which has been linked to the regulation of circadian rhythms and inducing sleep rhythms.

Membrane Transport: Movement of a polar solute across a membrane via a specific membrane protein (a transportor).

Membrane: A sheet-like composite of protein and lipid that is the boundary of cells and organelles.

Membrane Protein: A protein that is associated with a membrane, rather than found free in the cell. A membrane protein may be integral (embedded or buried) in the membrane, or peripheral (attached more loosely, by interactions with either lipid or intergral membrane proteins).

Membrane Transport: The facilitated transport of a molecule across a membrane.

Menopausal: Of, pertaining to, or characteristic of menopause.

Menopause: The period of permanent cessation of menstruation, usually occurring between the ages of 45 and 55.

Menstrual Cycle: A recurring cycle in which the endometrial lining of the uterus prepares for pregnancy; if pregnancy does not occur the

lining is shed at menstruation. The average menstrual cycle is 28 days, day 1 being the beginning of bleeding.

Menstruation: The periodic discharge of blood and mucosal tissue from the uterus, occurring approximately monthly from puberty to menopause in non-pregnant women.

Mesosome: An invagination of the bacterial cell membrane.

Messenger RNA (mRNA): A class of RNA molecules, each of which is complementary to one strand of DNA; carries the genetic message from the chromosome to the ribosomes for protein synthesis.

Metabolic Rate: The rate of metabolism; the amount of energy expended in a given period.

Metabolism: The entire set of enzyme-catalyzed transformations of organic molecules in living cells; the sum of anabolism and catabolism.

Metabolites: Substances necessary for taking part in a particular metabolic process, such as glucose in the metabolism of sugars and starches, and amino acids in the biosynthesis of proteins.

Metabolism: The sum total of the enzyme-catalyzed reactions that occur in a living organism.

Micelle: An aggregate of lipids in which the polar head groups face outward and the hydrophobic tails face inward; no solvent is trapped in the center.

Michaelis Constant (Km): The substrate concentration at which an enzyme-catalyzed reaction proceeds at one-half of the maximum velocity.

Michaelis-Menten Equation: (also known as the Henri-Michaelis-Menten equation). An equation relating the reaction velocity to the substrate concentration of an enzyme.

Micronized: To reduce to particles that are only a few microns in diameter.

Microsomes: Membranous vesicles formed by fragmentation of the endoplasmic reticulum of eukaryotic cells; recovered by differential centrifugation.

Microtubules: Thin tubules, made from globular proteins, that serve multiple purposes in eukaryotic cells.

Mitochondrion: An organelle, found in eukaryotic cells, in which oxidative phosphorylation takes place. It contains its own genome and unique ribosomes to carry out protein synthesis of only a fraction of the proteins located in this organelle.

Mobile Genetic Element: A segment of the genome that can move as a unit from one location on the genome to another, without any requirement for sequence homology.

Molecule: A single very small particle, made up of atoms, which is indivisible.

Molecular Seive Chromatography: See Gel filtration chromatography.

Molecular Weight: See Gram molecular weight.

Monolayer: A single layer of oriented lipid molecules.

Monomer: One unit of a protein or other structure.

N

NAD, NADP (nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate): Nicotinamide-containing coenzymes functioning as carriers of hydrogen atoms and electrons in some oxidation-reduction reactions.

Negative Feedback: Regulation of a biochemical pathway achieved when a reaction product inhibits an earlier step in the pathway.

Neurotransmitter: Any of several chemical substances, such as epinephrine or acetylcholine, that transmit nerve impulses across a synapse to

a postsynaptic element, such as another nerve, muscle or gland.

Nitric Oxide: Works as a signaling molecule in the cardiovascular system. Cells of a blood vessel's inner walls use nitric oxide to signal the vessel to relax and dilate, increasing blood flow.

Nitrogen Cycle: The passage of nitrogen through various valence states, as the result of reactions carried out by a wide variety of different organisms.

Nitrogen Fixation: Conversion of atmospheric nitrogen into a form that can be converted by biochemical reactions to an organic form. This reaction is carried out by a very limited number of microorganisms.

Nitrogenous Base: An aromatic nitrogen-containing molecule with basic properties. Such bases include purines and pyrimidines.

Noncompetitive Inhibitor: An inhibitor of enzyme activity whose effect is not reversed by increasing the concentration of substrate molecule.

Nonessential Amino Acids: Amino acids that can be made by humans and other vertebrates from simpler precursors, and are thus not required in the diet.

Nonheme Iron Proteins: Proteins, usually acting in oxidation –reduction reactions, containing iron but no porphyrin groups.

Nonpolar: Hydrophobic; describes molecules or groups that are poorly soluble in water.

Norepinephrine: Norepinephrine is an excitatory neurotransmitter that is important for attention and focus. Norepinephrine is synthesized from dopamine by means of the enzyme dopamine beta-hydroxylase, with oxygen, copper, and vitamin C as co-factors.

Nuclease: An enzyme that cleaves phosphodiester bonds of nucleic acids.

Nucleic Acids: Polymers of the ribonucleotides or deoxyribonucleotides.

Nucleoid: In bacteria, the nuclear zone that contains the chromosome but has no surrounding membrane.

Nucleolus: A spherical structure visible in the nucleus during interphase. The nucleolus is associated with a site on the chromosome that is involved in ribosomal RNA synthesis.

Nucleoside: An organic molecule containing a purine or pyrimidine base and a five-carbon sugar (ribose or deoxyribose).

Nucleosome: A complex of DNA and an octamer of histone proteins in which a small stretch of the duplex is wrapped around a molecular bead of histone.

Nucleotide: An organic molecule containing a purine or pyrimidine base, a five-carbon sugar (ribose or deoxyribose), and one or more phosphate groups. A phosphoester of a nucleoside.

Nucleus: In eukaryotic cells, the centrally-located organelle that encloses most of the chromosomes. Minor amounts of chromosomal substance are found in some other organelles, most notably the mitochondria and the chloroplasts.

O

Obesity: Obesity is an excess of calorie storage in the form of fat. It is an increasing public health and medical problem associated with reduced quality and quantity of life.

Oestradiol: Oestradiol is a powerful reproductive hormone that has a wide range of actions in both men and women.

Oestriol: Oestriol is a hormone made during pregnancy that can be used to measure foetal health and predict when birth may happen.

Oestrogen: A type of sex steroid hormone found in men and women responsible for secondary sexual characteristics. There are three oestrogens – oestrone, oestradiol and oestriol - the most potent of which is oestradiol.

Oestrone: Oestrone is a hormone produced by the ovaries. It is the primary oestrogen in postmenopausal women.

Oligonucleotide: A polynucleotide containing a small number of nucleotides. The linkages are the same as in a polynucleotide; the only distinguishing feature is the small size.

Oligosaccharide: A molecule containing a small number of sugar residues joined in a linear or a branched structure by glycosidic bonds.

Optical Activity: The property of a molecule that leads to rotation of the plane of polarization of plane-polarized light when the latter is transmitted through the substance. Chirality is a necessary and sufficient property for optical activity.

Organelle: A subcellular membrane-bounded body with a well-defined function.

Osmosis: Bulk flow of water through a semipermeable membrane into another aqueous compartment containing solute at a higher concentration.

Osmotic Pressure: The pressure generated by the mass flow of water to that side of a membrane-bounded structure that contains the higher concentration of solute molecules. A stable osmotic pressure is seen in systems in which the membrane is not permeable to some of the solute molecules.

Osteoporosis: Osteoporosis is a bone disorder caused by loss of bone mass resulting in abnormal bone structure and an increased risk of fracture.

Ovaries: The ovaries produce and release eggs (oocytes) into the female reproductive tract at the mid-point of each menstrual cycle. They also produce the female hormones oestrogen and progesterone.

Ovulation: The process by which an egg is released from the ovary at the mid-point of the menstrual cycle (around day 14). It is triggered by a surge in luteinising hormone from the pituitary gland; this hormone is measured in ovulation kits.

Oxidation: The loss of electrons from a compound.

Oxidation-Reduction Reaction: A reaction in which electrons are transferred from a donor to an acceptor molecule; also called a redox reaction.

Oxidative Phosphorylation: The formation of ATP as the result of the transfer of electrons to oxygen.

Oxido-Reductase: An enzyme that catalyzes oxidation-reduction reactions.

Oxytocin: Oxytocin is a hormone which acts on organs in the body (including the breast and uterus) and as a chemical messenger in the brain controlling key aspects of the reproductive system including childbirth and lactation, and aspects of human behaviour.

P

Parkinson's Disease: A degenerative disorder of the central nervous system characterized by tremor and impaired muscular coordination.

Pentose Phosphate Pathway: The pathway involving the oxidation of glucose-6-phosphate to pentose phosphates and further reactions of pentose phosphates.

Pentose: A sugar with five carbon atoms.

Peptide Bond: A substituted amide linkage between the α-amino group of one amino acid and the α-carboxyl group of another, with the elimination of the elements of water.

Peptide: An organic molecule in which a covalent amide bond is formed between the α-amino group of one amino acid and the α-carboxyl group of another amino acid, with the elimination of a water molecule. The resulting connection is called a peptide bond.

Peptidoglycan: The main component of the bacterial cell wall, consisting of a two-dimensional network of heteropolysaccharides running

in one direction, cross-linked with polypeptides running in the perpendicular direction.

Perimenopause: The period around the onset of menopause that is often marked by various physical changes, such as hot flashes and menstrual irregularity. Also called peri-menopause, early menopause or pre-menopause.

Periplasm: The region between the inner (cytoplasmic) membrane and the cell wall or outer membrane of a bacterium.

Permeable: The property of allowing material to pass through, as a permeable membrane.

Permease: A protein that catalyzes the transport of a specific small molecule across a membrane.

Peroxisomes: Subcellular organelles that contain flavin-requiring oxidases and that regenerate oxidized flavin by reaction with oxygen.

pH: The negative logarithm of the hydrogen ion concentration of an aqueous solution.

Phenotype: The observable trait(s) that result from the genotype in cooperation with the environment.

Phenylketonuria: A human disease caused by a genetic deficiency in the enzyme that converts phenylalanine to tyrosine. The immediate cause of the disease is an excess of phenylalanine, which can be alleviated by a diet low in phenylalanine.

Phosphatases: Enzymes that hydrolyze a phosphate ester or anhydride, releasing inorganic phosphate, P_i.

Phosphodiester: A molecule containing two alcohols esterified to a single molecule of phosphate. For example, the backbone of nucleic acids is connected by 5′-3′ phosphodiester linkages between the adjacent individual nucleotide residues.

Phosphogluconate Pathway: Another name for the pentose phosphate pathway. This name derives from the fact that 6-phosphogluconate is an intermediate in the formation of pentoses from glucose.

Phospholipid: A lipid containing charged hydrophilic phosphate groups; a component of cell membranes.

Phosphorylation: The formation of a phosphate derivative of a biomolecule.

Photorespiration: The removal of O_2 from the atmosphere by Rubisco and the subsequent biochemical reactions that serve to recycle some of the reduced carbon.

Photon: The ultimate unit (a quantum) of light energy.

Photophosphorylation: The enzymatic formation of ATP from ADP coupled to the light–dependent transfer of electrons in photosynthetic cells.

Photosynthesis: The biosynthesis that directly harnesses the chemical energy resulting from the absorption of light. Frequently used to refer to the formation of carbohydrates from CO_2 that occurs in the chloroplasts of plants or the plastids of photosynthetic microorganisms.

Photosynthetic Membrane: A bilayer of lipid molecules in which are embedded proteins that transform light energy into chemical free energy. (Also known as the thylakoid membrane.)

Photosystem I: A protein complex located in the photosynthetic membrane. Photosystem I is one of two types of reaction centers found in higher plants, algae, and cyanobacteria. The photosystem I reaction center uses light energy to transfer an electron from a mobile electron transfer protein (plastocyanin or a cytochrome c) on one side of the photosynthetic membrane to a mobile electron transfer protein (ferredoxin) on the opposite side of the photosynthetic membrane.

Photosystem II: A protein complex found in the photosynthetic membrane. Photosystem II is one of two types of reaction centers found in higher plants, algae and cyanobacteria. The

photosystem II reaction center uses light energy to transfer electrons from water to plastoquinone. Photosystem II is the source of the molecular oxygen in the atmosphere.

Pituitary Gland: A gland at the base of the brain. The pituitary secretes several different hormones involved in key metabolic processes.

Plasma Membrane: The membrane that surrounds the cytoplasm.

Plasma Proteins: The proteins present in blood plasma.

Plastid: In plants, a self- replicating organelle; may differentiate into a chloroplast.

Plastoquinone: A small organic molecule involved in electron and proton transfer in photosynthesis.

Platelets: Small, enucleated cells that initiate blood clotting; they arise from cells called megakaryocytes in the bone marrow. Also known as thrombocytes.

Polar Group: A hydrophilic (water-loving) group.

Polarimeter: An instrument for determining the rotation of polarization of light as the light.

Polymerase: An enzyme that catalyzes the synthesis of a polymer from monomers.

Polynucleotide Phosphorylase: An enzyme that polymerizes ribonucleotide diphosphates. No template is required.

Polynucleotide: A chain structure containing nucleotides linked together by phosphodiester (5′–3′) bonds. The polynucleotide chain has a directional sense with a 5′ and a 3′ end.

Polypeptide: A linear polymer of amino acids held together by peptide linkages. The polypeptide has a directional sense, with an amino- and a carboxy-terminal end.

Polyribosome (Polysome): A complex of an mRNA and two or more ribosomes actively engaged in protein synthesis.

Polysaccharide: A linear or branched chain structure containing many sugar molecules linked by glycosidic bonds.

Porphyrin: A complex planar structure containing four substituted pyrroles covalently joined in a ring and frequently containing a central metal atom. For example, heme is a porphyrin with a central iron atom.

Post Menopause: All of the time in a woman's life that take place after her last period ever, or more accurately, all of the time that follows the point when her ovaries become inactive. A woman who still has her uterus can be declared to be in post menopause once she has gone 12 full months with no flow at all, not even any spotting.

Pregnenolone: An unsaturated hydroxyl steroid that is formed by the oxidation of steroids (like cholesterol) and yields progesterone on dehydrogenation.

Premenstrual Syndrome (PMS): A varied group of physical and psychological symptoms, including abdominal bloating, breast tenderness, headache, fatigue, irritability, anxiety and depression that occur from 2 to 7 days before the onset of menstruation and cease shortly after menses begins.

Preventive Medicine: Measures taken to prevent illness or injury, rather than curing them. Preventive care may include examinations and screening tests tailored to an individual's age, health and family history. Also called preventive care or preventative medicine.

Primary Structure: In a polymer, the sequence of monomers and the covalent bonds. In proteins, it refers to the amino acid sequence.

Progesterone: A hormone that prepares the uterus for the fertilized ovum and maintains pregnancy.

Prolactin: A protein hormone produced by the pituitary gland of mammals that acts with other hormones to initiate the secretion of milk by the mammary glands. It also acts to maintain the

corpus luteum of the ovary, which is the source of the female sex hormone progesterone. In males, high levels of prolactin can cause testosterone levels to decrease.

Prostate Specific Antigen (PSA): A protein produced by the cells of the prostate gland. PSA is present in small quantities in the serum of normal men and is often elevated in the presence of prostate cancer and in other prostate disorders.

Projection Formulas: A method for representing molecules to show the configuration of groups around chiral centers; also known as Fischer projection formulas.

Prokaryote: A unicellular organism that contains a single chromosome, no nucleus, no membrane-bound organelles, and has characteristic ribosomes and biochemistry.

Prostaglandin: An oxygenated eicosanoid that has a hormonal function. Prostaglandins are unusual hormones in that they usually have effects only in that region of the organism where they are synthesized.

Prosthetic Group: Synonymous with coenzyme except that a prosthetic group is usually more firmly attached to the enzyme it serves.

Protein Kinases: Enzymes that transfer the terminal phosphoryl group of ATP or another nucleoside triphosphate to a Ser, Thr, Tyr, Asp, or His side chain in a target protein, thereby regulating the activity or other properties of that protein.

Protein: A macromolecule composed of one or more polypeptide chains, each with a characteristic sequence of amino acids linked by peptide bonds.

Proteoglycan: A protein-linked heteropolysaccharide in which the heteropolysaccharide is usually the major component.

Protist: A relatively undifferentiated organism that can survive as a single cell.

Proton Acceptor: A functional group capable of accepting a proton from a proton donor molecule.

Protoplasm: A general term referring to the entire contents of a living cell.

Purine: A heterocyclic ring structure with varying functional groups. The purines adenine and guanine are found in both DNA and RNA.

Pyranose: A simple sugar containing the six-membered pyran ring.

Pyridine Nucleotide: A nucleotide coenzyme containing the pyridine derivative nicotinamide; NAD or NADP.

Pyridoxal Phosphate: A coenzyme containing the vitamin pyridoxine (vitamin B_6); functions in reaction involving amino group transfer.

Pyrimidine Dimer: A covalently joined dimer of two adjacent pyrimidine residues in DNA, induced by absorption of UV light; most commonly derived from two adjacent thymines (a thymine dimer).

Pyrimidine: A heterocyclic six-membered ring structure. Cytosine and uracil are the main pyrimidines found in RNA, and cytosine and thymine are the main pyrimidines found in DNA.

Pyrophosphatase: An enzyme that hydrolyzes a molecule of inorganic pyrophosphate to yield two molecules of (ortho) phosphate; also known as pyrophosphatase.

Pyrophosphate: A molecule formed by two phosphates in anhydride linkage.

Q

Quantum: The ultimate unit of energy.

Quaternary Structure: In a protein, the way in which the different folded subunits interact to form the multisubunit protein.

R

R Group: Shorthand for the side chain of an amino acid.

Racemic Mixture (Racemate): An equimolar mixture of the D and L stereoisomers of an optically active compound.

Radioimmunoassay (RIA): A sensitive and quantitative method for detecting trace amounts of a biomolecule, based on its capacity to displace a radioactive form of the molecule from combination with its specific antibody.

Rate-Limiting Step: (1) generally, the step in an enzymatic reaction with the greatest activation energy or the transition state of highest free energy. (2) The slowest step in a metabolic pathway.

Reaction Center: A protein complex that uses light energy to create a stable charge separation by transferring a single electron energetically uphill from a donor molecule to an acceptor molecule, both of which are located in the reaction center.

Receptors: A molecular structure or site on the surface or interior of a cell that binds with substances such as hormones.

Reducing Agent (Reductant): The election donor in an oxidation-reduction reaction.

Reducing Sugar: A sugar in which the carbonyl (anomeric) carbon is not involved in a glycosidic bond and can therefore undergo oxidation.

Reduction: The addition of one or more electrons to an atom or molecule. In the case of a molecule, protons may be involved as well, resulting in hydrogen being added.

Relaxin: Relaxin is a hormone produced by the ovary and the placenta with important effects in the female reproductive system and during pregnancy. In preparation for childbirth, it relaxes the ligaments in the pelvis and softens and widens the cervix.

Renaturation: The process of returning a denatured structure to its original native structure, as when two single strands of DNA are reunited to form a regular duplex, or an unfolded polypeptide chain is returned to its normal folded three-dimensional structure.

Reverse Triiodothyronine (Reverse T3): A molecule which is an isomer of triiodothyroniine and derived from thyroxine through the use of deiodinase. Blocks the action of T3.

Ribose: The five-carbon sugar found in RNA.

Ribosomal RNA (rRNA): The RNA parts of the ribosome.

Ribosomes: Small cellular particles made up of ribosomal RNA and protein. They are the site, together with mRNA, of protein synthesis.

Ribozymes: Ribonucleic acid molecules with catalytic activities; RNA enzymes.

Rickets: Rickets is a condition in children where there is abnormal softening of the bones due to lack of minerals to strengthen it. It is also known as osteomalacia in adults.

RNA (Ribonucleic Acid): A polynucleotide in which the sugar is ribose.

RNA Polymerase: An enzyme that catalyzes the formation of RNA from ribonucleotide triphosphates, using DNA as a template.

Rubisco: (D-ribulose 1, 5-bisphosphate carboxylase/oxygenase) A water soluble protein complex responsible for the removal of CO_2 from the atmosphere. The enzyme works by attaching CO_2 to a five-carbon compound (1, 5 ribulose bisphosphate) that is split into two identical three-carbon compounds (phosphoglycerate). In addition to catalyzing the removal of CO_2 from the atmosphere, Rubisco also catalyzes the removal of O_2 from the atmosphere (less efficiently). The removal of O_2 is thought to be a consequence of poor design and leads to a complex set of compensatory reactions known as photorespiration.

S

S-adenosylmethionine (adoMet): An enzymatic cofactor involved in methyl group transfers

Salvage Pathway: A family of reactions that permits, for instance, nucleosides as well as purine and pyrimidine bases resulting from the partial breakdown of nucleic acids to be re-utilized in nucleic acid synthesis.

Saponification: Alkaline hydrolysis of triacylglycerols to yield fatty acids as soaps.

Saturated Fatty Acid: A fatty acid containing a fully saturated alkyl chain.

Secondary Metabolism: pathways that lead to specialized products not found in every living cell.

Secondary Structure: In a protein or a nucleic acid, any repetitive folded pattern that results from the interaction of the corresponding polymeric chains. In proteins, the most common are β-strands (sheets) and α-helices.

Semipermeable: The characteristic of allowing only some molecules, usually smaller or uncharged ones, to pass through.

Serotonin: Serotonin is an inhibitory neurotransmitter synthesized by enzymes that act on tryptophan and/or 5-HTP. Serotonin is stored in presynaptic vesicles and released to transmit electrochemical signals across the synapse.

Serotonin Metabolite: 5-Hydroxyindoleacet ic acid (5-HIAA) is a major metabolite of serotonin, generated via a two step process, involving monoamine oxidase A (MAO-A) and aldehyde dehydrogenase.

Serum: The clear yellowish fluid obtained upon separating whole blood into its solid and liquid components after it has been allowed to clot.

Sex Hormone: Any of a class of steroid hormones that regulate the growth and function of the reproductive organs or stimulate the development of the secondary sexual characteristics.

Stereoisomers: Isomers that are nonsuperimposable mirror images of each other.

Steroids: Compounds that are derivatives of a tetracyclic structure composed of a cyclopentane ring fused to a substituted phenanthrene nucleus.

Steroid Hormones: Any hormone containing the characteristic steroid ring complex; a term often associated with hormones such as progesterone, testosterone, estrogens, DHEA, and others.

Sterols: A class of lipids containing the steroid nucleus.

Stress: Physical, mental, or emotional strain or tension; a specific response by the body to stimulus, as fear or pain, that disturbs or interferes with the normal physiological equilibrium of an organism.

Stressors: An agent, condition or other stimulus that causes stress to an organism.

Svedberg (S): A unit of measure of the rate at which a particle sediments in a centrifugal field.

Svedberg Unit (S): The unit used to express the sedimentation constant ($S = 10^{-13}$sec). The sedimentation constant S is proportional to the rate of sedimentation of a molecule in a given centrifugal field and is related to the size and shape of the molecule.

Synthetic Hormones: Hormones made from plant progesterone and animal chemicals that are bio-similar but not identical to the hormones your body uses. Generally, an extra covalent bond or molecules are added so that it can be patented or to alter it's chemical properties or clinical effects. Being non-identical to the body may cause significant harmful side effects.

T

Taurine: Taurine is an inhibitory neurotransmitter involved in neuromodulatory and neuroprotective actions. Supplementing with taurine can have a specific effect on GABA function. There are two primary ways in which taurine affects GABA.; First, it can inhibit GABA transaminase, an enzyme that metabolizes GABA. This allows GABA to stay in the synaptic cleft longer to bind to the postsynaptic receptor. Second, taurine can bind to the GABAA receptor mimicking the effects of GABA.

TCA Cycle: See tricarboxylic acid cycle.

Terpenes: A diverse group of lipids made from isoprene precursors.

Tertiary Structure: In a protein or nucleic acid, the final folded form of the polymer chain.

Testosterone: A potent Androgenic hormone produced chiefly by the testes, which stimulates the development of male sex organs, secondary sexual traits and sperm.

Tetramer: Structure resulting from the association of four subunits.

Thioester: An ester of a carboxylic acid with a thiol or mercaptan.

Thromboxanes: A class of molecules derived from arachidonate and involved in platelet aggregation during blood clotting.

Thylakoid: Closed cisterna, or disk, formed by the pigment–bearing internal membranes of chloroplasts.

Thymidine: One of the four nucleosides found in DNA.

Thymine: A pyrimidine base found in DNA.

Thyroid: The gland located in the center and anterior aspect of the neck responsible (amongst other things) for temperature regulation.

Thyroid Peroxidase (TPO): An enzyme mainly expressed in the thyroid that liberates iodine for addition onto tyrosine residues on thyroglobulin for the production of T4, T3 and reverse T3.

Thyroid Stimulating Hormone (TSH): A glycoprotein hormone secreted by the anterior portion of the pituitary gland that stimulates and regulates the activity of the thyroid gland. Also called thyrotropin.

Thyroxin (T4): The thyroid gland iodine-containing hormone that regulates the metabolic rate of the body; used in the treatment of hypothyroidism.

Transamination: Enzymatic transfer of an amino group from an α-amino acid to an α-keto acid.

Transfection: An artificial process of infecting cells with naked viral DNA.

Transferrin: A blood plasma protein for iron ion delivery.

Tricarboxylic Acid (TCA) Cycle: The cyclical process whereby acetate is completely oxidized to CO_2 and water, and electrons are transferred to NAD^+ and flavine. The TCA cycle is localized to the mitochondria in eukaryotic cells and to the plasma membrane in prokaryotic cells. Also called the Krebs or citric acid cycle.

Triodothyronine (T3): A thyroid hormone derived from thyroxine but several times more potent; used in treating hypothyroidism.

Tyramine: Tyramine (4-hydroxy-phenethylamine) is a naturally occurring monoamine compound formed by the enzymatic decarboxylation of the aromatic amino acid tyrosine.

U

Ultracentrifuge: A high-speed centrifuge that can attain speeds up to 60,000 rpm and centrifugal fields of 500,000 times gravity. Useful for characterizing and/or separating macromolecules.

Urea Cycle: A metabolic pathway in the liver that leads to the synthesis of urea from amino groups and CO_2. The function of the pathway is to convert the ammonia resulting from catabolism to a nontoxic form, which is then secreted.

V

Viroids: Pathogenic agents, mostly of plants, that consist of short (usually circular) RNA molecules.

Virus: A complex of nucleic-acid and protein, that can infect and replicate inside a specific host cell to make more virus particles.

Vitamin: A trace organic substance required in the diet of some species. Many vitamins are precursors of coenzymes.

Vitamin D: Vitamin D is a hormone produced by the kidneys that helps to control the concentration of calcium in the blood and is vital for the development of strong bones.

W

Watson-Crick Base Pairs: The type of hydrogen-bonded base pairs found in DNA, or comparable base pairs found in RNA. The base pairs are A-T, G-C, and A-U.

Wild Type: The normal (unimutated) phenotype.

X

Xenoestrogen: Substances or pollutants originating outside the body that have estrogen-like activities. Exposure to these substances can have a profound impact on a person's natural hormonal balance.

Xenohormones: Substances or pollutants originating outside the body that have hormone-like activities. Exposure to these substances can have a profound impact on a person's natural hormonal balance.

X-ray Crystallography: A technique for determining the structure of molecules from the X-ray diffraction patterns that are produced by crystalline arrays of the molecules.

Y

Ylid: A compound in which adjacent, covalently-bonded atoms, both having an electronic octet, have opposite charges.

Z

Zwitterion: A dipolar ion with spatially-separated positive and negative charges. For example, most amino acids are zwitterions, having a positive charge on the α-amino group and a negative charge on the α-carboxyl group but no net charge on the overall molecule.

Z Form: A duplex DNA structure in which there is the usual type of hydrogen bonding between the base pairs but in which the helix formed by the two polynucleotide chains is left-handed rather than right-hande.

Zygote: A cell that results from the union of haploid male and female sex cells. Zygotes are diploid.

Zymogen: An inactive precursor of an enzyme. For example, trypsin exists in the inactive form trypsinogen before it is converted to its active form, trypsin.

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