KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)

(Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

SYLLABUS

DEPARTMENT OF CHEMISTRY

STAFF NAME: Dr.M.R.EZHILARASI
SUBJECT NAME: Analytical Clinical biochemistry
SUB.CODE:17CHU404B
SEMESTER: IV
CLASS: II B.Sc (CHEMISTRY)

Semester-IV

17CHU404B ANALYTICAL CLINICAL BIOCHEMISTRY 3H 3C

Instruction Hours/week: L:3 T:0 P:0 Marks: Internal: 40 External: 60 Total:100

Scope

The course deals with the basic understanding of the structures, properties and functions of carbohydrates, lipids and proteins

Objectives

This course enables the student to

1. Understand the basic structure of carbohydrates
2. Understand the basic structures of protein
3. Understand the basic structures of lipids
4. Understand the basic structures of hormones
5. Understand the biochemistry of diseases.

Methodology

Blackboard teaching, Power point presentation and group discussion.

Unit I

Carbohydrates: Biological importance of carbohydrates, Metabolism, Cellular currency of energy (ATP), Glycolysis, Alcoholic and Lactic acid fermentations, Krebs cycle. Isolation and characterization of polysachharides.

Unit II

Proteins: Classification, biological importance; Primary and secondary and tertiary structures of proteins: α-helix and β- pleated sheets, Isolation, characterization, denaturation of proteins. *Enzymes:* Nomenclature, Characteristics (mention of Ribozymes), Classification; Active site, Mechanism of enzyme action, Stereospecificity of enzymes, Coenzymes and cofactors, Enzyme inhibitors, Introduction to Biocatalysis: Importance in "Green Chemistry" and Chemical Industry.

Unit III

Lipids: Classification. Biological importance of triglycerides and phosphoglycerides and cholesterol; Lipid membrane, Liposomes and their biological functions and underlying applications. Lipoproteins.

Unit IV

Properties, functions and biochemical functions of steroid hormones. Biochemistry of peptide hormones.

Structure of DNA (Watson-Crick model) and RNA, Genetic Code, Biological roles of DNA and RNA: Replication, Transcription and Translation, Introduction to Gene therapy.

Enzymes: Nomenclature, classification, effect of pH, temperature on enzyme activity, enzyme inhibition.

Unit V

Biochemistry of disease: A diagnostic approach by blood/ urine analysis.

Blood: Composition and functions of blood, blood coagulation. Blood collection and preservation of samples. Anaemia, Regulation, estimation and interpretation of data for blood sugar, urea, creatinine, cholesterol and bilirubin.

Urine: Collection and preservation of samples. 6. Formation of urine. Composition and estimation of constituents of normal and pathological urine.

Suggested Readings

Text Books:

[1] Cooper, T.G. (1977). *Tool of Biochemistry.* John Wiley and Sons.
[2] Keith Wilson & John Walker.(1994). *Practical Biochemistry*. Cambridge University Press.
[3] Alan H Gowenlock,(2005). Varley's. *Practical Clinical Biochemistry*.CBS Publisher.
[4] Thomas M. Devlin.(2009). *Textbook of Biochemistry*. Academic Internet Publishers.
[5] Berg, J.M., Tymoczko, J.L. & Stryer, L. (2002). *Biochemistry*. W.H. Freeman.
[6] Vladimir Bartos by Clinical *Biochemistry* First Edition by Charles University Prague.

Reference Books

1. Nelson, D. L. & Cox, M. M.(2008). *Lehninger's Principles of Bioch*emistry. 7th Ed. W. H. Freeman.
2. Harwood. (1990). *Series on Analytical Chemistry*. John Wiley & Sons.

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LECTURE PLAN

DEPARTMENT OF CHEMISTRY

STAFF NAME: Dr.M.R.EZHILARASI

SUBJECT NAME: ANALYTICAL CLINICAL BIOCHEMISTRY
SUB.CODE:17CHU404B
SEMESTER: IV CLASS: II B.Sc (CHEMISTRY)

Total Lecture hours: 30 Hours

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
	UNIT-I		
1	1	***Carbohydrates:*** Biological importance of carbohydrates, Metabolism, Cellular currency of energy (ATP)	R1: 521
2	1	Glycolysis	R1: 522
3	1	Alcoholic and Lactic acid fermentations	R1: 538
4	1	Krebs cycle	T2: 237
5	1	Isolation and characterization of polysachharides.	T1: 300

6	1	Recapitulation and discussion of important questions	
	Total No of Hours Planned For Unit 1=6		
	UNIT-II		
1	1	*Proteins:* Classification, biological importance; Primary and secondary and tertiary structures of proteins: α-helix and β- pleated sheets	T2: 116
2	1	Isolation, characterization, denaturation of proteins	T4: 69,67
3	1	*Enzymes:* Nomenclature, Characteristics (mention of Ribozymes), Classification; Active site	T3: 581, T1: 84,96
4	1	Mechanism of enzyme action, Stereospecificity of enzymes, Coenzymes and cofactors, Enzyme inhibitors,	T1: 102, t2: 201
5	1	Introduction to Biocatalysis: Importance in "Green Chemistry" and Chemical Industry.	W1
6	1	Recapitulation and discussion of important questions	
	Total No of Hours Planned For Unit II=6		
	UNIT-III		
1	1	*Lipids:* Classification	T5: 1062
2	1	Biological importance of triglycerides and phosphoglycerides and cholesterol	T2: 95, r1: 345,349,354

3	1	Lipid membrane, Liposomes and their biological functions and underlying applications	T1: 131, R1:348
4	1	Lipoproteins	T4: 56
5	1	Recapitulation and discussion of important questions	
	Total No of Hours Planned For Unit III=5		
		UNIT-IV	
1	1	Properties, functions and biochemical functions of steroid hormones.	T1: 357, T3: 257,T4: 1079
2	1	Biochemistry of peptide hormones.	T2: 263
3	1	*Structure of DNA* (Watson-Crick model) and RNA, Genetic Code, Biological roles of DNA and RNA: Replication, Transcription and Translation	T1: 173,193,241,195, R1: 1034
4	1	Introduction to Gene therapy.	T2: 261
5	1	*Enzymes*: Nomenclature, classification, effect of pH, temperature on enzyme activity, enzyme inhibition.	T2: 190,192
6	1	Recapitulation and discussion of important questions	
	Total No of Hours Planned For Unit IV=6		
		UNIT-V	
1	1	*Blood:* Composition and functions of blood, blood coagulation. Blood collection and preservation of samples. Anaemia, Regulation	T3: 642, T5: 406

2	1	Estimation and interpretation of data for blood sugar, urea, creatinine, cholesterol and bilirubin.	W2
3	1	*Urine:* Collection and preservation of samples. 6. Formation of urine	T5: 48
4	1	Composition and estimation of constituents of normal and pathological urine.	T5: 57
5	1	Recapitulation and discussion of important questions	
6	1	Discussion of previous ESE question papers	
7	1	Discussion of previous ESE question papers	
	Total No of Hours Planned for unit V=7		
Total Planned Hours	**30**		

Suggested Readings

Text Books:

.T1: David Hames And Nigel Hooper by *Biochemistry* IIIrd Edition, 2005,Ttaylor and Fransis, UK
T2: Keith Wilson & John Walker.(1994). *Principles and Techniques of Biochemistry and Molecular Biology*. 7 th Edition. Cambridge University Press.
T3: Demystified by *Biochemistry*. Mc Graw Hill Publications
T4: Berg, J.M., Tymoczko, J.L. & Stryer, L. (2002). *Biochemistry*. W.H. Freeman.
T5: Vladimir Bartos by Clinical *Biochemistry* First Edition by Charles University Prague.

Reference Books

R1: Nelson, D. L. & Cox, M. M.(2008). *Lehninger's Principles of Bioch*emistry. 7th Ed. W. H. Freeman.

Website:

W1: http//www.Introduction to Biocatalysis: Importance in “Green Chemistry” and Chemical Industry.
W2: http//www. Estimation and interpretation of data for blood sugar, urea, creatinine, cholesterol and bilirubin

LECTURE NOTES

UNIT-I

SYLLABUS

Carbohydrates: Biological importance of carbohydrates, Metabolism, Cellular currency of energy (ATP), Glycolysis, Alcoholic and Lactic acid fermentations, Krebs cycle. Isolation and characterization of polysachharides.

Introduction:

Glucose occupies a central position in the metabolism of plants, animals, and many microorganisms. It is the complete oxidation of glucose to carbon dioxide and water proceeds with a standard free-energy change of _2,840 kJ/mol. By storing glucose as a high molecular weight polymer such as starch or glycogen, a cell can stockpile large quantities of hexose units while maintaining a relatively low cytosolic osmolarity. When energy demands increase, glucose can be released from these intracellular storage polymers and used to produce ATP either aerobically or anaerobically.

Glucose is not only an excellent fuel, it is also a remarkably versatile precursor, capable of supplying a huge array of metabolic intermediates for biosynthetic reactions. A bacterium such as *Escherichia coli* can obtain from glucose the carbon skeletons for every amino acid, nucleotide, coenzyme, fatty acid, or other metabolic intermediate it needs for growth. A comprehensive study of the metabolic fates of glucose would encompasshundreds or thousands of transformations. In animals and vascular plants, glucose has three major fates: it may be stored (as a polysaccharide or as sucrose); oxidized to a three-carbon compound (pyruvate) via glycolysis to provide ATP and metabolic intermediates; or oxidized via the pentose phosphate (phosphogluconate) pathway to yield ribose 5-phosphate for nucleic acid synthesis and NADPH for reductive biosynthetic processes (Fig. 14–1).

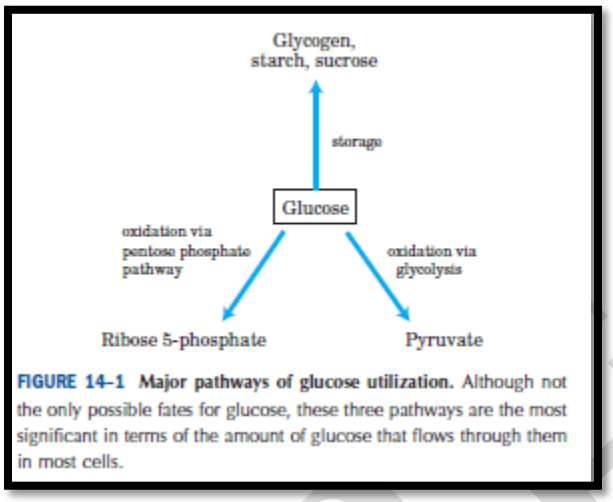


FIGURE 14–1 Major pathways of glucose utilization. Although not the only possible fates for glucose, these three pathways are the most significant in terms of the amount of glucose that flows through them in most cells.

Organisms that do not have access to glucose from other sources must make it. Photosynthetic organisms make glucose by first reducing atmospheric CO2 to trioses, then converting the trioses to glucose. Nonphotosyntheticcells make glucose from simpler three and four-carbon precursors by the process of gluconeogenesis, effectively reversing glycolysis in a pathway that uses many of the glycolytic enzymes. In this chapter we describe the individual reactions of glycolysis, gluconeogenesis, and the pentose phosphate pathway and the functional significance of each pathway. We also describe the various fates of the pyruvate produced by glycolysis; they include the fermentations that are used by many organisms in anaerobic niches to produce ATP and that are exploited industrially as sources of ethanol, lactic acid, and other.

The problem of alcoholic fermentation, of the origin and nature of that mysterious and apparently spontaneous change, which converted the insipid juice of the grape into stimulating wine, seems to have exerted a fascination over the minds of natural philosophers from the very earliest times.

Glycolysis

In **glycolysis** (from the Greek *glykys,* meaning "sweet," and *lysis,* meaning "splitting"), a molecule of glucose is degraded in a series of enzyme-catalyzed reactions to yield two molecules of the three-carbon compound pyruvate. During the sequential reactions of glycolysis, some of

the free energy released from glucose is conserved in the form of ATP and NADH. Glycolysis was the first metabolic pathway to be elucidated and is probably the best understood. From Eduard Buchner's discovery in 1897 of fermentation in broken extracts of yeast cells until the elucidation of the whole pathway in yeast (by Otto Warburg and Hans von Euler-Chelpin) and in muscle (by Gustav Embden and Otto Meyerhof) in the 1930s, the reactions of glycolysis in extracts of yeast and muscle were a major focus of biochemical research. The philosophical shift that accompanied these discoveries was announced by Jacques Loeb in 1906: Through the discovery of Buchner, Biology was relieved of another fragment of mysticism. The splitting up of sugar into CO2 and alcohol is no more the effect of a "vital principle" than the splitting up of cane sugar by invertase. The history of this problem is instructive, as it warns us against considering problems as beyond our reach because they have not yet found their solution.

The development of methods of enzyme purification, the discovery and recognition of the importance of coenzymes such as NAD, and the discovery of the pivotal metabolic role of ATP and other phosphorylated compounds all came out of studies of glycolysis. The glycolytic enzymes of many species have long since been purified and thoroughly studied.

Glycolysis is an almost universal central pathway of glucose catabolism, the pathway with the largest flux of carbon in most cells. The glycolytic breakdown of glucose is the sole source of metabolic energy in some mammalian tissues and cell types (erythrocytes, renal medulla, brain, and sperm, for example). Some plant tissues that are modified to store starch (such as potato tubers) and some aquatic plants (watercress, for example) derive most of their energy from glycolysis; many anaerobic microorganisms are entirely dependent on glycolysis.

Fermentation is a general term for the *anaerobic* degradation of glucose or other organic nutrients to obtain energy, conserved as ATP. Because living organisms first arose in an atmosphere without oxygen, anaerobic breakdown of glucose is probably the most ancient biological mechanism for obtaining energy from organic fuel molecules. In the course of evolution, the chemistry of this reaction sequence has been completely conserved; the glycolytic enzymes of vertebrates are closely similar, in amino acid sequence and three-dimensional

structure, to their homologs in yeast and spinach. Glycolysis differs among species only in the details of its regulation and in the subsequent metabolic fate of the pyruvate formed. The thermodynamic principles and the types of regulatory mechanisms that govern glycolysis are common to all pathways of cell metabolism.

A study of glycolysis can therefore serve as a model for many aspects of the pathways discussed throughout this book. Before examining each step of the pathway in some detail, we take a look at glycolysis as a whole.

An Overview: Glycolysis Has Two Phases

The breakdown of the six-carbon glucose into two molecules of the three-carbon pyruvate occurs in ten steps, the first five of which constitute the *preparatory phase* (Fig. 14–2a).

In these reactions, glucose is first phosphorylated at the hydroxyl group on C-6 (step 1). The D-glucose 6-phosphate thus formed is converted to Dfructose 6-phosphate (step 2), which is again phosphorylated, this time at C-1, to yield D-fructose 1,6-bisphosphate (step 3). For both phosphorylations, ATP is the phosphoryl group donor. As all sugar derivatives in glycolysis are the D isomers, we will usually omit the D designation except when emphasizing stereochemistry. Fructose 1,6-bisphosphate is split to yield two three-carbon molecules, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (step 4); this is the "lysis" step that gives the pathway its name. The dihydroxyacetone phosphate is isomerized to a second molecule of glyceraldehyde 3-phosphate (step 5), ending the first phase of glycolysis. From a chemical perspective, the isomerization in step 2 is critical for setting up the phosphorylation and COC bond cleavage reactions in steps 3 and 4 , as detailed later. Note that two molecules of ATP are invested before the cleavage of glucose into two three-carbon pieces; later there will be a good return on this investment. To summarize: in the preparatory phase of glycolysis the energy of ATP is invested, raising the free-energy content of the intermediates, and the carbon chains of all the metabolized hexoses are converted into a common product, glyceraldehyde 3-phosphate. The energy gain comes in the *payoff phase* of glycolysis (Fig. 14–2b).

Each molecule of glyceraldehydes 3-phosphate is oxidized and phosphorylated by inorganic phosphate (*not* by ATP) to form 1,3-bisphosphoglycerate (step 6). Energy is then released as the two molecules of 1,3-bisphosphoglycerate are converted to two molecules of pyruvate (steps 7 through 10). Much of this energy is conserved by the coupled phosphorylation of four molecules of ADP to ATP. The net yield is two molecules of ATP 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.

Fates of Pyruvate With the exception of some interesting variations in the bacterial realm, the pyruvate formed by glycolysis is further metabolized via one of three catabolic routes. In aerobic organisms or tissues, under aerobic conditions, glycolysis is only the first stage in the complete degradation of glucose (Fig. 14–3). Pyruvate is oxidized, with loss of its carboxyl group as CO_2, to yield the acetyl group of acetyl-coenzyme A; the acetyl group is then oxidized completely to CO_2 by the citric acid cycle (Chapter 16). The electrons from these oxidations are passed to O_2 through a chain of carriers in the mitochondrion, to form H_2O. The energy from the electron-transfer reactions drives the synthesis of ATP in the mitochondrion (Chapter 19).

The second route for pyruvate is its reduction to lactate via **lactic acid fermentation.** When vigorously contracting skeletal muscle must function under lowoxygen conditions **(hypoxia),** NADH cannot be reoxidized to NAD+, but NAD+ is required as an electron acceptor for the further oxidation of pyruvate. Under these conditions pyruvate is reduced to lactate, accepting electrons from NADH and thereby regenerating the NAD^+ necessary for glycolysis to continue. Certain tissues and cell types (retina and erythrocytes, for example) convert glucose to lactate

even under aerobic conditions, and lactate is also the product of glycolysis under anaerobic conditions in some microorganisms (Fig. 14–3).

The third major route of pyruvate catabolism leads to ethanol. In some plant tissues and in certain invertebrates, protists, and microorganisms such as brewer's yeast, pyruvate is converted under hypoxic or anaerobic conditions into ethanol and CO2, a process called **ethanol (alcohol) fermentation** (Fig. 14–3). The oxidation of pyruvate is an important catabolic process, but pyruvate has anabolic fates as well. It can, for example, provide the carbon skeleton for the synthesis of the amino acid alanine. We return to these anabolic reactions of pyruvate in later chapters.

ATP Formation Coupled to Glycolysis During glycolysis some of the energy of the glucose molecule is conserved in ATP, while much remains in the product, pyruvate. The overall equation for glycolysis is

$$\text{Glucose} + 2\text{NAD}^+ + 2\text{ADP} + 2\text{P}_i \longrightarrow 2\ \text{pyruvate} + 2\text{NADH} + 2\text{H}^+ + 2\text{ATP} + 2\text{H}_2\text{O} \quad (14\text{–}1)$$

For each molecule of glucose degraded to pyruvate, two molecules of ATP are generated from ADP and Pi. We can now resolve the equation of glycolysis into two processes—the conversion of glucose to pyruvate, which is exergonic:

$$\text{Glucose} + 2\text{NAD}^+ \longrightarrow 2\ \text{pyruvate} + 2\text{NADH} + 2\text{H}^+ \quad (14\text{–}2)$$
$$\Delta G_1'^{\circ} = -146\ \text{kJ/mol}$$

and the formation of ATP from ADP and Pi, which is endergonic:

$$2\text{ADP} + 2\text{P}_i \longrightarrow 2\text{ATP} + 2\text{H}_2\text{O} \quad (14\text{–}3)$$
$$\Delta G_2'^{\circ} = 2(30.5\ \text{kJ/mol}) = 61.0\ \text{kJ/mol}$$

The sum of Equations 14–2 and 14–3 gives the overall

standard free-energy change of glycolysis, $\Delta G_s'^{\circ}$:

$$\Delta G_s'^{\circ} = \Delta G_1'^{\circ} + \Delta G_2'^{\circ} = -146 \text{ kJ/mol} + 61.0 \text{ kJ/mol} = -85 \text{ kJ/mol}$$

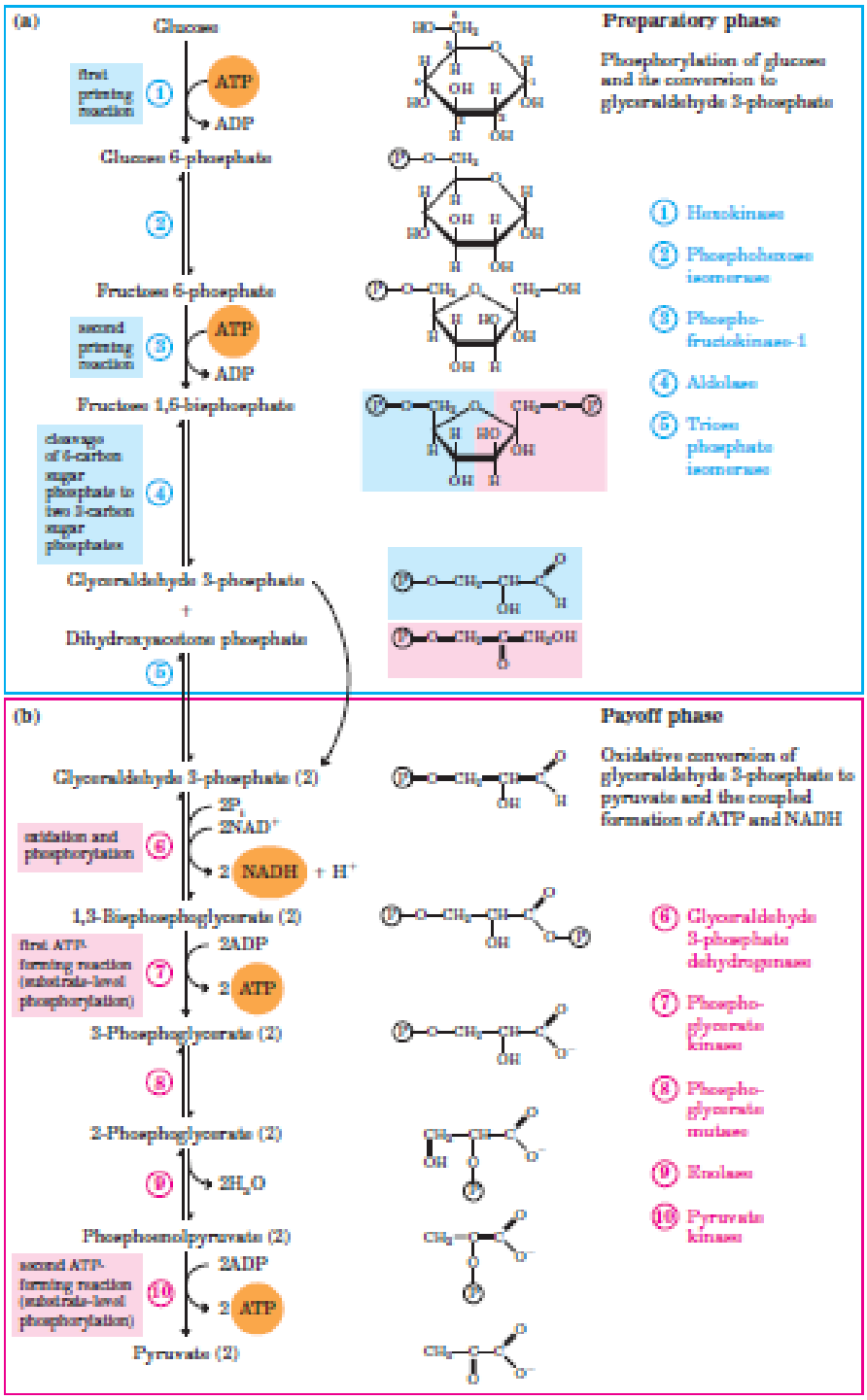


FIGURE 14–2 The two phases of glycolysis. For each molecule of glucose that passes through the preparatory phase (a), two molecules of glyceraldehyde 3-phosphate are formed; both pass through the payoff phase (b). Pyruvate is the end product of the second phase of glycolysis. For each glucose molecule, two ATP are consumed in the preparatory phase and four ATP are produced in the payoff phase, giving a net yield of two ATP per molecule of glucose converted to pyruvate. The numbered reaction steps are catalyzed by the enzymes listed on the right, and also correspond to the numbered headings in the text discussion. Keep in mind that each phosphoryl group, represented here as Ⓟ, has two negative charges ($-PO_3^{2-}$).

Under standard conditions and in the cell, glycolysis is an essentially irreversible process, driven to completion by a large net decrease in free energy. At the actual intracellular concentrations of ATP, ADP, and Pi (see Box 13–1) and of glucose and pyruvate, the energy released in glycolysis (with pyruvate as the end product) is recovered as ATP with an efficiency of more than 60%.

Energy Remaining in Pyruvate Glycolysis releases only a small fraction of the total available energy of the glucose molecule; the two molecules of pyruvate formed by glycolysis still contain most of the chemical potential energy of glucose, energy that can be extracted by oxidative reactions in the citric acid cycle (Chapter 16) and oxidative phosphorylation (Chapter 19).

Importance of Phosphorylated Intermediates

Each of the nine glycolytic intermediates between glucose and pyruvate is phosphorylated (Fig. 14–2). The phosphoryl groups appear to have three functions.

1. Because the plasma membrane generally lacks transporters for phosphorylated sugars, the phosphorylated glycolytic intermediates cannot leave the cell. After the initial phosphorylation, no further energy is necessary to retain phosphorylated intermediates in the cell, despite the large difference in their intracellular and extracellular concentrations.

2. Phosphoryl groups are essential components in the enzymatic conservation of metabolic energy. Energy released in the breakage of phosphoanhydride bonds (such as those in ATP) is partially conserved in the formation of phosphate esters such as glucose 6-phosphate. High-energy phosphate compounds formed in glycolysis (1,3-bisphosphoglycerate and phosphoenolpyruvate) donate phosphoryl groups to ADP to form ATP.

3. Binding energy resulting from the binding of phosphate groups to the active sites of enzymes lowers the activation energy and increases the specificity of the enzymatic reactions (Chapter 6). The phosphate groups of ADP, ATP, and the glycolytic intermediates form complexes with

Mg2+, and the substrate binding sites of many glycolytic enzymes are specific for these Mg2+ complexes. Most glycolytic enzymes require $Mg2^+$ for activity.

The Preparatory Phase of Glycolysis Requires ATP

In the preparatory phase of glycolysis, two molecules of ATP are invested and the hexose chain is cleaved into two triose phosphates. The realization that *phosphorylated* hexoses were intermediates in glycolysis came slowly and serendipitously. In 1906, Arthur Harden and William Young tested their hypothesis that inhibitors of proteolytic enzymes would stabilize the glucose fermenting enzymes in yeast extract. They added blood serum (known to contain inhibitors of proteolytic enzymes) to yeast extracts and observed the predicted stimulation of glucose metabolism. However, in a control experiment intended to show that boiling the serum destroyed the stimulatory activity, they discovered that boiled serum was just as effective at stimulating glycolysis.

Careful examination and testing of the contents of the boiled serum revealed that inorganic phosphate was responsible for the stimulation. Harden and Young soon discovered that glucose added to their yeast extract was converted to a hexose bisphosphate (the "Harden- Young ester," eventually identified as fructose 1,6- bisphosphate). This was the beginning of a long series of investigations on the role of organic esters of phosphate in biochemistry, which has led to our current understanding of the central role of phosphoryl group transfer in biology.

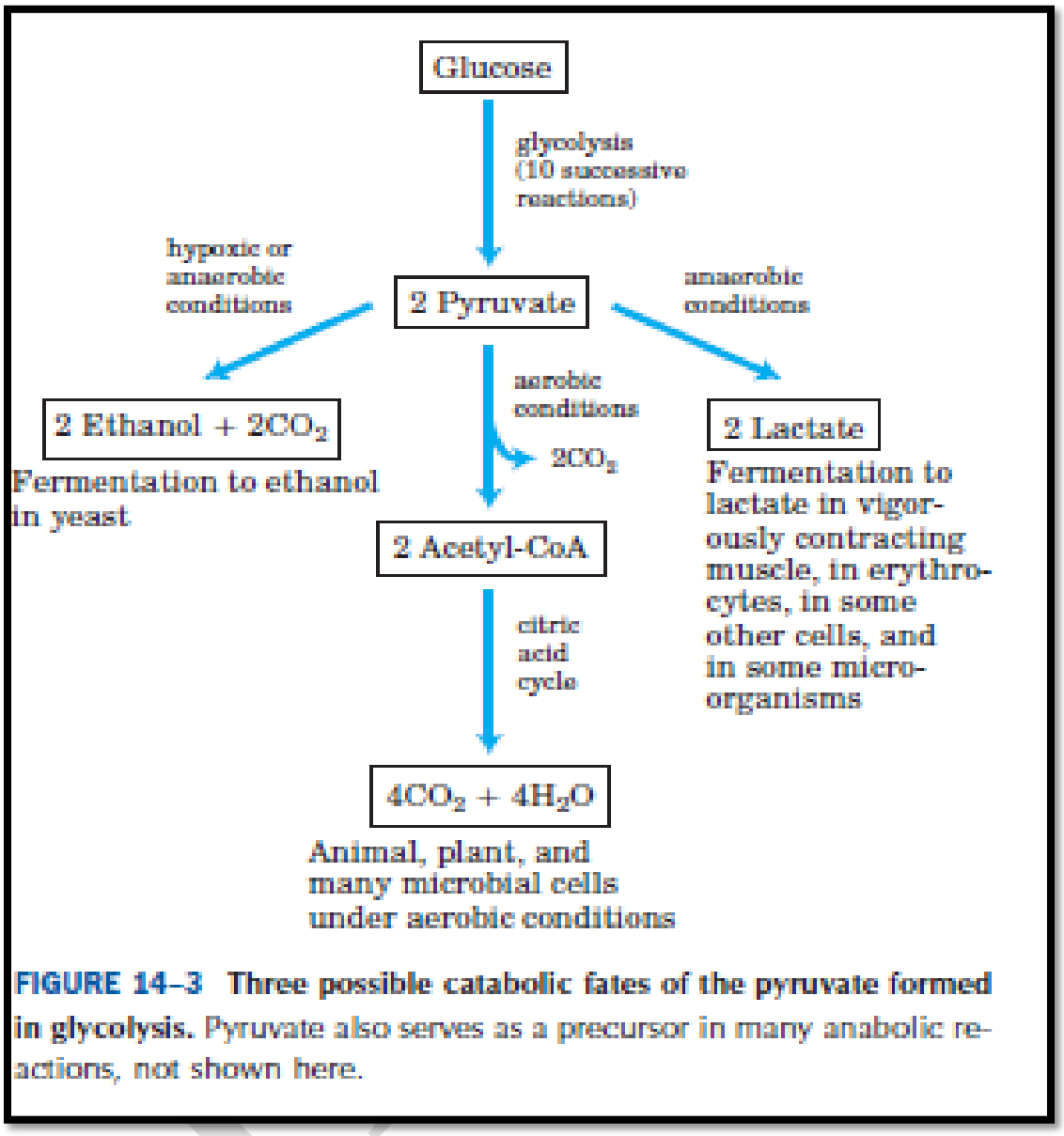
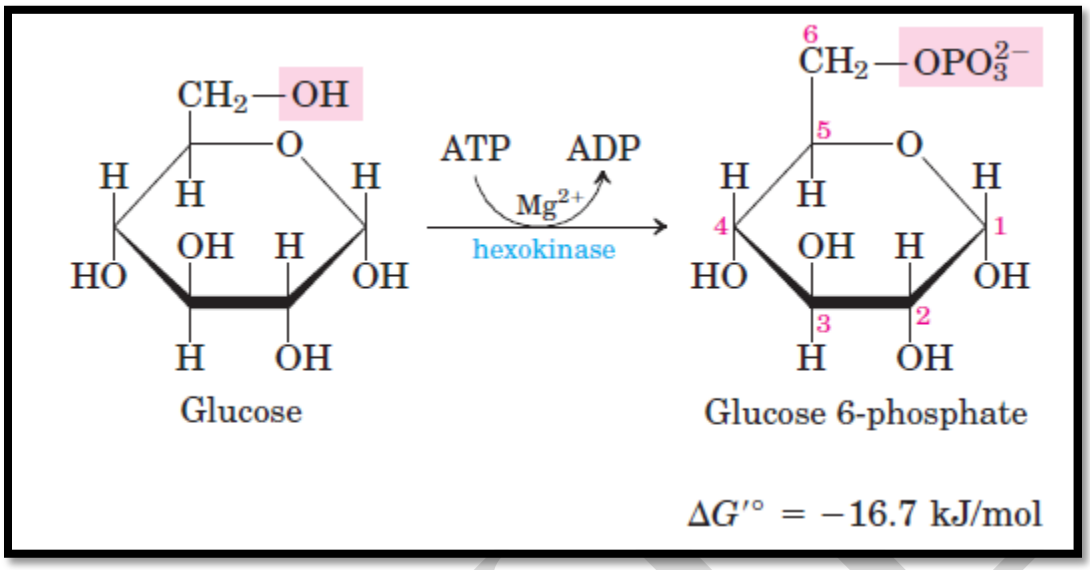


FIGURE 14–3 Three possible catabolic fates of the pyruvate formed in glycolysis. Pyruvate also serves as a precursor in many anabolic reactions, not shown here.

1. Phosphorylation of Glucose

In the first step of glycolysis, glucose is activated for subsequent reactions by its phosphorylation at C-6 to yield **glucose 6-phosphate,** with ATP as the phosphoryl donor:



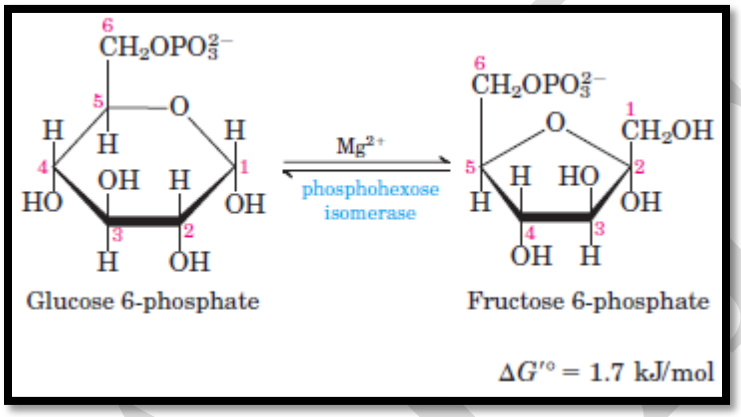
This reaction, which is irreversible under intracellular conditions, is catalyzed by **hexokinase.** Recall that kinases are enzymes that catalyze the transfer of the terminal phosphoryl group from ATP to an acceptor nucleophile (see Fig. 13–10). Kinases are a subclass of transferases (see Table 6–3). The acceptor in the case of hexokinase is a hexose, normally D-glucose, although hexokinase also catalyzes the phosphorylation of other common hexoses, such as D-fructose and D-mannose.

Hexokinase, like many other kinases, requires Mg^{2+} for its activity, because the true substrate of the enzyme is not ATP^{4+} but the $MgATP^{2+}$ complex (see Fig. 13–2).Mg^{2+} shields the negative charges of the phosphoryl groups in ATP, making the terminal phosphorus atom an easier target for nucleophilic attack by an OOH of glucose. Hexokinase undergoes a profound change in shape, an induced fit, when it binds glucose; two domains of the protein move about 8 Å closer to each other when ATP binds (see Fig. 6–22). This movement brings bound ATP closer to a molecule of glucose also bound to the enzyme and blocks the access of water (from the solvent), which might otherwise enter the active site and attack (hydrolyze) the phosphoanhydride bonds of ATP. Like the other nine enzymes of glycolysis, hexokinaseis a soluble, cytosolic protein.Hexokinase is present in all cells of all organisms. Hepatocytes also contain a form of hexokinase called hexokinase IV or glucokinase, which differs from other forms of hexokinase in kinetic and regulatory properties (see Box 15–2).

Two enzymes that catalyze the same reaction but are encoded in different genes are called **isozymes.**

2. Conversion of Glucose 6-Phosphate to Fructose 6-Phosphate

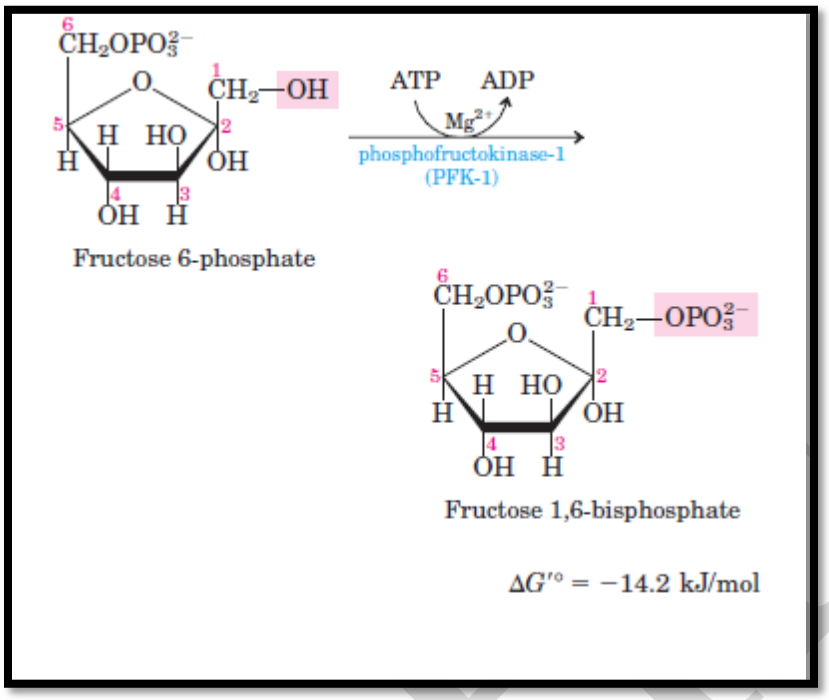
The enzyme **phosphohexose isomerase (phosphoglucose isomerase)** catalyzes the reversible isomerization of glucose 6-phosphate, an aldose, to **fructose 6-phosphate,** a ketose:



The mechanism for this reaction is shown in Figure 14–4. The reaction proceeds readily in either direction, as might be expected from the relatively small change in standard free energy. This isomerization has a critical role in the overall chemistry of the glycolytic pathway, as the rearrangement of the carbonyl and hydroxyl groups at C-1 and C-2 is a necessary prelude to the next two steps. The phosphorylation that occurs in the next reaction (step 3) requires that the group at C-1 first be converted from a carbonyl to an alcohol, and in the subsequent reaction (step 4) cleavage of the bond between C-3 and C-4 requires a carbonyl group at C-2 (p. 485).

3 .Phosphorylation of Fructose 6-Phosphate to Fructose 1,6- Bisphosphate

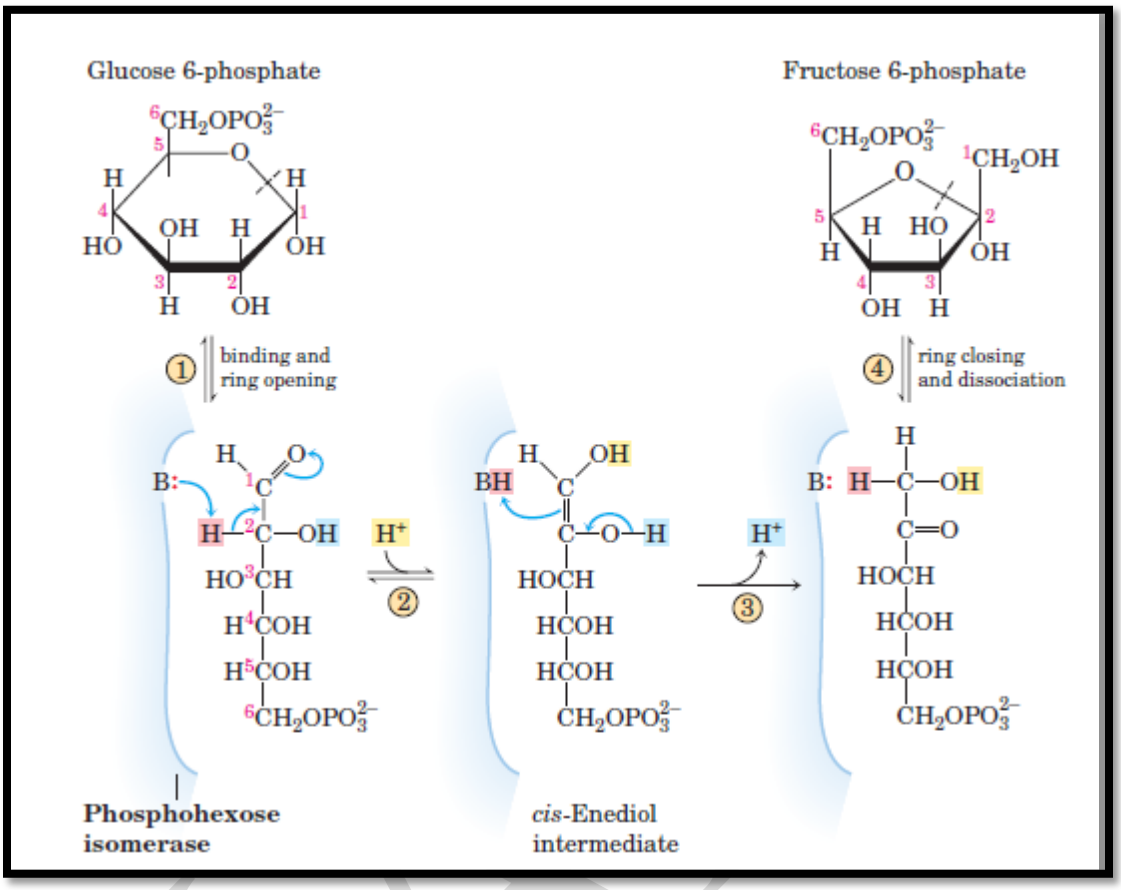
In the second of the two priming reactions of glycolysis, **phosphofructokinase-1 (PFK-1)** catalyzes the transfer of a phosphoryl group from ATP to fructose 6-phosphate to yield **fructose 1,6-bisphosphate**



Fructose 6-phosphate

Fructose 1,6-bisphosphate

$\Delta G'^{o} = -14.2$ kJ/mol



MECHANISM FIGURE 14–4

The phosphohexose isomerase reaction. *The ring opening and closing reactions (steps 1 and 4) are catalyzed by an active-site His residue, by mechanisms omitted here for simplicity. The movement of the proton between C-2 and C-1 (steps 2 and 3) is base-catalyzed by an active-site Glu residue (shown as B:). The proton (pink) initially at C-2 is made more easily abstract able byelectron withdrawal by the adjacent carbonyl and the nearby hydroxyl group. After its transfer from C-2 to the active-site Glu residue, the proton is freely exchanged with the surrounding solution; that is, the proton from C-2 in step 2 is not necessarily the same one that is added to C1 in step 3 . (The additional exchange of protons (yellow and blue) between the hydroxyl groups and solvent is shown for completeness. The hydroxyl groups are weak acids and can exchange protons with the surrounding water whether the isomerization reaction is underway or not.)*

Phosphohexose Isomerase Mechanism

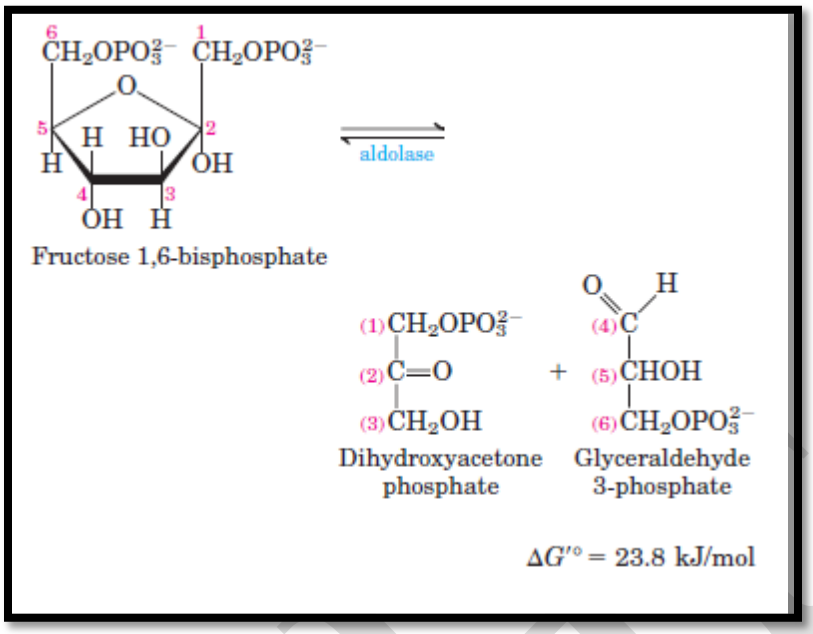
This enzyme is called PFK-1 to distinguish it from a second enzyme (PFK-2) that catalyzes the formation of fructose 2,6-bisphosphate from fructose 6-phosphate in a separate pathway. The PFK-1 reaction is essentially irreversible under cellular conditions, and it is the first "committed" step in the glycolytic pathway; glucose 6-phosphate and fructose 6-phosphate have other possible fates, but fructose 1,6-bisphosphate is targeted for glycolysis. Some bacteria and protists and perhaps all plants have a phosphofructokinase that uses pyrophosphate (PPi), not ATP, as the phosphoryl group donor in the synthesis of fructose 1,6-bisphosphate:

$$\text{Fructose 6-phosphate} + PP_i \xrightarrow{Mg^{2+}} \text{fructose 1,6-bisphosphate} + P_i \qquad \Delta G'^{\circ} = -14 \text{ kJ/mol}$$

Phosphofructokinase-1 is a regulatory enzyme (Chapter 6), one of the most complex known. It is the major point of regulation in glycolysis. The activity of PFK-1 is increased whenever the cell's ATP supply is depleted or when the ATP breakdown products, ADP and AMP (particularly the latter), are in excess. The enzymeis inhibited whenever the cell has ample ATP and is well supplied by other fuels such as fatty acids. In some organisms, fructose 2,6-bisphosphate (not to be confused with the PFK-1 reaction product, fructose 1,6- bisphosphate) is a potent allosteric activator of PFK-1. The regulation of this step in glycolysis is discussed in greater detail in Chapter 15.

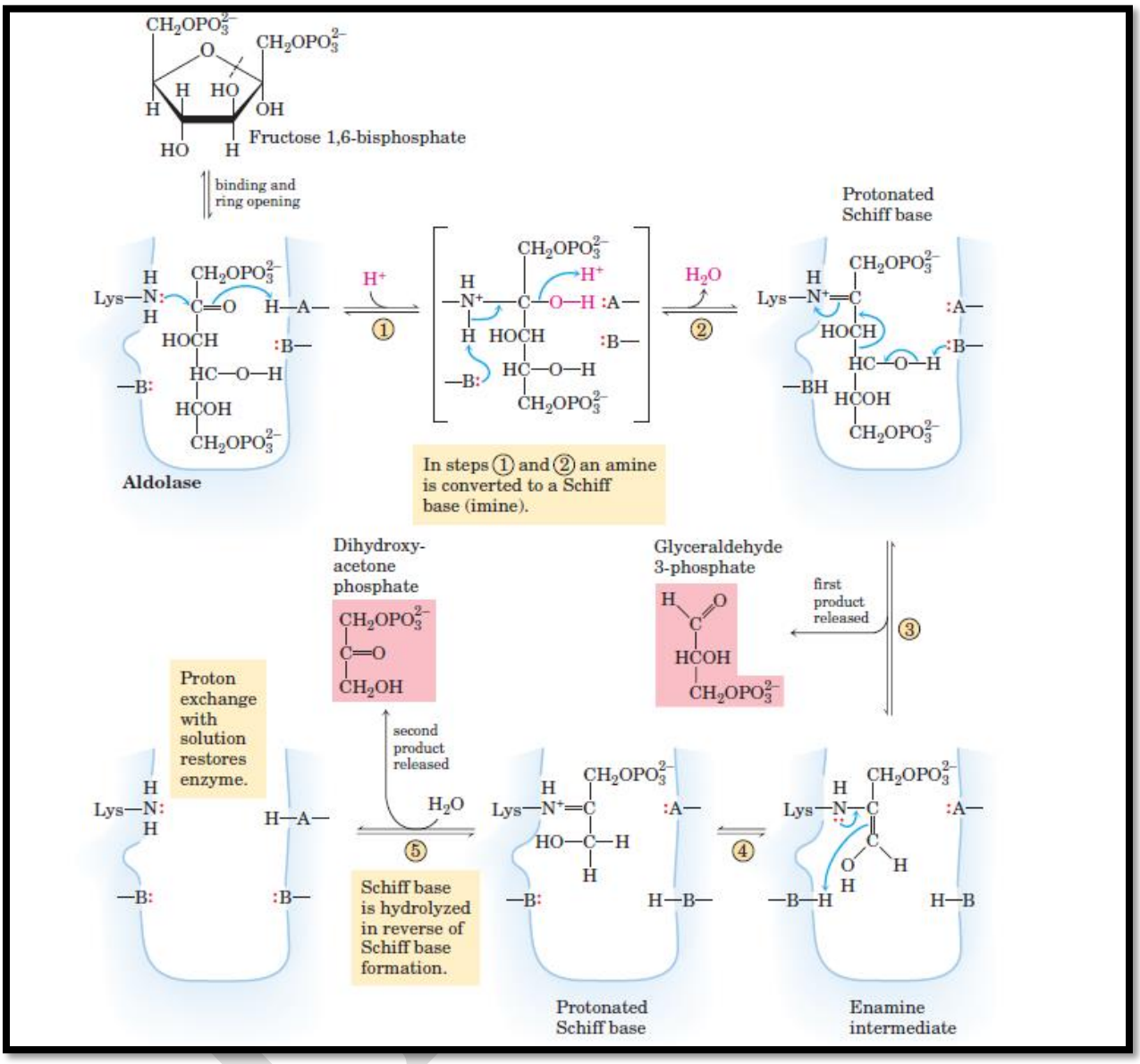
4. Cleavage of Fructose 1,6-Bisphosphate

The enzyme **fructose 1,6-bisphosphate aldolase,** often called simply **aldolase,** catalyzes a reversible aldol condensation (p. 485). Fructose 1,6-bisphosphate is cleaved to yield two different triose phosphates, **glyceraldehydes 3-phosphate,** an aldose, and **dihydroxyacetone phosphate,** a ketose:



There are two classes of aldolases. Class I aldolases, found in animals and plants, use the mechanism shown in Figure 14–5. Class II enzymes, in fungi and bacteria, do not form the Schiff base intermediate. Instead, a zinc ion at the active site is coordinated with the carbonyl oxygen at C-2; the Zn^{2+} polarizes the carbonyl group and stabilizes the enolate intermediate created in the COC bond cleavage step. Although the aldolase reaction has a strongly positive standard free-energy change in the direction of fructose 1,6-bisphosphate cleavage, at the lower concentrations of reactants present in cells, the actual free-energy change is small and the aldolase reaction is readily reversible. We shall see later that aldolase acts in the reverse direction during the process of gluconeogenesis (see Fig. 14–16).

5. Interconversion of the Triose Phosphates Only one of the two triose phosphates formed by aldolase, glyceraldehydes 3-phosphate, can be directly degraded in the subsequent steps of glycolysis. The other product, dihydroxyacetone phosphate, is rapidly and reversibly converted to glyceraldehyde 3-phosphate by the fifth enzyme of the sequence, **triose phosphate isomerase:**



MECHANISM FIGURE 14–5 The class I aldolase reaction. *The reaction shown here is the reverse of an aldol condensation. Note that cleavage between C-3 and C-4 depends on the presence of the carbonyl group at C-2. 1 and 2 The carbonyl reacts with an active-site Lys residue to form an imine, which stabilizes the carbanion generated by the bond cleavage—an imine delocalizes electrons even better than does a carbonyl. 3 Bond cleavage releasesglyceraldeyde 3-phosphate as the first product. 4 The resulting enamine covalently linked to the enzyme is isomerized to a protonated Schiff base, and 5 hydrolysis of the Schiff base*

generates dihydroxyacetone phosphate as the second product. A and B represent amino acid residues that serve as general acid (A) or base (B).

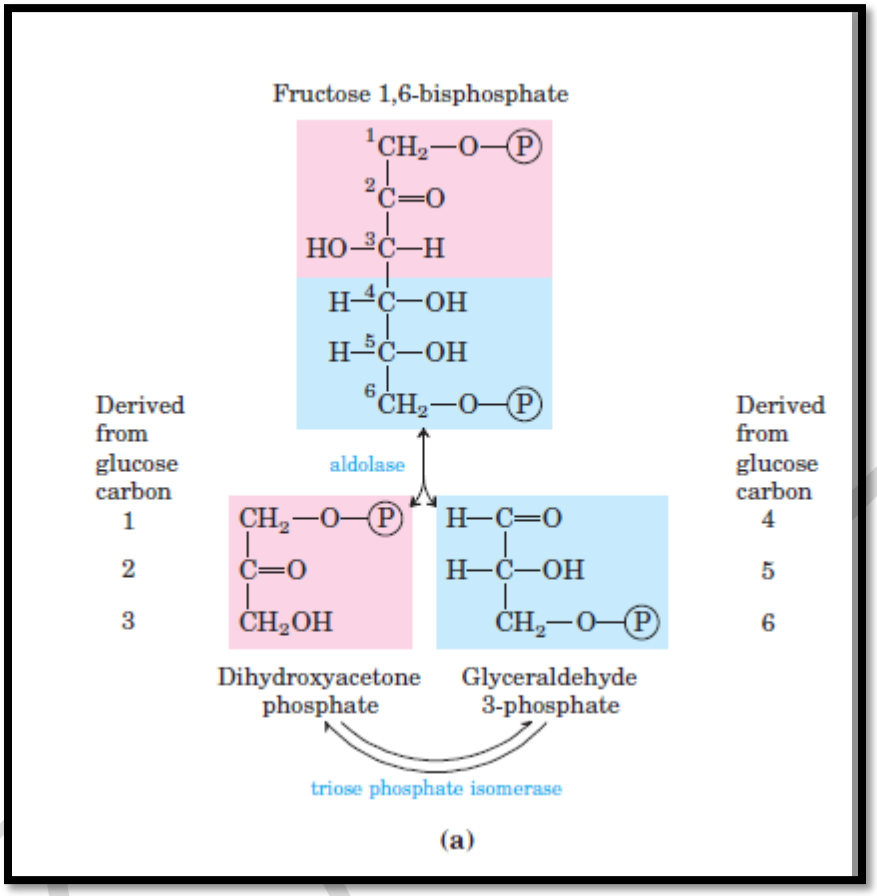
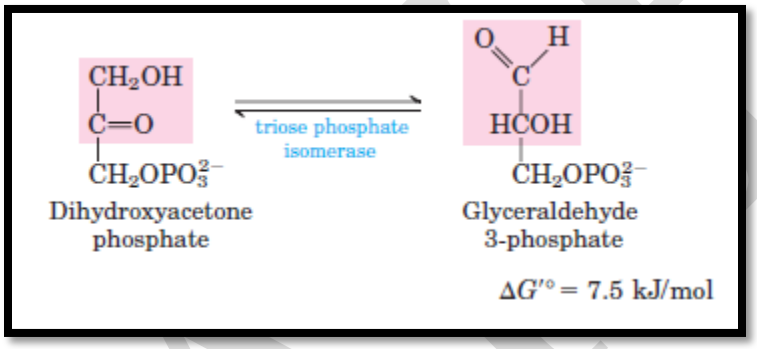


FIGURE 14–6 Fate of the glucose carbons in the formation of glyceraldehydes 3-phosphate. ***(a)*** *The origin of the carbons in the two threecarbon products of the aldolase and triose*

phosphate isomerase reactions. The end product of the two reactions is glyceraldehydes 3-phosphate (two molecules). ***(b)*** *Each carbon of glyceraldehydes 3-phosphate is derived from either of two specific carbons of glucose. Note that the numbering of the carbon atoms of glyceraldehydes 3-phosphate differs from that of the glucose from which it is derived. In glyceraldehyde 3-phosphate, the most complex functional group (the carbonyl) is specified as C 1. This numbering change is important for interpretingexperiments with glucose in which a single carbon is labeledwith a radioisotope. (See Problems 3 and 5 at the end of this chapter.)*



The reaction mechanism is similar to the reaction promoted by phosphohexose isomerase in step 2 of glycolysis (Fig. 14–4). After the triose phosphate isomerase reaction, C-1, C-2, and C-3 of the starting glucose are chemically indistinguishable from C-6, C-5, and C-4, respectively (Fig. 14–6), setting up the efficient metabolism of the entire six-carbon glucose molecule. This reaction completes the preparatory phase of glycolysis. The hexose molecule has been phosphorylated at C-1 and C-6 and then cleaved to form two molecules of glyceraldehyde 3-phosphate.

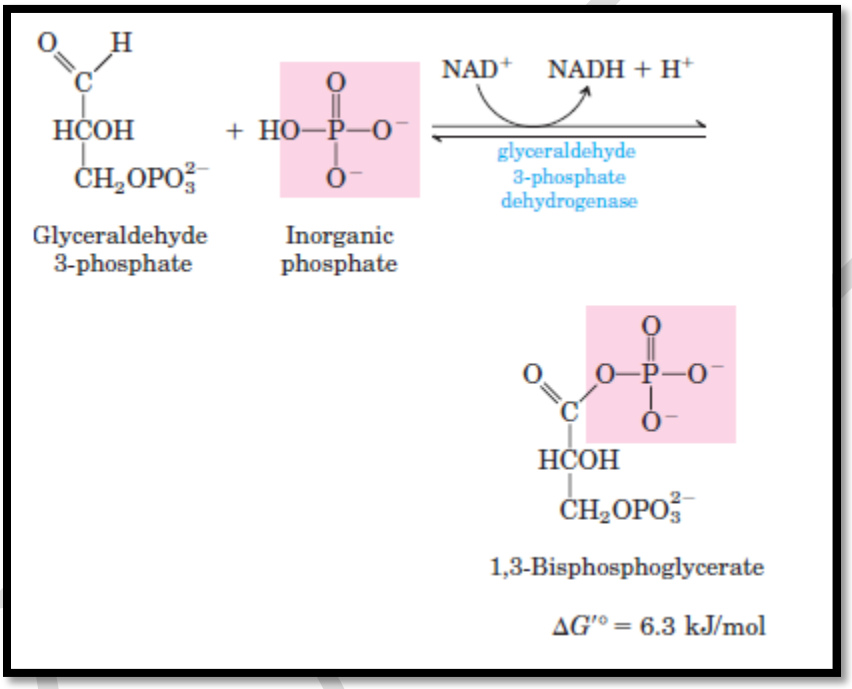
The Payoff Phase of Glycolysis Yields ATP and NADH

The payoff phase of glycolysis (Fig. 14–2b) includes the energy-conserving phosphorylation steps in which some of the free energy of the glucose molecule is conserved in the form of ATP. Remember that one molecule of glucose yields two molecules of glyceraldehyde 3-phosphate; both halves of the glucose molecule follow the same pathway in the second phase of glycolysis. The conversion of two molecules of glyceraldehyde 3-phosphate to two molecules of pyruvate is

accompanied by the formation of four molecules of ATP from ADP. However, the net yield of ATP per molecule of glucose degraded is only two, because two ATP were invested in the preparatory phase of glycolysis to phosphorylate the two ends of the hexose molecule.

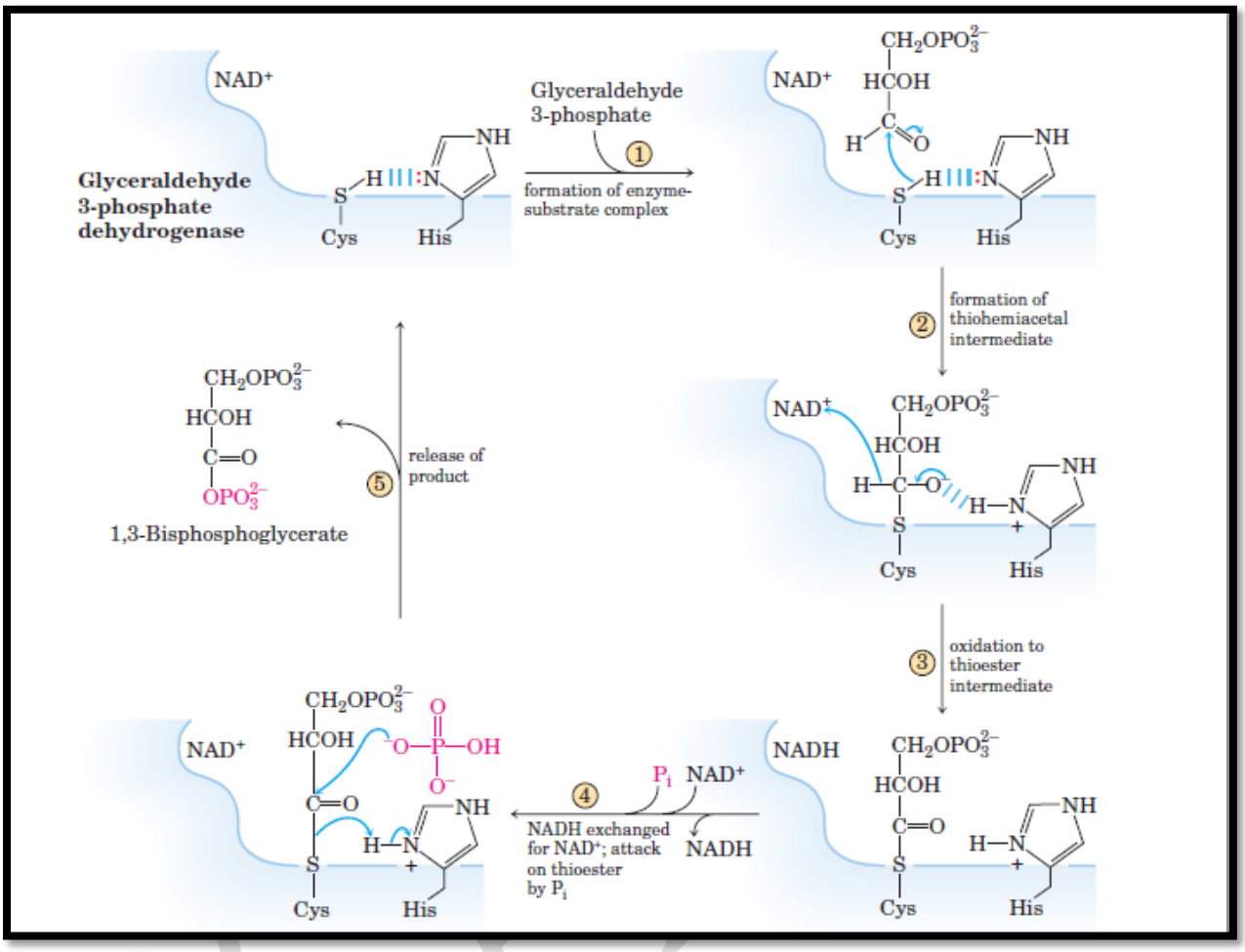
6. Oxidation of Glyceraldehyde 3-Phosphate to 1,3-Bisphosphoglycerate

The first step in the payoff phase is the oxidation of glyceraldehyde 3-phosphate to **1,3-bisphosphoglycerate,** catalyzed by **glyceraldehyde 3- phosphate dehydrogenase:**



This is the first of the two energy-conserving reactions of glycolysis that eventually lead to the formation of ATP. The aldehyde group of glyceraldehyde 3-phosphate is oxidized, not to a free carboxyl group but to a carboxylic acid anhydride with phosphoric acid. This type of anhydride, called an **acyl phosphate,** has a very high standard free energy of hydrolysis ($\Delta G'^{\circ}$= -49.3 kJ/mol; see Fig. 13–4, Table 13–6). Much of the free energy of oxidation of the aldehyde group of glyceraldehyde 3- phosphate is conserved by formation of the acyl phosphate group at C-1 of 1,3-bisphosphoglycerate. The acceptor of hydrogen in the glyceraldehyde 3- phosphate dehydrogenase reaction is NAD^+ (see Fig. 13–15), bound to a Rossmann fold as shown in Figure

13–16. The reduction of NAD^+ proceeds by the enzymatic transfer of a hydride ion ($:H^-$) from the aldehyde group of glyceraldehyde 3-phosphate to the nicotinamide ring of NAD+, yielding the reduced coenzyme NADH. The other hydrogen atom of the substrate molecule is released to the solution as H+. Glyceraldehyde 3-phosphate is covalently bound to the dehydrogenase during the reaction (Fig. 14–7). The aldehyde group of glyceraldehyde 3-phosphate reacts with the OSH group of an essential Cys residue in the active site, in a reaction analogous to the formation of a hemiacetal (see Fig. 7–5), in this case producing a *thio*hemiacetal. Reaction of the essential Cys residue with a heavy metal such as Hg^{2+} irreversibly inhibits the enzyme. Because cells maintain only limited amounts of NAD^+, glycolysis would soon come to a halt if the NADH formed in this step of glycolysis were not continuously reoxidized. The reactions in which NAD^+ is regenerated anaerobically are described in detail in Section 14.3, in our discussion of the alternative fates of pyruvate.

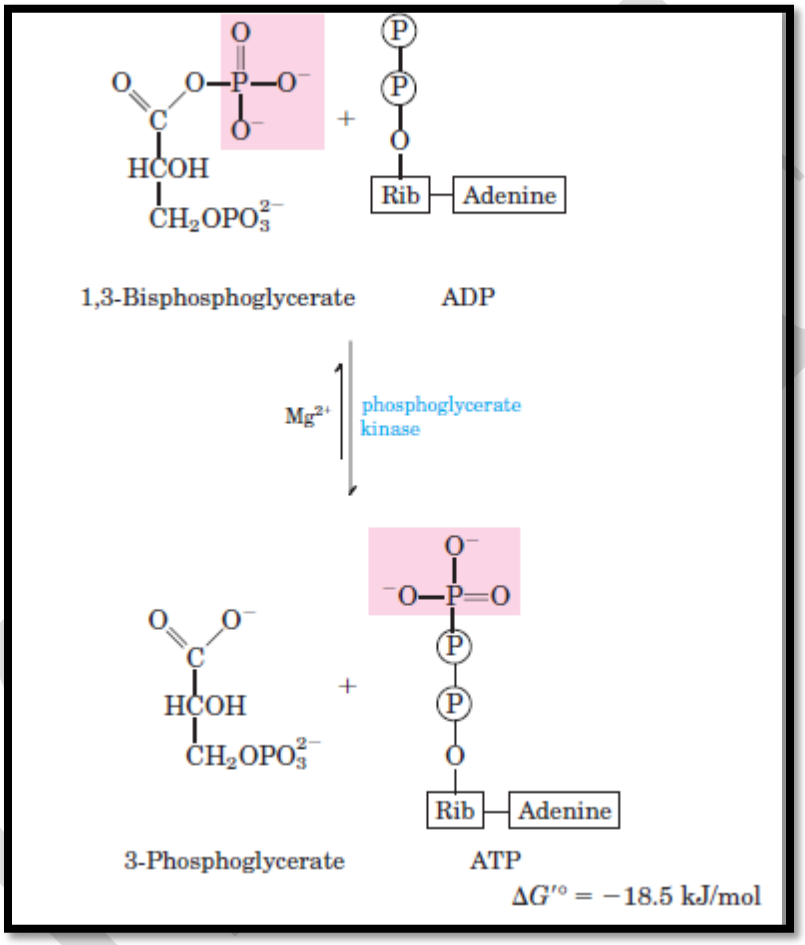


MECHANISM FIGURE 14–7 The glyceraldehyde 3-phosphate dehydrogenase reaction. *After 1 formation of the enzyme-substrate complex, 2 a covalent thiohemiacetal linkage forms between the substrate and the OSH group of a Cys residue—facilitated by acid-base catalysis with a neighboring base catalyst, probably a His residue. 3 This enzyme-substrate intermediate is oxidized by NAD_ bound to the active site, forming a covalent acyl-enzyme intermediate, a thioester. 4 The newly formed NADH leaves the active site and is replaced by another NAD_ molecule. The bond between the acyl group and the thiol group of the enzyme has a very high standard free energy of hydrolysis. 5 This bond undergoes phosphorolysis (attack by Pi), releasing the acyl phosphate product, 1,3-bisphosphoglycerate. Formation of this product*

conserves much of the free energy liberated during oxidation of the aldehyde group of glyceraldehyde 3-phosphate.

7. Phosphoryl Transfer from 1,3-Bisphosphoglycerate to ADP

The enzyme **phosphoglycerate kinase** transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and **3- phosphoglycerate:**



Notice that phosphoglycerate kinase is named for the reverse reaction. Like all enzymes, it catalyzes the reaction in both directions. This enzyme acts in the direction suggested by its name during gluconeogenesis (see Fig. 14–16) and during photosynthetic CO2 assimilation (see Fig. 20–4). Steps 6 and 7 of glycolysis together constitute an energy-coupling process in which 1,3-bisphosphoglycerate is the common intermediate; it is formed in the first reaction (which would

be endergonic in isolation), and its acyl phosphate group is transferred to ADP in the second reaction (which is strongly exergonic). The sum of these two reactions is

$$\text{Glyceraldehyde 3-phosphate} + \text{ADP} + P_i + \text{NAD}^+ \rightleftharpoons \text{3-phosphoglycerate} + \text{ATP} + \text{NADH} + \text{H}^+ \qquad \Delta G'^\circ = -12.5 \text{ kJ/mol}$$

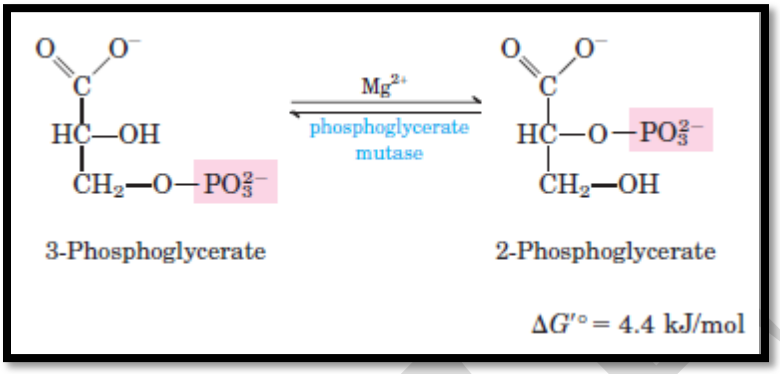
Thus the overall reaction is exergonic. Recall from Chapter 13 that the actual free-energy change, ΔG, is determined by the standard free-energy change, $\Delta G'^\circ$, and the mass-action ratio, Q, which is the ratio [products]/[reactants] (see Eqn 13–3). For step 6

$$\Delta G = \Delta G'^\circ + RT \ln Q = \Delta G'^\circ + RT \ln \frac{[\text{1,3-bisphosphoglycerate}][\text{NADH}]}{[\text{glyceraldehyde 3-phosphate}][P_i][\text{NAD}^+]}$$

Notice that $[H^+]$ is not included in Q. In biochemical calculations, $[H^+]$ is assumed to be a constant (10^{-7} M), and this constant is included in the definition of $\Delta G'^\circ$ (p. 491). When the mass-action ratio is less than 1.0, its natural logarithm has a negative sign. Step 7, by consuming the product of step 6 (1,3-bisphosphoglycerate), keeps [1,3-bisphosphoglycerate] relatively low in the steady state and thereby keeps Q for the overall energy coupling process small. When Q is small, the contribution of ln Q can make ΔG strongly negative. This is simply another way of showing how the two reactions, steps 6 and 7, are coupled through a common intermediate. The outcome of these coupled reactions, both reversible under cellular conditions, is that the energy released on oxidation of an aldehyde to a carboxylate group is conserved by the coupled formation of ATP from ADP and Pi. The formation of ATP by phosphoryl group transfer from a substrate such as 1,3-bisphosphoglycerate is referred to as a **substrate-level phosphorylation,** to distinguish this mechanism from **respiration-linked phosphorylation.** Substrate-level phosphorylations involve soluble enzymes and chemical intermediates (1,3-bisphosphoglycerate in this case). Respiration-linked phosphorylations, on the other hand, involve membrane-bound enzymes and transmembrane gradients of protons (Chapter 19).

8 .Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate

The enzyme **phosphoglycerate mutase** catalyzes a reversible shift of the phosphoryl group between C-2 and C-3 of glycerate; Mg^{2+} is essential for this reaction:



The reaction occurs in two steps (Fig. 14–8). A phosphoryl group initially attached to a His residue of the mutase is transferred to the hydroxyl group at C-2 of 3- phosphoglycerate, forming 2,3-bisphosphoglycerate (2,3-BPG). The phosphoryl group at C-3 of 2,3-BPG is then transferred to the same His residue, producing 2- phosphoglycerate and regenerating the phosphorylated enzyme. Phosphoglycerate mutase is initially phosphorylated by phosphoryl transfer from 2,3-BPG, which is required in small quantities to initiate the catalytic cycle and is continuously regenerated by that cycle. Although in most cells 2,3-BPG is present in only trace amounts, it is a major component (~5 mM) of erythrocytes, where it regulates the affinity of hemoglobin for oxygen (see Fig. 5–17; note that in the context of hemoglobin regulation, 2,3-bisphosphoglycerate is usually abbreviated as simply BPG).

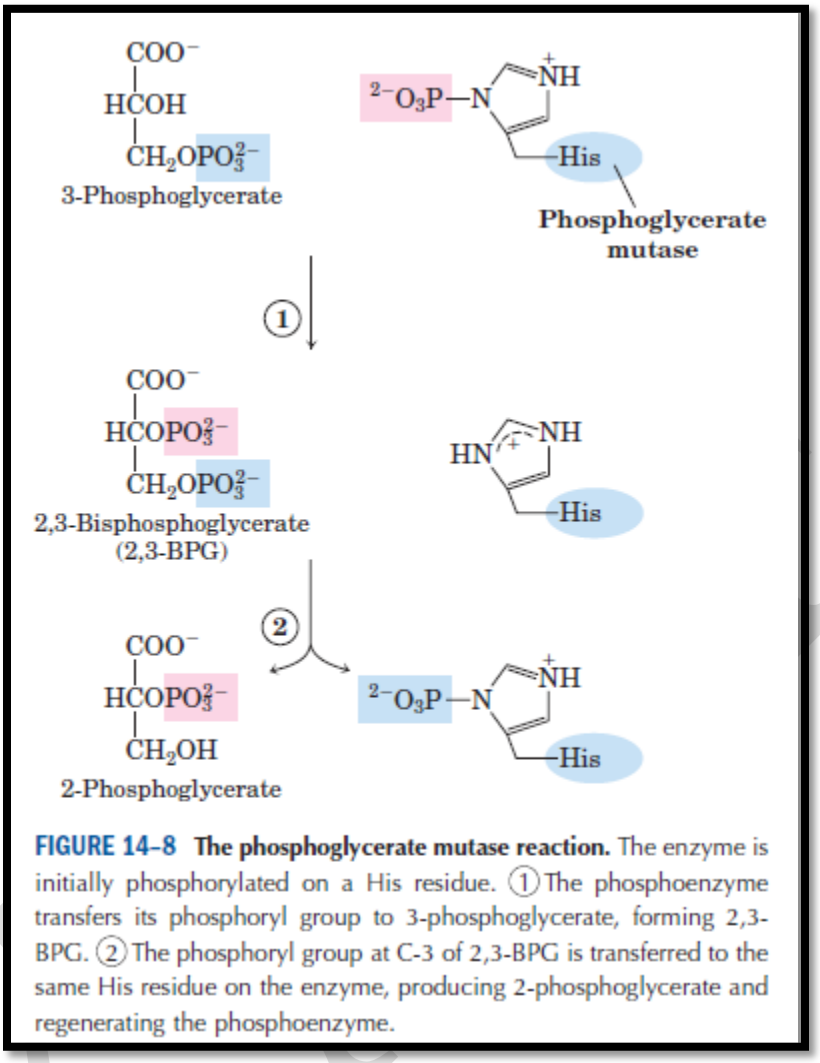
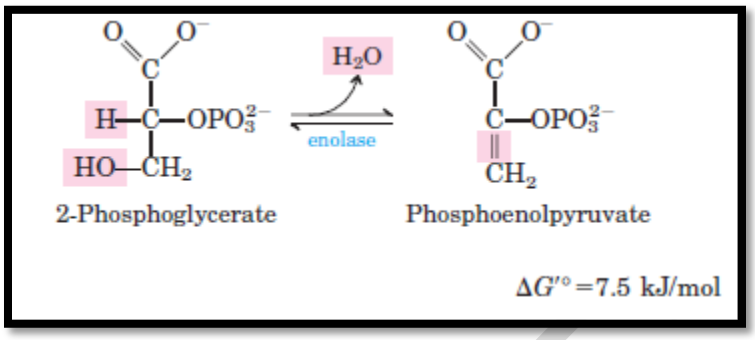


FIGURE 14–8 The phosphoglycerate mutase reaction. The enzyme is initially phosphorylated on a His residue. (1) The phosphoenzyme transfers its phosphoryl group to 3-phosphoglycerate, forming 2,3-BPG. (2) The phosphoryl group at C-3 of 2,3-BPG is transferred to the same His residue on the enzyme, producing 2-phosphoglycerate and regenerating the phosphoenzyme.

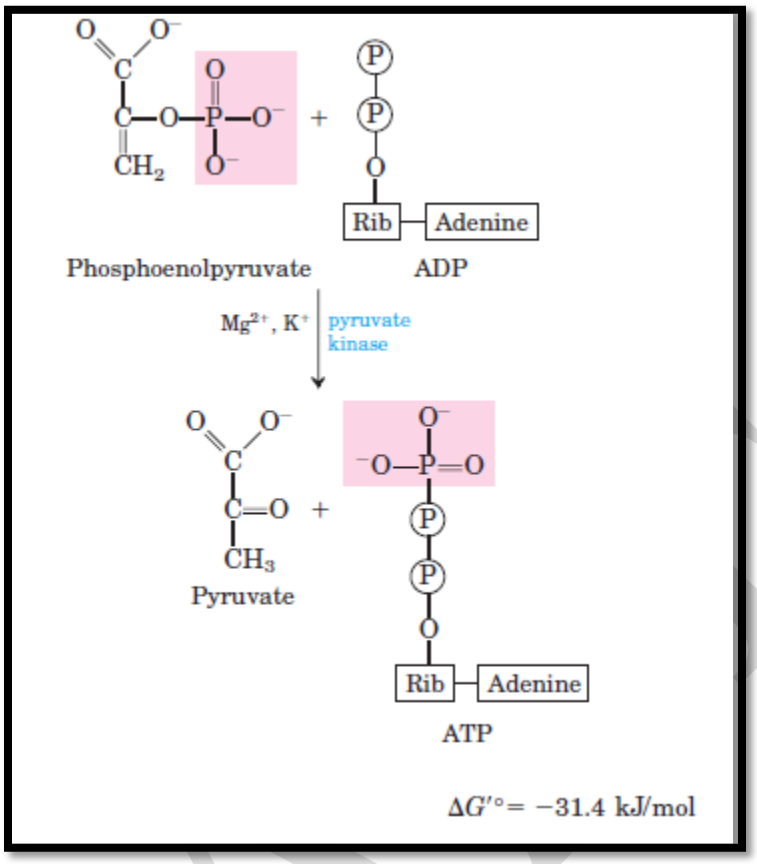
9 .Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

In the second glycolytic reaction that generates a compound with high phosphoryl group transfer potential, **enolase** promotes reversible removal of a molecule of water from 2-phosphoglycerate to yield **phosphoenolpyruvate (PEP):**

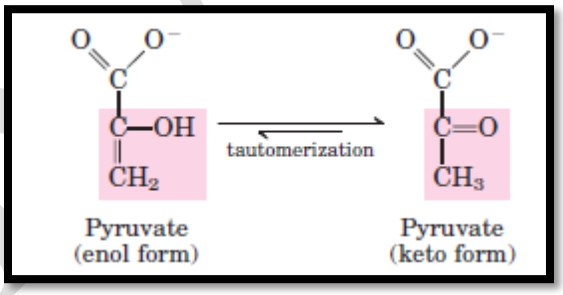


The mechanism of the enolase reaction is presented in Figure 6–23. Despite the relatively small standard free energy change of this reaction, there is a very large difference in the standard free energy of hydrolysis of the phosphoryl groups of the reactant and product: -17.6 kJ/mol for 2-phosphoglycerate (a low-energy phosphate ester) and _61.9 kJ/mol for phosphoenolpyruvate (a compound with a very high standard free energy of hydrolysis) (see Fig. 13–3, Table 13–6). Although 2-phosphoglycerate and phosphoenolpyruvate contain nearly the same *total* amount of energy, the loss of the water molecule from 2-phosphoglycerate causes a redistribution of energy within the molecule, greatly increasing the standard free energy of hydrolysis of the phosphoryl group.

10. Transfer of the Phosphoryl Group from Phosphoenolpyruvate to ADP The last step in glycolysis is the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, catalyzed by **pyruvate kinase,** which requires K^+ and either $Mg2^+$ or $Mn2^+$:



In this substrate-level phosphorylation, the product **pyruvate** first appears in its enol form, then tautomerizes rapidly and nonenzymatically to its keto form, which predominates at pH 7:



The overall reaction has a large, negative standard free energy change, due in large part to the spontaneous conversion of the enol form of pyruvate to the keto form (see Fig. 13–3). The _*G*__ of phosphoenolpyruvate hydrolysis is _61.9 kJ/mol; about half of this energy is conserved in the formation of the phosphoanhydride bond of ATP (Δ*G*'°= -30.5 kJ/mol), and the rest (-31.4

kJ/mol) constitutes a large driving force pushing the reaction toward ATP synthesis. The pyruvate kinase reaction is essentially irreversible under intracellular conditions and is an important site of regulation, as described in Chapter 15.

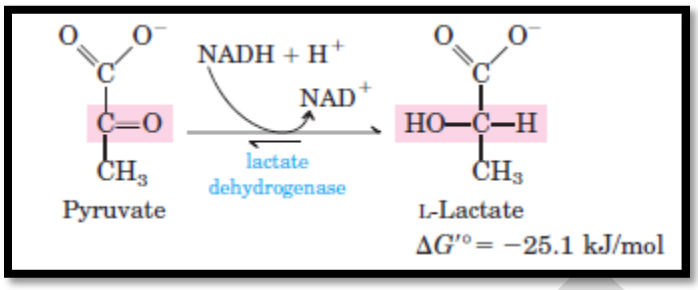
Fates of Pyruvate under Anaerobic Conditions: Fermentation

Pyruvate occupies an important junction in carbohydrate catabolism (Fig. 14–3). Under aerobic conditions pyruvate is oxidized to acetate, which enters the citric acid cycle and is oxidized to CO_2 and H_2O, and NADH formed by the dehydrogenation of glyceraldehyde 3- phosphate is ultimately reoxidized to NAD^+ by passage of its electrons to O_2 in mitochondrial respiration. However, under hypoxic conditions, as in very active skeletal muscle, in submerged plant tissues, or in lactic acid bacteria, NADH generated by glycolysis cannot be reoxidized by O_2. Failure to regenerate NAD^+ would leave the cell with no electron acceptor for the oxidation of glyceraldehyde 3-phosphate, and the energy-yielding reactions of glycolysis would stop. NAD^+ must therefore be regenerated in some other way.

The earliest cells lived in an atmosphere almost devoid of oxygen and had to develop strategies for deriving energy from fuel molecules under anaerobic conditions. Most modern organisms have retained the ability to constantly regenerate NAD^+ during anaerobic glycolysis by transferring electrons from NADH to form a reduced end product such as lactate or ethanol.

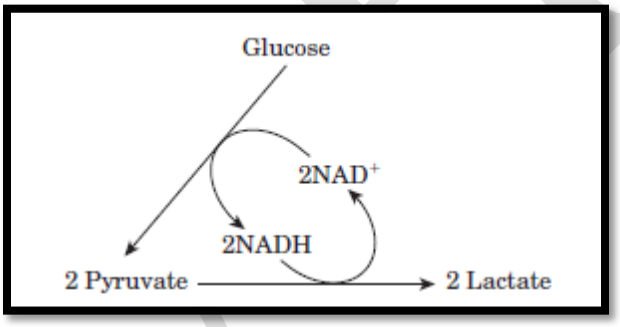
Pyruvate Is the Terminal Electron Acceptor in LacticAcid Fermentation

When animal tissues cannot be supplied with sufficient oxygen to support aerobic oxidation of the pyruvate and NADH produced in glycolysis, NAD_ is regenerated from NADH by the reduction of pyruvate to **lactate.** As mentioned earlier, some tissues and cell types (such as erythrocytes, which have no mitochondria and thus cannot oxidize pyruvate to CO2) produce lactate from glucose even under aerobic conditions. The reduction of pyruvate is catalyzed by **lactate dehydrogenase,** which forms the L isomer of lactate at pH 7:



The overall equilibrium of this reaction strongly favors lactate formation, as shown by the large negative standard free-energy change.

In glycolysis, dehydrogenation of the two molecules of glyceraldehyde 3-phosphate derived from each molecule of glucose converts two molecules of NAD_ to two of NADH. Because the reduction of two molecules of pyruvate to two of lactate regenerates two molecules of NAD_, there is no net change in NAD_ or NADH:



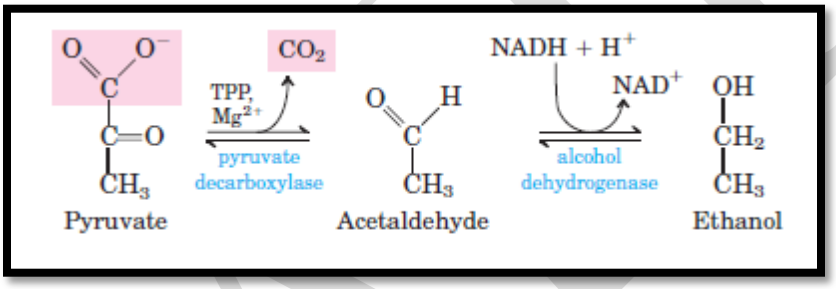
The lactate formed by active skeletal muscles (or by erythrocytes) can be recycled; it is carried in the blood to the liver, where it is converted to glucose during the recovery from strenuous muscular activity. When lactate is produced in large quantities during vigorous muscle contraction (during a sprint, for example), the acidification that results from ionization of lactic acid in muscle and blood limits the period of vigorous activity. The best-conditioned athletes can sprint at top speed for no more than a minute (Box 14–1). Although conversion of glucose to lactate includes two oxidation-reduction steps, there is no net change in the oxidation state of carbon; in glucose ($C_6H_{12}O_6$) and lactic acid (C_3H6O_3), the H: C ratio is the same. Nevertheless,

some of the energy of the glucose molecule has been extracted by its conversion to lactate—enough to give a net yield of two molecules of ATP for every glucose molecule consumed.

Fermentation is the general term for such processes, which extract energy (as ATP) but do not consume oxygen or change the concentrations of NAD^+ or NADH. Fermentations are carried out by a wide range of organisms, many of which occupy anaerobic niches, and they yield a variety of end products, some of which find commercial uses.

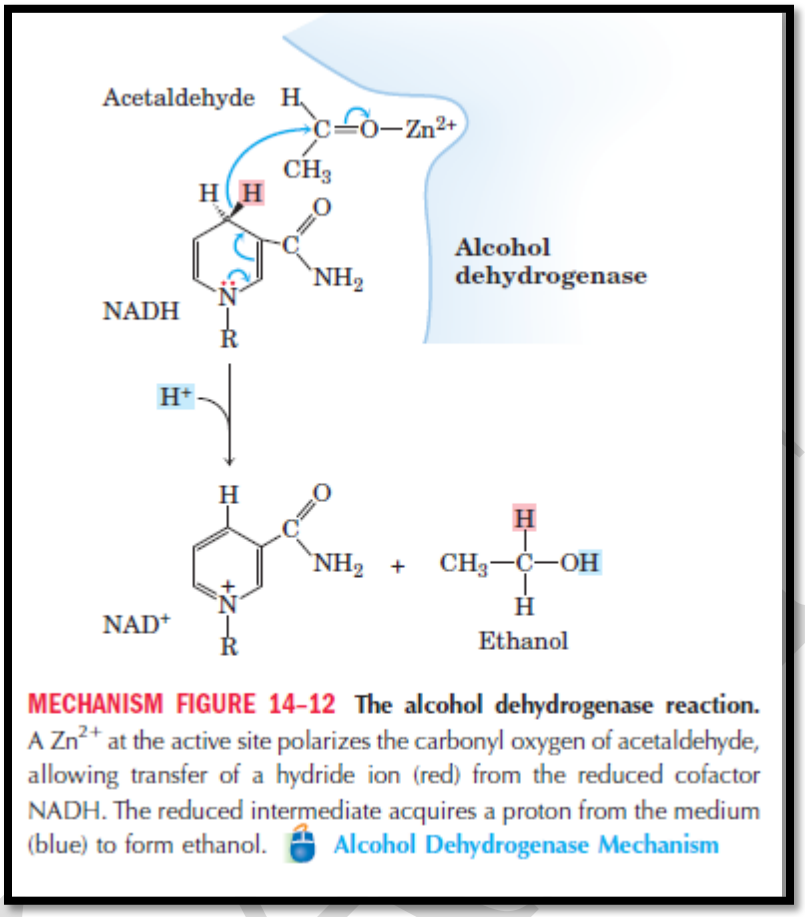
Ethanol Is the Reduced Product in Ethanol Fermentation

Yeast and other microorganisms ferment glucose to ethanol and CO_2, rather than to lactate. Glucose is converted to pyruvate by glycolysis, and the pyruvate is converted to ethanol and CO_2 in a two-step process:



In the first step, pyruvate is decarboxylated in an irreversible reaction catalyzed by **pyruvate decarboxylase.** This reaction is a simple decarboxylation and does not involve the net oxidation of pyruvate. Pyruvate decarboxylase requires Mg2+ and has a tightly bound coenzyme, thiamine pyrophosphate, discussed below.

In the second step, acetaldehyde is reduced to ethanol through the action of **alcohol dehydrogenase,** with the reducing power furnished by NADH derived from the dehydrogenation of glyceraldehyde 3-phosphate. This reaction is a well-studied case of hydride transfer from NADH (Fig. 14–12).



MECHANISM FIGURE 14–12 **The alcohol dehydrogenase reaction.** A Zn^{2+} at the active site polarizes the carbonyl oxygen of acetaldehyde, allowing transfer of a hydride ion (red) from the reduced cofactor NADH. The reduced intermediate acquires a proton from the medium (blue) to form ethanol. Alcohol Dehydrogenase Mechanism

Ethanol and CO2 are thus the end products of ethanol fermentation, and the overall equation is

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i \longrightarrow 2\ \text{ethanol} + 2\text{CO}_2 + 2\text{ATP} + 2\text{H}_2\text{O}$$

As in lactic acid fermentation, there is no net change in the ratio of hydrogen to carbon atoms when glucose (H: C ratio =12/6 = 2) is fermented to two ethanol and two CO_2 (combined H:C ratio = 12/6 = 2). In all fermentations, the H: C ratio of the reactants and products remains the same.

Pyruvate decarboxylase is present in brewer's and baker's yeast and in all other organisms that ferment glucose to ethanol, including some plants. The CO_2 produced by pyruvate decarboxylation in brewer's yeast is responsible for the characteristic carbonation of champagne.

The ancient art of brewing beer involves a number of enzymatic processes in addition to the reactions of ethanol fermentation (Box 14–2). In baking, CO_2 released by pyruvate decarboxylase when yeast is mixed with a fermentable sugar causes dough to rise. The enzyme is absent in vertebrate tissues and in other organisms that carry out lactic acid fermentation. Alcohol dehydrogenase is present in many organisms that metabolize ethanol, including humans. In human liver it catalyzes the oxidation of ethanol, either ingested or produced by intestinal microorganisms, with the concomitant reduction of NAD+ to NADH.

The Citric Acid Cycle

We begin our discussion of the citric acid cycle with the production of *acetyl-CoA*, which is the fuel used to drive the cycle. For example, acetyl-CoA is produced from pyruvate using the enzyme pyruvate dehydrogenase, coenzyme A (CoASH), and one NAD+. The product of this reaction is acetyl-CoA with the liberation of 1 CO_2 molecule + NADH. The acetyl-CoA molecule is the input molecule for the citric acid cycle, but *oxaloacetate* is also required. The latter molecule is not considered "fuel" for the cycle because it is a recycled product used in the reaction.

We can summarize the essential facts of the citric acid cycle in the following way:

• The citric acid cycle is an eight-step reaction.
• It requires 8 enzymes.
• The fi nal product is oxaloacetate.
• Two NADH molecules are produced.
• One GTP molecule is produced.
• One FADH2 molecule is produced.
• The cycle is accompanied by the liberation of 2 CO2 molecules.

The eight enzymes utilized in the citric acid cycle are:

1. Citrate synthase
2. Aconitase
3. Isocitrate dehydrogenase
4. *a*-Ketoglutarate dehydrogenase
5. Succinyl-CoA synthetase

6. Succinate dehydrogenase
7. Fumarase
8. Malate dehydrogenase

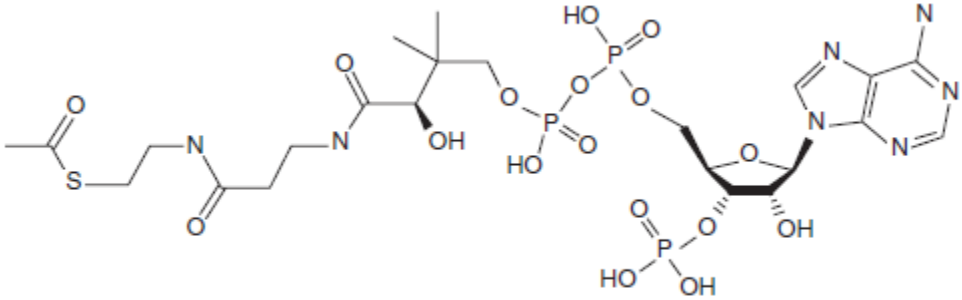


Figure 11-1 Acetyl-CoA, the input or "fuel" for the citric acid cycle.

The production of acetyl-CoA for the citric acid cycle must occur inside the mitochondria because it is unable to cross the mitochondrial membrane from the cytosol. As a result, other molecules must be utilized. It turns out that pyruvate, fatty acids, and some amino acids are able to cross the mitochondrial membrane where they can be used by the mitochondria to produce acetyl-CoA.

STEPS IN THE CITRIC ACID CYCLE

Now let's consider the eight steps of the citric acid cycle.

Step 1: Acetyl-CoA → Citrate

In the fi rst step of the cycle, the enzyme citrate synthase catalyzes the condensation of acetyl-CoA (Fig. 11-1) with oxaloacetate (Fig. 11-2). This produces a molecule called *citrate*, and liberates coenzyme A. One water molecule is also required. Note that citrate is a tertiary alcohol that cannot be oxidized very easily (Fig. 11-3), so it must be further processed. This is done in the second step of the cycle.

Step 2: Citrate → Isocitrate

In step 2 of the cycle, there are two substeps used to generate isocitrate, which is easier to oxidize. First, the enzyme aconitase catalyzes the dehydration of the citrate molecule producing an intermediate molecule called *cis-aconitate.*

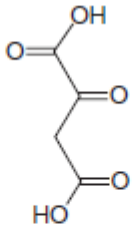


Figure 11-2 Oxaloacetate, a molecule required in the first step of the citric acid cycle and produced in the final or eighth step.

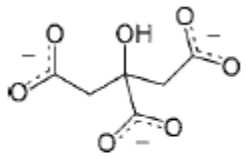


Figure 11-3 The citrate molecule.

The cis-aconitate molecule, remaining bound to the enzyme, is then hydrated to produce the product of the second step, isocitrate. The net effect of this reaction is that the hydroxyl group on citrate is moved from the third carbon atom to the fourth carbon atom, producing an isomer of citrate that is easier to oxidize.

Step 3: Isocitrate → α-Ketoglutamate

The next step in the citric acid cycle also has two substeps or phases. First, isocitrate is oxidized by the enzyme isocitrate dehydrogenase producing *oxalosuccinate.* Like cis-aconitate in step 2, this molecule is an intermediate that never dissociates from the enzyme. Instead it undergoes further processing. In the second phase of step two, the enzyme decarboxylates oxalosuccinate to

produce *a*-ketoglutamate. In step 3, one NADH is produced in the fi rst phase and a molecule of CO2 is release in the second phase.

Step 4: _-Ketoglutamate → Succinyl-CoA

The next step in the reaction, which is catalyzed by *a*-ketoglutamate dehydrogenase, decarboxylates *a*-ketoglutamate producing succinyl-CoA (Fig. 11-4). One NAD+ and 1 molecule of coenzyme A are required for this step of the reaction, which produces 1 NADH and 1 molecule of CO2.

Step 5: Succinyl-CoA → Succinate

In this step, a high energy GTP molecule is produced. This is substrate level phosphorylation. The reaction is catalyzed by the molecule succinyl-CoA synthetase, and the coenzyme A molecule consumed in the production of succinyl-CoA is released (see Fig. 11-5).

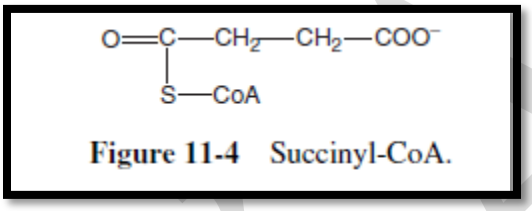


Figure 11-4 Succinyl-CoA.

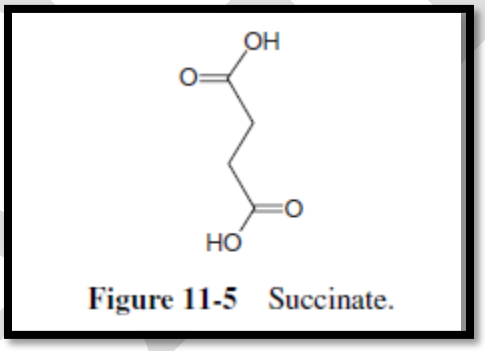


Figure 11-5 Succinate.

Steps 1 to 5 can be considered to be the fi rst part of the citric acid cycle. Two NADH, 1 GTP and 2 CO_2 molecules have been produced. In the second part of the cycle, succinate will be oxidized back to the starting product in the reaction, oxaloacetate, which can then be used in another round of the cycle. This oxidation requires three steps and produces 1 FADH2 and 1 NADH molecule.

Step 6: Succinate → Fumarate

In this step, succcinate dehydrogenase oxidizes the succinate molecule producing fumarate. One FAD is utilized in this reaction, and succinate dehydrogenase eliminates 2 hydrogens in the oxidation of the central single bond of the succinate molecule. The coenzyme FAD is therefore reduced to FADH2 in the process (see Fig. 11-6).

Step 7: Fumarate → L-Malate

In this step, fumarate hydratase or *fumarase* catalyzes a reversible reaction in which fumarate is transformed into malate. One molecule of water is required, in a process which transforms the central double bond of fumarate into a single bond. The top carbon of the double bond is changed from CH ‡ CHOH and the bottom carbon of the double bond is transformed from CH ‡ CH2 (see Fig. 11-7).

Step 8: Malate → Oxaloacetate

We have now reached the fi nal step in the cycle. In this last step, the molecule oxaloacetate is regenerated from malate, allowing the cycle to start anew (provided there is a supply of acetyl-CoA). The enzyme which catalyzes this step is malate dehydrogenase, and 1 NADH molecule is produced.

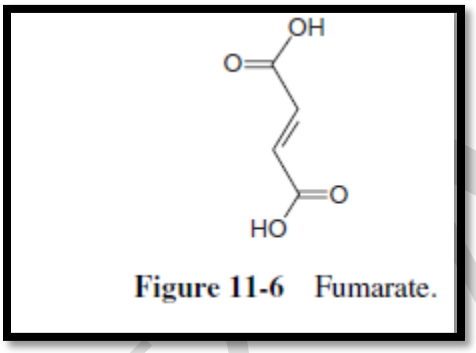


Figure 11-6 Fumarate.

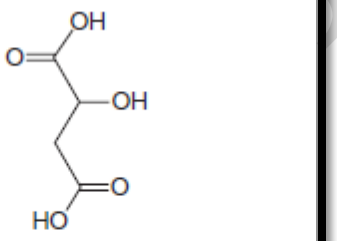


Figure 11-7 Malate.

NOTE: The only input molecule to the citric acid cycle is the acetyl-CoA molecule, whose carbon atoms are lost as CO2 molecules. All other molecules in the cycle are regenerated intermediates. Acetyl-CoA is the *fuel* for the cycle which must be input from the outside.

ENERGETICS OF THE CITRIC ACID CYCLE

The citric acid cycle is an *exergonic* reaction with $\Delta G^{\circ\prime} = -60$ kJ/mol

Two steps in the reaction are *endergonic*. These are:

• Step 2, conversion of citrate to isocitrate with $\Delta G^{\circ\prime} = +5$ kJ/mol.

• Step 8, the conversion of malate to oxaloacetate with $\Delta G^{\circ\prime} = +30$ kJ/mol.

The most exergonic steps in the cycle are

• Step 1, the conversion of acetyl-CoA to citrate with $\Delta G^{\circ\prime} = -32$ kJ/mol.

• Step 4, the conversion of *a*-ketoglutamate to succinyl-CoA, with $\Delta G^{\circ\prime} = -30$ kJ/mol.

Since they are endergonic, steps 2 and 8 of the citric acid cycle are *unfavored*. It is possible for them to proceed because they are followed by the energetically favorable steps 3 and 1. In step 2, at equilibrium, the reversible reaction which converts citrate to isocitrate actually favors the formation of citrate. However, step 3 of the reaction is exergonic and hence is energetically favorable. So when isocitrate is formed, it is removed and utilized in step 3. Similarly, at equilibrium the reversible reaction which converts malate to oxaloacetate favors the formation of malate. However oxaloacetate is an input molecule for step 1 of the cycle, which is exergonic (and hence energetically favorable). So when oxaloacetate forms then step 1 of the cycle will proceed removing the molecule.

CONTROL OF THE CITRIC ACID CYCLE

The citric acid cycle is controlled by two factors:

• Regulatory enzymes

• The energy requirements of mitochondria

There are four regulatory enzymes in the citric acid cycle. These are

1. Citrate synthase
2. Isocitrate dehydrogenase
3. 2-Oxogulta dehydrogenase
4. Succinate dehydrogenase

Citrate synthase catalyzes the first step of the citric acid cycle, which is the condensation reaction of acetyl-CoA and oxaloacetate using an induced fit process.

Citrate synthase has an oxaloacetate binding site. Initially, the enzyme exists in an *open* state with a cleft where we fi nd the binding site. It first binds a molecule of oxaloacetate to this site. Upon binding, oxaloacetate induces a change in the structure of the citrate synthase enzyme into a *closed* state that produces a binding site for acetyl-CoA. This is the induced fit aspect of the action of this enzyme.

An intermediate compound is formed from acetyl-CoA and oxaloacetate called *citroyl-CoA*. This molecule remains bound to the enzyme, and the hydrolysis of a thioester bond results in the production of citrate and coenzyme A. The first step in the citric acid cycle is inhibited by

NADH and succinyl-CoA. In the first case, energy requirements of mitochondria are a limiting factor of the initiation of the citric acid cycle. Succinyl-CoA can also bind to the active acetyl-CoA binding site and so competes with acetyl-CoA in the initiation of the first step. Step 2 of the citric acid cycle is an important regulatory step as well. This is because the enzyme which catalyzes the reaction, isocitrate dehydrogenase, is an allosteric enzyme whose activity is inhibited by the presence of high-energy compounds. That is, it is inhibited by the presence of ATP and NADH, and conversely it is activated by ADP and NAD+. When compounds with high-energy bonds are present, isocitrate dehydrogenase is inhibited, leading to a "shut down" process that works as follows. Without the action of the enzyme, isocitrate begins to accumulate in the mitochondria. This leads to an equilibrium situation, which favors the conversion of isocitrate to citrate, with 7% isocitrate and 93% citrate at equilibrium. Citrate begins to accumulate in the mitochondria but a transport protein in the membrane of the mitochondria allows it to exit to the cytoplasm. Here it acts to inhibit the enzymes pyruvate kinase and phosphofructokinase, essentially shutting off metabolism.

Isocitrate dehydrogenase is dependent on NAD+ for its activity. In addition, it requires a cofactor which can be either Mn2+ or Mg2+. The next regulatory enzyme in the citric acid cycle is 2-oxoglutarate dehydrogenase, which is inhibited by high levels of NADH and succinyl-CoA. Hence a high energy state in the mitochondria inhibits the action of this enzyme, which acts in step 4 of the citric acid cycle to produce succinyl-CoA.

The final regulatory enzyme in the citric acid cycle is succinate dehydrogenase. This enzyme acts on step 6, where succinate is converted into fumarate with the transformation (reduction) of an FAD molecule to FADH2. The enzyme is actually inhibited by the last step in the cycle, because an accumulation of oxaloacetate inhibits the enzyme. As such we say that a *feedback loop* exists between steps 8 and 6 of the cycle, controlling the production of fumarate when oxaloacetate levels get too high. As the energy levels of the mitochondria go up with the correlated increase in oxaloacetate concentration, succinate dehydrogenase is inhibited putting the breaks on the citric acid cycle. An interesting feature of succinate dehydrogenase is that an FAD molecule is permanently attached to the enzyme via a covalent bond to an HIS residue of the enzyme. The FAD molecule acts as the electron acceptor in step 6 of the citric acid cycle.

Oxidation of an alkane to an alkene is accomplished by FAD, and succinate is an alkane, while fumarate is an alkene. Coenzyme Q reoxidizes succinate dehydrogenase in the electron transport chain.

Isolation and characterization of Polysachharides:

Feeder Pathways for Glycolysis

Many carbohydrates besides glucose meet their catabolic fate in glycolysis, after being transformed into one of the glycolytic intermediates. The most significant are the storage polysaccharides glycogen and starch; the disaccharides maltose, lactose, trehalose, and sucrose; and the monosaccharides fructose, mannose, and galactose(Fig. 14–9).

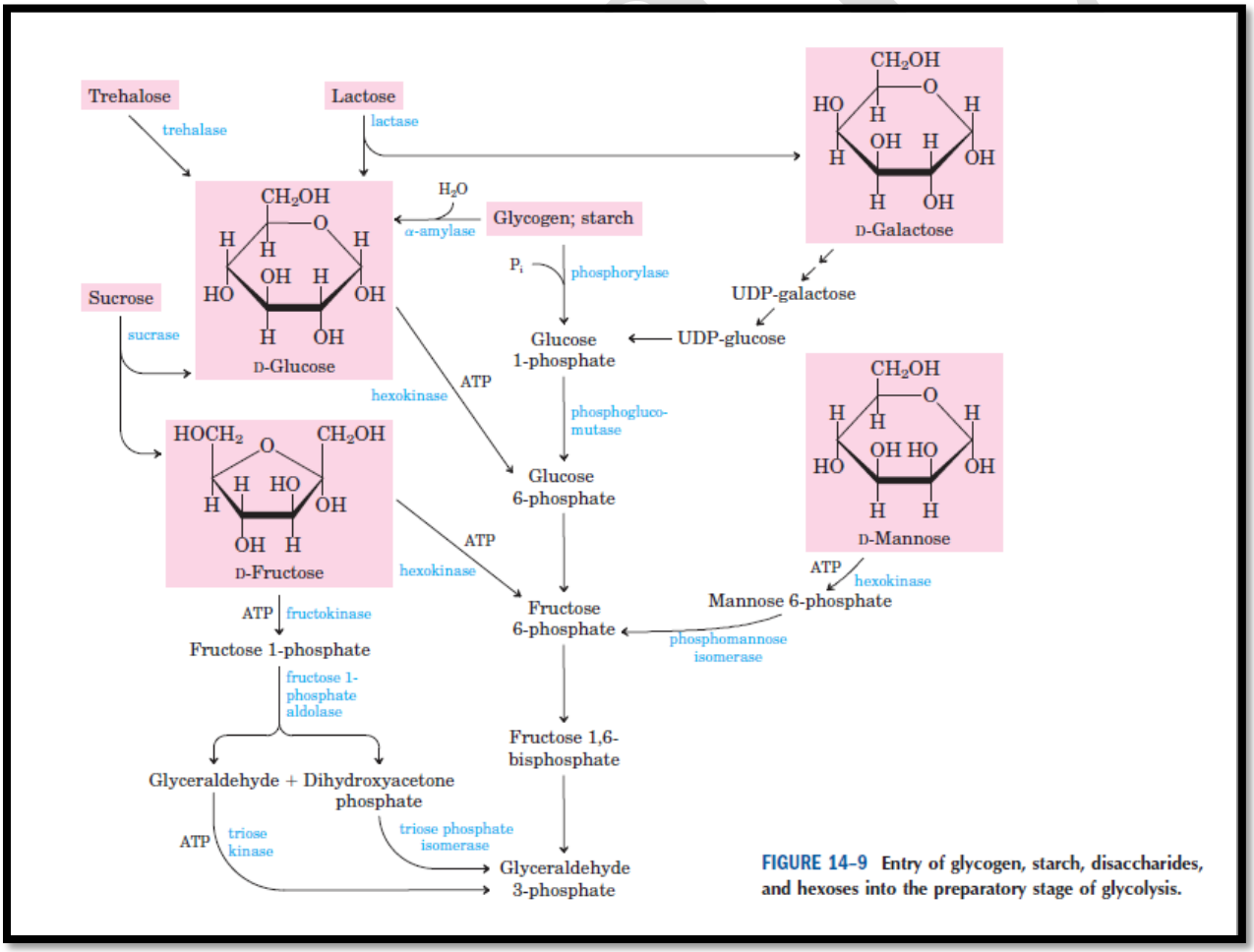


FIGURE 14–9 Entry of glycogen, starch, disaccharides, and hexoses into the preparatory stage of glycolysis.

Glycogen and Starch Are Degraded by Phosphorolysis

Glycogen in animal tissues and in microorganisms (and starch in plants) can be mobilized for use within the same cell by a phosphorolytic reaction catalyzed by **glycogen phosphorylase** (**starch phosphorylase** in plants). These enzymes catalyze an attack by Pi on the (α1→4) glycosidic linkage that joins the last two glucose residues at a non reducing end, generating glucose 1-phosphate and a polymer one glucose unit shorter (Fig. 14–10). *Phosphorolysis* preserves some of the energy of the glycosidic bond in the phosphate ester glucose 1-phosphate. Glycogen phosphorylase (or starch phosphorylase) acts repetitively until it approaches an (α1→6) branch point (see Fig. 7–15), where its action stops. A **debranching enzyme** removes the branches.

The mechanisms and control of glycogen degradation are described in detail in Chapter 15.

Glucose 1-phosphate produced by glycogen phosphorylase is converted to glucose 6-phosphate by **phosphoglucomutase,** which catalyzes the reversible reaction

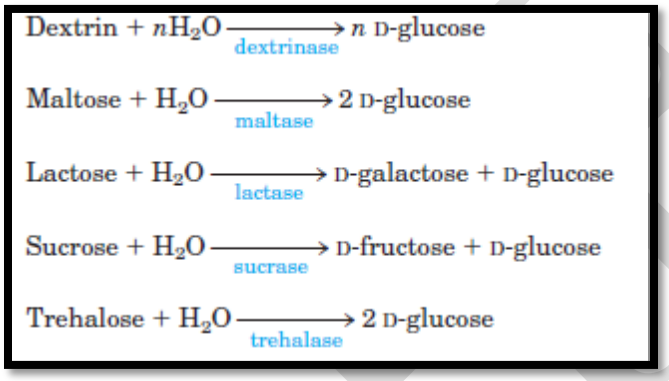
The glucose 6-phosphate thus formed can enter glycolysis or another pathway such as the pentose phosphate pathway, described in Section 14.5. Phosphoglucomutase employs essentially the same mechanism as phosphoglycerate mutase (p. 531). The general name **mutase** is given to enzymes that catalyze the transfer of a functional group from one position to another in the same molecule. Mutases are a subclass of **isomerases,** enzymes that interconvert stereoisomers or structural or positional isomers (see Table 6–3).

Dietary Polysaccharides and Disaccharides Undergo Hydrolysis to Monosaccharides

For most humans, starch is the major source of carbohydrates in the diet. Digestion begins in the mouth, where salivary α-**amylase** (Fig. 14–9) hydrolyzes the internal glycosidic linkages of starch, producing short polysaccharide fragments or oligosaccharides. (Note that in this *hydrolysis* reaction, water, not Pi, is the attacking species.) In the stomach, salivary α-amylase is inactivated by the low pH, but a second form of α-amylase, secreted by the pancreas into the small intestine, continues the breakdown process. Pancreatic α-amylase yields mainly maltose and maltotriose (the di- and trisaccharides of α(1→4) glucose) and oligosaccharides called limit dextrins, fragments of amylopectin containing α(1→6) branch points. Maltose and dextrins are

degraded by enzymes of the intestinal brush border (the fingerlike microvilli of intestinal epithelial cells, which greatly increase the area of the intestinal surface). Dietary glycogen has essentially the same structure as starch, and its digestion proceeds by the same pathway

Disaccharides must be hydrolyzed to monosaccharides before entering cells. Intestinal disaccharides and dextrins are hydrolyzed by enzymes attached to the outer surface of the intestinal epithelial cells:



The monosaccharides so formed are actively transported into the epithelial cells (see Fig. 11–44), then passed into the blood to be carried to various tissues, where they are phosphorylated and funneled into the glycolytic sequence.

Lactose intolerance, common among adults of most human populations except those originating

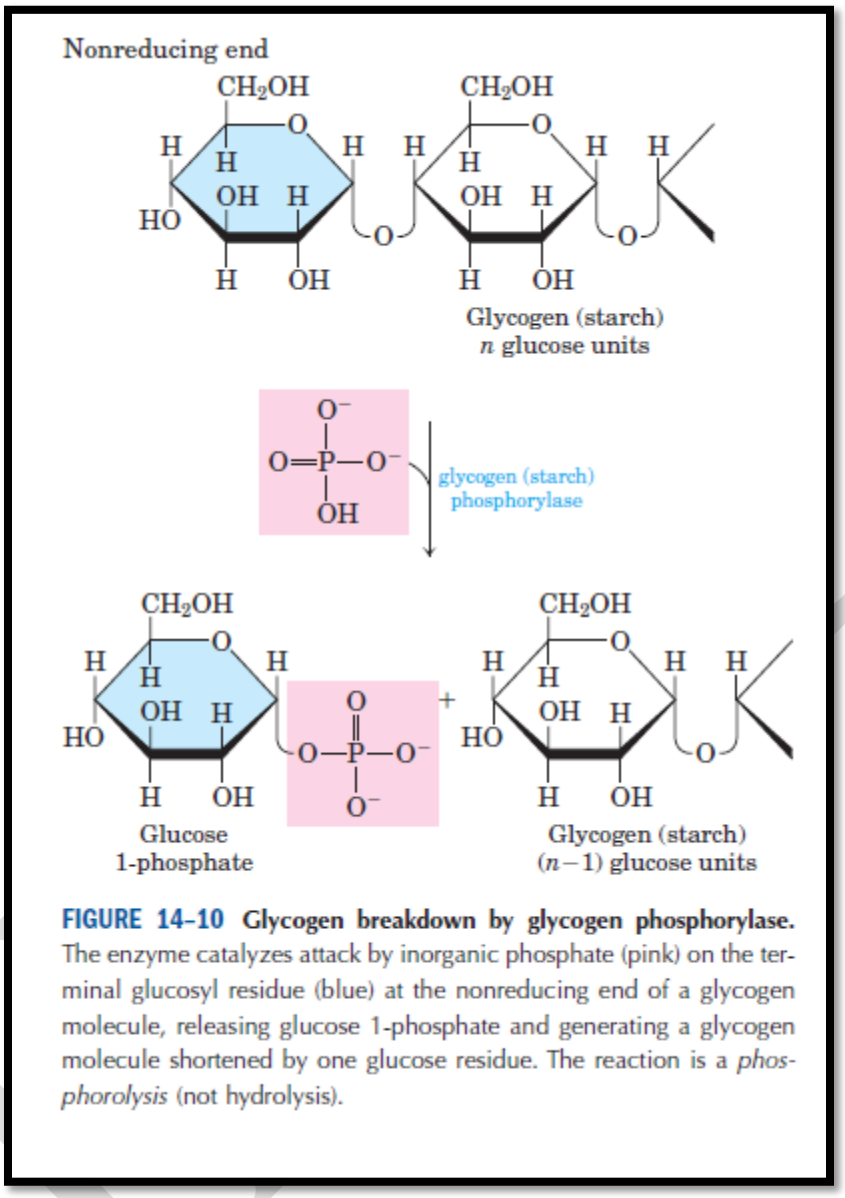


FIGURE 14–10 Glycogen breakdown by glycogen phosphorylase. The enzyme catalyzes attack by inorganic phosphate (pink) on the terminal glucosyl residue (blue) at the nonreducing end of a glycogen molecule, releasing glucose 1-phosphate and generating a glycogen molecule shortened by one glucose residue. The reaction is a *phosphorolysis* (not hydrolysis).

in Northern Europe and some parts of Africa, is due to the disappearance after childhood of most or all of the lactase activity of the intestinal cells. Lactose cannot be completely digested and absorbed in the small intestine and passes into the large intestine, where bacteria convert it to toxic products that cause abdominal cramps and diarrhea. The problem is further complicated because undigested lactose and its metabolites increase the osmolarity of the intestinal contents, favoring the retention of water in the intestine. In most parts of the world where lactose

intolerance is prevalent, milk is not used as a food by adults, although milk products predigested with lactase are commercially available in some countries. In certain human disorders, several or all of the intestinal disaccharidases are missing. In these cases, the digestive disturbances triggered by dietary disaccharides can sometimes be minimized by a controlled diet.

Possible Questions

Part-A

1. Hexokinase

(a) is primarily used in the liver to initiate glycolysis.

(b) is only used by neurons to initiate glycolysis.

(c) is used to trap glucose for glycolysis.

(d) None of the above.

Answer:c

2. Glucokinase

(a) is used by skeletal muscle to initiate glycolysis.

(b) is used by the liver, and has a low affi nity for glucose.

(c) is used by the liver, and has a high affi nity for glucose.

(d) is not an isozyme of hexokinase.

Answer:b

3. The NET production of ATP in glycolysis is

(a) 2 ATP molecules. (b) 4 ATP molecules.

(c) 6 ATP molecules. (d) 38 ATP molecules.

Answer:a

4. The initial investment of ATP required in glycolysis is

(a) 4 ATP molecules.

(b) 2 ATP molecules.

(c) 1 ATP molecule.

(d) No ATP is required.

Answer:b

5. If a cell needs to continue using glycolysis for energy, it must replenish its supply of

(a) NAD+ molecules. (b) NADH molecules. (c) Protons. (d) H+ molecules

Answer:a

6. Lactate fermentation

(a) is used by yeast to replenish NAD+.

(b) is used by animal cells to replenish NAD+.

(c) is used by animal cells to replenish NADH.

(d) is used by animal cells to replenish NAD+, under aerobic conditions

Answer:b

7. In the pay-off phase of glycolysis, the total number of ATP molecules produced is

(a) 1. (b) 2. (c) 4. (d) 6.

Answer:c

8. Low levels of ATP in a cell

(a) activate phosphofructo-kinase by driving it to the L conformation.
(b) are correlated with high levels of ADP and AMP, which activate phosphofructo-kinase.
(c) only activate phosphofructo-kinase if levels of AMP are constant.
(d) have no effect on phosphofructo-kinase.
Answer:b

9. Fructose can be utilized in glycolysis

(a) by entry at step 3 in muscle cells, and step 5 in neurons.

(b) by entry at step 5 in muscle cells, and step 3 in liver cells.

(c) by entry at step 2 in muscle cells, and step 5 in liver cells.

(d) by entry at step 3 in muscle cells, and step 5 in liver cells.

Answer: d

10. The Warburg effect describes

(a) the tendency of cancer cells to rely on glycolysis, even under aerobic conditions.

(b) the tendency of cancer cells to quickly abandon glycolysis once oxygen is reintroduced.

(c) the heavy reliance of cancer cells on lactate dehydrogenase.

(d) None of the above.

Answer:a

11. In the fi rst step of the citric acid cycle

(a) acetyl-CoA is bound to the enzyme citrate synthase, and then it undergoes a condensation reaction with oxaloacetate.

(b) acetyl-CoA undergoes a free condensation reaction with oxaloacetate.

(c) oxaloacetate is bound to the enzyme citrate synthase and then it undergoes a condensation reaction with acetyl-CoA.

(d) oxaloacetate is bound to the enzyme acetate dehydrogenase, and then it undergoes a condensation reaction with acetyl-CoA.

Answer: c

12. In the citric acid cycle

(a) all steps are exergonic.

(b) all steps except 2 are exergonic.

(c) all steps except 2 are endergonic.

(d) all steps are endergonic.

Answer: b

13. The fi nal intermediate product in the citric acid cycle is

(a) L-malate.

(b) Acetyl-CoA.

(c) Oxaloacetate.

(d) Fumarate.

Answer:c

14. GTP is synthesized in the citric acid cycle

(a) in the transformation of succinyl-CoA to succinate.

(b) in the transformation of succinate to fumarate.

(c) in the transformation of succinate to succinyl-CoA.

(d) GTP is *not* synthesized in the citric acid cycle, 1 molecule of ATP is.

Answer:a

15. Isocitrate dehydrogenase

(a) is activated by high concentrations of ATP and NADH.

(b) is activated by high concentrations of ATP and NADPH.

(c) is unaffected by high concentrations of NADPH.

(d) is inhibited by high concentrations of high-energy compounds.

Answer:d

16. The fuel of the citric acid cycle could be described as

(a) acetyl-CoA plus oxaloacetate, which participate in the first step of the cycle.

(b) only acetyl-CoA, oxaloacetate is a recycled intermediate.

(c) acetyl-CoA and H2O.

(d) acetyl-CoA and GDP.

Answer:b

17. The electron transport chain

(a) creates a proton gradient across the inner mitochondrial membrane, with a low proton concentration in the mitochondrial matrix.

(b) creates a proton gradient across the inner mitochondrial membrane, with a low proton concentration in the intermembrane space.

(c) creates a proton gradient across the outer mitochondrial membrane, with a high proton concentration in the intermembrane space.

(d) creates a proton gradient across the outer mitochondrial membrane, with a low proton concentration in the intermembrane space.

Answer:a

18. The largest amount of ATP production is obtained from

(a) the oxidation of succinate dehydrogenase.

(b) the oxidation of NADH molecules.

(c) the oxidation of FADH2 molecules.

(d) through the electron chain.

Answer:b

19. In the electron transport chain, complex II

(a) does not contribute to the proton gradient, but mediates the transfer of electrons from succinate to cytochrome a.

(b) does not contribute to the proton gradient, but mediates the transfer of electrons from succinate to cytochrome c.

(c) does not contribute to the proton gradient, but mediates the transfer of electrons from succinate to coenzyme Q.

(d) contributes to the proton gradient, and mediates the transfer of electrons from succinate to coenzyme Q.

Answer:c

20. The transport of protons back into the mitochondrial matrix appears to

(a) cause a conformational change in a *b*-subunit that changes it from the L to the T state allowing ADP and inorganic phosphate to bind forming ATP.

(b) cause a conformation change in a *g*-subunit that changes it from the L to the T state allowing ADP and inorganic phosphate to bind forming ATP.

(c) cause a conformational change in a *b*-subunit that changes it from the T to the O state releasing all ATP molecules.

(d) cause a conformational change in a *b*-subunit that changes it from the O to the L state allowing ADP and inorganic phosphate to bind forming ATP.

Answer:a

21. In the citric acid cycle, the conversion of citrate to isocitrate

(a) is done to make the molecule more suitable for reduction.

(b) is slightly endergonic.

(c) is done to make the molecule more suitable for oxidation.

(d) is heavily exergonic.

Answer:c

22. The enzymatic activity of citrate synthase can be described as

(a) an induced fi t process.

(b) as an uncompetitive inhibition process.

(c) as a competitive inhibition process.

(d) as a lock and key process.

Answer:a

23. Isocitrate dehydrogenase is considered a regulatory enzyme in the citric acid cycle because

(a) its activity is inhibited by the presence of citrate.

(b) its activity is inhibited by the presence of ADP.

(c) its activity is inhibited by the presence of high-energy compounds.

(d) its activity is regulated by cofactors produced in the cycle.

Answer:c

24. Succinate dehydrogenase is a regulatory enzyme in the citric acid cycle

primarily because

(a) its activity is inhibited by an accumulation of oxaloacetate.

(b) its activity is inhibited by an accumulation of malate.

(c) its activity is inhibited by high-energy compounds.

(d) its activity is inhibited by an accumulation of citrate.

Answer:a

25. Cataplerotic reactions are beneficial because

(a) they catalyze the production of an extra GTP molecule.

(b) they catalyze the production of an extra NADH molecule.

(c) they utilize intermediate compounds in the citric acid cycle, preventing their buildup in the mitochondria.

(d) they extract extra energy from the citric acid cycle.

Answer:c

26. The electron transport chain

(a) generates 5 ATP molecules.

(b) prepares a proton gradient which makes ATP production possible.

(c) utilizes large amounts of oxygen, so is damaging to the cells.

(d) establishes an electron gradient, making ATP production possible.

Answer:b

27. Which out of the following statements is true about regulation of metabolic pathway?

a) Most of the metabolic pathways are regulated

b) Most of the metabolic pathways are not regulated

c) Regulation of metabolic pathways always involves changing the amount of enzymes

d) Metabolic regulation always depends on control by hormones

Answer: a

28. The rate of breakdown of metabolites is termed as

a) Metabolic state

b) Metabolism

c) Steady state

d) Homeostasis

Answer: c

29. Diminished delivery of oxygen to tissues is termed as

a) Hypoxia

b) Ischemia

c) Homeostasis

d) Metabolism

Answer: a

30. Diminished flow of blood to tissues is termed as

a) Hypoxia

b) Ischemia

c) Homeostasis

d) Metabolism

Answer: b.

31. Which of the following statements is true about the control of muscle glycogen phosphorylase?

a) It is activated by phosphorylation by an active phosphorylase kinase

b) It is allosterically activated by ATP

c) It is allosterically activated by cAMP

d) Normally it exists in active form

Answer: a

32. Which of the following is not a factor determining the activity of an enzyme?

a) Association with regulatory protein

b) Sequestration

c) Allosteric regulation

d) Nucleotides

Answer: d

33. Which of the following statements is true?

a) High insulin/glucagon ratio activates lipolysis in muscle

b) High insulin/glucagon ratio inhibits lipolysis in liver

c) High insulin/glucagon ratio activates lipolysis in adipocytes

d) Low insulin/glucagon ratio activates lipolysis in adipocytes

Answer: d

34. Which of the following type of metabolite is used for generating glucose under severe starvation conditions?

a) Amino acids

b) Fats

c) Glycogen

d) Starch

Answer: a

35. Which of the following statements is true about brain metabolism in starvation?

a) The brain can use glucogenic amino acids for energy

b) The brain can only use glucose as fuel

c) Up to a quarter of energy requirement of the brain can come from fatty acids

d) Up to a half of energy requirement of the brain can come from ketone bodies

Answer: a

36. One of the following statements about the control of enzyme activity by phosphorylation is correct

a) Phosphorylation of an enzyme results in conformational change

b) Phosphorylation of an enzyme occurs only at specific tyrosine residues

c) Phosphorylation of an enzyme is carried out by phosphoprotein phosphatases

d) Enzyme control by phosphorylation is irreversible

Answer: a

37. Which of the following enzyme catalyzes the first step of glycolysis?

a) Hexokinase

b) Pyruvate kinase

c) Glucokinase

d) Phosphofructokinase-1

Answer: a.

38. The general term used for the anaerobic degradation of glucose to obtain energy is

a) Anabolism

b) Oxidation

c) Fermentation

d) Metabolism

View Answer

Answer: c

39. Whenever the cell's ATP supply is depleted, which of the following enzyme's activity is increased?

a) Hexokinase

b) Pyruvate kinase

c) Glucokinase

d) Phosphofructokinase-1

View Answer

Answer: d

40. Cleavage of Fructose 1, 6-biophosphate yields

a) Two aldoses

b) Two ketoses

c) An aldose and a ketose

d) Only a ketose

Answer: c

41. Dihydroxyacetone phosphate is rapidly and reversibly converted to

a) Glyceraldehyde 3-phosphate

b) 1, 3-bis-phosphoglycerate

c) Fructose 1, 6-bisphosphate
d) Fructose 6-phosphate

Answer: a

42. The first step in the payoff phase of glycolysis is
a) Reduction of 1, 3-bisphosphoglycerate to glyceraldehyde 3-phosphate
b) Oxidation of glyceraldehyde 3-phosphate to 1, 3-bisphosphoglycerate
c) Reversible conversion of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate
d) Irreversible conversion of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate

Answer: b

43. The substrate used in the last step of glycolysis is
a) Glyceraldehyde 3-phosphate
b) Pyruvate
c) Phosphoenolpyruvate
d)1, 3-bisphosphoglycerate

Answer: c

44. High concentration of glucose 6-phosphate is inhibitory to
a) Hexokinase
b) Pyruvate kinase
c) Glucokinase
d) Phosphofructokinase-1

Answer: a

45. The product formed in the first substrate level phosphorylation in glycolysis is
a) Pyruvate
b) 3-phosphoglycerate
c) 1, 3-bisphosphoglycerate
d) 2-phosphoglycerate

Answer: b

46. Glycolysis converts
a) Glucose into pyruvate
b) Glucose into phosphoenolpyruvate
c) Fructose into pyruvate
d) Fructose into phosphoenolpyruvate
View Answer

Answer: a

47. Gluconeogenesis responds to which of the following?
a) Hormonal control
b) pH control
c) Temperature control
d) Blood control

Answer: a

48. When blood sugar levels fall, glycolysis is halted in liver to allow
a) Homeostasis
b) Anaerobic respiration
c) Aerobic respiration
d) Gluconeogenesis

Answer: d

49. How many steps are catalyzed by same enzymes in both glycolysis and gluconeogenesis?
a) 6
b) 7
c) 8
d) 9

Answer: b

50. How many steps are catalyzed by different enzymes in glycolysis and gluconeogenesis?
a) 2
b) 3

c) 4

d) 5

Answer: b

51. Three reactions of glycolysis are so exergonic, which are not catalyzed by

a) Hexokinase

b) PFK-1

c) Pyruvate kinase

d) Pyruvate dehydrogenase

Answer: d

52. What are the effects of increased concentration of citrate?

a) Increases the inhibitory effect of ATP

b) Decreases the inhibitory effect of ATP

c) Increases the activity of ATP

d) Increases the activity of AMP

Answer: a

53. The second control point in gluconeogenesis is the reaction catalyzed by

a) Pyruvate kinase

b) Pyruvate dehydrogenase

c) FBPase-1

d) PFK-1

Answer: c

54. Which of the following statements is true about PFK-1?

a) It is stimulated by AMP and ADP

b) It is stimulated by citrate and ATP

c) It is inhibited by AMP and ADP

d) It is stimulated by citrate and ADP

Answer: a

55. Which of the following statements is true regarding acetyl co-A?

a) It stimulates pyruvate dehydrogenase

b) It stimulates pyruvate carboxylase
c) It inhibits pyruvate carboxylase
d) It stimulates hexokinase

Answer: b

56. Which of the following is a potent regulator of glycolysis and gluconeogenesis?
a) Fructose 2, 6-bisphosphate
b) Fructose 1, 6-bisphosphate
c) Fructose 6-phosphate
d) Glucose 1, 6-bisphosphate

Answer: a

57. Glucagon and epinephrine stimulate glycogen breakdown to glucose 6-phosphate
a) Directly by binding to glycogen phosphorylase
b) Indirectly by first stimulating adenylate cyclase to make cAMP
c) Only in the liver
d) Only in muscle cells

Answer: b

58. The compounds responsible for coordinated regulation of glucose and glycogen metabolism are
a) NADH
b) NAD^+
c) Acetyl co-A
d) Fructose 2, 6-bisphosphate

Answer: d

59. Glycogen synthase a is activated by
a) Phosphorylation catalyzed by GSK3
b) Dephosphorylation catalyzed by GSK3
c) Phosphorylation catalyzed by pyruvate kinase
d) Phosphorylation catalyzed by pyruvate carboxylase

Answer: a

60. Glucagon is released from
a) Muscle
b) Pancreas
c) Kidneys
d) Epithelial tissues

Answer: b

61. Glucose 6-phosphatase is present only in
a) Liver
b) Muscle
c) Epithelial tissues
d) Kidneys

Answer: a

62. Which of the following enzymes is involved in the positive regulation of glycolysis/gluconeogenesis?
a) Hexokinase II
b) Hexokinase IV
c) PFK-2/FBPase-2
d) Pyruvate kinase

Answer: c

63. Which of the following enzymes is involved in fatty acid synthesis?
a) Hexokinase II
b) Hexokinase IV
c) PFK-2/FBPase-2
d) Pyruvate dehydrogenase

Answer: d

64. Which of the following is involved in triacylglycerol synthesis?
a) Hexokinase II
b) Acyl co-A glycerol transferases
c) PFK-2/FBPase-2
d) Pyruvate dehydrogenase

Answer: b

65. Which of the following is involved in pentose phosphate pathway?
a) Glucose 6-phosphate dehydrogenase
b) Acyl co-A glycerol transferases
c) PFK-2/FBPase-2
d) Pyruvate dehydrogenase

Answer: a

66. Which of the following enzyme's gene expression is slowed by insulin?
a) Hexokinase II
b) Hexokinase IV
c) PEP carboxykinase
d) Pyruvate kinase

Answer: c

67. Which of the following is true about the enzyme producing NADH from a triose phosphate in the glycolytic pathway?
a) It produces 1, 3-biphosphoglycerate and NADH
b) It catalyzes irreversible reaction
c) It uses NAD^+ and dihydroxyacetone phosphate as substrates
d) It uses $FADH_2$ and glyceraldehyde 3-phosphate as substrates

Answer: a

68. The major factor(s) determining whether glucose is oxidized by aerobic or anaerobic glycolysis is/are
a) Ca^{+2}
b) $FADH_2$

c) NADH and the ATP/ADP ratio

d) Presence of high AMP

Answer: c

69. When glucose is converted to lactate by anaerobic glycolysis, equivalent number of ATPs derived is?

a) 1

b) 2

c) 3

d) 4

Answer: b

70. When one molecule of glucose is oxidized to two molecules of lactate during anaerobic glycolysis, which of the following statements is false?

a) Glyceraldehyde 3-P dehydrogenase reaction produces 2 ATP molecules

b) Lactate dehydrogenase reaction produces no ATP

c) Pyruvate kinase reaction produces 2 ATP molecules

d) Phosphofructokinase-1 reaction uses 1 ATP molecule

Answer: a

71. In the reduction of pyruvate to lactate, which of the following is regenerated?

a) H^+

b) NADH

c) NAD^+

d) Na^+

Answer: c

72. For its activity, pyruvate decarboxylase requires

a) Mg^{+2}

b) Ca^{+2}

c) Na^+

d) H^+

Answer: a

73. TPP (thiamine pyrophosphate) is derived from

a) Vitamin A

b) Vitamin B1

c) Vitamin C

d) Vitamin B2

Answer: b

74. The end products in ethanol fermentation are

a) Ethanol and CO_2

b) Ethanol and O_2

c) Ethanol, H_2 and CO_2

d) Ethanol, O_2 and CO_2

Answer: a

75. Enzyme involved in the pathway of ethanol fermentation?

a) Hexokinase

b) Pyruvate decarboxylase

c) Pyruvate dehydrogenase

d) Pyruvate kinase

Answer: b

76. Enzyme involved in the pathway of synthesis of acetyl-coA

a) Hexokinase

b) Pyruvate decarboxylase

c) Pyruvate dehydrogenase

d) Pyruvate kinase

Answer: c

Part-B 5 x 2 =10 marks

1. **What is metabolism?**

2. **What is cori cycle?**
3. **What is fermentation?**
4. **How many ATP produces in the glycolysis pathway?**
5. **Explain the conversion of glucoe -6-phospate to glucose 1,6 biphospate.**

Part-C 5 x6 = 30 marks

1. **Write a note on Glycolysis pathway?**
2. **Explain alcoholic fermentation?**
3. **Write a note on Citric acid cycle?**
4. **Explain Lactate fermentation.**
5. **Write the biological importance of carbohydrate metabolism?**

KARPAGAM ACADEMY OF HIGHER EDUCATION

COIMBATORE-21

DEPARTMENT OF CHEMISTRY

SUBJECT: ANALYTICAL CLINICAL BIOCHEMISTRY

SUBJECT CODE: 17CHU404B

UNIT-I

PART-A

MULTIPLE CHOICE QUESTIONS (EACH QUESTIONS CARRY ONE MARK)

ONLINE EXAMINATION (20 X1 =20 MARKS)

1. Hexokinase

(a) is primarily used in the liver to initiate glycolysis.

(b) is only used by neurons to initiate glycolysis.

(c) is used to trap glucose for glycolysis.

(d) None of the above.

Answer:c

2. Glucokinase

(a) is used by skeletal muscle to initiate glycolysis.

(b) is used by the liver, and has a low affi nity for glucose.

(c) is used by the liver, and has a high affi nity for glucose.

(d) is not an isozyme of hexokinase.

Answer:b

3. The NET production of ATP in glycolysis is

(a) 2 ATP molecules. (b) 4 ATP molecules.

(c) 6 ATP molecules. (d) 38 ATP molecules.

Answer:a

4. The initial investment of ATP required in glycolysis is

(a) 4 ATP molecules.

(b) 2 ATP molecules.

(c) 1 ATP molecule.

(d) No ATP is required.

Answer:b

5. If a cell needs to continue using glycolysis for energy, it must replenish its supply of

(a) NAD+ molecules. (b) NADH molecules. (c) Protons. (d) H+ molecules

Answer:a

6. Lactate fermentation

(a) is used by yeast to replenish NAD+.

(b) is used by animal cells to replenish NAD+.

(c) is used by animal cells to replenish NADH.

(d) is used by animal cells to replenish NAD+, under aerobic conditions

Answer:b

7. In the pay-off phase of glycolysis, the total number of ATP molecules produced is

(a) 1. (b) 2. (c) 4. (d) 6.

Answer:c

8. Low levels of ATP in a cell

(a) activate phosphofructo-kinase by driving it to the L conformation.
(b) are correlated with high levels of ADP and AMP, which activate phosphofructo-kinase.
(c) only activate phosphofructo-kinase if levels of AMP are constant.
(d) have no effect on phosphofructo-kinase.

Answer:b

9. Fructose can be utilized in glycolysis

(a) by entry at step 3 in muscle cells, and step 5 in neurons.

(b) by entry at step 5 in muscle cells, and step 3 in liver cells.

(c) by entry at step 2 in muscle cells, and step 5 in liver cells.

(d) by entry at step 3 in muscle cells, and step 5 in liver cells.

Answer: d

10. The Warburg effect describes

(a) the tendency of cancer cells to rely on glycolysis, even under aerobic conditions.

(b) the tendency of cancer cells to quickly abandon glycolysis once oxygen is reintroduced.

(c) the heavy reliance of cancer cells on lactate dehydrogenase.

(d) None of the above.

Answer:a

11. In the fi rst step of the citric acid cycle

(a) acetyl-CoA is bound to the enzyme citrate synthase, and then it undergoes a condensation reaction with oxaloacetate.

(b) acetyl-CoA undergoes a free condensation reaction with oxaloacetate.

(c) oxaloacetate is bound to the enzyme citrate synthase and then it undergoes a condensation reaction with acetyl-CoA.

(d) oxaloacetate is bound to the enzyme acetate dehydrogenase, and then it undergoes a condensation reaction with acetyl-CoA.

Answer: c

12. In the citric acid cycle

(a) all steps are exergonic.

(b) all steps except 2 are exergonic.

(c) all steps except 2 are endergonic.

(d) all steps are endergonic.

Answer: b

13. The fi nal intermediate product in the citric acid cycle is

(a) L-malate.

(b) Acetyl-CoA.

(c) Oxaloacetate.

(d) Fumarate.

Answer:c

14. GTP is synthesized in the citric acid cycle

(a) in the transformation of succinyl-CoA to succinate.

(b) in the transformation of succinate to fumarate.

(c) in the transformation of succinate to succinyl-CoA.

(d) GTP is *not* synthesized in the citric acid cycle, 1 molecule of ATP is.

Answer:a

15. Isocitrate dehydrogenase

(a) is activated by high concentrations of ATP and NADH.

(b) is activated by high concentrations of ATP and NADPH.

(c) is unaffected by high concentrations of NADPH.

(d) is inhibited by high concentrations of high-energy compounds.

Answer:d

16. The fuel of the citric acid cycle could be described as

(a) acetyl-CoA plus oxaloacetate, which participate in the first step of the cycle.

(b) only acetyl-CoA, oxaloacetate is a recycled intermediate.

(c) acetyl-CoA and H2O.

(d) acetyl-CoA and GDP.

Answer:b

17. The electron transport chain

(a) creates a proton gradient across the inner mitochondrial membrane, with a low proton concentration in the mitochondrial matrix.

(b) creates a proton gradient across the inner mitochondrial membrane, with a low proton concentration in the intermembrane space.

(c) creates a proton gradient across the outer mitochondrial membrane, with a high proton concentration in the intermembrane space.

(d) creates a proton gradient across the outer mitochondrial membrane, with a low proton concentration in the intermembrane space.

Answer:a

18. The largest amount of ATP production is obtained from

(a) the oxidation of succinate dehydrogenase.

(b) the oxidation of NADH molecules.

(c) the oxidation of FADH2 molecules.

(d) through the electron chain.

Answer:b

19. In the electron transport chain, complex II

(a) does not contribute to the proton gradient, but mediates the transfer of electrons from succinate to cytochrome a.

(b) does not contribute to the proton gradient, but mediates the transfer of electrons from succinate to cytochrome c.

(c) does not contribute to the proton gradient, but mediates the transfer of electrons from succinate to coenzyme Q.

(d) contributes to the proton gradient, and mediates the transfer of electrons from succinate to coenzyme Q.

Answer:c

20. The transport of protons back into the mitochondrial matrix appears to

(a) cause a conformational change in a *b*-subunit that changes it from the L to the T state allowing ADP and inorganic phosphate to bind forming ATP.

(b) cause a conformation change in a *g*-subunit that changes it from the L to the T state allowing ADP and inorganic phosphate to bind forming ATP.

(c) cause a conformational change in a *b*-subunit that changes it from the T to the O state releasing all ATP molecules.

(d) cause a conformational change in a *b*-subunit that changes it from the O to the L state allowing ADP and inorganic phosphate to bind forming ATP.

Answer:a

21. In the citric acid cycle, the conversion of citrate to isocitrate

(a) is done to make the molecule more suitable for reduction.

(b) is slightly endergonic.

(c) is done to make the molecule more suitable for oxidation.

(d) is heavily exergonic.

Answer:c

22. The enzymatic activity of citrate synthase can be described as

(a) an induced fi t process.

(b) as an uncompetitive inhibition process.

(c) as a competitive inhibition process.

(d) as a lock and key process.

Answer:a

23. Isocitrate dehydrogenase is considered a regulatory enzyme in the citric acid cycle because

(a) its activity is inhibited by the presence of citrate.

(b) its activity is inhibited by the presence of ADP.

(c) its activity is inhibited by the presence of high-energy compounds.

(d) its activity is regulated by cofactors produced in the cycle.

Answer:c

24. Succinate dehydrogenase is a regulatory enzyme in the citric acid cycle primarily because

(a) its activity is inhibited by an accumulation of oxaloacetate.

(b) its activity is inhibited by an accumulation of malate.

(c) its activity is inhibited by high-energy compounds.

(d) its activity is inhibited by an accumulation of citrate.

Answer:a

25. Cataplerotic reactions are beneficial because

(a) they catalyze the production of an extra GTP molecule.

(b) they catalyze the production of an extra NADH molecule.

(c) they utilize intermediate compounds in the citric acid cycle, preventing their buildup in the mitochondria.

(d) they extract extra energy from the citric acid cycle.

Answer:c

26. The electron transport chain

(a) generates 5 ATP molecules.

(b) prepares a proton gradient which makes ATP production possible.

(c) utilizes large amounts of oxygen, so is damaging to the cells.

(d) establishes an electron gradient, making ATP production possible.

Answer:b

27. Which out of the following statements is true about regulation of metabolic pathway?

a) Most of the metabolic pathways are regulated

b) Most of the metabolic pathways are not regulated

c) Regulation of metabolic pathways always involves changing the amount of enzymes

d) Metabolic regulation always depends on control by hormones

Answer: a

28. The rate of breakdown of metabolites is termed as

a) Metabolic state

b) Metabolism

c) Steady state

d) Homeostasis

Answer: c

29. Diminished delivery of oxygen to tissues is termed as

a) Hypoxia

b) Ischemia

c) Homeostasis

d) Metabolism

Answer: a

30. Diminished flow of blood to tissues is termed as

a) Hypoxia

b) Ischemia

c) Homeostasis

d) Metabolism

Answer: b.

31. Which of the following statements is true about the control of muscle glycogen phosphorylase?

a) It is activated by phosphorylation by an active phosphorylase kinase

b) It is allosterically activated by ATP

c) It is allosterically activated by cAMP

d) Normally it exists in active form

Answer: a

32. Which of the following is not a factor determining the activity of an enzyme?

a) Association with regulatory protein

b) Sequestration

c) Allosteric regulation

d) Nucleotides

Answer: d

33. Which of the following statements is true?

a) High insulin/glucagon ratio activates lipolysis in muscle

b) High insulin/glucagon ratio inhibits lipolysis in liver

c) High insulin/glucagon ratio activates lipolysis in adipocytes

d) Low insulin/glucagon ratio activates lipolysis in adipocytes

Answer: d

34. Which of the following type of metabolite is used for generating glucose under severe starvation conditions?

a) Amino acids

b) Fats

c) Glycogen

d) Starch

Answer: a

35. Which of the following statements is true about brain metabolism in starvation?

a) The brain can use glucogenic amino acids for energy

b) The brain can only use glucose as fuel

c) Up to a quarter of energy requirement of the brain can come from fatty acids
d) Up to a half of energy requirement of the brain can come from ketone bodies

Answer: a

36. One of the following statements about the control of enzyme activity by phosphorylation is correct
a) Phosphorylation of an enzyme results in conformational change
b) Phosphorylation of an enzyme occurs only at specific tyrosine residues
c) Phosphorylation of an enzyme is carried out by phosphoprotein phosphatases
d) Enzyme control by phosphorylation is irreversible

Answer: a

37. Which of the following enzyme catalyzes the first step of glycolysis?
a) Hexokinase
b) Pyruvate kinase
c) Glucokinase
d) Phosphofructokinase-1

Answer: a.

38. The general term used for the anaerobic degradation of glucose to obtain energy is
a) Anabolism
b) Oxidation
c) Fermentation
d) Metabolism
View Answer

Answer: c

39. Whenever the cell's ATP supply is depleted, which of the following enzyme's activity is increased?
a) Hexokinase
b) Pyruvate kinase
c) Glucokinase
d) Phosphofructokinase-1
View Answer
Answer: d

40. Cleavage of Fructose 1, 6-biophosphate yields

a) Two aldoses

b) Two ketoses

c) An aldose and a ketose

d) Only a ketose

Answer: c

41. Dihydroxyacetone phosphate is rapidly and reversibly converted to

a) Glyceraldehyde 3-phosphate

b) 1, 3-bis-phosphoglycerate

c) Fructose 1, 6-bisphosphate

d) Fructose 6-phosphate

Answer: a

42. The first step in the payoff phase of glycolysis is

a) Reduction of 1, 3-bisphosphoglycerate to glyceraldehyde 3-phosphate

b) Oxidation of glyceraldehyde 3-phosphate to 1, 3-bisphosphoglycerate

c) Reversible conversion of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate

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Answer: b

43. The substrate used in the last step of glycolysis is

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Answer: c

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c) Pyruvate kinase reaction produces 2 ATP molecules

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Answer: a

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a) H^+

b) NADH

c) NAD^+

d) Na^+

Answer: c

72. For its activity, pyruvate decarboxylase requires

a) Mg^{+2}

b) Ca^{+2}

c) Na^+

d) H^+

Answer: a

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a) Vitamin A

b) Vitamin B1

c) Vitamin C

d) Vitamin B2

Answer: b

74. The end products in ethanol fermentation are

a) Ethanol and CO_2

b) Ethanol and O_2

c) Ethanol, H_2 and CO_2

d) Ethanol, O_2 and CO_2

Answer: a

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b) Pyruvate decarboxylase

c) Pyruvate dehydrogenase

d) Pyruvate kinase

Answer: b

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b) Pyruvate decarboxylase

c) Pyruvate dehydrogenase

d) Pyruvate kinase

Answer: c

UNIT-2

Lecture Notes

Syllabus

Unit II

Proteins: Classification, biological importance; Primary and secondary and tertiary structures of proteins: α-helix and β- pleated sheets, Isolation, characterization, denaturation of proteins. Enzymes: Nomenclature, Characteristics (mention of Ribozymes), Classification; Active site, Mechanism of enzyme action, Stereospecificity of enzymes, Coenzymes and cofactors, Enzyme inhibitors, Introduction to Biocatalysis: Importance in "Green Chemistry" and Chemical Industry.

Protein Structure

Introduction

A *protein* is a large organic molecule constructed of a chain of amino acids. The word protein, derived from the Greek *proteios*, loosely means "holding fi rst place"— an apt name because proteins regulate the life machine. They orchestrate the business of life by controlling multiple bioprocesses including metabolism, cell growth, and neurotransmission. And, although proteins provide structure and can act as energy source, the main reason they are so important is their role as enzymes—enabling chemical reactions that are critical to life. Proteins can do this because they have an ability to move electrons and protons around biosystems.

The sequence of amino acids that form a protein is encoded in our genes, held in the DNA. The collection of proteins produced by a given organism is called the organism's *proteome*. The differences among proteomes of different organisms create different life forms. It is no surprise that researchers throughout the bioscience community are focusing on the huge repertoire of proteins, approximately, 100,000 distinct molecules, produced by the human genome.

To obtain a basic understanding of proteins, you need to know:

• General characteristics of proteins
• The structure of amino acids (the building blocks of proteins)
• How amino acids are linked together
• How the sequence of amino acids on a polypeptide chain results in the threedimentional structure of the molecule

General Characteristics of Proteins

Biomolecules exhibit a pattern of modular assembly, meaning that they are built from repeating fundamental units. In the case of a protein, the primary structure is a string of *amino acids*. An amino acid molecule is characterized by the presence of an *amine* (NH2) and a carboxyl group (COOH) on the first (alpha [*a*]) carbon (Fig. 6-1). The other groups bonded to the *a*-carbon are represented by the symbol R.
The exception is proline, discussed below.

One of the truly remarkable features of proteins is the fact that all of these diverse chemicals are built from only 20 major amino acids. That's really pretty simple, considering the huge diversity of protein function in the life machine. You should be grateful for this, biochemist, since this means *just 20 different molecules* control almost all of our life functions. Not only ours, as human beings, but for *all life forms* on earth. We are all made of the same stuff, from the tiniest mycoplasma to the president of the United States. Note that while there are only 20 major amino
acids, there are hundreds of *minor* amino acids. These are amino acids that are either short lived or are simple derivatives of the major amino acids.

To understand why nature can keep things so simple, think of the English alphabet as an analogy. There are only 26 letters in the alphabet that almost any child can learn. Despite this small number, the letters of the alphabet can be arranged in a myriad of different ways to produce wildly different strings of characters or messages.

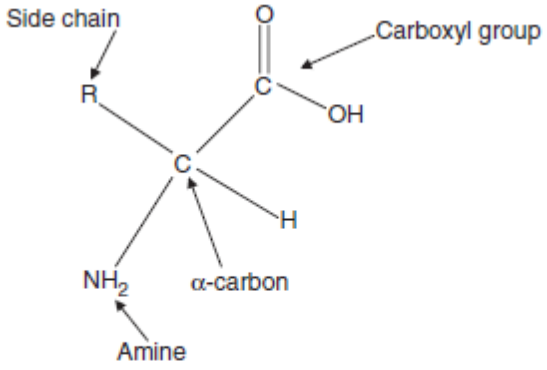


Figure 6-1 Structure of an amino acid.

For example, how many messages can you construct that contain 100 letters?
Since there are 26 letter choices for *each* character in the message, there are on the order of

$$26 \times 26 \times \cdots \times 26 \approx 10^{141}$$

possible character messages! This is a very large number indeed. Similar logic applies to the construction of proteins. There are a very large number of molecules that can be constructed by arranging the 20 major amino acids in chains—about two quadrillion, or 2,000,000,000,000,000

to be exact. This diversity is possible because each point in the peptide chain can hold any one of the 20 amino acids. Also, the length of a protein can be up to several thousand amino acids.

Returning to our analogy with the 26-letter alphabet, the astute reader will note that not all 10141 character messages are valid. In fact *most* randomly assembled messages would be completely meaningless. The construction of proteins from amino acids works this way as well. Not all of the two quadrillion combinations of amino acids produce functioning proteins. To form a polypeptide chain, the carboxyl group of one *a*-carbon on one amino acid links with the amine group of the *a*-carbon of the next amino acid. This happens via the removal of an OH from the carboxyl group and an H atom from the amine to form H2O. The result is a *peptide bond* as shown in Fig. 6-2.

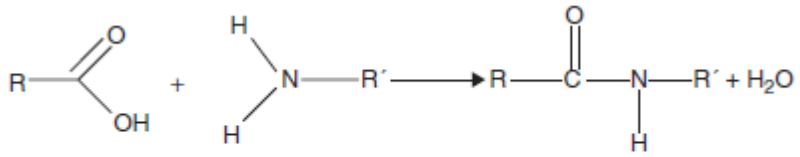


Figure 6-2 Peptide bond.

As is typical of the assembly of biomolecules, the formation of a peptide bond requires energy. The result of the initial step of attaching amino acids together through peptide bonds is a long, linear chain of *a*-carbons in a nice row, called a *polypeptide.* Proteins are initially produced as polypeptides of 300 to 1000 amino acids. Functional proteins may consist of a single, long chain. However, many proteins are composed of two or more chains. Also, some bioactive molecules consist of only about 10 amino acids and are called *oligopeptides*.

Most proteins contain between 50 and 2000 amino acids. When we discuss the configuration of the protein, it will be important to remember that the main chain of the linked *a*-carbons forms a flat and planar backbone for the protein. The basic structure can bend to an extent—rather like a stiff piece of poster board—but cannot rotate around the peptide bond axis with the freedom of rotation enjoyed by their side chains.

In addition, the backbone carbon chain is itself rich in hydrogen-bonding potential because each residue has a carbonyl group C==O which is a good hydrogen-bond acceptor (attracts electrons) and an HN group which is a good hydrogen-bond donor (partially gives up electrons). Remember that a hydrogen bond doesn't involve an exchange of electrons, which is simply due to the creation of electromagnetic dipoles within molecules. This capacity to form hydrogen bonds means that, in the backbone of a polypeptide chain, weak attachments can be formed between the amino acids, *tending to bend or twist the chain*.

The *a*-carbon of an amino acid also bears a single hydrogen and the diverse group symbolized as "R." "R" stands for the side chains. Different amino acids have different R groups attached to their *a*-carbon. In the primary structure of a protein, the molecules are put together by

the ribosomes in a strand, like strings of pearls, and the side chains protrude from the long row of *a*-carbons. After release from the ribosomes, the R groups interact causing the strand to twist and distort. The final configuration of the protein depends on the order of the amino acids, which determines the interactions between the side groups.

DETAILS OF AMINO ACIDS

Amino acids in solution at neutral pH exist mainly as *zwitterions* rather than as unionized molecules. Zwitterions have both a positive and a negative charge (Fig. 6-3). The amino group carries a positive charge (NH_3^+) and the carboxyl group carries a negative charge (CO_2^-). The presence of both charges means that the molecule can act as both an acid and a base, that is, *amphoteric*. For reasons unknown to us, all naturally occurring amino acids are in the "L" configuration, meaning simply that, in solution, they rotate a beam of light to the left.

Note that if you produce amino acids in a laboratory, you will produce a *racemic mixture* of both L and D confi gurations. This may be a problem to those of you that plan to use your laboratory-produced molecules in a biological system, because the *unnatural* configuration may not function properly. So biomolecules exhibit *handedness*. (Some bacteria have small amounts of the D-amino acids.)

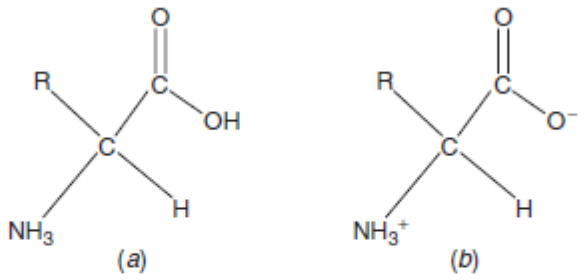


Figure 6-3 The general form of amino acids (*a*) written as a neutral amino acid, and (*b*) written in the zwitterionic form.

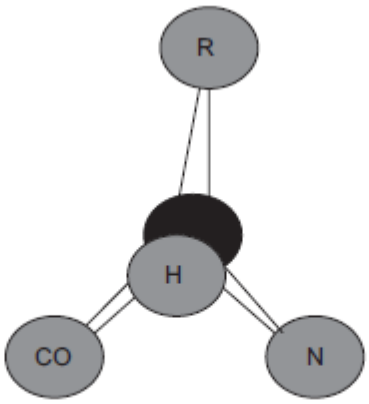


Figure 6-4 Orientation of atoms around the α-carbon of amino acids.

A handy acronym will help you to remember how the groups are arranged around the *a*-carbon of amino acids (Fig. 6-4). Looking at the molecule from the top with the hydrogen atom sticking toward you, you will observe that the other groups are arranged (from left to right) as C==O, R and NH_2. Reading from left to right and taking the CO, the R and the N, you spell CORN.

We will divide the 20 major amino acids into three groups:

1. Nonpolar side chains
2. Uncharged polar side chains
3. Charged polar side chains

The characteristics of the major amino acids are summarized in Table 6-1. Before we start examining amino acids in detail, it would be wise to review some basic terms in organic chemistry provided in App. A.

In a nutshell, the nonpolar amino acids lack amine, hydroxyl, or sulfhydryl groups in their side chains. The polar side chains have such groups. Whether or not these groups are actually ionized at a physiological pH depends on the pKa for the dissociation of the protein member of the group.

Nonpolar Side Chains

With the exception of glycine, proteins with nonpolar side chains avoid the watery environment of the cell. These consist of amino acids whose side chains are long chains of methyl and methylene groups and are hydrophobic. These molecules do not ionize and, because the carbon and the hydrogen share the electron equally, these molecules do not exhibit electromagnetic poles that create hydrogen bonding. There are no ionizable protons. These are *glycine, valine, alanine, leucine, isoleucine, proline, phenylalanine,* and *tryptophan*. Their side groups contain just carbon and hydrogen, as methyl or methylene groups. These hydrophobic groups tend to cluster

Table 6-1 Summary of Characteristics of Major Amino Acids

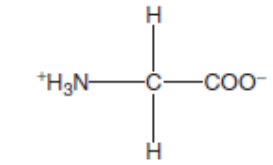
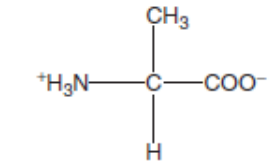
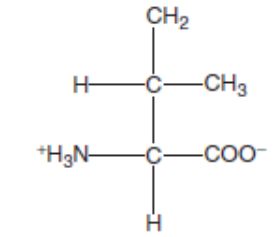
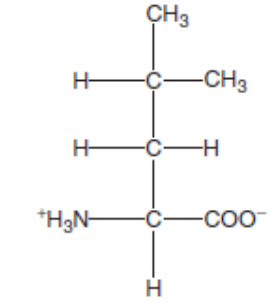
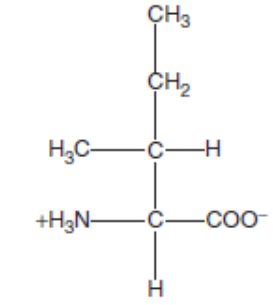
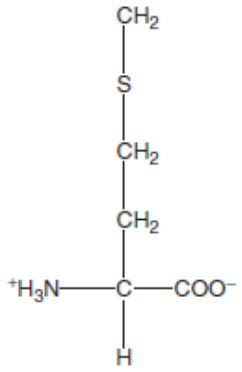
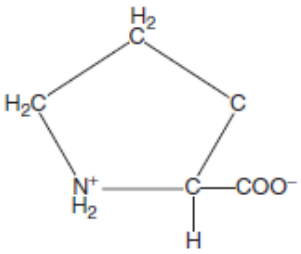
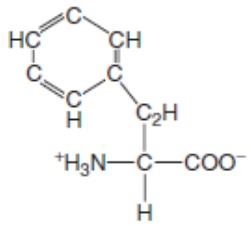
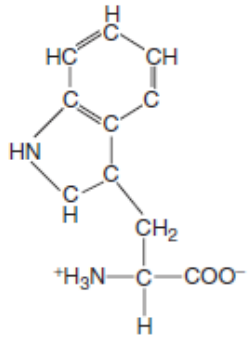
Name	Abbreviation(s)	Unique Features	Structure
Nonpolar, hydrophobic side chains—found in protein interiors			
Glycine	Gly, G	• Smallest • R is a single proton • Flexible • Neuroinhibitor • Role in biosynthesis of many compounds, such as purines	
Alanine	Ala, A	• Methane • α-Keto homologue is pyruvate • Role in nitrogen transport from tissues to the liver	
Valine	Val, V	• Butyl group • Branched chain amino acid found in high concentration in muscles	
Leucine	Leu, L	• Branched chain amino acid found in high concentration in muscles	
Isoleucine	Ile, I	• Isomer of leucine • Branched chain amino acid found in high concentration in muscles	

Table 6-1 Summary of Characteristics of Major Amino Acids (*Continued*)

Name	Abbreviation(s)	Unique Features	Structure
Nonpolar, hydrophobic side chains—found in protein interiors			
Methionine	Met, M	• One of two amino acids containing sulfur • Contains a sulfur as an ester • Sulfur easily oxidized • As s-adenosyl-L-methionine (SAM), a methyl donor in many bioreactions	
Proline	Pro, P	• α-Carbon forms a ring containing primary amine • Inflexible • Forms kinks in secondary structures	
Phenylalanine	Phe, F	• Alanine plus a phenyl • Converted to tyrosine, which is, in turn, converted to L-dopa • Interferes with the production of serotonin	
Tryptophan	Trp, W	• Bulky, aromatic side chains • Indole group • Precursor for serotonin and niacin	

(*Continued*)

Table 6-1 Summary of Characteristics of Major Amino Acids (*Continued*)

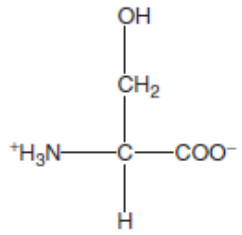
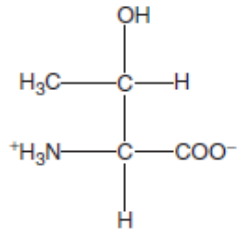
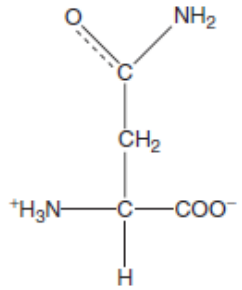
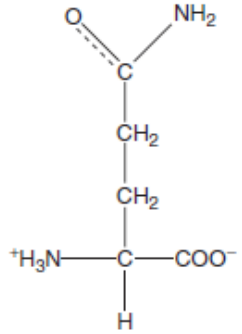
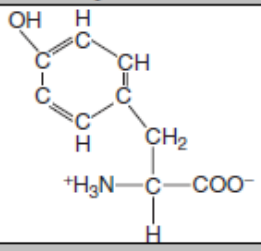
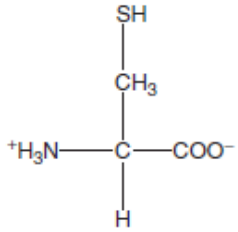
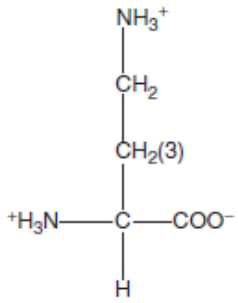
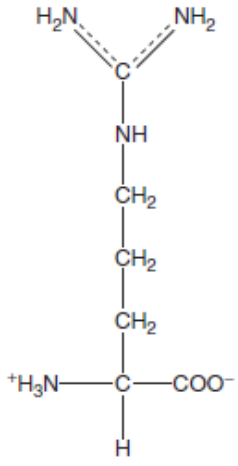
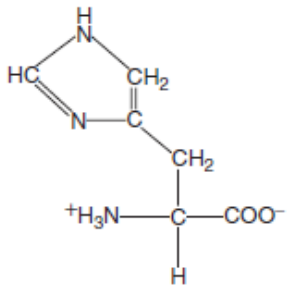
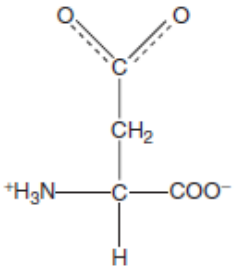
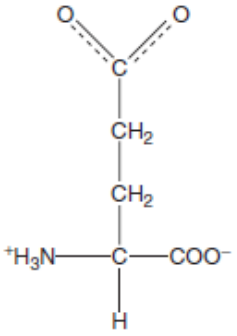
Name	Abbreviation(s)	Unique Features	Structure
Uncharged polar side chains—metabolically active and located on the exterior of proteins			
Serine	Ser, S	• Hydroxyl group • Found at the active site of enzymes • Aids in glycoprotein formation	
Threonine	Thr, T	• Methyl and hydroxyl group • Found at the active site of enzymes • Aids in glycoprotein formation	
Asparagine	Asn, N	• Methyl group and carboxyl group with a highly polar uncharged amine that readily forms hydrogen bonds • Found at the ends of alpha helices and beta sheets • Aids in formation of glycoproteins • Input to urea cycle • α-Keto homologue is oxaloacetate	
Glutamine	Gln, Q	• Two methyl groups and carboxyl group with a highly polar uncharged amine • Forms isopeptide linkages • Central role as nitrogen donor in synthesis of nonessential amino acids • Provides nitrogen transport to the liver	

Table 6-1 Summary of Characteristics of Major Amino Acids (*Continued*)

Name	Abbreviation(s)	Unique Features	Structure
Uncharged polar side chains—metabolically active and located on the exterior of proteins			
Tyrosine	Tyr, Y	• Similar to phenylalanine but with polar hydroxyl group on phenyl ring • Important metabolically because ionization altered by micro pH changes	
Charged polar side chains—reactive			
Cysteine	Cys, C	• Sulfhydryl (thiol) group • Forms disulfide bridges • Found at the active site of enzymes • Binds iron	
Lysine	Lys, K	• Long aliphatic chain terminating in an amine • Nucleophilic • Forms ionic bonds	
Arginine	Arg, R	• Long aliphatic chain containing an amine and terminating in two amines • Nucleophilic • Forms ionic bonds • Generated in the urea cycle	

(*Continued*)

Name	Abbreviation(s)	Unique Features	Structure
Charged polar side chains—reactive			
Histidine	His, H	• Methyl • Imidazole group • Ionic bonds found at the active site of enzymes • Crucial in the structure of hemoglobin	
Aspartate or aspartic acid	Asp, D	• Donates an amine to become oxaloacetate • Active in proteolytic enzymes	
Glutamate or glutamic acid	Glu, E	• Central role as nitrogen donor in synthesis of nonessential amino acids • Provides nitrogen transport to the liver	

together in the interior of the molecule and thereby to shield themselves against the aqueous environment. Glycine, however, can be found in the interior or the exterior of a protein. Two members of this group have a great effect on the final configuration of a protein. Glycine, the simplest amino acid, has an R group that is simply a proton. Glycine's small size enables it to fit into tight spots. Remember this when we talk about collagen.

In contrast, proline has a side chain of methylenes covalently bonded to the nitrogen atom of the backbone. This forms a five-membered pyrrolidine ring that rigidly holds the amino acid in a single conformation. The presence of proline can cause kinks in an otherwise smoothly folded peptide chain. Proline initiates some of the structures regularly found in proteins such as the *a*-helix. Also, no hydrogen bonding is possible with the *a*-carbon because no amide hydrogen is
present.

Uncharged Polar Side Chains

The second group consists of amino acids whose side chains are hydrophilic because they have either hydroxyl (OH), sulfhydryl (SH), or amine (NH_2) groups. But, even though they contain ionizable groups, these groups are minimally ionized at the neutral pH of the cell. The group contains *serine, threonine, asparagine, glutamine, tyrosine,* and *cysteine.* Remember that these side groups are not charged but exhibit electromagnetic dipoles and can participate in hydrogen bonds. These dipoles are created because hydrogen and oxygen, hydrogen and sulfur, and hydrogen and nitrogen do not share electrons equally. Because they are uncharged, these amino acids also tend to occur on the inner side of the protein molecule. However, unlike the nonpolar amino acids, they stabilize protein structure and enable folding by participating in interior hydrogen bonds. Also, the ability to exchange protons or electrons in cellular microenvironments of varying pH makes these amino acids potentially active in the fundamental business of regulating bioprocesses.

Cysteine is very important in infl uencing the structure of a protein. In cysteinerich proteins, two cysteine residues are frequently covalently linked to each other (through oxidation of the SH groups) to produce a disulfi de bridge. The resulting unit of two molecules of cysteine is called *cystine* (Fig. 6-5).

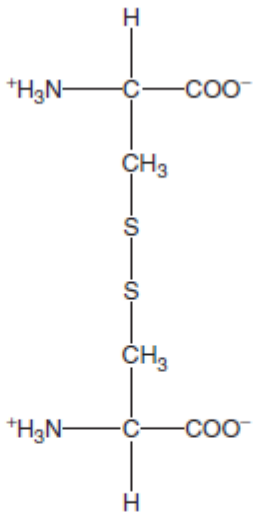


Figure 6-5 Formation of cystine.

Disulfide bonds crosslink proteins and stabilize the protein structure. Proteins are held together primarily by relatively weak hydrogen bonds, so disulfide bonds can have a very large effect on the stability of the protein structure. Extracellular proteins often have disulfide bonds whereas intracellular proteins usually lack them. Sulfhydryl groups are very important in other ways. The sulfhydryl group commonly occurs at the active site of enzymes. Also, the sulfhydryl

groups can form a complex with iron (Fe), forming Fe-S proteins, or *ferredoxins*. Serine and threonine contain carboxylic acids (COOH). The hydroxyl groups can accept or donate a proton giving them an important role in the biosystem machinery. They are important in a number of active functions, as well as participating in structural H-bonding. Serine is located at active site of a number of enzymes, where the hydroxyl group participates in hydrolytic reactions. Serine and threonine also aid in the binding of nonamino acid molecules to proteins. For example, the hydroxyl group at the surface of the protein can be used to build oxygen bridges and to bind sugars, making glycoproteins.

The hydroxyl group of tyrosine is poorly ionized at neutral pH because the ring structure tends to hold on to the electron. However, ionization increases at more basic pHs which can occur in a microenvironment of a biochemical reaction. Tyrosine is found at the active site of some enzymes. The ionization of the side chain can be increased by manipulation of the local pH to create basic conditions.

Unlike serine and threonine, asparagine, and glutamine are amides rather than carboxylic acids. Their side chains do not ionize and are not very reactive. However, the amide group is very polar and participates in hydrogen bonds as both hydrogen bond donor and acceptor.

Glutamine participates in linkages between protein chains in a special covalent linkage, called an isopeptide bond. Isopeptide bonds are similar to peptide bonds except the amino group participating in the bond is not the *a*-amino group. This link leads to proteins that are tightly bound, like keratin molecules in hair and in formation of a fi brin blood clot.

Asparagine is used to bind sugars to proteins to form glycoproteins. Interestingly, unlike normal cells, some cancer cells cannot make asparagines and must acquire this amino acid from the diet. It is thought that if cancer cells can be denied a source of asparagine, this could offer an innovative cancer treatment option.

Charged Polar Side Chains

The amino acids with charged side chains at neutral pH are *lysine, arginine, histidine, aspartic acid,* and *glutamic acid. Lysine* and *arginine* are basic because of the presence of amines. Lysine is an active *nucleophile* (donate a pair of electrons to a chemical bond) and participates in a variety of reactions. Arginine contains a guanidine (i.e., has three nitrogen atoms) and is also strongly basic.

Histidine has an *imidazole* ring in the side chain (a ring structure containing nitrogen). This makes it nucleophilic (electron rich) and basic like other amines. In the nonprotonated form of imidazole, the nitrogen-hydrogen is a hydrogen bond donor while the carbon-bonded nitrogen is a hydrogen bond acceptor. The imidazole ring renders histidine very versatile. Histidine is found in the active sites of enzymes because the imidazole ring can bind and release protons in the course of enzymatic reactions. Aspartic and glutamic acid are important in many biosynthetic reactions. The conjugate base of glutamic acid, glutamate, plays a crucial role in nitrogen

metabolism as a nitrogen donor to form nonessential amino acids and as a nitrogen acceptor in transporting nitrogen to the kidneys.

In summary, the 20 major amino acids provide great versatility within the protein structure. The carbon backbone itself is rich in structures that form hydrogen bonds. The hydrophobic side chains of some amino acids cause portions of the molecule to behave like a lipid and seek the inside of the molecule structure. Others are ionized and found on the outside of the protein molecule where they can interact with their environment. Many of the side chains form hydrogen bonds within a protein. Two can form strong covalent bonds either within one polypeptide chain or between polypeptide chains, one involving sulfi de atoms (cysteine) and one utilizing a carboxyl and amide groups of the side chains in a bond, like the peptide bond (glutamine). Those amino acids possessing surface hydroxyl groups or highly basic nitrogen groups tend to be found at the active side of enzymes because of their ability to accept or donate electrons.

FORMATION OF PROTEINS FROM AMINO ACIDS

Proteins are constructed on the ribosomes, where the genetic code is implemented via strands of RNA. The way this is done is discussed in detail in a later chapter. For our purposes, suffice it to say that the sequence of amino acids is dictated by the genetic code and read out by the ribosomes. As you go through this discussion, look at it like an entrepreneur—an entrepreneur who is interested in the production of proteins.

The key to the proper function of many proteins is their *shape*. The shape is formed as the protein folds. Proteins with exactly the same amino acids, but arranged in different order, can be shaped differently and hence perform a different function. And, as we will see below, identical proteins may or may not share the same function, depending on how they are folded. If folded improperly, proteins may fail to function or may accumulate into insoluble aggregates known as *inclusion bodies.*

The shape of a protein develops in stages. The structure of the original protein as it is created on the ribosome is known as the *primary structure.* The primary structure is a long string of amino acids forming *polypeptide chains.*

When complete, the primary polypeptide chain looks rather like a lengthy caterpillar—only it is flat. Extruding from this creature are numerous protrusions. Each segment of this caterpillar bares quite different protrusions. The protrusions are endowed with forces that affect the movement of the caterpillar. One protrusion is very tiny and can fit into a small space, should the creature curl around tightly. One is unusually bulky and tends to spread things out. One forms a loop upon itself and creates a knot. Some seek protection from their environment. These tend to be found inside the creature when it curls up. Others exhibit an attraction to one another (through hydrogen bonds) and induce the creature to curl. Some form attachments either between protrusions (because they are ionized through presence of hydroxyl or amine groups). One

protrusion bonds with a like protrusion, creating an unusually strong force to hold the creature in a given confi guration.

Protein structures generally are described at four levels: primary, secondary, tertiary, and quaternary. A *primary structure* is simply the two-dimentional linear sequence of amino acids in the peptide chain. See Fig. 6-6.

The secondary structure is formed as the protein begins to twist in accordance with chemical forces within the primary chain. The hydrogen in the amine groups and the oxygen in the carboxyl groups of the backbone form hydrogen bonds that stabilize the secondary structures.

Secondary structure commonly takes one of two forms. One form is a left-handed helix that is developed as the molecule twines around itself. This form is called an *a-helix* (Fig. 6-7).

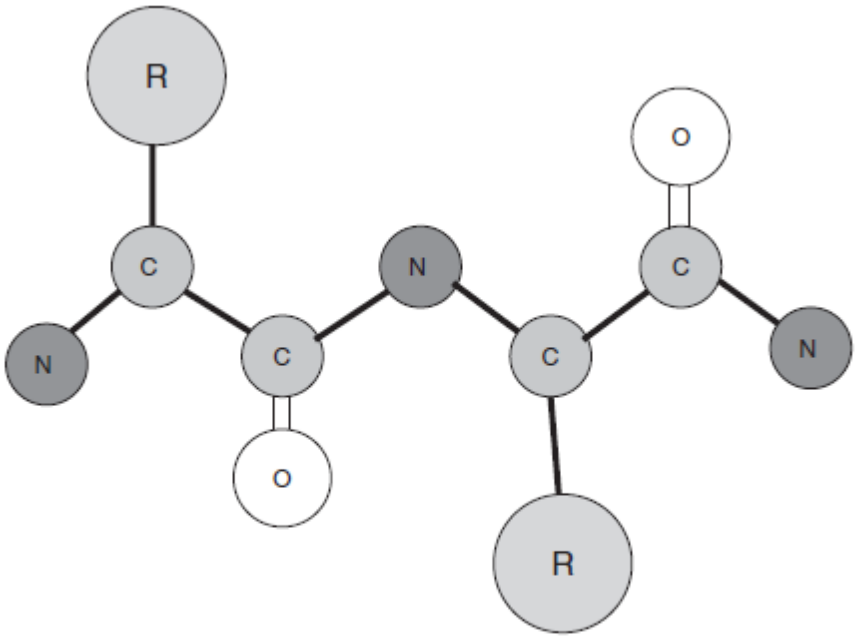


Figure 6-6 Protein primary structure.

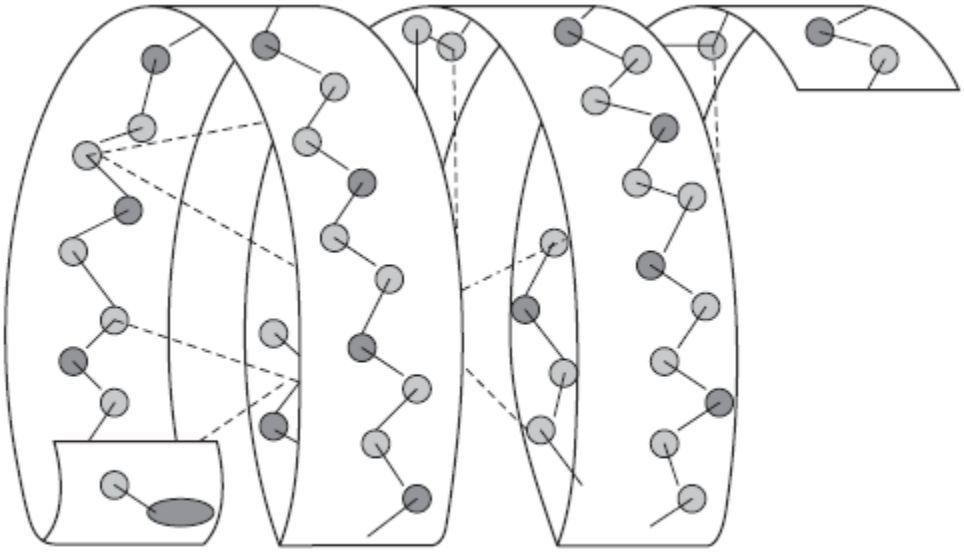


Figure 6-7 α-Helix.

As is usual with biological terminology the alpha designation has nothing whatsoever to do with this confi guration, but is part of the name because this was the first configuration discovered. In the *a*-helix, stabilizing hydrogen bonds formed between amine and carboxyl groups on the carbon backbone are tight. The side chains protrude from the exterior of the helix.

Globular proteins typically contain the *a*-helix form. As the name implies, globular proteins are globelike in form and do not have structural strength. Hormones are globular proteins. Also, some structural proteins also consist of *a*-helices. For example, *keratin* is a family of structural *fibrous proteins* that include *a*-keratin. Hair and other *a*-keratins consist of coiled single protein strands, which are then further coiled into superhelical structures. These structures can stretch out and return to the original confi guration. The supercoils contain a high percentage of cysteine residues that form stabilizing cross-links. The sulfur from the cysteine accounts for the distinct odor of burning hair. Extensive cysteine cross-links between *a*-coils form the hard structures of hooves and nails.

The other secondary form is a crimped shape called a *β-pleated sheet.* A *β-pleated* sheet looks a little like a piece of tin (Fig. 6-8). The initial polypeptide chains form links between carboxyl and amine groups on adjacent chains. *β-Sheets* can consist of peptide chains that run in opposite directions (antiparallel) or in the same direction (parallel) (Fig. 6-9). Either way, the hydrogen of amine groups on one chain form hydrogen bonds with the oxygen of carboxyl groups on another chain. *β*-Sheets contain a high percentage of the smallest amino acids, glycine, and alanine. The lack of bulky side chains usually allows tight linkage of adjacent peptide

strands. *β*-Sheets are usually found in structural proteins, such as silk and the *β-keratin proteins* found in claws, beaks, and shells of turtles.

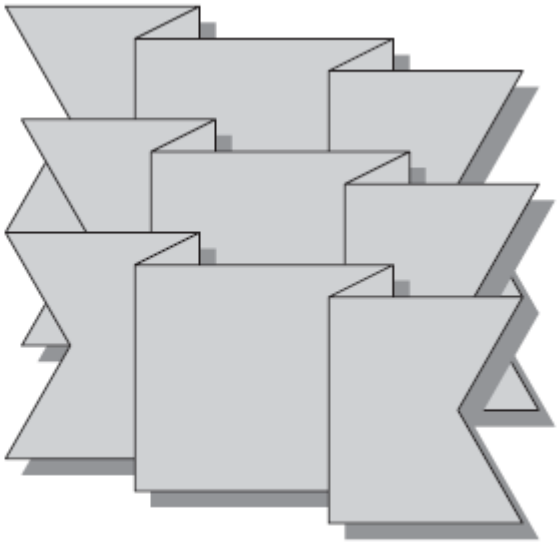


Figure 6-8 *β*-Pleated sheet.

The more intricate folding begins with the tertiary structure as the protein folds back on itself (Fig. 6-10). As the helix or sheet bends back on itself in a precise order, bonds are formed between the side chains, including hydrogen and ionic bonds.

A quaternary structure may be formed from the association of two or more peptide strands. The formation of disulfide bonds is important in the quaternary structure of many proteins. The quaternary structure of many proteins includes both *α*-helixes and *β*-sheets.

If a protein is disassembled such that the structure disintegrates, the protein is said to be *denatured*. Examples of factors that can denature proteins include heat and changes in pH. Note that even though secondary and tertiary structures are gone, the primary structure remains, with the peptide bonds intact. When denatured,

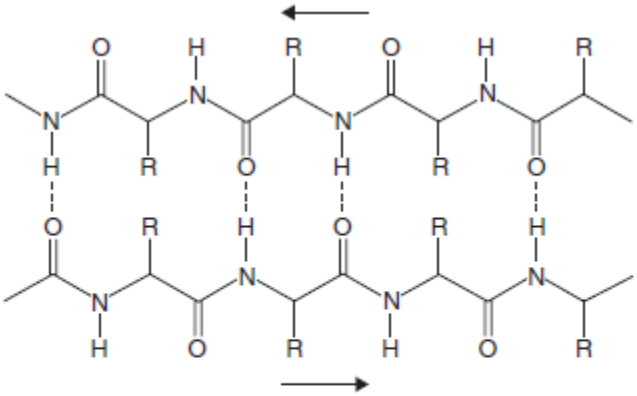


Figure 6-9 Antiparallel β-sheet.

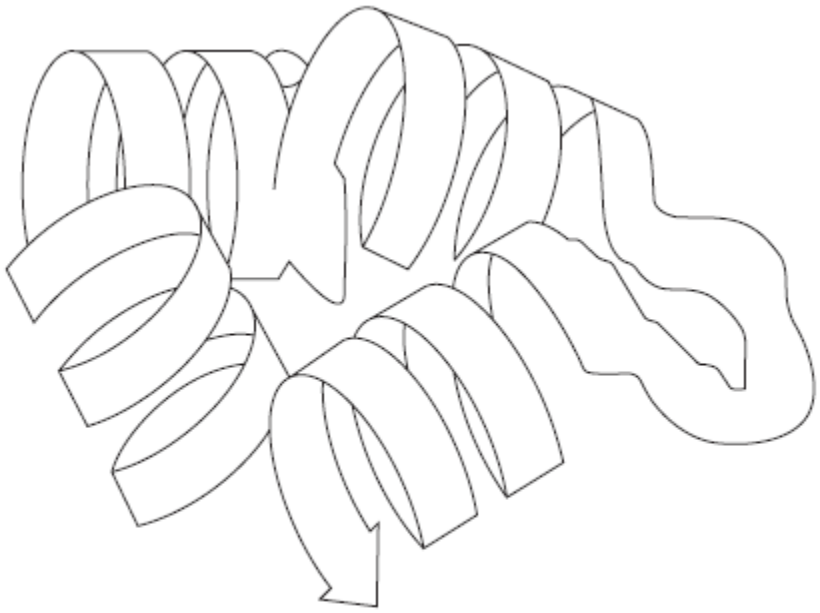


Figure 6-10 Tertiary structure.

the basic polypeptide strand forms loose loops called a *random coil*. If conditions are right, this random coil may spontaneously reassume the appropriate shape through self-assembly. Many proteins are built from discernable units that can assemble and disassemble independently of the rest of the protein. These building units are known as *domains*. Some of these domains are common building units that recur across protein families. For example, many enzymes contain a substrate binding site called the α/β-barrel. The α/β-barrel is also called the TIM barrel after triose phosphate isomerase, which was the first such structure to be described. The barrel is formed through linked

α-helixes and β-sheets that curve around in such a way as to form a fat barrel, or donut, shape. The β-strands form the inner wall of the donut and the α-helices form the outer wall of the donut. The active site is on the outside of the "barrel." The center of the barrel is stuffed with hydrophobic amino acid side chains. See Fig. 6-11. Some proteins contain unique structures. An example is collagen, the fibrous protein found in skin, cartilage, bone, and other connective tissue and the most common human body protein.

Collagen, which composes more than a third of the body's total protein content, is important enough to spend a little time in description. Collagen is formed from subunits called *tropocollagen,* a rod made up of three polypeptide strands (Fig. 6-12). These three left-handed helices are twisted together into a right-handed triple helix stabilized by numerous hydrogen bonds. The result is a versatile molecule that, depending on the assembly, can provide varying degrees of flexibility to body structures. If the triple helix structure is disrupted, as by heat, the result is, literally, *gelatin.*

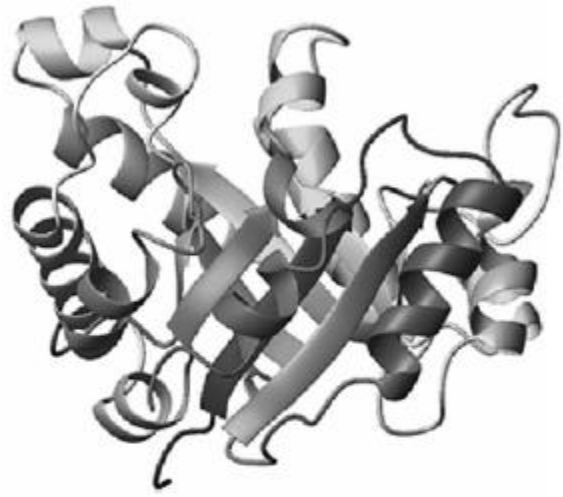


Figure 6-11 Example of quaternary structure—TIM barrel.
(*Courtesy of Wikipedia commons.*)

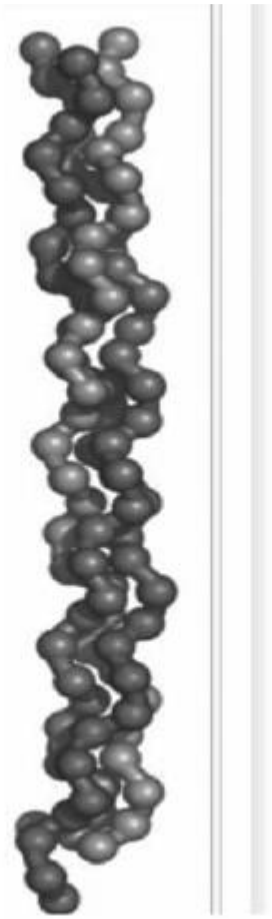


Figure 6-12 Tropocollogen. (*Courtesy of Wikipedia commons.*)

You may remember the discussion on amino acids and the fact that glycine's small shape allows it to fit into tight spots and the proline's ring structure tends to constrain mobility. Collagen consists of approximately a third glycine and about 10% proline. The glycine allows for tight coupling of adjacent strands and the proline provides rigidity at strategic points within a strand. Collagen also contains *hydroxyproline*, which is derived from proline and *hydroxylysine*, which is derived from lysine. Synthesis of both of these derivative amino acids requires vitamin C. This role explains why lack of vitamin C causes a painful joint condition called *scurvy*.

Enzymes

CHARACTERISTICS AND NOMENCLATURE

Specificity and nomenclature

Enzymes are nature's biological catalysts possessing the ability to promote specific chemical reactions under the mild conditions that prevail in most living organisms. They are all

proteins but range widely in their size from as few as 60–70 amino acid residues as in RNase to as many as several thousand. Generally they are much larger than their substrates and bind with them by means of active sites created by the specific three-dimensional folding of the protein. Interaction of specific functional groups in a small number of amino acid residues lining the active site with the substrate results in the formation of a transition state for which the activation energy barrier is significantly reduced relative to the non-enzyme-catalysed reaction. As a result, the reaction rate is increased by a factor of many millions relative to the uncatalysed reaction. Enzymes do not alter the position of equilibrium of reversible reactions that they catalyse but they do accelerate the establishment of the position of equilibrium for the reaction.

Many enzymes are members of coordinated metabolic or signalling pathways that collectively are responsible for maintaining a cell's metabolic needs under varying physiological conditions (Sections 15.5 and 17.4.5). The over- or under-expression of an enzyme can lead to cell dysfunction which we may recognise as a particular disease state. Enzyme inhibitors are widely used as therapeutic agents for the treatment of such conditions (Sections 15.2 and 18.1). Organ damage, for example heart muscle as a result of deprivation of oxygen following a heart attack, or the liver as a result of chemical damage as in alcoholic cirrhosis, results in the release of cellular enzymes into extracellular fluids and eventually into the blood. Such release can be clinically monitored to aid diagnosis of the organ damage and to make a prognosis for the patient's future recovery (Section 16.3).

Enzymes are believed to catalyse over 4 000 different reactions but individual enzymes are characterised by their specificity for a particular type of chemical reaction. As a generalisation, enzymes involved in biosynthetic or signalling reactions show a higher specificity than ones involved in degradation reactions. Bond specificity is characteristic of enzymes such as peptidases and esterases that hydrolyse specific bond types. The specificity of these enzymes is determined by the presence of specific functional groups within the substrate adjacent to the bond to be cleaved. Group specificity is characteristic of enzymes that promote a particular reaction on a structurally related group of substrates. As an example, the kinases catalyse the phosphorylation of substrates that have a common structural feature such as a particular amino acid (e.g. the tyrosine kinases, see Section 17.4.4) or sugar (e.g. hexokinase). DNA polymerase has a high specificity not only copying the base sequence of the DNA but also checking the product for accuracy afterwards. Enzymes may also display stereospecificity and be able to distinguish between optical and geometrical isomers of substrates. Enzymes have a high capacity for regulation in that the activity of enzymes that control the rate of a particular metabolic or signaling pathway can be enhanced or reduced in response to changing intracellular and extracellular demands. A range of regulatory mechanisms operates to allow short, medium and long-term changes in activity (Section 15.5.2).

Nomenclature and classification

By international convention, each enzyme is classified into one of six groups on the basis of the type of chemical reaction that it catalyses. Each group is divided into subgroups according to the nature of the chemical group and coenzymes involved in the reaction. In accordance with the Enzyme Commission (EC) rules, each enzyme can be assigned a unique four-figure code and an unambiguous systematic name based upon the reaction catalysed. The six groups are:

• Group 1: Oxidoreductases, which transfer hydrogen or oxygen atoms or electrons from one substrate to another. This group includes the dehydrogenases, reductases, oxidases, dioxidases, hydroxylases, peroxidases and catalase.

• Group 2: Transferases, which transfer chemical groups between substrates. The group includes the kinases, aminotransferases, acetyltransferases and carbamyltransferases.

• Group 3: Hydrolases, which catalyse the hydrolytic cleavage of bonds. The group includes the peptidases, esterases, phosphatases and sulphatases.

• Group 4: Lyases, which catalyse elimination reactions resulting in the formation of double bonds. The group includes adenylyl cyclase (also known an adenylate cyclase), enolase and aldolase.

• Group 5: Isomerases, which interconvert isomers of various types by intramolecular rearrangements. The group includes phosphoglucomutase and glucose-6-phosphate isomerase.

• Group 6: Ligases (also called synthases), which catalyse covalent bond formation with the concomitant breakdown of a nucleoside triphosphate, commonly ATP. The group includes carbamoyl phosphate synthase and DNA ligase.

As an example of the operation of these rules, consider the enzyme alcohol dehydrogenase which catalyses the reaction: alcohol þ NADþ _!_aldehyde or ketone þ NADH þ Hþ It has the systematic name alcohol :NAD oxidoreductase and the classification number 1:1:1:1. The first 1 indicates that it is an oxidoreductase, the second 1 that it acts on a CH–OH donor, the third 1 that NADþ or NADPþ is the acceptor and the fourth 1 that it is the first enzyme named in the 1:1:1 subgroup. Systematic names tend to be user unfriendly and for day-to-day purposes recommended trivial names are preferred.

When correctly used they give a reasonable indication of the reaction promoted by the enzyme in question but they fail to identify fully all the reactants involved. For example, glyceraldehyde-3-phosphate dehydrogenase fails to identify the involvement of orthophosphate and NADþ and phosphorylase kinase fails to convey the information that it is the b form of phosphorylase that is subject to phosphorylation involving ATP.

Cofactors

The catalytic properties of an enzyme are often dependent upon the presence of nonpeptide molecules called cofactors or coenzymes. These may be either weakly or tightly bound to the enzyme; in the latter case they are referred to as a prosthetic group. Examples of

coenzymes include NADþ, NADPþ, FMN and FAD, whilst examples of prosthetic groups include haem and oligosaccharides, and simple metal ions such as Mg2þ, Fe2þ and Zn2þ. DNA and RNA polymerases and many nucleases, for example, require two divalent cations for their active site. The cations correctly orientate the substrate and promote acid–base catalysis.

Isoenzymes and multienzyme complexes

Isoenzymes

Some enzymes exist in multiple forms called isoenzymes or isoforms that differ in amino acid sequence. An example is lactate dehydrogenase (LD) (EC 1:1:1:27) which exists in five isoforms. LD is a tetramer which can be assembled from two subunits, H (for heart) and M (for muscle). The five forms are therefore H4, H3M, H2M2, HM3 and M4 which can be separated by electrophoresis and shown to have different affinities for their substrates, lactate and pyruvate, and for analogues of these two compounds. They also have different maximum catalytic activities and tissue distributions, and as a consequence are important in diagnostic enzymology (Section 16.3).

Multienzyme complexes

Some enzymes that promote consecutive reactions in a metabolic pathway associate to form a multienzyme complex. Examples include the fatty-acid synthase (EC 2:3:1:86) (seven catalytic centres), pyruvate dehydrogenase (EC 2:7:1:99) (three catalytic centres) and DNA polymerase (EC 2:7:7:7) (three catalytic centres). Multienzyme complexes have a number of advantages over individual enzymes including a reduction in the transit time for the diffusion of the product of one enzyme to the catalytic site of the next, a reduction in the possibility of the product of one enzyme being acted upon by another enzyme not involved in the pathway, and the possibility of one enzyme activating an adjacent enzyme (Section 15.5.4).

Units of enzyme activity

Units of enzyme activity are expressed either in the SI units of katals (defined as the number of moles of substrate consumed or product formed per second) or international units (number of m moles of substrate consumed or product formed per minute). Allied to activity units is specific activity which expresses the number of international units per mg protein or katals per kg protein (note: 60 international units per mg protein is equivalent to 1 katal (kg protein)_1).

ENZYME STEADY-STATE KINETICS

Monomeric enzymes

Initial rates

When an enzyme is mixed with an excess of substrate there is an initial short period of time (a few hundred microseconds) during which intermediates leading to the formation of the product gradually build up (Fig. 15.1). This so-called pre-steady state requires special techniques for study and these are discussed in Section 15.3.3. After this pre-steady state, the reaction rate and the concentration of intermediates change relatively slowly with time and so-called steady-state kinetics exist. Measurement of the progress of the reaction during this phase gives the relationships shown in Fig. 15.2. Tangents drawn through the origin to the curves of substrate concentration and product concentration versus time allow the initial rate, v_0, to be calculated. This is the maximum rate for a given concentration of enzyme and substrate under the defined experimental conditions. Measurement of the initial rate of an enzyme-catalysed reaction is a prerequisite to a complete understanding of the mechanism by which the enzyme works, as well as to the estimation of the activity of an enzyme in a biological sample. Its numerical value is influenced by many factors, including substrate and enzyme concentration, pH, temperature and the presence of activators or inhibitors.

For many enzymes, the initial rate, v_0, varies hyperbolically with substrate concentration for a fixed concentration of enzyme (Fig. 15.3). The mathematical equation expressing this hyperbolic relationship between initial rate and substrate concentration is known as the Michaelis–Menten equation:

$$\nu_0 = \frac{V_{max}[S]}{K_m + [S]} \qquad (15.1)$$

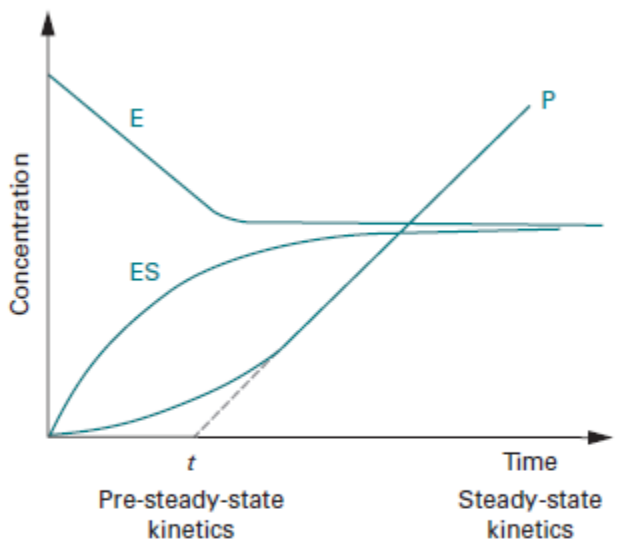


Fig. 15.1 Pre-steady-state progress curve for the interaction of an enzyme (E) with its substrate (S). P, product; *t*, induction time.

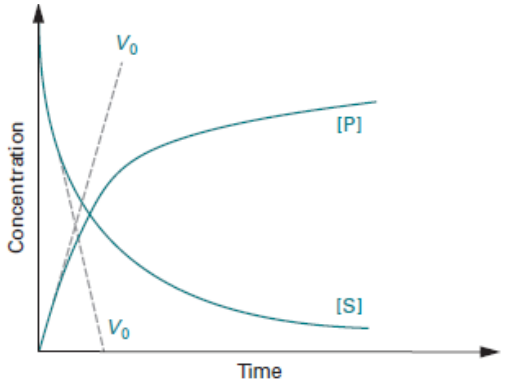


Fig. 15.2 Calculation of initial rate (ν_0) from the time-dependent change in the concentration of substrate (S) and product (P) of an enzyme-catalysed reaction.

where ν max is the limiting value of the initial rate when all the active sites are occupied, Km is the Michaelis constant and [S] is the substrate concentration. At low substrate concentrations the occupancy of the active sites on the enzyme molecules is low and the reaction rate is directly related to the number of sites occupied. This approximates to first-order kinetics in that the rate is proportional to substrate concentration. At high substrate concentrations effectively all of the active sites are occupied and the reaction becomes independent of the substrate concentration since no more enzyme–substrate complex can be formed and zero-order or saturation kinetics are observed. Under these conditions the reaction rate is only dependent upon the conversion of the enzyme– substrate complex, ES, to products and the diffusion of the products from the enzyme. It can be seen from equation 15.1 that when ν_0= 0.5 Vmax, Km= [S]. Thus Km is numerically equal to the substrate concentration at which the initial rate is one-half of

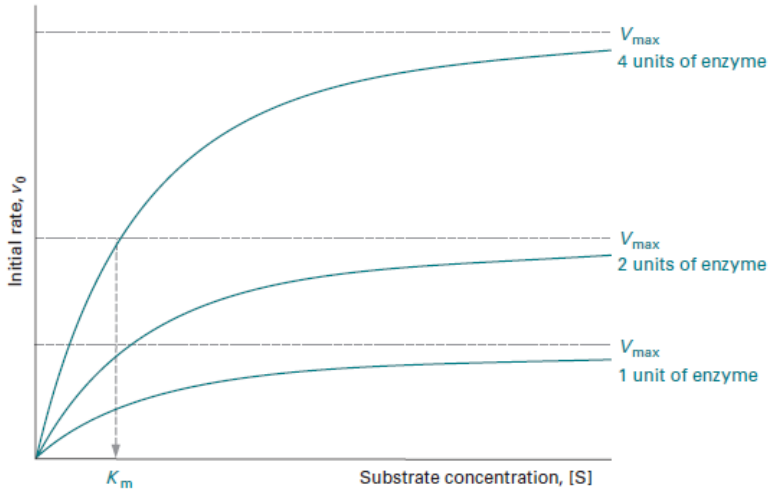


Fig. 15.3 The effect of substrate concentration on the initial rate of an enzyme-catalysed reaction in the presence of three different concentrations of enzyme. Doubling the enzyme concentration doubles the maximum initial rate, V_{max}, but has no effect on K_m.

the maximum rate (Fig. 15.3) and has units of molarity. Values of Km are usually in the range 10^{-2} to 10^{-5} M and are important because they enable the concentration of substrate required to saturate all of the active sites of the enzyme in an enzyme assay to be calculated. When [S]>> »Km, equation 15.1 reduces to $v_0 \approx$ Vmax, but a simple calculation reveals that when [S]=10Vmax, n0 is only 90% Vmax and that when [S]=100Km, $v_{0=}$ 99%Vmax. Appreciation of this relationship is vital in enzyme assays.

As previously stated, enzyme-catalysed reactions proceed via the formation of an enzyme substrate complex in which the substrate (S) is non-covalently bound to the active site of the enzyme (E). The formation of this complex for the majority of enzymes is rapid and reversible and is characterised by the dissociation constant, Ks, of the complex:

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES$$

where k_{+1} and k_{-1} are the rate constants for the forward and reverse reactions.

At equilibrium, the rates of the forward and reverse reactions are equal and the Law of Mass Action can be applied to the reversible process:

$$k_{+1}[E][S] = k_{-1}[ES] \qquad (15.2)$$

hence:

$$K_s = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_{+1}} = \frac{1}{K_a}$$

where Ka is the association (or affinity) constant.

It can be seen that when Ks is numerically large, the equilibrium is in favour of unbound E and S, i.e. of non-binding, whilst when Ks is numerically small, the equilibrium is in favour of the formation of ES, i.e. of binding. Thus Ks is inversely proportional to the affinity of the enzyme for its substrate.

The conversion of ES to product (P) can be most simply represented by the irreversible equation:

$$ES \xrightarrow{k_{+2}} E + P$$

where k_{+2} is the first-order rate constant for the reaction.

In some cases the conversion of ES to E and P may involve several stages and may not necessarily be essentially irreversible. The rate constant k_{+2} is generally smaller than both k_{+1} and $k_{-1}$and in some cases very much smaller. In general, therefore, the conversion of ES to products is the rate-limiting step such that the concentration of ES is essentially constant but not necessarily the equilibrium concentration. Under these conditions the Michaelis constant, Km, is given by:

$$K_m = \frac{k_{+2} + k_{-1}}{k_{+1}} = K_s + \frac{k_{+2}}{k_{+1}} \qquad (15.3)$$

It is evident that under these circumstances, Km must be numerically larger than Ks and only when k_{+2} is very small do Km and Ks approximately equal each other. The relationship between these two constants is further complicated by the fact that for some enzyme reactions two products are formed sequentially, each controlled by different rate constants:

$$E + S \underset{}{\overset{k_{+2}}{\longleftrightarrow}} ES \rightarrow P_1 + EA \overset{k_{+3}}{\rightarrow} E + P_2$$

where P_1 and P_2 are products, and A is a metabolic product of S that is further metabolised to P_2. In such circumstances it can be shown that:

$$K_m = K_s \frac{k_{+3}}{k_{+2} + k_{+3}} \qquad (15.4)$$

so that Km is numerically smaller than Ks. It is obvious therefore that care must be taken in the interpretation of the significance of Km relative to Ks. Only when the complete reaction mechanism is known can the mathematical relationship between Km and Ks be fully appreciated and any statement made about the relationship between Km and the affinity of the enzyme for its substrate.

Although the Michaelis–Menten equation can be used to calculate Km and Vmax, its use is subject to the difficulty of experimentally measuring initial rates at high substrate concentrations and hence of extrapolating the hyperbolic curve to give an accurate value of Vmax. Linear transformations of the Michaelis–Menten equation are therefore commonly used alternatives. The most popular of these is the Lineweaver–Burk equation obtained by taking the reciprocal of the Michaelis–Menten equation:

$$\frac{1}{v_0} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \qquad (15.5)$$

Introduction to biocatalysis:

Importance in Green Chemistry and Chemical Industry:

Biocatalysis have many advantages over chemocatalysis in the context of green chemistry, which include mild reaction conditions (physiological pH and temperature), the use of environmentally compatible catalysts (enzymes) and solvents (usually water), high catalytic activity and good regio- and chemo-selectivities for multifunctional molecules. As a result, the use of enzymes can avoid the need for functional group activation, or unnecessary protection/deprotection steps.

This simple overview demonstrates that biocatalytic transformations can potentially satisfy eight out of the twelve principles of green chemistry. They often result in a shorter, less wasteful, environmentally and economically appealing processes when compared to conventional chemical syntheses. Biocatalysis has already become widespread in industrial organic synthesis with over 130 commercialised processes. Some of the major themes in biocatalysis are as follows:

- Biocatalysts (enzymes/whole cells) can **replace** chemo-catalysts in synthetic routes.
- Biocatalysts can enable **new synthetic pathways** which may be shorter, more efficient and more sustainable.
- Combining **chemo- and bio-catalysis** generates opportunities for the design of synthetic routes.
- Biocatalysts with a **broad substrate scope** that are **active and stable** under the conditions of a chemical process are needed.
- There are a range of **emerging technologies for biocatalyst development** (directed evolution/pathway engineering).

POSSIBLE QUESTION:

PART-A 20 x 1= 20 marks

Each question carries one mark

1. The backbone of a protein forms the basis of the secondary structure stabilized by
(a) sulfhydryl covalent bonds.
(b) ionic bonds.
(c) hydrogen bonds between the side chains.
(d) hydrogen bonds between groups on the *a*-carbon.
Answer: d

2. In a folded protein, one would expect
(a) hydrophilic groups on the outside where they interact with theirenvironment.
(b) hydrophobic groups on the inside where they stabilize the protein byionic bonds.
(c) both hydrophilic and hydrophobic groups on the inside forminghydrogen bonds.
(d) hydrophilic groups on the inside where they stabilize the protein byionic bonds.
Answer: a

3. Proline is likely to be
(a) ionized at physiological pH.

(b) found in areas where protein structure exhibits rigidity.
(c) found at active sites of enzymes.
(d) important in formation of hydrogen bonds in the protein backbone.
Answer: b

4. Histidine is
(a) nonpolar.
(b) electron rich.
(c) a kink in a polypeptide chain.
(d) found on the inside of protein structures.
Answer:b

5. *a*-Helices and *b*-sheets are different because
(a) *a*-helices are formed within a single polypeptide chain and *b*-sheets are formed between polypeptide chains.
(b) *a*-helices are secondary structures and *b*-sheets are tertiary.

(c) only *b*-sheets are found in keratin.
(d) *b*-sheets are held together by cysteine linkages, whereas, *a*-helices are formed by hydrogen bounds.
Answer:a

6. Gelatin is
(a) denatured collagen.
(b) an *a*-helix.
(c) common domain.
(d) tropocollagen.
Answer:a

7. Glycine is
(a) found in rigid portions of the protein.
(b) found at active sites of enzymes.
(c) found in tight curves.
(d) polar but nonionized at physiological pH.
Answer:c

8. The following statement is not true:
(a) Vitamin C is required for the synthesis of amino acids found in collagen.
(b) Many proteins self-assemble into the correct shape.

(c) Proteins in general require chaperonins for proper folding.
(d) Secretory proteins contain a unique sequence recognized by special chaperonins.
(e) Heat shock proteins increase in times of cellular stress.
Answer:c

9. The following is not true about heat-shock proteins:
(a) Are found in greater abundance in cells under thermal stress.
(b) Are harvested by heat-shocking the cells and collecting the precipitate.
(c) Are important in protein folding.
(d) Are important in protecting cells from the impact of stressful environments.
Answer:b

10. The following statement is not true:
(a) Hydrogen bonds are much weaker than the bond that forms cystine.
(b) pH in the cell microenvironment may be different than the rest of the cell.
(c) Many secretory proteins require refolding prior to insertion into the cell membrane.
(d) There are only 20 distinct amino acids.
(e) A denatured protein forms a random coil.

Answer:d

11. All Proteins contain the
a.same 20 amino acids b. different amino acids c. 300 amino acids occurring in nature d. Only a few amino acids
Answer: a

12. Proteins contain
a. Only L-α- amino acids b. Only D-amino acids c. DL-amino acids d. Both a and b
Answer: a

13. The optically inactive amino acid is
a. Glycine b. Serine c. Threonine d. Valine
Answer: a

14. Sulphur containing amino acid is
(A) Methionine (B) Leucine

(C) Valine (D) Asparagine
Answer:a

15. At neutral pH, a mixture of amino acids in solution would be predominantly:
(A) Dipolar ions (B) Nonpolar molecules (C) Positive and monovalent
(D) Hydrophobic
Answer: a

16. pH (isoelectric pH) of alanine is
(A) 6.02 (B) 6.6 (C) 6.8 (D) 7.2
Answer:a

17. An essential amino acid in man is
(A) Aspartate (B) Tyrosine (C) Methionine (D) Serine
Answer:c

18. Non essential amino acids
(A) Are not components of tissue proteins
(B) May be synthesized in the body from essential amino acids
(C) Have no role in the metabolism
(D) May be synthesized in the body in diseased States
Answer: b

19. Which one of the following is semiessential amino acid for humans?
(A) Valine (B) Arginine (C) Lysine (D) Tyrosine
Answer: b

20. An example of polar amino acid is
(A) Alanine (B) Leucine (C) Arginine (D) Valine
Answer: c

21.During the formation of the peptide bond which of the following takes place?
a) Hydroxyl group is lost from its carboxyl group of one amino acid and a hydrogen atom is lost from its amino group of another amino acid

b) Hydrogen atom is lost from its carboxyl group of one amino acid and a hydroxyl group is lost from its amino group of another amino acid

c) Hydroxyl group is lost from its carboxyl group of one amino acid and a hydroxyl group is lost from its amino group of another amino acid

d) Hydrogen atom is lost from its carboxyl group of one amino acid and a hydrogen atom is lost from its amino group of another amino acid

Answer: a

22. Peptide bond is a

a) Covalent bond

b) Ionic bond

c) Metallic bond

d) Hydrogen bond

Answer: a

23. A tripeptide has

a) 3 amino acids and 1 peptide bond

b) 3 amino acids and 2 peptide bonds

c) 3 amino acids and 3 peptide bonds

d) 3 amino acids and 4 peptide bonds

Answer: b

24. The factor which does not affect pK_a value of an amino acid is

a) The loss of charge in the α-carboxyl and α-amino groups

b) The interactions with other peptide R groups

c) Other environmental factors

d) Molecular weight

Answer: d

25. Which of the following is a 39-residue hormone of the anterior pituitary gland?

a) Corticotropin

b) Glucagon

c) Insulin

d) Bradykinin

Answer: a

26. The average molecular weight of an amino acid residue in a protein is about

a)128

b)118

c)110

d)120

Answer: c

.

27. Which of the following is not the classified form of conjugated proteins?

a) Lipoproteins

b) Glycoproteins

c) Metalloproteins

d) Complete proteins

Answer: d

28. Which part of the amino acid gives it uniqueness?

a) Amino group

b) Carboxyl group

c) Side chain

d) None

Answer: c

29. Which of the following information is responsible to specify the three-dimensional shape of a protein?

a) The protein's peptide bond

b) The protein's amino acid sequence

c) The protein's interaction with other polypeptides

d) The protein's interaction with molecular chaperons

Answer: b

30. Unfolding of a protein can be termed as

a) Renaturation

b) Denaturation
c) Oxidation
d) Reduction
Answer: b

31. Which of the following does not affect the stability of a α-helix?
a) Electrostatic repulsion
b) Bulkiness
c) Interaction between R groups spaced three residues apart
d) Occurrence of alanine and glycine residues
Answer: d

32. Which of the following is not true about secondary protein structure?
a) The hydrophilic/hydrophobic character of amino acid residues is important to secondary structure.
b) The ability of peptide bonds to form intramolecular hydrogen bonds is important to secondary structure.
c) The alpha helix, beta pleated sheet and beta turns are examples of protein secondary structure.
d) The steric influence of amino acid residues is important to secondary structure.
View Answer
Answer: a

33. β-pleated sheets are the examples of
a) Primary structure
b) Secondary structure
c) Tertiary structure
d) Quaternary structure
Answer: b.

34. A coiled peptide chain held in place by hydrogen bonding between peptide bonds in the same chain is
a) Primary structure
b) α-helix

c) β-pleated sheets
d) Tertiary structure

Answer: b

35. A structure that has hydrogen bonds between polypeptide chains arranged side by side is
a) Primary structure
b) α-helix
c) β-pleated sheets
d) Tertiary structure

Answer: c

36. Which of the following are known as helix breakers?
a) Proline and glycine
b) Isoleucine and leucine
c) Valine
d) Threonine

Answer: a

37. Which of the following is false about NMR spectroscopy?
a) NMR is an abbreviated form of Nuclear Magnetic Resonance
b) The intramolecular magnetic field around an atom in a molecule changes the resonance frequency giving structural information about the atom
c) The intermolecular magnetic field around an atom in a molecule changes the resonance frequency giving structural information about the atom
d) It is a technique that exploits magnetic properties of atomic nuclei

Answer: c

38. Which of the statements is false about multiple sequence alignment?
a) Both protein and nucleic acid secondary structures can be used
b) More useful in RNA
c) These alignments can be made more accurate by the inclusion of secondary structure information
d) A significant increase in accuracy

Answer: b

39. Secondary structure is defined by
a) Hydrogen bonding
b) Vander Waals forces
c) Covalent bonding
d) Ionic bonding
Answer: a

40. Which of the following is false statement?
a) α-Keratin is α helical
b) Collagen is α helical
c) Hemoglobin has a quaternary structure
d) α-Keratin is β pleated structure
View Answer
Answer: d

Part-B 5x2= 10 marks

1. What is co enzyme?
2. What are factors that affect the enzyme activity?
3. What is enzyme active site?
4. Draw the secondary structure of protein?
5. Explain coenzyme and cofactors?
6. What is meant by apoenzyme?
7. Explain the primary structure of protein.
8. How do enzymes function as biological catalysts?
9. What is enzyme specificity?

Part-C 5 x6 = 30 marks

1. Write a note on biological importance of Proteins?
2. Discuss the structure of protein?
3. Explain the nomenclature and characteristics of enzyme?
4. Write a note on enzyme inhibitors?
5. Explain the biocatalysis important in greenchemistry.
6. Explain the mode of enzyme action.
7. What are the names and main functions of the six different classes of enzymes?

KARPAGAM ACADEMY OF HIGHER EDUCATION

COIMBATORE-21

DEPARTMENT OF CHEMISTRY

SUBJECT: ANALYTICAL CLINICAL BIOCHEMISTRY

SUBJECT CODE: 17CHU404B

UNIT-II

PART-A

MULTIPLE CHOICE QUESTIONS (EACH QUESTIONS CARRY ONE MARK)

ONLINE EXAMINATION (20 X1 =20 MARKS)

1. During the formation of the peptide bond which of the following takes place?
a) Hydroxyl group is lost from its carboxyl group of one amino acid and a hydrogen atom is lost from its amino group of another amino acid
b) Hydrogen atom is lost from its carboxyl group of one amino acid and a hydroxyl group is lost from its amino group of another amino acid
c) Hydroxyl group is lost from its carboxyl group of one amino acid and a hydroxyl group is lost from its amino group of another amino acid
d) Hydrogen atom is lost from its carboxyl group of one amino acid and a hydrogen atom is lost from its amino group of another amino acid

Answer: a

2. Peptide bond is a
a) Covalent bond
b) Ionic bond
c) Metallic bond
d) Hydrogen bond

Answer: a

3. A tripeptide has
a) 3 amino acids and 1 peptide bond
b) 3 amino acids and 2 peptide bonds
c) 3 amino acids and 3 peptide bonds
d) 3 amino acids and 4 peptide bonds

Answer: b

4. The factor which does not affect pK_a value of an amino acid is
a) The loss of charge in the α-carboxyl and α-amino groups
b) The interactions with other peptide R groups
c) Other environmental factors
d) Molecular weight

Answer: d

5. Which of the following is a 39-residue hormone of the anterior pituitary gland?
a) Corticotropin
b) Glucagon
c) Insulin
d) Bradykinin

Answer: a

6. The average molecular weight of an amino acid residue in a protein is about
a) 128
b) 118
c) 110
d) 120

Answer: c

7. Which of the following is not the classified form of conjugated proteins?
a) Lipoproteins
b) Glycoproteins
c) Metalloproteins
d) Complete proteins

Answer: d

8. Which part of the amino acid gives it uniqueness?
a) Amino group
b) Carboxyl group
c) Side chain
d) None

Answer: c

9. Which of the following information is responsible to specify the three-dimensional shape of a protein?
a) The protein's peptide bond
b) The protein's amino acid sequence
c) The protein's interaction with other polypeptides
d) The protein's interaction with molecular chaperons

Answer: b

10. Unfolding of a protein can be termed as
a) Renaturation
b) Denaturation
c) Oxidation
d) Reduction

Answer: b

11. What are the following is not a factor responsible for denaturation of proteins?
a) pH change
b) Organic solvents
c) Heat
d) Charge

Answer: d

12. The salt which produces salting out effect during extraction of proteins is
a) $NH_4\ SO_4$
b) $(NH_4)_2\ SO_4$
c) $(NH_4)_3\ SO_4$
d) NaCl

Answer: b

13. Mobile phase can be
a) Only solid
b) Only gas
c) Solid or liquid
d) Liquid or gas

Answer: d

14. The pattern on paper in chromatography is called
a) Chroming

b) Chroma
c) Chromatograph
d) Chromatogram

Answer: d

15. Which of the following statements about column chromatography is correct?
a) Resolution increases as the length of the column increases
b) Mobile phase is a porous solid material with appropriate chemical properties held in the column
c) Stationary phase is a buffered solution that percolates through mobile phase
d) Large proteins emerge from the column sooner than small ones

Answer: a

16. Which of the following statements is true about size-exclusion chromatography?
a) During the separation of a mixture of proteins, protein with smallest molecular weight is eluted first
b) During the separation of a mixture of proteins, protein with largest molecular weight is eluted first
c) During the separation of a mixture of proteins, protein with largest molecular weight is eluted last
d) During the separation of a mixture of proteins, protein with largest molecular weight flow around the beads

Answer: b

17. Which of the following statements is true about affinity chromatography?
a) During the separation of a mixture of proteins, the protein which does not bind to ligand is eluted first
b) During the separation of a mixture of proteins, the protein which does not bind to ligand is eluted last
c) During the separation of a mixture of proteins, the protein which binds to ligand is eluted first
d) Unwanted proteins are eluted by ligand solution

Answer: a

18. Which of the following statements is true about ion-exchange chromatography?
a) It separates proteins according to their size
b) The column matrix with bound anionic groups is called cationic exchanger
c) The column matrix with bound anionic groups is called anionic exchanger
d) The column matrix with bound cationic groups is called cationic exchanger

Answer: b

19. Which of the following statements is true about SDS polyacrylamide chromatography?
a) SDS polyacrylamide gel electrophoresis separates proteins on the basis of size
b) SDS polyacrylamide gel electrophoresis separates proteins on the basis of charge

c) SDS binds to proteins non-covalently with a stoichiometry of around one SDS molecule per three amino acids
d) SDS binds to proteins non-covalently with a stoichiometry of around one SDS molecule per one amino acid

Answer: b

20. Which of the following statements is true about two-dimensional electrophoresis?
a) Separates proteins of identical molecular weight, same pI but different charge
b) Separates proteins of different molecular weight and different pI
c) Separates proteins of identical molecular weight that differ in pI
d) Isoelectric focusing is also termed as two-dimensional electrophoresis

Answer: c

21. Which of the following statements is false?
a) The term activity refers to the total units of enzyme in a solution
b) The specific activity is a measure of enzyme purity
c) Specific activity increases during purification of an enzyme and becomes maximal and constant when the enzyme is pure
d) Specific activity decreases during purification of an enzyme and becomes maximal and constant when the enzyme is pure

Answer: d

22. Which of the following statements is false?
a) Primary structure of a protein determines how it folds up into a unique three dimensional structure
b) Secondary structure of a protein determines how it folds up into a unique three dimensional structure
c) Three dimensional structure of a protein determines the function of a protein
d) Amino acid sequence is absolutely invariant for a particular protein

Answer: b

23. Who deduced the double-helical structure of DNA?
a) Frederick Sanger
b) Mendel
c) Watson and Francis Crick
d) Anton van Leeuwenhoek

Answer: c

24. Two chains of amino acids in an insulin molecule are held together by
a) Sulfide bridges
b) Disulfide bridges
c) Peptide bond
d) Covalent linkage

Answer: b

25. Tertiary conformation of proteins is maintained by 3 types of bonds namely ionic, hydrogen and
a) Sulfide
b) Disulfide
c) Covalent
d) Peptide

Answer: b

26. Hemoglobin is a
a) Monomer
b) Dimer
c) Trimer
d) Tetramer

Answer: d

27. Which of the following is false?
a) The two main types of secondary structure are the α helix and β pleet structures
b) α helix is a right handed coiled strand
c) The hydrogen bonding in a β-sheet is between strands rather than within strands
d) The hydrogen bonding in a β-sheet is within strands rather than between strands

Answer: d

28. Native state of a protein can be disrupted by
a) Temperature
b) pH
c) Removal of water
d) Presence of hydrophilic surfaces

Answer: d

29. Which of the following is true?
a) The disulfide bridges formed by reduction of the sulfhydryl groups on cysteine stabilizes protein

tertiary structure
b) The disulfide bridges formed by oxidation of the sulfhydryl groups on cysteine destabilizes protein tertiary structure
c) The disulfide bridges formed by oxidation of the sulfhydryl groups on cysteine stabilizes protein tertiary structure
d) The disulfide bridges formed by reduction of the sulfhydryl groups on cysteine destabilizes protein tertiary structure

Answer: c.

30. Identify the wrong statement
a) Hemoglobin is a globular protein
b) Hemoglobin is a fibrous protein
c) Fibrous proteins are insoluble in water
d) Collagen is a fibrous protein
View Answer

Answer: b

31. In 3° structure of proteins, folding and shaping is done by
a) Hydrophobic interactions
b) Polar interactions
c) Hydrogen bonding
d) None of the mentioned

Answer: a

32. Amino acids sequence in DNA can be determined by the order of their
a) rRNA
b) tRNA
c) Nucleotides
d) mRNA

Answer: c

33. Which of the following is a Sanger's reagent?
a) 1-fluoro-2, 4-dinitrobenzene
b) 1-fluoro-2, 3-dinitrobenzene
c) 1-fluoro-2, 4-trinitrobenzene
d) 1-fluoro-2, 3-trinitrobenzene

Answer: a

34. The amino acid sequences of thousands of different proteins from many species have been determined using principles first developed by
a) Edman
b) Sanger
c) Mendel
d) Watson and Crick

Answer: b

35. Which of the following compound is not involved in Edman degradation?
a) Phenylisothiocyanate
b) CF_3 COOH
c) FDNB
d) Phenylthiocarbonyl

Answer: c

36. Which of the following statements is false?
a) Oxidation of cysteine residue with performic acid is done to break disulfide bond in proteins
b) Reduction of cysteine residue with dithiothreitol is done to break disulfide bond in proteins
c) Reduction of cysteine residue with performic acid is done to break disulfide bond in proteins
d) Reduced cysteine is further acetylated by iodoacetate

Answer:c

37. Cleaving of peptide chain is done by
a) Trypsin
b) Tyrosine
c) Tryptophan
d) Arginine

Answer: a

38. Which of the following is the correct order of sequencing?
a) Cleaving, sequencing and ordering
b) Sequencing, ordering and cleaving
c) Ordering, cleaving and sequencing
d) Ordering, sequencing and cleaving

Answer: a

39. Edman degradation is used for
a) Identifying N-terminal amino acids
d) Identifying C-terminal amino acids
c) Identifying amino acid
d) Identifying carbohydrates

Answer: a

40. What best summarizes the MALDI method by which gas phase ions are produced for mass spectrometry?
a. Sample is hit by a low energy xenon beam.
b. Sample is forced through a narrow capillary tube and solvent rapidly evaporates.
c. Sample is embedded in a crystalline matrix and bombarded by laser beams.
d. Sample is heated and then bombarded by electrons

Answer: c.

41. Which of the following is Edman reagent?
a) Phenylisothiocyanate
b) CF_3 COOH
c) FDNB
d) Phenylthiocarbonyl

Answer: a

42. Which of the following does not affect the stability of a α-helix?
a) Electrostatic repulsion
b) Bulkiness
c) Interaction between R groups spaced three residues apart
d) Occurrence of alanine and glycine residues

Answer: d

43. Which of the following is not true about secondary protein structure?
a) The hydrophilic/hydrophobic character of amino acid residues is important to secondary structure.
b) The ability of peptide bonds to form intramolecular hydrogen bonds is important to secondary structure.
c) The alpha helix, beta pleated sheet and beta turns are examples of protein secondary structure.
d) The steric influence of amino acid residues is important to secondary structure.

Answer: a

44. β-pleated sheets are the examples of
a) Primary structure
b) Secondary structure
c) Tertiary structure
d) Quaternary structure

Answer: b

45. A coiled peptide chain held in place by hydrogen bonding between peptide bonds in the same chain is
a) Primary structure
b) α-helix
c) β-pleated sheets
d) Tertiary structure

Answer: b

46. A structure that has hydrogen bonds between polypeptide chains arranged side by side is
a) Primary structure
b) α-helix
c) β-pleated sheets
d) Tertiary structure

Answer: c

47. Which of the following are known as helix breakers?
a) Proline and glycine
b) Isoleucine and leucine
c) Valine
d) Threonine

Answer: a

48. Which of the following is false about NMR spectroscopy?
a) NMR is an abbreviated form of Nuclear Magnetic Resonance
b) The intramolecular magnetic field around an atom in a molecule changes the resonance frequency giving structural information about the atom
c) The intermolecular magnetic field around an atom in a molecule changes the resonance frequency giving structural information about the atom
d) It is a technique that exploits magnetic properties of atomic nuclei

Answer: c

49. Which of the statements is false about multiple sequence alignment?
a) Both protein and nucleic acid secondary structures can be used
b) More useful in RNA
c) These alignments can be made more accurate by the inclusion of secondary structure information
d) A significant increase in accuracy

Answer: b

50. Secondary structure is defined by
a) Hydrogen bonding
b) Vander Waals forces
c) Covalent bonding
d) Ionic bonding

Answer: a

51. Which of the following is false statement?
a) α-Keratin is α helical
b) Collagen is α helical
c) Hemoglobin has a quaternary structure
d) α-Keratin is β pleated structure

Answer: d

52. Fibroin is rich in
a) Alanine and Glycine
b) Alanine
c) Glycine
d) Pro
Answer:a

53. Which of the following bonds are not involved in tertiary type of protein structure?
a) Disulfide bond
b) Hydrogen bonding
c) Salt bridges
d) Hydrophilic interactions

Answer: d

54. Which of the following does not possess a quaternary structure?
a) Myoglobin

b) Lactate dehydrogenase
c) Immunoglobin M
d) Creatine Phospho Kinase

Answer: a

55. Which of the following is abundantly found in collagen?
a) Glycine
b) Serine
c) Alanine
d) Tryptophan

Answer: a

56. Which of the following is first determined as oligomer?
a) Myoglobin
b) Collagen
c) Keratin
d) Hemoglobin

Answer: d

57. Which of the following is false?
a) Lysozyme has S-S linkage
b) Ribonuclease has S-S linkage
c) Heme group in cytochrome c is covalently linked to the protein on two sides
d) Ribonuclease has SH-SH linkage

Answer: d

58. Which of the following enzyme is secreted by the pancreas?
a) Ribonuclease
b) Lysozyme
c) Cytochrome c
d) Myoglobin

Answer: a

59. Which of the following is a component of mitochondria?
a) Ribonuclease
b) Lysozyme
c) Cytochrome c
d) Myoglobin

Answer: c

60. Which of the following serves as bactericidal agent?
a) Ribonuclease
b) Lysozyme
c) Cytochrome c
d) Myoglobin

Answer: b

61. Which of the following is false about fibrous protein?
a) It is in rod or wire like shape
b) Keratin and collagen are the best examples
c) Hemoglobin is the best example
d) It provides structural support for cells and tissues

Answer: c

62. Which of the following forces is favorable for protein folding?
a) Hydrophobic interactions
b) Hydrogen bonding
c) Vander Waals forces
d) Ionic bonding
Answer: a

63. A process by which a protein structure assumes its functional shape or conformation is
a) Denaturing
b) Folding
c) Synthesis
d) Hydrolysis
Answer: b

64. Process of folding does not depend on
a) Concentration of salts
b) pH
c) Solute
d) Solvent
Answer: c

65. Which of the following cannot denature a protein?
a) Iodoacetic acid

b) SDS detergent
c) Urea
d) Heating to 90°C

Answer: a

66. Which of the following is a function of chaperone protein?
a) It degrades proteins that have folded improperly
b) It provide a template for how the proteins should fold
c) It rescues proteins that have folded improperly and allows them to refold properly
d) It degrades proteins that have folded properly

Answer: c

67. As folding progresses which of the following does not take place?
a) Entropy decreases
b) Amount of protein in native state increases
c) Free energy increases
d) Amount of protein in native state decreases

Answer: d

68. Which of the following are chaperons in E.coli?
a) Hsp70
b) Hsp40
c) DnaA
d) DnaK and DnaJ
Answer: d

69. Which of the following about spontaneous folding is false?
a) It involves initial formation of highly compact structure
b) It involves initial formation of a local secondary structure
c) It is essentially a random process
d) It may be defective in some human diseases

Answer: c
70. Protein A will fold into its native state only when protein B is also present in the solution. However protein B can fold itself into native confirmation without the presence of protein A. Which of the following is true?
a) Protein B serves as precursor for protein A
b) Protein B serves as molecular chaperon for protein A
c) Protein B serves as ligand for protein A
d) Protein B serves as structural motif for protein A

Answer: b

71. Which of the following is true about ribonucease?
a) Native state which is catalytically inactive is denatured
b) Unfolded state is inactive
c) Renatured ribonuclease is inactive
d) Renaturation involves reestablishment of the correct disulfide cross links

Answer: a

Lecture Notes

Unit-III

Syllabus

Lipids: Classification. Biological importance of triglycerides and phosphoglycerides and cholesterol; Lipid membrane, Liposomes and their biological functions and underlying applications. Lipoproteins.

Introduction:

Lipids are a class of compounds distinguished by their *insolubility* in water and *solubility* in nonpolar solvents. Lipids are important in biological systems because they form the *cell membrane*, a mechanical barrier that divides a cell from the external environment. Lipids also provide energy for life and several essential vitamins are lipids. Lipids can be divided in two major classes, *nonsaponifiable lipids* and *saponifiable lipids.* A nonsaponifiable lipid cannot be broken up into smaller molecules by hydrolysis, but a saponifiable lipid contains one or more ester groups allowing it to undergo hydrolysis in the presence of an acid, base, or enzyme. Within these two major classes of lipids, there are several specific types of lipids important to life, including *fatty acids, triglycerides, glycerophospholipids, sphingolipids*, and *steroids*. Saponifi able lipids include *triglycerides, waxes, phospholipids*, and *sphingolipids*.

Each of these categories can be further broken down. Nonsaponifiable lipids include steroids, prostaglandins, and terpenes.

Nonpolar lipids, such as triglycerides, are used for energy storage and fuel. Polar lipids, which can form a barrier with an external water environment, are used in membranes. Polar lipids include glycerophospholipids and sphingolipids. Fatty acids are important components of all of these lipids.

Fatty Acids

A *fatty acid* is a molecule characterized by the presence of a carboxyl group attached to a long hydrocarbon chain. Therefore these are molecules with a formula R–COOH where R is a hydrocarbon chain. Fatty acids can be said to be carboxylic acids, and come in two major varieties.

• **Saturated fatty acids:** This is a fatty acid that does not have any double bonds. We say that a fatty acid is saturated when every carbon atom in the hydrocarbon chain is bonded to as many hydrogen atoms as possible (the carbon atoms are saturated with hydrogen). Saturated fatty acids are solids at room temperature. Animal fats are a source of saturated fatty acids. In addition, fatty acids pack easily and form rigid structures (e.g., fatty acids are found in membranes).

• **Unsaturated fatty acids:** An unsaturated fatty acid can have one or more double bonds along its hydrocarbon chain. A fatty acid with one double bond is called *monounsaturated.* If it contains two or more double bonds, we say that the fatty acid is *polyunsaturated.* Fatty acids only contain cis double bonds (see later for a discussion of *cis* and *trans*). The melting point of a fatty acid is influenced by the number of double bonds that the molecule contains and by the length of the hydrocarbon tail. The more double bonds it contains, the lower the melting point. As the length of the tail increases, the melting point increases. The melting point *decreases* as the number of double bonds *increases* because of the cis geometry of the double bonds. This introduces kinks in the hydrocarbon chain that *decreases* the number of vander Waals interactions. Conversely, as the length of the tail is *increased*, the number of vander Waals interactions is *increased*, raising the melting point. Obviously only the latter factor will affect the melting point of saturated fatty acids. Unsaturated fatty acids are liquids at room temperature. In the solid state, unsaturated fatty acids do not pack as well as straight-chained saturated fatty acids. As a result, intermolecular forces between molecules are lower, and it is easier to separate the molecules by raising the temperature, resulting in a lower melting point. Plants are the source of unsaturated fatty acids.

Common saturated fatty acids contain between 12 and 20 carbon atoms. Table 4-1 lists some common saturated fatty acids. Note the increase in melting point with the increase in the length of the hydrocarbon chain.

The straight-chain structure of saturated fatty acids is illustrated in Fig. 4-1 which displays lauric acid.

In Fig. 4-2, we show an illustration of oleic acid, an unsaturated fatty acid, which shows the prominent kink in the molecule.

Table 4-1 Common Saturated Fatty Acids

Fatty Acid	Carbon Atoms	Formula	Melting Point (°C)
Lauric	12	$CH_3(CH_2)_{10}COOH$	44
Myristic	14	$CH_3(CH_2)_{12}COOH$	54
Palmitic	16	$CH_3(CH_2)_{14}COOH$	63
Stearic	18	$CH_3(CH_2)_{16}COOH$	70
Arachidic	20	$CH_3(CH_2)_{18}COOH$	77

Some common unsaturated fatty acids are listed in Table 4-2. Notice that this table illustrates the melting point of a fatty acid as it relates to its characteristics. Oleic acid and palmitoleic acid have the same number of double bonds, but oleic acid has a longer hydrocarbon chain. Hence the melting point is increased. Now compare the linoleic and linolenic fatty acids. They both have the same number of carbon atoms, hence their hydrocarbon chains are the same length. But linolenic acid has *more* double bonds, therefore its melting point is *lower*.

OMEGA FATTY ACIDS

Unsaturated fatty acids can also be classified according to the location of the closest double bond to the methyl end of the carbon chain furthest from the carboxyl group. This is done by specifying an *omega number* for the fatty acid. Looking at Table 4-2, we examine the formula of the fatty acid and count the number of carbon atoms starting with the CH_3 on the left up to the first double bond. Hence

- Palmitoleic acid is an omega-7 fatty acid.
- Oleic acid is an omega-9 fatty acid.
- Linoleic acid is an omega-6 fatty acid.
- Linolenic acid is an omega-3 fatty acid.

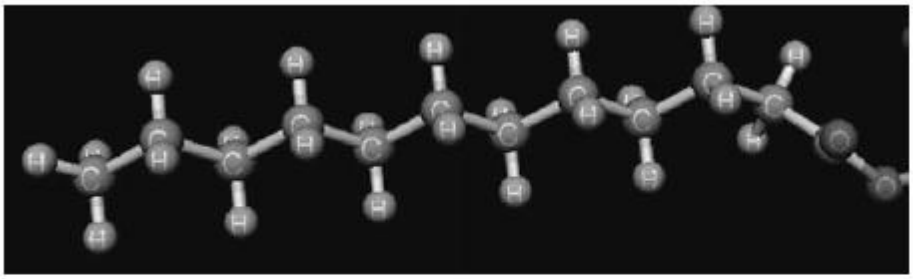


Figure 4-1 A rendering of lauric acid. This is a saturated fatty acid which has a straight hydrocarbon chain.

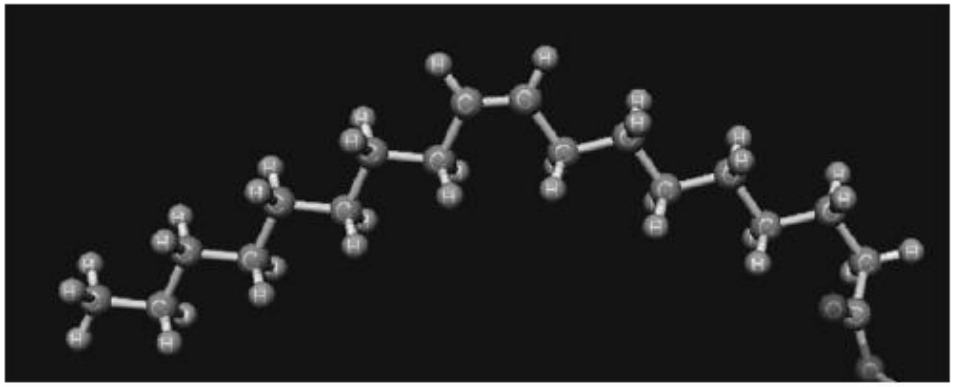


Figure 4-2 A rendering of oleic acid, an unsaturated fatty acid. Note the kink in the molecule, as opposed to the straight-chain nature of the saturated fatty acid shown in Fig. 4-1.

Omega-3 and omega-6 fatty acids are believed to be very important for cardiovascular health. Linolenic acid is an essential fatty acid because other omega-3 fatty acids needed by the human body are synthesized from it.

CIS- AND TRANS-FATTY ACIDS

Naturally occurring fatty acids have cis bonds. *Trans-fatty acids* are created artificially using a process called *hydrogenation.* A trans-fatty acid has a trans configuration rather than cis configuration at each double bond. This causes the molecule to straighten.

These two stereoisomers can be distinguished in the following way:

• In a *cis* stereoisomer, two similar groups attached to the carbon double bond are found on the same side.

• In a *trans* stereoisomer, two similar groups attached to the carbon double bond are found on opposite sides.

Table 4-2 Common Unsaturated Fatty Acids

Fatty Acid	Carbon Atoms	Formula	Melting Point (°C)
Palmitoleic	16	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	1
Oleic	18	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	13
Linoleic	18	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6COOH$	–5
Linolenic	18	$CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6COOH$	–11
Arachidonic	20	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$	–49

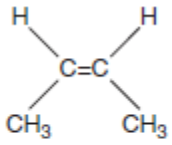


Figure 4-3 The cis configuration of 2-butene has the methyl groups on the *same* side of the double bond.

The designation of a stereoisomer as cis or trans is a geometrical designation. The atoms are arranged differently in space and there is a high energy barrier between the two configurations. As a result, two compounds that are differentiated as cis or trans are *different compounds.* We can understand the difference between cis and trans configurations by considering a simple example. Butene comes in two varieties, shown in Figs. 4-3 and 4-4.

Trans bonds, unlike cis bonds, do not introduce kinks in the hydrocarbon chain of a fatty acid. As a result, the effect of double bonds (in lowering the melting point) does not occur in the trans isomer of a given fatty acid. Trans-fatty acids behave more like saturated fatty acids, so the melting point is dependent on the length of the hydrocarbon chain. Trans-fatty acids have much higher melting points than the corresponding unsaturated cis-fatty acid. Although they are created by saturating vegetable oils, trans-fatty acids are considered unhealthy and are believed to lead to cardiovascular disease if consumed in large amounts.

The key features of a trans-fatty acid are that the hydrocarbon chain contains a double bond, but the chain is straight instead of containing kinks like an unsaturated fatty acid. A trans-fatty acid is shown in Fig. 4-5.

ESSENTIAL AND NONESSENTIAL FATTY ACIDS AND THEIR ROLE IN DIET

If a fatty acid can only be obtained from the diet (for humans), we say that the fatty acid is an *essential fatty acid.* Two fatty acids cannot be synthesized in the human body and are therefore essential. These are *linoleic* and *linolenic* fatty acids, which are both unsaturated. Nonessential fatty acids can be made by the human body and so do not need to be obtained from diet alone. These are made from carbohydrates and proteins or from other fatty acids.

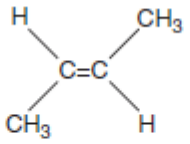


Figure 4-4 In the trans configuration of 2-butene, the methyl groups are on the *opposite* side of the double bond.

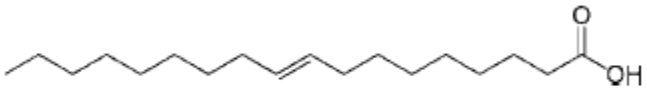


Figure 4-5 An example of a trans-fatty acid, *elaidic acid.* Note that although it has a double bond, since this is a trans isomer the hydrocarbon chain is straight.

Fatty acids are an important source of energy. While carbohydrates or proteins only provide 4 kcal/g, fatty acids provide more than twice the energy per unit weight at 9 kcal/g. This is one reason why a high-fat diet can lead to obesity.

Triglycerides

A triglyceride (often called *tryglycerol*—we will use both terms interchangeably) is a fatty acid trimester of glycerol, which is illustrated in Fig. 4-6. Triglycerides are important for human health in that they provide most of the lipids in our diet. Notice that glycerol has three hydroxyl groups. Fatty acids can be attached at these three sites forming a triglyceride.

One important characteristic of a tryglycerol is its state at room temperature.

The degree of saturation and the length of their chains attached to the glycerol backbone both determine their state at room temperature. So we see that not surprisingly, the state of the tryglycerol is determined by how the fatty acid chains it contains behave. Hence

• Short-chain unsaturated triglycerides are *liquid* at room temperature.

• Long-chain saturated triglycerides are *solid* at room temperature.

Animal fats contain a high amount of saturated triglycerides while plant oils contain a high amount of unsaturated triglycerides. Think *lard* when thinking about a saturated triglyceride, and think *vegetable oil* when thinking about an unsaturated triglyceride. While neither is healthy when consumed in excess, vegetable oils are far healthier than lard, so a biochemist can keep in the back of their mind that fats that are solid at room temperature are not as healthy as those that are liquid.

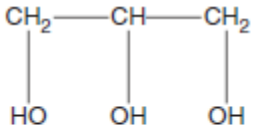


Figure 4-6 An illustration of a glycerol molecule.

Figure 4-7 A triglyceride.

THE ROLE OF TRIGLYCERIDES IN HEALTH

While fat may seem bad, triglycerides play many important roles in the body. For example, triglycerides can be used for energy storage in animals. This food reserve can be called upon during periods of starvation, with the high-calorie content of the fatty acids adding to the value of storing fat and providing much needed energy. In addition, triglycerides can provide insulation for animals in the form of body fat, which allows them to survive in colder temperatures. These two roles played by fat in the body, which arose over eons of evolution, are now deemed undesirable in modern industrialized society where humans no longer face starvation or have to deal directly with cold weather.

THE FORMATION OF TRYGLYCEROLS

Trigylcerides are formed when each of the OH groups in glycerol reacts with the COOH group of a fatty acid to create an ester group. Three water molecules are liberated in the process. The resulting molecule is illustrated in general in Fig. 4-7.

Note the fatty acid chains which extend from the glycerol backbone.

If all three R groups are the same, that is, the fatty acid that leads to the formation of each ester group in the resulting tryglycerol is the same; we say that the compound is a *simple tryglycerol.* In nature, the R groups will be different. In that case we call the resulting compound a *complex tryglycerol*.

Sphingolipids

A *sphingolipid* is an important constituent of the cell membrane which is based on a backbone molecule called *sphingosine* rather than glycerol. Sphingolipids can be found throughout the nervous systems of mammals, where they form a component of the *myelin sheath,* which is a fatty layer that provides insulation for the axons of neurons.

Components can be incorporated with a sphingosine molecule via reactions at the NH_2 and OH groups. For example, to obtain a *sphingomyelin,* a fatty acid is attached at the location of the NH_2 group of sphingosine. Another type of sphingolipid called a cerebroside has a saccharide unit attached at the location of the OH group of sphingosine. Cerebrosides are also abundant in the nervous system. An illustration of a sphingolipid is shown in Fig. 4-8.

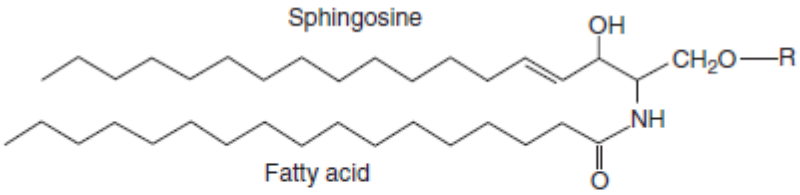


Figure 4-8 A sphingolipid consists of a fatty acid bound to sphingosine and an R group. (*Image courtesy of Wikipedia*)

The structure of a sphingolipid can be described as consisting of an unbranched 18-carbon alcohol with one trans double bond between carbons 4 and 5. These compounds have an NH_3^+ group bonded to the second carbon, and hydroxyl groups located at carbons 1 and 3. Besides playing a role in the central nervous system, it is believed that sphingolipids function as cell surface markers, providing ABO blood type antigens, for example.

Nonsaponifiable Lipids

The defining characteristic of a lipid is that it is insoluble in water and it is soluble in a nonpolar solvent. A nonsaponifiable lipid is one that cannot be broken down by hydrolysis. These characteristics bring several important biomolecules under the lipid umbrella that you may not think of as "fats." We begin our discussion of nonsaponifiable lipids by taking a brief look at *steroids*.

A *steroid* is a biologically important lipid that cannot be broken down into smaller molecules by the process of hydrolysis because it lacks ester groups. The defining characteristic of a steroid is the presence of a four-ring system that gives it structure. This ring system contains a single five-membered carbon ring together with three six-membered carbon rings.

The four-ring structure characteristic of steroids is illustrated in Fig. 4-9.

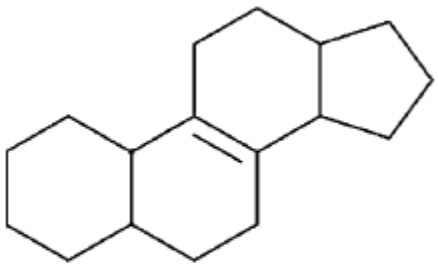


Figure 4-9 A steroid has a four-ring structure. Note that three of the rings have six members, and the remaining ring, found on the right, has five members.

Perhaps the most fundamental and famous steroid is cholesterol, which we discuss in the next section. Here we discuss several other steroids that are important in the body. These include the following:

• **Androgens** These are "male sex hormones" that regulate the development of the male reproductive system and the secondary sexual characteristics in males.

• **Progesterone, estrone, and estradiol** These are "female sex hormones" that regulate the development of the female reproductive system and are responsible for the maintenance of secondary sexual characteristics in females.

• **Aldosterone** This steroid controls water and electrolyte balances.

• **Cortisone** This compound is involved in metabolism and in controlling inflammation.

• **Bile salts** Facilitates the digestion of certain lipids and the absorption of fat-soluble vitamins.

• **Vitamin D** An important steroid that controls calcium absorption and deposition in the bone. Recent research also suggests that vitamin D plays a fundamental role in the prevention of many cancers. High consumption of vitamin D and sun exposure appear to reduce cancer risk.

Prostaglandins are nonsaponifiable lipids that are involved in several body functions. They consist of a 20-carbon chain that includes a five-membered ring at the end. One of the most important roles of prostaglandins is in the regulation of blood pressure. They also control blood clotting and induce labor.

Terpenes are large molecules constructed out of an *isoprene*, which is a branched carbon-5 unit (see Fig. 4-10). You know terpenes best as several common vitamins.

Some biologically important terpenes include

• **Vitamin A** Important for healthy vision, in particular night vision

• **Vitamin E** An important antioxidant that is involved in the maintenance of cell membrane integrity

• **Vitamin K** Involved in blood clotting

An example of a terpene is shown in Fig. 4-11, which displays vitamin K.

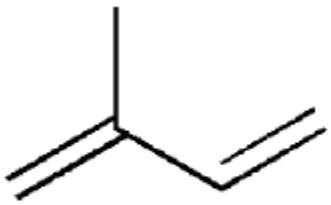


Figure 4-10 An isoprene is a five-membered carbon branch. Isoprenes can be assembled into vitamins called terpenes.

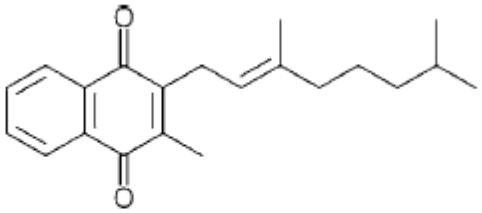


Figure 4-11 An example of a terpene, a variant of vitamin K.

Cholesterol

Cholesterol is an important lipid found in the cell membrane. It is a *sterol*, which means that cholesterol is a combination of a steroid and an alcohol. It is an important component of cell membranes and is also the basis for the synthesis of other steroids, including the sex hormones estradiol and testosterone, as well as other steroids such as cortisone and vitamin D. An illustration of cholesterol is shown in Fig. 4-12.

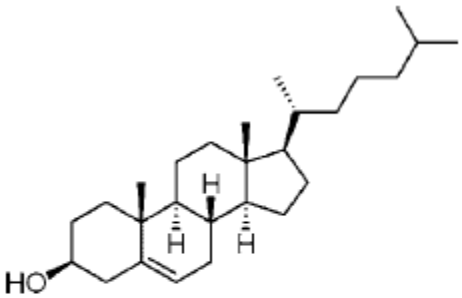


Figure 4-12 Cholesterol, which consists of an OH bound to a steroid ring formation and a hydrocarbon chain.

In the cell membrane, the steroid ring structure of cholesterol provides a rigid hydrophobic structure that helps boost the rigidity of the cell membrane. Without cholesterol the cell membrane would be too fluid.

In the human body, cholesterol is synthesized in the liver. Cholesterol is insoluble in the blood, so when it is released into the blood stream it forms complexes with *lipoproteins*. Cholesterol can bind to two types of lipoprotein, called *high-density lipoprotein* (*HDL)* and *low-density lipoprotein* (*LDL)*. A lipoprotein is a spherical molecule with water soluble proteins on the exterior. Therefore, when cholesterol is bound to a lipoprotein, it becomes blood soluble and can be transported throughout the body.

HDL cholesterol is transported back to the liver. If HDL levels are low, then the blood level of cholesterol will increase. High levels of blood cholesterol are associated with plaque formation in the arteries, which can lead to heart disease and stroke.

While most cholesterol in the body is synthesized in the liver, dietary cholesterol also adds to the total blood levels. Cholesterol intake from the diet enters the bloodstream in the LDL form. This helps explain why consumption of foods with high-cholesterol content can lead to increased blood levels of cholesterol which is bad for health. So reducing the cholesterol in the diet can lower the blood level of cholesterol. This can reduce the amount of plaque formation. Aerobic exercise also contributes to health by increasing HDL levels in the blood. Hence more cholesterol is returned to the liver leading to a lower blood level of cholesterol, and reduced plaque formation.

CHOLESTEROL'S ROLE IN THE CELL MEMBRANE

A cholesterol molecule has a hydroxyl group which acts to bind it to phospholipids in the cell membrane. Meanwhile, the steroid portion of the molecule acts as an anchor of sorts, interacting with the fatty acid chains of phospholipids. It is embedded directly in the cell membrane, helping guard it from too much fluidity.

An Overview of the Cell Membrane

A *cell membrane* is a structure constructed out of lipids, cholesterol, proteins, and carbohydrates that divides the cytoplasm and intracellular components of a cell from the external environment. The primary characteristic of the cell membrane, whose main component is constructed out of lipids, is that it consists of a *lipid bilayer* which functions to keep the external aqueous environment out and internal water in. You can think of a cell membrane by analogy as a plastic bag.

The physical division that the cell membrane provides is accomplished with phospholipids that have a hydrophilic head and hydrophobic tails. The outer layer of the cell membrane is formed by closely packed phospholipids, with the hydrophilic head facing outward, into the external watery environment. The tails, which are hydrophobic, extend downward from the heads inward where they bond to the tails of other phospholipids that form the inner side of the lipid bilayer. Both external layers of the cell membrane are water friendly and hence are in contact with aqueous solutions.

When a molecule contains both a hydrophilic and a hydrophobic component, we say that it is *amphipathic.* An example of a lipid membrane is shown in Fig. 4-13—this is a single lipid layer showing the hydrophilic heads and the hydrophobic tails.

When placed in an aqueous environment, amphipathic lipids will spontaneously arrange themselves into a spherical lipid bilayer structure. This arrangement is such that the hydrophobic tails are protected from the external aqueous environment, while the hydrophilic heads face it, well head-on. The formation of the spherical structure traps some aqueous solution inside the lipid bilayer, providing an internal environment

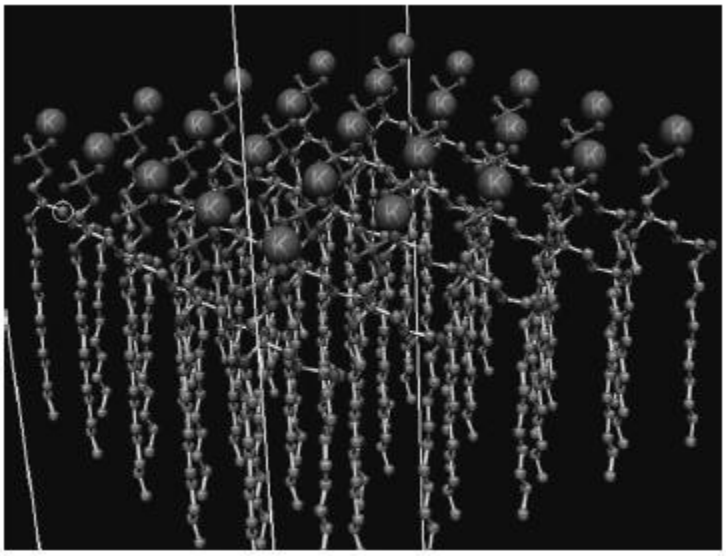


Figure 4-13 A lipid layer consists of water-loving (hydrophilic) heads and water-hating (hydrophobic) tails. The tails extend below the heads which face a watery or aqueous environment. See Fig. 4-14.

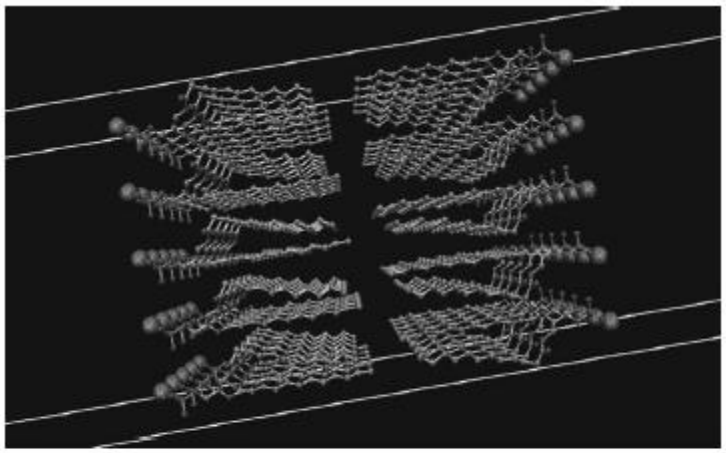


Figure 4-14 A lipid bilayer consists of two lipid layers of the type shown in Fig. 4-13. The hydrophobic tails join in the middle forming a bilayer-membrane structure.

faced by the internal layer of hydrophilic heads. Life may have gotten started by the spontaneous formation of lipid bilayer spheres, which may have trapped other chemicals such as RNA that led to the construction of more complicated proto-organisms. The side of the lipid bilayer that faces the external environment is called the *extracellular side,* while the side of the lipid bilayer that faces the internal cell environment is called the *intracellular side.* This division of external and internal is what makes life possible, without it life would be a meaningless phenomenon. The cell membrane is the first component that gives identity to an organism by distinguishing or separating it from its environment. This allows metabolic processes to take place within the cell; it allows the cell to store genetic information in the form of RNA and DNA; and it allows the cell to maintain necessary pH and ionic composition inside its internal fluids. Moreover, the cell membrane functions to dispose of waste materials to the outside environment.

The specific lipid content in a cell membrane consists of *phosphoglycerides* (this is a phospholipid containing a glycerol phosphate), *plasmalogens* (a lipid with an ether-linked alkene at the fi rst carbon of the glycerol), sphingomyelins, glycolipids, and cholesterol. A phospholipid has a phosphate ester group and ionic charges, with a fatty acid chain that contains an even number of carbon atoms which typically ranges between 14 and 24. The fatty acid chains can be saturated or unsaturated, with the unsaturated fatty acids adding to the fl exibility of the cell membrane. A cell membrane is a liquid-like structure that has a certain amount of flexibility.

As mentioned above, that flexibility is provided by the presence of unsaturated fatty acids. If you recall from the opening section of this chapter, unsaturated fatty acids (in the natural state) have cis double bonds among one or more of their carbon atoms that add a kink to the molecule. When they are packed together or with other molecules, these kinks serve to prevent the lipid molecules in the biliary from getting too close to one another. The end result is that intermolecular interactions among components of the membrane are decreased, giving it a flexible structure.

Of course, you would not want the cell membrane to be too flexible, otherwise it would just fall apart. Stability can be added to the cell membrane via the presence of cholesterol, as mentioned in the previous section. The hydrophobic portion of a cholesterol molecule is its four-ring system which is rigid as compared with the lipid bilayer. Adding cholesterol to the cell membrane keeps it stable by preventing it from being too fluid.

Proteins also play an important role in the cell membrane. A cell membrane can consist of anywhere between 20 and 75% protein. An *integral membrane protein* is one that is embedded directly in the lipid bilayer, sometimes extending from the extracellular side down into the intracellular side. Integral proteins act as a *gate* that can allow certain molecules (such as glucose) to enter the interior of the cell. More specifically, integral proteins function as ion channels and proton pumps. They may also act as receptors. They determine what comes in and what leaves a cell.

A *peripheral protein* is one that is associated with the cell membrane but that is not embedded directly in it. This association is usually temporary, and can involve an association with an integral protein. Peripheral proteins may be involved in some cellular process, for example, an enzyme might act as a peripheral protein driving some chemical reaction. Hormones can also act as peripheral proteins.

Molecules and ions need to be transported across the cellular membrane in order for the cell to function. This can be accomplished in one of the following ways:

• **Diffusion** If a concentration gradient exists from inside to outside the cell or vice versa, relatively small hydrophobic molecules are able to diffuse across the cell membrane passing through the bilayer of hydrophobic tails. An example of this is O_2 diffusion.

• **Active transport** Integral proteins can actively move molecules in and out of the cell against the concentration gradient, a process that requires energy input.

• **Passive transport: This** involves the movement of molecules along a concentration gradient, from high to low concentration. The movement of the molecules is done with the assistance of an integral protein which acts as a *channel*. Large polar uncharged molecules are transported across cell membranes via passive transport, such as glucose. Ions are also transported across cell membranes via passive transport. An example is the movement of ions across neural axons.

A complete picture of a cell membrane is shown in Fig. 4-15 which includes proteins, the lipid bilayer, and cholesterol.

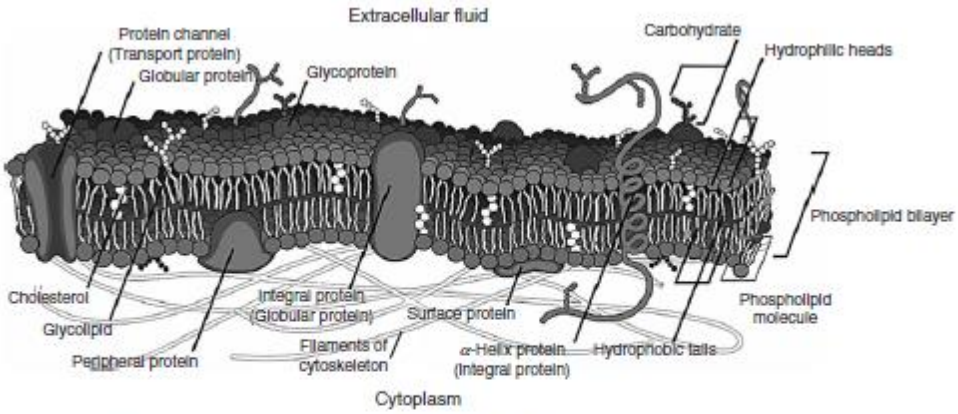


Figure 4-15 An illustration of the cell membrane, showing embedded proteins, cholesterol, and carbohydrate components. (*Image courtesy of Wikipedia*)

Lipid Metabolism

Lipids, in the form of triaglycerol and lipoproteins among other biomolecules, are a major component of the diet. Triaglycerols, in particular, are an important calorie source that stores a large amount of energy. While metabolism of carbohydrates and proteins produces around 4 to 5 kcal/g, the metabolism of triaglycerols produces nearly twice that amount at 9 kcal/g. The digestion of fats requires the action of enzymes produced in the pancreas and *bile salts* produced by the gall bladder. In this chapter, we give a basic overview of lipid metabolism.

Lipases and Phospholipases

A *lipase* is a hydrolytic enzyme that works in the small intestine to aid in the digestion of lipids. In particular, a lipase acts on dietary triaglycerol by hydrolyzing ester bonds between a fatty acid and a glycerol. Lipases are produced in the pancreas.

To catalyze the cleavage of the molecule, the enzyme must work in the presence of water. Some key facts about lipid digestion are:

• Triaglycerols are water insoluble.

• Digestive enzymes are water soluble.

• Therefore, digestive enzymes must act at water-lipid interfaces.

The products produced by the action of a lipase are:

• 2-Monoacylglycerol

• Two fatty acid molecules

The digestion process of triacylglycerol works as follows:

• Lipids are digested by lipase enzymes.

• The products of digestion become bound to *bile salt micelles* (see below).

• Fatty acids are absorbed from the micelles to cells in the small intestine.

• The fatty acids are transported to the liver.

• They are bound as lipoproteins that can be transported to cells to be used as needed.

Recall from our chapter on lipids that many fat molecules exist as *phospholipids*. A *phospholipase* is an enzyme which hydrolyzes a single ester bond of a phospholipids between a fatty acid and a glycerol. There are four types or classes of phospholipases.

These are:

• Phospholipase A This class has two subclasses. Phospholipase A1 hydrolyzes the ester bond between glycerol and a fatty acid at position 1 of the carbon chain of a phosphoglyceride, while phospholipase A2 hydrolyzes the ester bond between glycerol and a fatty acid at position 2 of the carbon chain of a phosphoglyceride.

• Phospholipase B hydrolyzes the ester bond at both positions 1 and 2 of a phosphoglyceride.

• Phospholipase C cleaves the molecule at the phosphate.

• Phospholipase D cleaves the molecule after the phosphate.

The points of action of each phospholipase enzyme are indicated in Fig. 5-1. The cleavage of a lipid or phospholipid produces fatty acids and lysophospho glycerides, respectively. These molecules act as *detergents* in the digestive system. That is, they act to break

up large droplets of fat into small droplets which are easier to digest. *Bile salts* aid in the digestive process by helping to increase the surface area of the water-lipid interface, and they act as detergents.

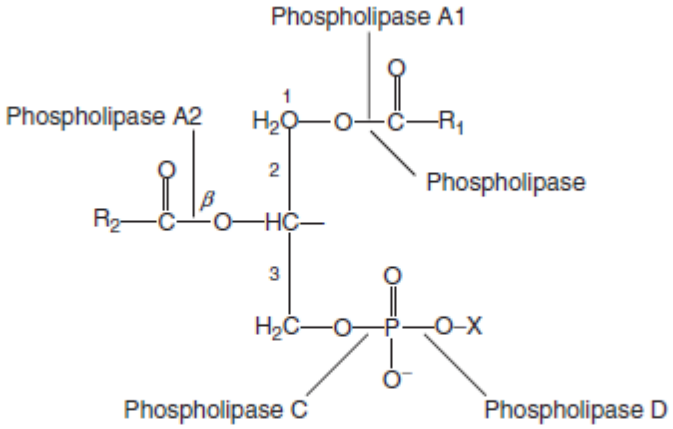


Figure 5-1 An illustration of where phospholipase enzymes act to cleave a phospholipid molecule.

Bile salts increase the surface area of the water-lipid interface by *emulsifying* the lipids, which means they are broken up into smaller droplets. So bile salts also act as detergents, which aid in the digestive process. Moreover, the bile salts form micelles which are single-layered structures (spherical in the case of bile salts) with the hydrophobic tails pointing inward. These micelles incorporate triacylglycerol, cholesterol, fat-soluble vitamins, fatty acids and 2-monoacylglycerol. When this happens we say that the micelle is a *mixed micelle*. These mixed micelles migrate to the intestinal wall where they release fatty acids, 2-monoacylglycerol, and fat-soluble vitamins that are absorbed by epithelial cells of the intestine. Bile salts are a derivative of cholesterol made in

the liver and released by the gall bladder in the form of *bile* to the small intestine.

Bile salts are required for the absorption of fat-soluble vitamins. The fat-soluble vitamins that are digested in this way include:

- Vitamin A
- Vitamin D
- Vitamin E
- Vitamin K

LIPID TRANSPORT

Lipids are moved through the blood stream by a complex between a protein and a lipid called a *lipoprotein*. These molecules are classified by their density. Since lipids are low-density molecules, the lower the density of the lipoprotein, the higher the ratio of lipid to protein in the molecule. These molecules include:

• **Chylomicrons** the lowest density lipoprotein
• **VLDL** very low-density lipoproteins
• **LDL** low-density lipoproteins
• **HDL** high-density lipoproteins
• **IDL** intermediate-density lipoproteins (derived from VLDLs in the formation of LDLs)

As noted above, chylomicrons are the lowest density lipoprotein. These molecules are composed primarily of triacylglycerols and are in fact 95% lipids. They serve to transport lipids for storage in adipose tissue. Once there, the chylomicrons are degraded and taken up by the liver. Next on the scale we find the very low-density lipoproteins (VLDL). These molecules are between 90 and 95% fat. Like chylomicrons, they also act to transport triacylglycerols to adipose tissue. VLDLs are degraded into LDLs.

We have already "met" LDL and HDL lipoproteins in our discussion of cholesterol. The primary transport protein for cholesterol is LDL. An LDL molecule is composed of about 85% lipid, with cholesterol being its primary lipid constituent. LDLs carry cholesterol to the cells.

HDLs are only composed of 50% lipid. They transport phospholipids and cholesterol. HDL molecules actually transport cholesterol back to the liver.

LIPOPROTEIN LIPASE

Once transported to their destination, fatty acids must be released from the lipoprotein complex. This is done in adipose tissue by an enzyme called *lipoprotein lipase*. The enzyme acts to break triacylglycerol into 2 fatty acid molecules and 2-monoacylglycerol. These molecules then diffuse into fat cells, which reassemble them into triacylglycerol molecules for storage.

Cholesterol Release

Cholesterol carried by LDL is transported to the cells where it can be utilized. Cells have LDL receptors on their surface, which can bind LDL and bring it into the cell by a process of endocytosis. Organelles called lysosomes fuse with the vesicles containing LDL and release lysozymes that hydrolyze the protein-lipid bond. The protein complex is degraded in the cell to

its constituent amino acids. At this point, the cholesterol exists in the form of *cholesteryl esters*, which are hydrolyzed into fatty acids and free cholesterol molecules.

β Oxidation of Fatty Acids

Fat is stored as triacylglycerol. To extract energy from the molecule, it must be broken down once again by a lipase enzyme. This enzyme catalyzes the hydrolysis of the triacylglycerol molecule into glycerol and fatty acids. Then, the fatty acids can be utilized to obtain energy in a process that occurs in three steps.

1. Activation
2. Transport to mitochondria
3. Oxidation to acetyl-CoA

We now consider each of these in turn.

ACTIVATION

Activation is a process by which a fatty acid is converted to a *coenzyme a derivative*. This process takes place on the outer surface of the mitochondria. The activation reaction is catalyzed by *acyl-CoA synthetase*. There are three different acyl-CoA synthetase enzymes.

• One to activate acetate, propionate, and butyrate-2 carbon to 4-carbon molecules.

• One that activates medium chain-length fatty acids, which are 4-carbon to 12-carbon molecules.

• Finally, one that activates long-chain fatty acids.

The activation of a fatty acid requires one adenosine triphosphate (ATP) molecule and Coenzyme A (CoASH). *Two phosphate* bonds of ATP are hydrolyzed in the process, leaving behind AMP + PP_i (two atoms of inorganic phosphate). This reaction is illustrated in Fig. 5-2.

TRANSPORT

A transport protein passes acyl-CoA fatty acid derivatives across the mitochondrial membrane to the matrix where they can be oxidized. This is done by *carnitine*, which is an *acyl-group carrier*. Acyl groups are bound to a hydroxyl group of carnitine using an enzyme called *carnitine acyltransferase*. There are actually two carriers, *carrier I* resides on the outer side of the inner mitochondrial membrane, while *carrier II* resides on the inner side of the inner mitochondrial membrane.

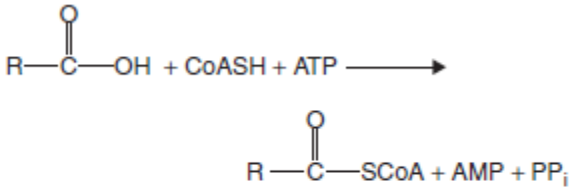


Figure 5-2 The activation of a fatty acid.

A *translocase enzyme* moves the acyl-carnitine complex to *acyltransferase II*, which releases the acyl-CoA derivative into the mitochondrial matrix. Free carnitine is transported in the opposite direction, toward the intermembrane space.

OXIDATION

Once inside the mitochondrial matrix, the molecule can be oxidized. This process works by shortening the acyl-CoA fatty acid derivatives two carbons at a time, yielding acetyl-CoA as the end product. This process is called β-oxidation because the bond between C-2 and C-3 of the chain is the β bond. The oxidation process requires four steps.

Step 1: Acyl-CoA → Trans-Δ^2-enoylacyl-CoA (enoyl-CoA)

This reaction reduces an FAD molecule FAD → FADH2. Enoyl-CoA serves as a substrate for water.

Step 2: Enoyl-CoA → 3-hydroxyacyl-CoA

In this step, water is added across the carbon-carbon double bond.

Step 3: 3-Hydroxyacyl-CoA → 3-Keoacyl-CoA

This step reduces NAD+ to NADH.

Step 4: 3-keoacyl-CoA → Acyl-CoA + Acetyl-CoA

This step requires the presence of a CoA molecule. Acetyl-CoA is released from the fatty acid by thiolytic cleavage.

The β-oxidation process continues, removing the first two carbon units of the fatty acid, until no more carbon units are available. For example, palmitoyl-CoA has 16 carbons. Seven cleavages are used which produce 8 molecules of acetyl-CoA which can then be utilized by the mitochondria to produce energy. Under normal conditions, intermediates used in the citric acid cycle are produced by carbohydrates. When glucose is not readily available (such as on a high-protein diet or during vigorous exercise) different mechanism must be used to digest fatty acids.

This is done using *ketone bodies*, molecules produced from acetyl-CoA by mitochondria found only in the liver.

Energy Yield

The energy yield from fatty acids is considerably higher than that obtained from glucose. The complete oxidation of one molecule of 16-carbon palmitic acid yields a net of 129 ATP molecules. One molecule of palmitic acid yields 8 molecules of acetyl-CoA from β-oxidation, 7 molecules of fl avine adenine dinucleotide and its reduced form ($FADH_2$), and 7 molecules of nicotinamide adenine dinucleotide and its reduced form (NADH). The $FADH_2$ and NADH molecules are oxidized in the electron transport chain. Each molecule of $FADH_2$ yields 2 ATP, thus 14 total ATP are produced from $FADH_2$. Each NADH molecule yields 3 ATP, giving a total of 21 ATP. Each acetyl-CoA yields 1 GTP (guanosine triphosphate) and 12 ATP. With 8 molecules of acetyl-CoA produced from palmitic acid, a total of $8 \times 12 = 96$ molecules of ATP are produced. A total of 131 molecules of ATP are produced, but there is an energy cost of 2 ATP in the metabolism of palmitic acid, giving the net of 129 ATP molecules. This is about three times the energy liberated from a molecule of glucose.

Possible Questions:

Part-A 20 x 1 = 20 marks

Each questions caries one mark.(Online examinations)

1. In an unsaturated fatty acid

(a) melting point is increased by the length of the hydrocarbon tail and is increased by the degree of unsaturation.

(b) melting point is decreased by the length of the hydrocarbon tail and is increased by the degree of unsaturation.

(c) melting point is increased by the length of the hydrocarbon tail and is decreased by the degree of unsaturation.

(d) melting point is decreased by the length of the hydrocarbon tail and is decreased by the degree of unsaturation.

Answer: c

2. Which of the following statements about trans fatty acids is false?

(a) Trans-fatty acids have straight hydrocarbon chains, while the cis variety has one or more kinks.

(b) Trans-fatty acids can be derived from plant oils using a process called hydrogenation.

(c) Trans-fatty acids have lower melting points than the corresponding cis confi guration.

(d) A trans stereoisomer differs from a cis stereoisomer in that similar groups attached to the carbon double bond are found on opposite sides.

Answer: c

3. Consider *erucic acid* whose formula is CH_3 (CH_2)7CH==CH(CH_2)11COOH. It is

(a) an omega-9 fatty acid.

(b) an unsaturated fatty acid.

(c) an omega-7 unsaturated fatty acid.

(d) Both (a) and (b) are correct.

Answer:d

4. Nonessential fatty acids

(a) include the groups linoleic and linolenic fatty acids.

(b) can be synthesized by the human body, so do not need to be directly consumed in the diet.

(c) are not required by the human body, so do not need to be directly consumed in the diet.

(d) include only saturated fatty acids.

Answer: b

5. Which of the following is not a true statement about triglycerides.

(a) Short chain, unsaturated triglycerides are liquid at room temperature.

(b) Long chain, saturated triglycerides tend to be solid at room temperature.

(c) Short chain, saturated triglycerides are solid at room temperature.

(d) Triglycerides are formed with a glycerol backbone and two or three fatty acids. They can be solid or liquid at room temperature depending on the length of the chains and the degree of saturation.

Answer: c

6. Which of the following statements about steroids is false?

(a) A steroid is a nonsaponifiable lipid meaning that it cannot be broken down by hydrolysis.

(b) A steroid contains a four-ring system.

(c) Steroids are important regulators of may bodily functions including the regulation of secondary sexual characteristics.

(d) A steroid is a nonsaponifiable lipid meaning that it can be broken down by hydrolysis.

Answer: d

7. Dietary cholesterol can be an important factor in heart health because

(a) it is not absorbed by the liver.

(b) it enters the blood stream bound to a low-density lipoprotein, so can raise blood cholesterol levels.

(c) it enters the blood stream bound to a high-density lipoprotein, so it can raise blood cholesterol levels.

(d) it is absorbed in the small intestine.

Answer: b

8. The structure of cholesterol is best described as

(a) a fi ve-ring system, bound to a hydroxyl group.

(b) a four-ring system, bound to a hydroxyl group.

(c) a four-ring system with one fi ve-membered ring, bound to a hydroxyl group and a hydrocarbon chain.

(d) a four-ring system with two fi ve-membered rings, bound to a hydroxyl group and a hydrocarbon chain.

Answer: c

9. A sphingolipid

(a) is structurally similar to a tryglycerol, but has a sphingosine backbone instead of a glycerol backbone.

(b) has two NH2 groups.

(c) is an important regulator in cholesterol metabolism.

(d) None of the above.

Answer:a

10. The cell membrane

(a) is made more flexible by the presence of unsaturated fatty acids because the kinks in the cis double bonds minimize close packing of molecules in the lipid bilayer.

(b) is made more flexible by the presence of cholesterol, whose four-ring structure minimizes the close packing of molecules in the lipid bilayer.

(c) is made less flexible by the presence of unsaturated fatty acids, whose presence increases intermolecular attraction among components in the lipid bilayer.

(d) is made more flexible by the presence of unsaturated fatty acids because the kinks in the trans double bonds minimize close packing of molecules in the lipid bilayer.

Answer:a

11. ----------is a process by which a fatty acid is converted to a coenzyme a derivative.

a.Activation b. Tranport c. Oxidation d. Reduction

Answer: a

12. A lipase breaks a triacylglycerol molecule down into

(a) 2-monoacylglycerol plus one fatty acid.

(b) 2-monoacylglycerol plus two fatty acids.

(c) acetyl-glycerol plus two fatty acids.

(d) 2-monoacylglycerol plus three fatty acids.

Answer: b

13. The lowest density lipoproteins can be best described as

(a) transporting cholesterol back to the liver.

(b) transporting cholesterol to cells.

(c) transporting fat molecules to adipose tissue.

(d) transporting cholesterol to the small intestine.

Answer: c

14. Chylomicrons are molecules that

(a) transport CoA lipid derivatives into the mitochondrial matrix.

(b) are lipoprotein complexes consisting of 95% lipids.

(c) are lipoprotein complexes consisting of 85% lipids.

(d) are transport molecules which move fatty acids from the small intestine to the liver.

Answer: b

15. The caloric value of lipid is

a. 6.0 Kcal/g b. 9.0 Kcal/g c. 15.0 Kcal/g d. 12.0 Kcal/g

Answer: b

16. Acrolic test is given by

a. Cholesterol b. Glycerol c. Glycosides d. Sphingol

Answer: b

17. Which of the following is non essential fatty acid?

a. Oleic acid b. Linoleic acid c. Arachidonic acid d. Linolenic acid

Answer: a

18. The essential fatty acid retard

a. Atherosclerosis b. Diabetes mellitus c. Nepritis d. Oedema

Answer: a

19. Bile is produced by

a. Liver b. Gall-bladder c. Pancreas d. Intestine

Answer: a

20. Lipase can act only at pH

a. 2.5-4 b. 3.5-5 c. 4 to 5 d. 5 – 7

Answer: d

Part-B 5 x 2= 10 marks

1. What is the role of triglycerides in health?
2. What is *lipoprotein lipase?*
3. Expain the essential and nonessential fatty acids and their role in diet.
4. What is Lipid metabolism?
5. Expain polar and nonpolar lipids.
6. Exapain the classes of lipids.
7. Explain -cholesterol's role in the cell membrane**.**

Part-C 5 x 6 = 30 marks

1. Explain the oxidation of mitochondrial matrix.
2. Write a note on Lipases and Phospholipases?
3. Write a note on β-oxidation of fatty acids?
4. Write a note on Lipid transport?
5. Write a note on fatty acids?
6. Write a note on cell membarane?
7. Explain the following terms a) Omega fatty acids. b) cis- trans fatty acids
8. Explain the formation of Tryglycerols.

KARPAGAM ACADEMY OF HIGHER EDUCATION

COIMBATORE-21

DEPARTMENT OF CHEMISTRY

SUBJECT: ANALYTICAL CLINICAL BIOCHEMISTRY

SUBJECT CODE: 17CHU404B

UNIT-III

PART-A

MULTIPLE CHOICE QUESTIONS (EACH QUESTIONS CARRY ONE MARK)

ONLINE EXAMINATION (20 X1 =20 MARKS)

1. Which of the following is an essential fatty acid?
a) Linolenic
b) Palmitic
c) Oleic
d) Stearic

Answer: a

2. Which of the following is a polar derivative of cholesterol?
a) Bile salt
b) Oestrogen
c) Vitamin D
d) Progesterone

Answer: a

3. Which of the following fatty acid has the least melting point?
a) Palmitic acid
b) Stearic acid
c) Arachidonic acid
d) Timnodonic acid

Answer: d

4. Out of the following which is not a source of glycerol?
a) Adipolysis

b) Glycolysis
c) Glycogenolysis
d) Diet

Answer: c

5. Which of the following is false about fatty acids?
a) Melting point of fatty acids decreases with increase in degree of saturation
b) Lipids in tissues that are subjected to cooling are more unsaturated
c) Naturally occurring unsaturated long-chain fatty acids are nearly Trans-configuration
d) The membrane lipids contain mostly unsaturated fatty acids

Answer: c

6. Which of the following fatty acid has 16 carbon atoms?
a) Linolenic acid
b) Oleic acid
c) Palmitic acid
d) Stearic acid

Answer: c

7. Which of the following is a hydroxyl fatty acid?
a) Linoleic acid
b) Palmitic acid
c) Linolenic acid
d) Cerebronic acid

Answer: d

8. Out of the following, which is not an essential amino acid?
a) Linolic acid
b) Linolenic acid
c) Arachidic acid
d) Arachidonic acid

Answer: c

9. The number of milligrams of KOH required to neutralize the free and combined fatty acid in one gram of a given fat is called
a) Saponification number

b) Iodine number
c) Acid number
d) Polenske number

Answer: a

10. Which of the following is a storage form of lipid?
a) Glycolipid
b) Phospholipid
c) Sufolipid
d) Triacyl glycerol

Answer: d

11. Out of the following, cholesterol does not serve as a precursor for which compounds?
a) Vitamin D
b) Sex hormones
c) Bile salts
d) Bile pigments

Answer: d

12. Which of the following is a sphingophospholipid?
a) Lecithin
b) Sphingomyelin
c) Plasmolegen
d) Cardiolipin

Answer: b

13. Which of the following glycerophospholid acts as a lipotropic agent?
a) Cardiolipin
b) Phosphatidylserine
c) Phosphatidylinositol
d) Phosphatidylcholine

Answer: d

14. Which of the following phospholipids is a component of inner mitochondrial membrane?
a) Plasmologen
b) Cephalin
c) Lecithin
d) Cardiolipin

Answer: d

15. In which of the following glycerophospholipids two phosphatidic acids share a single glycerol?
a) Cardiolipin
b) Phosphatidylserine
c) Phosphatidylinositol
d) Phosphatidylcholine

Answer: a

16. Platelet activating factor stimulates the release of which of the following compounds?
a) Vasopressin
b) Serotonin
c) Adrenaline
d) Cortisol

Answer: b

17. Which of the following group of membrane lipids predominate in plant cells?
a) Galactolipids
b) Sphingolipids
c) Glycerophospholipids
d) Archaebacterial ether lipids

Answer: a

18. Which of the following membrane lipids have a direct glycosidic linkage between the head-group sugar and the backbone glycerol?
a) Glycolipids
b) Phospholipids
c) Sphingolipids
d) Ether lipids

Answer: a

19. The backbone of phospholipids is

a) L-glycerol 1-phosphate

b) L-glycerol 3-phosphate

c) D-glycerol 3-phosphate

d) sn-glycerol 1-phosphate

Answer: b

20. What is the head-group alcohol in plasmolegen and platelet-activating factor?

a) Alkene

b) Choline

c) Alkane

d) Acetic acid

Answer: b

21. Phosphorylation of phosphatidylinositol yields

a) Phosphatidylinositol 4, 5-biphosphate

b) Phosphatidylinositol 3, 5-biphosphate

c) Phosphatidylinositol 3, 4-biphosphate

d) Phosphatidylinositol 5, 6-biphosphate

Answer: a

22. Which of the following vitamin is derived from cholesterol?

a) A

b) B

c) C

d) D

Answer: d

23. What leads to the activation of protein kinase C?

a) Release of intracellular Ca^{+2} + diacylglycerol

b) Release of intracellular Mg+ + diacylglycerol

c) Release of intracellular Ca^{+2} + glycerol

d) Release of intracellular Ca^{+2} + triacylglycerol

Answer: a

24. Which of the following serves as a specific binding site for the proteins involved in membrane fusion during exocytosis?

a) Phosphatidylinositol

b) Phosphatidylinositol 4, 5-biphosphate

b) Phosphatidylinositol 3, 5-biphosphate

c) Phosphatidylinositol 3, 4-biphosphate

Answer: b

5. Which of the following hormone is responsible for the activation of phospholipase C?

a) Serotonin

b) Cortisol

c) Vasopressin

d) Adrenaline

Answer: c

26. An example of glycerophospholipid involved in cell signaling is

a) Cardiolipin

b) Phosphatidic acid

c) Phosphatidylcholine

d) Phosphatidylinositol

Answer: d

27. Which of the following type structure contains all the three glycosphingolipids?

a) B structure

b) A structure

c) O structure

d) AB structure

Answer: c

28. The lipids with potent bio activities derived from isoprenoid precursors are common in

a) Vitamin A, K, ubiquinone and dolichol

b) Vitamin A, D, ubiquinone and dolichol

c) Vitamin A, B, D and K

d) Vitamin A, B, K and dolichol

Answer: a

29. Which of the following vitamin is responsible for Ca^{+2} and phosphate metabolism?
a) A
b) K
c) E
d) D

Answer: d

30. How many products are obtained by the hydrolysis of phosphatidylinositol by phospholipase C?
a) 1
b) 2
c) 3
d) 4

Answer: b

31. Which of the following is false about lipids?
a) They are either strongly hydrophobic or amphipathic
b) They are more soluble in water
c) Extraction of lipids from tissues require organic solvents
d) They are insoluble in water

Answer: b

32. Which would move faster in thin layer chromatography?
a) Beeswax
b) Phosphatidylinositol
c) Cholesterol
d) Steroid

Answer: a

33. In which type of chromatography, solvents of increasing polarity are passed through a column of silica gel?
a) High performance liquid chromatography
b) Thin layer chromatography

c) Adsorption chromatography
d) Gas-liquid chromatography

Answer: c

34. Phosphatidylinositol, phosphatidylglycerol and phosphatidylserine are easily separated by
a) Absorption chromatography
b) TLC
c) HPLC
d) Gas-liquid chromatography

Answer: b

35. For the determination of fatty acid composition, transesterification is done in a warm aqueous solution of
a) KCl+methanol
b) KOH+methanol
c) NaOH+methanol
d) H_2O+methanol

Answer: c

36. A mixture of fatty acyl methyl esters are separated based on
a) Charge
b) Chain length and degree of saturation
c) Molecular weight
d) Ionic size

Answer: b

37. Which technique is preferred in the separation of fatty acyl methyl esters from a mixture?
a) Gas-liquid chromatography
b) Absorption chromatography
c) TLC
d) Centrifugation

Answer: a

38. The dye used in TLC for detecting separated lipids by spraying the plate is
a) Mordant
b) Alizarin

c) Rhodamine
d) Fuchsin

Answer: c

39. Which of the following is not a phospholipase?
a) A
b) C
c) D
d) K

Answer: d

40. In which type of chromatography lipids are carried up a silica gel coated pate by a rising solvent front, less polar travels farther than the more polar ones?
a) Absorption chromatography
b) Thin layer chromatography
c) Gas-liquid chromatography
d) HPLC

Answer: b

41. Conversion of acetyl co-A to malonyl co-A requires which of the following?
a) NADPH
b) H_2O
c) Folic acid
d) Biotin

Answer: d

42. The prosthetic group of acyl carrier protein is
a) 4'-phosphopantetheine
b) 3'-phosphopantetheine
c) 2'-phosphopantetheine
d) 1'-phosphopantetheine

Answer: a

43. Which of the following carries acyl groups in thio-ester linkage?
a) Acyl carrier protein
b) Acetyl co-A ACP transacetylase

c) Enoyl-ACP reductase
d) Malonyl co-A ACP transferase

Answer: a

44. Which of the following transfers acyl group from co-A to cys residue of KS?
a) Acyl carrier protein
b) Acetyl co-A ACP transacetylase
c) Enoyl-ACP reductase
d) Malonyl co-A ACP transferase

Answer: b

45. Which of the following condenses acyl and malonyl groups?
a) Acyl carrier protein
b) Acetyl co-A ACP transacetylase
c) β-ketoacyl ACP synthase
d) Malonyl co-A ACP transferase

Answer: c

46. Which of the following transfers malonyl group from co-A to ACP?
a) Acyl carrier protein
b) Acetyl co-A ACP transacetylase
c) Enoyl-ACP reductase
d) Malonyl co-A ACP transferase

Answer: d

47. Which of the following reduces β-keto group to β-hydroxyl group?
a) β-ketoacyl ACP reductase
b) β-hydroxyacyl ACP dehydratase
c) Enoyl ACP reductase
d) Malonyl co-A ACP transferase

Answer: a

48. Which of the following removes H_2O from β-hydroxyl ACP, creating double bond?
a) β-ketoacyl ACP reductase
b) β-hydroxyacyl ACP dehydratase

c) Enoyl ACP reductase

d) Malonyl co-A ACP transferase

Answer: b

49. Which of the following reduces double bond, forming saturated acyl ACP?

a) β-ketoacyl ACP reductase

b) β-hydroxyacyl ACP dehydratase

c) Enoyl ACP reductase

d) Malonyl co-A ACP transferase

Answer: c

50. Which of the following converts PGH_2 to thromboxane A_2?

a) Enoyl-ACP reductase

b) β-ketoacyl ACP reductase

c) Cyclooxygenase

d) Thromboxane synthase

Answer: d

51. Which of the following is not true regarding synthesis of triacyl glycerol in adipose tissue?

a) Phosphatidate is hydrolyzed

b) Glycerol 3-phosphate dehydrogenase plays an important role

c) Glycerol kinase plays an important role

d) It is synthesized from dihydroxyacetone phosphate

Answer: c

52. Hydrolysis of phosphatidic acid by phosphatidic acid phosphatase yields

a) 1, 2-diacylglycerol

b) 1, 3-diacylglycerol

c) 1, 4-diacylglycerol

d) 1, 5-diacylglycerol

Answer: a

53. In animal tissues, triacylglycerols and glycerophospholipids share two precursors

a) Fatty acyl co-A and L-glycerol 3-phosphate

b) L-glycerol 3-phosphate and L-glycerol 2-phosphate

c) Diacylglycerol 3-phosphate and L-glycerol 3-phosphate

d) L-glycerol 3-phosphate and diacylglycerol 2-phosphate

Answer: a

54. Which of the following is more commonly called phosphatidic acid?
a) Diacylglycerol 3-phosphate
b) Fatty acyl co-A
c) L-glycerol 3-phosphate
d) L-glycerol 2-phosphate

Answer: a

55. Where does triacylglycerol form?
a) Liver
b) Kidneys
c) Adipose tissue
d) Heart

Answer: a

56. In adipose tissue, glyceroneogenesis couples with
a) Reesterification
b) Esterification
c) Glycolysis
d) Phosphorylation

Answer: a

57. Which of the following class of drugs reduce the levels of fatty acids circulating in the blood?
a) Thiazolidinediones
b) Amphetamines
c) Cathinones
d) Synthetic cannabinoids

Answer: a

58. The higher activity of which of the following enzymes leads to increased synthesis of the precursors of glyceroneogenesis
a) PEP carboxykinase
b) Acyl transferase

c) Acyl co-A synthase

d) Phosphatidic acid phosphatase

Answer: a

59. Dihydroxyacetone phosphate precursor of glycerol 3-phosphate is derived from

a) Glycerol

b) Triacylglycerol

c) Glycerol 3-phosphate

d) Pyruvate

Answer: d

60. Biological steroid derived from cholesterol is

a) Clenbuterol

b) Cortisol

c) Winstrol

d) Dianabol

Answer: b

CLASS: I MSC CHEMISTRY COURSE NAME: ANALYTICAL CLINICAL BIOCHEMISTRY

COURSE CODE: 17CHU404B UNIT: IV (Steroid harmones and DNA and RNA)

Batch-2017-20

Lecture Notes

Unit IV

Syllabus

Properties, functions and biochemical functions of steroid hormones. Biochemistry of peptide hormones.

Structure of DNA (Watson-Crick model) and RNA, Genetic Code, Biological roles of DNA and RNA: Replication, Transcription and Translation, Introduction to Gene therapy.

Enzymes: Nomenclature, classification, effect of pH, temperature on enzyme activity, enzyme inhibition.

Introduction:

In a multicellular organism, it is necessary for cells to communicate with each other to regulate various processes. While there are some synapses in the brain which operate using direct electrical contact, the primary means of communication in the body is through the use of chemical messengers. There are two basic ways that chemical messengers act to carry signals in the body: via *hormones* and *neurotransmitters.* A hormone is a chemical messenger that carries a signal from one cell to another, often from one part of the body to a cell in a distant location. Neurotransmitters are substances that are released by neurons to control or alter the behavior of their neighbors.

Cells respond to their environment by altering their biochemical processes. For all chemical messengers, be they hormones or neurotransmitters, the interface between the demands of the environment and the inner workings of the cell is accomplished through molecular signals that typically attach to the cell membrane. When a messenger attaches to the cell membrane

through signal transduction it affects the biochemistry of the cell. We begin the chapter with a discussion of hormones.

Hormones

A hormone is a chemical that acts as a messenger transmitting a signal from one cell to another. When it binds to another cell which is the target of the message, the hormone can alter several aspects of cell function, including cell growth, metabolism, or other function. Hormones can turn various bioprocesses on or off, and as such play a fundamental role in the livelihood of a multicellular organism. Hormones can be classified in a wide variety of ways. The first way that we can characterize a hormone is by looking at the distance over which the hormone acts.

There are three primary ways we can classify hormones in this way:

• *Autocrine:* A hormone is classified as an autocrine hormone if it acts on the same cell that released it.

• *Paracrine:* A paracrine hormone is one that acts on cells which are nearby relative to the cell which released it. An example of paracrine hormones includes *growth factors*, which are proteins that stimulate cellular proliferation and differentiation. Specifically, consider the binding of white blood cells to T cells. When the white blood cell binds to a T cell, it releases a protein growth factor called *interleukin-1*. This causes the T cell to proliferate and differentiate.

• *Endocrine:* An endocrine hormone is one that is released into the bloodstream by *endocrine glands.* The receptor cells are distant from the source. This is probably how most people think of hormones. An example of an endocrine hormone is *insulin*, which is released by the pancreas into the bloodstream where it regulates glucose uptake by liver and muscle cells.

Hormones can also be classified by the type of molecule the hormone is. There are three major classifications you should be aware of:

• *Steroids:* Steroid hormones are for the most part derivatives of cholesterol.

• *Amino acid derivatives:* Several hormones (and neurotransmitters) are derived from amino acids.

• *Polypeptides:* Many hormones are chains of amino acids. There are a large number of hormones that act in the body, and unfortunately we will only be able to discuss a small number of them. We now consider each of the major hormone types in turn.

STEROID HORMONES

Most steroid hormones are derived from cholesterol (see Fig. 12-1). Cholesterol is converted into a steroid hormone via the cleavage of a 6 carbon residue, producing a compound called *pregnenolone*, which is shown in Fig. 12-2. Pregnenolone is a precursor to C18, C19, and C21 steroids. We classify these steroids as follows:

• *Estrane:* An estrane is a steroid consisting of 18 carbon atoms.

• *Androstane:* An androstane is a steroid consisting of 19 carbon atoms.

• *Pregnane:* A pregnane is a steroid consisting of 21 carbon atoms.

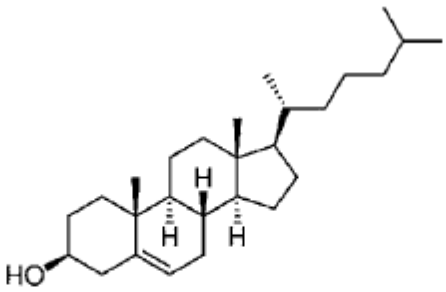


Figure 12-1 Steroid hormones are derived from cholesterol.

EXAMPLES OF STEROID HORMONES

A steroid hormone derived directly from pregnenolone is called *progesterone* (see Fig. 12-3). This hormone, which is secreted primarily by the *corpus luteum*, is a precursor for many other hormones. The corpus luteum is a temporary structure in females, derived from an ovarian follicle during the menstrual cycle. Progesterone is also produced by the adrenal glands. In addition to acting as a precursor molecule

in the synthesis of other hormones, progesterone plays several fundamental roles, the most familiar of which deal with the female reproductive system, where it prepares the female body for pregnancy. In ovulating women, it is released during the second 2 weeks of the menstrual cycle.

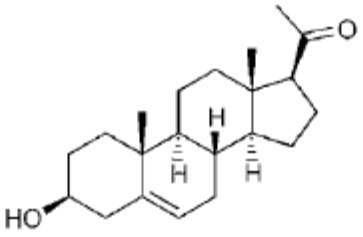


Figure 12-2 Pregnenolone is a precursor to many hormones.

Specifi cally, three effects of progesterone when it is secreted after ovulation can be identified:

• It prepares the endometrium for pregnancy. Progesterone levels drop if pregnancy does not occur after ovulation.

• It inhibits contraction of smooth muscle in the uterus.

• It inhibits the development of new follicles.

Progesterone also plays key roles in pregnancy, labor, and after birth. During pregnancy the placenta secretes progesterone, taking over for the corpus luteum.

Levels of progesterone are elevated during pregnancy, and they act to inhibit ovulation and lactation, but progesterone causes the growth of milk producing glands. A drop in progesterone levels may be involved in inducing labor and in the triggering of lactation.

In addition to its central role in pregnancy, progesterone plays other roles as well. It is known that the brain contains large number of progesterone receptors, and progesterone may be involved in memory and learning in the brain.

Aldosterone is a hormone derived directly from progesterone. This hormone is manufactured in the adrenal cortex, and is an example of a class of steroids we call *mineralocorticoids.* A mineralocorticoid is a hormone which is involved in the regulation of fluid volume and sodium in the body. In the case of aldosterone, three effects can be identified:

• It increases the reabsorption of sodium in the blood.

• It stimulates the release of potassium.

• It increases fluid volume.

From these three primary effects, it can be seen that the overall impact of aldosterone is a rise in blood pressure. If levels of aldosterone are too high in the body, high blood pressure and muscle cramps can result. *Renin* is an enzyme released by the kidneys in response to low blood

volume or low sodium concentrations, and it stimulates the production of aldosterone. Pregnant women often have high levels of aldosterone.

The major *sex hormones* are also derived from progesterone. These include *testosterone*, which is synthesized primarily in the testes of males but also in the adrenal glands and ovaries of females. Testosterone is responsible for the so-called secondary sex characteristics in males, such as facial hair and deepening of the voice. It is derived directly from a 19 carbon hormone called *androstenedione*. In the female, secondary sexual characteristics are regulated by a hormone called *estradiol*, shown in Fig. 12-3, which is a derivative of testosterone. Estradiol is also produced in smaller amounts in males. Interestingly, estradiol plays a role in the regulation of gene transcription. It has an effect on bone and is also involved in the production of lipoproteins in the liver.

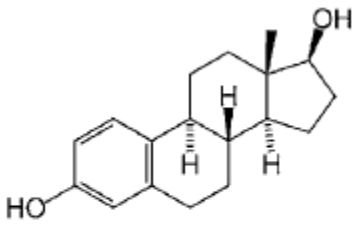


Figure 12-3 Estradiol is a hormone which plays a role in the development of female secondary sexual characteristics, among other functions.

Cortisol is another hormone synthesized from progesterone that is produced in the adrenal cortex of the brain. It is classifi ed as a *glucocorticoid*, meaning that it is a steroid which is involved in the metabolism of glucose. However, cortisol has wide and varied effects throughout the body. Cortisol may be involved in the regulation of the daily cycle of the body, as it helps restore homeostasis after periods of sleep. In the early morning, levels of cortisol are at their highest, while levels of cortisol are at their lowest about 3 hours after the onset of sleep. Cortisol also inhibits bone formation, has effects on the immune system, and increases sodium uptake. As a result cortisol increases blood pressure. Levels of cortisol are regulated

in the body by two enzymes known as *enzyme 11*-β *hydroxysteroid dehydrogenase type I* (11-β HSD1) and *type II* (11-β HSD2). Local concentrations of cortisol are increased by the action of 11-β HSD1, which converts a biologically inactive compound called *cortisone* into cortisol. The reverse process, mediated by 11-β HSD2, converts cortisol back into cortisone decreasing cortisol levels.

Vitamin D

One other steroid hormone is of particular interest. This is *vitamin D*, a steroid that is obtained either from the diet or from sun exposure. It comes in two primary forms, called *vitamin D2* (obtained from the diet) and *vitamin D3* (*cholecalciferol*— obtained from sun exposure synthesized in the skin from 7-dehydrocholesterol).

Vitamin D is not by itself useful to the body. It must be converted into a biologically active form by a two-step process that takes place in the liver and kidneys. Vitamin D3 is far more effective than vitamin D_2 in the production of the biologically active steroid (may be up to 10 times as effective). In the case of vitamin D_3, the molecule is hydroxylated in a reaction catalyzed by *25-hydroxylase* yielding a molecule called *25-hydroxycholecalciferol*. The production of this intermediate molecule is not strongly regulated in the body, so levels of this chemical reflects levels of vitamin D consumption in the diet and sun exposure. The fi nal step in synthesis of the biologically active form of vitamin D take place in the kidneys, where the conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol is mediated by an enzyme called *1-α-hydroxylase*. The fi rst three steps in the synthesis of the biologically active form of vitamin D are illustrated in Figs. 12-4 to 12-6.

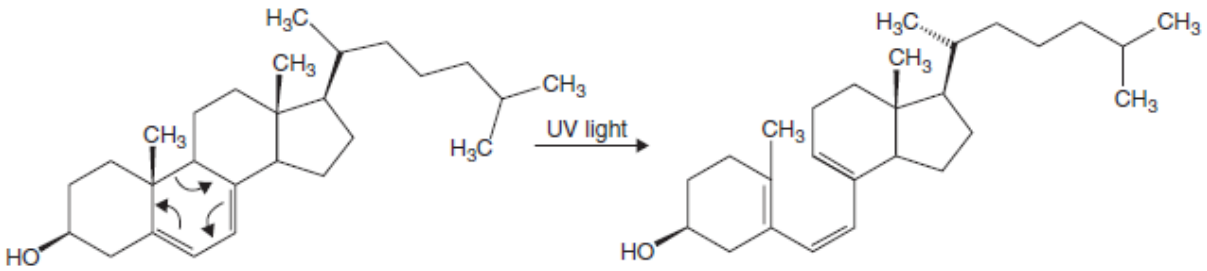


Figure 12-4 Exposure to UV light causes the molecule 7-dehydrocholesterol to transform into a molecule loosely called *pre-vitamin* D_3.

The major function of vitamin D in the body is the promotion of the intestinal absorption of calcium. It does this by promoting the manufacture of proteins in the cell that transport calcium from the intestine to the bloodstream. As such, vitamin D plays a key role in the maintenance of healthy bones. It plays a key role in several bone diseases. In *rickets*, a vitamin D deficiency in childhood leads to weakened and deformed bones. In adults vitamin D deficiency plays a role in osteomalacia and osteoporosis.

Recent research indicates that vitamin D plays an even wider role in the body. Vitamin D receptors exist throughout the body, including in the brain, gonads, heart, skin, prostate gland, and breasts. Vitamin D influences the growth and differentiation of cells, and may be implicated in cancer. In fact, vitamin D deficiency has been shown to correlate with increased risk for many types of cancer.

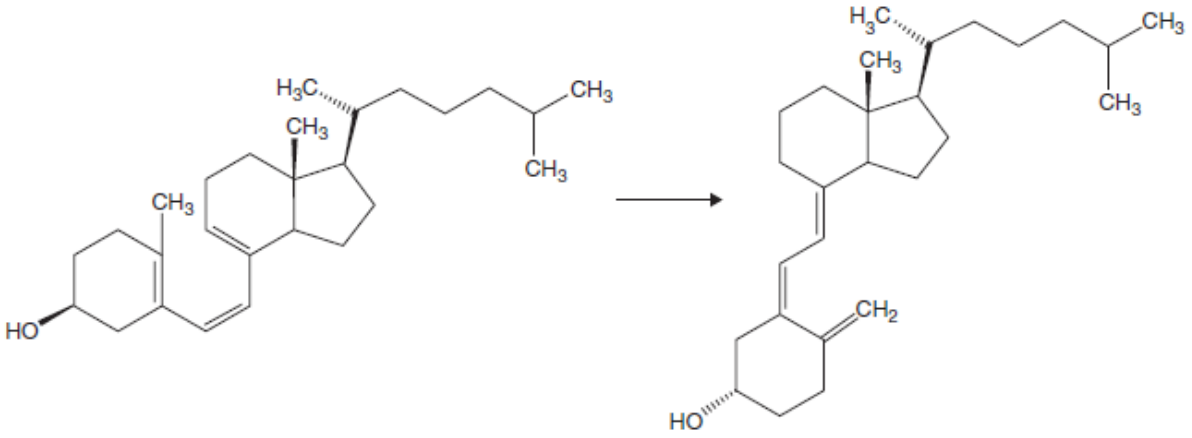


Figure 12-5 Pre-vitamin D_3 then spontaneously converts into vitamin D_3.

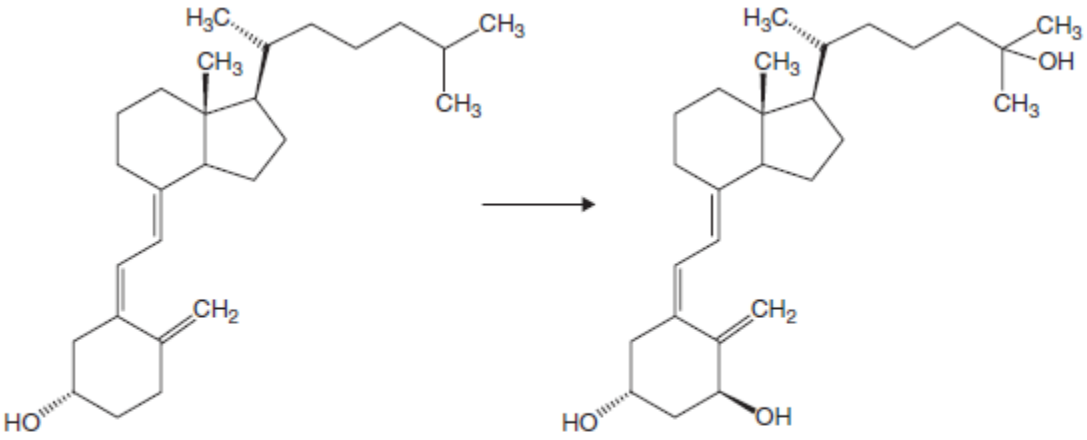


Figure 12-6 Conversion of vitamin D_3 into 25-hydroxycholecalciferol.

MECHANISM OF ACTION OF STEROID HORMONES

The way that hormones exert their effect on the cell is one of the most fascinating processes in the body. The primary method of action of steroid hormones is surprisingly to stimulate or inhibit the transcription of genes by the cell. Steroid hormones are lipids. As such

they pass directly through the plasma membrane of the cell where they can bind to *intracellular receptors* which couple directly to nucleotide sequences called *hormone response elements* (HRES). Hormone receptors can be found in the cytosol or actually inside the nucleus of the cell. An intracellular receptor is a polypeptide chain. On one end, we find a carboxyl group which marks the location of a *ligand-binding domain* to which the hormone binds. Upstream from the ligand-binding domain, there exists a *DNA-binding domain* which consists of amino acids that will bind the receptor to a specifi c DNA sequence.

Finally, at the other end we fi nd a region which activates transcription, this amino acid sequence is terminated by an amino group and so is known as the *amino terminus.* When a hormone binds to a receptor, it causes conformational changes that give the receptor the ability to bind to DNA. We say that the hormone *activates* the receptor. The activated receptor then binds to a promoting region of the DNA specific to genes that are regulated by the hormone. After binding, the transcription of the gene is stimulated in most cases (hormones can also act to inhibit gene transcription).

PEPTIDE HORMONES

Several important hormones are short chains of amino acids called *peptide hormones.* These hormones are typically endocrine hormones which are secreted into the blood stream so that they can have infl uences far from their site of secretion. The *pituitary gland* plays a major role in the secretion of these substances. For example, *vasopressin* is a hormone secreted by the pituitary gland that has wide ranging effects, including reduction of urine volume, stimulation of water reabsorption by the kidneys, and stimulation of moderate vasoconstriction. These actions indicate that vasopressin acts to increase blood pressure, and it is also known as the *antidiuretic hormone.* A drop in blood pressure or blood volume will stimulate the release of vasopressin. This short chain polypeptide consists of 9 amino acids. Surprisingly, vasopressin may act in the brain to enhance pair bonding among mates in mammals.

Another example of a peptide hormone is *oxytocin*, which is a polypeptide made from 9 amino acids (Fig. 12-7). This hormone, which is made in the hypothalamus and secreted by the pituitary gland, also plays a wide range of roles in mammals. It is thought to be involved in social recognition and pair bonding, and is also secreted in large amounts in females during labor. It stimulates smooth muscle in the uterus, leading to contractions that deliver the fetus. After birth oxytocin levels in the cerebrospinal fluid of mothers is high, and it has been

established that this hormone acts to promote bonding of the mother with her infant. Stimulation of the nipples of a nursing mother will cause the hypothalamus to release oxytocin. Oxytocin is also released in the brains of both sexes during orgasm, possibly enhancing pair bonding.

Furthermore, it is believed to aid in the regulation of the circadian rhythm. During periods of high stress, the effects of oxytocin are reduced because oxytocin neurons in the brain are suppressed by *catecholamines*, which are neurotransmitters released by the adrenal gland during stress (see below).

Prolactin is a much longer polypeptide, consisting of about 195 amino acids together in a single chain. This hormone is also synthesized in the pituitary, but is also made by other cells such as cells in the immune system. Prolactin release is triggered in lactating females by infant stimulation of the nipples.

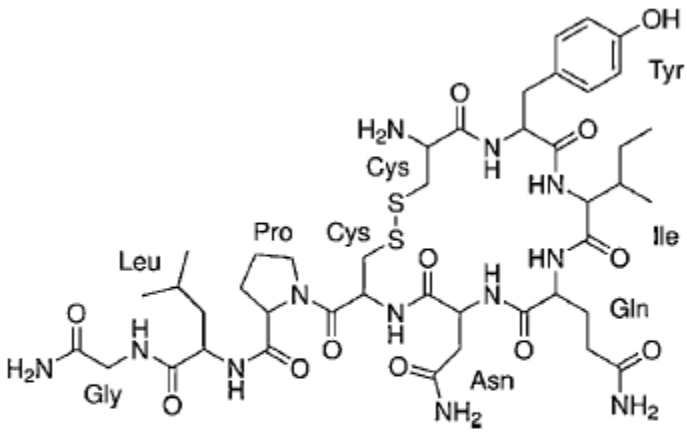


Figure 12-7 Oxytocin is a peptide hormone consisting of a chain of 9 amino acids.

The primary function of the hormone is to stimulate development of the mammary glands and to trigger milk production. It has other actions as well; recent research indicates that during sexual arousal prolactin acts against dopamine to cause the so-called refractory period. In males, prolactin may cause impotence and loss of sexual desire in some cases.

Another important polypeptide hormone is *insulin*, which acts to stimulate the uptake of glucose by liver and muscle cells so that they can store it as glycogen. Insulin is synthesized in the pancreas by β-*cells*, which construct a single chain molecule called *proinsulin*. Enzymes

excise a portion of the proinsulin molecule called the *C peptide*, producing the actual insulin molecule. When conditions warrant it, the β-cells will release insulin together with the c peptide into the blood stream via exocytosis. The role of insulin in the body is well known, with its primary role being to control the uptake of glucose by liver and muscle cells.

Areas of Hormone Production

We have seen that certain areas of the body (such as the adrenal and pituitary glands) are involved in hormone production. Hormones are actually produced in many areas throughout the body, which we now summarize. Due to limited space, this list is necessarily incomplete, but is provided to give the reader an idea of the wide range of organs involved in hormone production and release.

ADRENAL CORTEX

The adrenal cortex is involved in the production of two types of steroid hormones, glucocorticoids which affect metabolism and decrease inflammation, and mineralocorticoids which act to maintain salt and water balances.

ADRENAL MEDULLA

The *medulla* or core of the adrenal gland is responsible for the production of two important hormones which are amino acid derivatives. These include *epinephrine*, which causes the contraction of the smooth muscles, increases hear rate and blood pressure, and stimulates glycogenolysis in liver and muscle cells. The adrenal medulla also synthesizes norepinephrine which stimulates arteriole contraction and decreases peripheral circulation among other effects. We will discuss both hormones in more detail when we discuss neurotransmitters, below.

INTESTINE

Hormones are also produced in the intestines. Two examples include *cholecystokinin* (CCK), which is a polypeptide hormone that causes the pancreas to release digestive enzymes. In addition, it also stimulates the gall bladder causing it to empty.

LIVER

The liver is involved in the synthesis and secretion of several hormones. Insulin-like growth factor-1 (IGF-1) or somatomedin is a polypeptide hormone which stimulates cartilage

growth. Cells in the bone marrow are also affected by this hormone. It can bind to cells and trigger mitosis. The release of IGF-1 is triggered by the binding of growth hormone to liver cells. Another important hormone produced by the liver is *thrombopoietin,* is a 332 amino acid chain polypeptide. This hormone acts in the bone marrow, where it causes cells to differentiate into *megakaryocytes* which generate blood platelets.

PLACENTA

We have already seen that the steroid progestin hormones are produced in the placenta, where they play a role in regulation of the menstrual cycle and pregnancy. The placenta also produces a polypeptide hormone called *chorionic gonadotropin* that acts to cause the release of progesterone.

STOMACH

The stomach also produces several hormones, which are peptides. An example is *gastrin,* which triggers cells in the stomach to secrete gastric juice.

THYROID

The thyroid gland produces several vital hormones. The polypeptide *calcitonin,* which consists of a chain of 32 amino acids, acts to lower the amount of calcium in the bloodstream. It does this by inhibiting calcium uptake by the intestines. The thyroid also produces two important hormones which are amino acid derivatives, *triiodothyronine* (T3) and *thyroxine* (T4). These hormones act to control the rate of metabolic processes, with T3 acting as a metabolic stimulator.

NEUROTRANSMITTERS

The nervous system is composed of cells called *neurons* which are linked together by structures called *synapses* which enable the cells to "talk" to each other. A synapse actually contains a small gap between the cells called a *synaptic cleft,* which is a gap on the order of 20 nm wide. Nerve cells can be viewed as having a treelike structure, with branches at the top connected to the cell body containing the nucleus, a trunk like structure called the *axon* and a system of "roots" at the bottom which connect to the branches of another nearby neuron with which it can communicate. A nerve cell works to transmit information via an electrical signal that travels down the axon. However, the existence of the gap (synaptic cleft) requires nerve cells to use chemical messengers to transmit the signal to the next neuron. These chemical messengers are waiting in vesicles that are released into the synaptic cleft via exocytosis. They diffuse across the gap and bind to receptors on the next neuron. We call the chemical messengers

neurotransmitters, which are chemicals that serve to relay a signal from one neuron to another. This action serves to alter the behavior of neighboring neurons, causing them to "fire" or inhibiting them from doing so. The type of neurotransmitter released by a cell depends on the cell type and its location in the brain or peripheral nervous system.

There are several different classes of neurotransmitters. Several are *monoamines*, and include the famous chemicals *dopamine, serotonin,* and *norepinephrine* which are involved in the regulation of mood and emotion, among other functions. Polypeptides can also act as neurotransmitters; we have already met one in oxytocin. There are also smaller molecules which consist of a few amino acids such as *γ-aminobutyric acid* (GABA), an amino acid derivative which acts as a chemical messenger between neurons.

Many neurotransmitters also act as hormones in the bloodstream. However, they are not able to enter the brain this way because the brain is protected by a type of filter scientists call the *blood brain barrier*. The existence of this filtration system has important consequences in treating neurological disorders, because therapeutic agents must be administered as precursor molecules which can pass the blood brain barrier. Once inside the brain, they are processed into the desired chemical.

We now consider a few neurotransmitters in detail.

Epinephrine and Norepinephrine

Two important chemicals which act as neurotransmitters and hormones are epinephrine and norepinephrine, (also known by their older names adrenaline and noradrenaline) which are produced in the adrenal medulla. The base compound used in the synthesis of these two chemicals is the amino acid tyrosine. Norepinephrine, shown in Fig. 12-8, is an 8-carbon compound with the formula $C_8H_{11}NO_3$

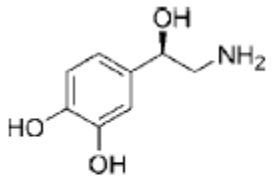


Figure 12-8 Norepinephrine is an important neurotransmitter.

One of the primary roles played by norepinephrine is in the production of the *flight or fight response*. In short, given a stimulation which may be threatening, ithelps ready the body for

action. A threatening stimulus will cause the release of large amounts of norepinephrine which act through the sympathetic nervous system (SNS) to

• Cause the release of glucose.

• Increase the heart rate.

• Stimulate the skeletal muscles.

For these reasons this compound is known as a *stress hormone*. The activation of this response takes place in the brain stem, and so is automatic. In the bloodstream, this chemical acts to cause vasoconstriction, an effect which also readies the body for physical action.

Norepinephrine is also a key neurotransmitter in the brain. It plays several roles, including

• The regulation of mood and emotion.

• Maintenance of attention and focus.

• Depression.

The role of norepinephrine in clinical depression is well established. Compounds called *reuptake inhibitors,* which delay norepinephrine from being taken up from the synaptic cleft and hence prolong the action of the chemical, enhance the mood of depressed patients.

Tyrosene is oxidized by tyrosine hydroxylase into a compound called *L-dopa*, which is the rate-limiting step in the production of norepinephrine. L-dopa then undergoes decarboxylation by pyridoxal phosphate and *dopa* decarboxylase to produce *dopamine*, which is itself an important neurotransmitter. An enzyme called *dopamine* β-*hydroxylase* then transforms dopamine into norepinephrine. When an animal is faced with a threat, the related compound epinephrine is also released, acting to trigger the fl ight or fi ght response. Using older or common terminology, epinephrine is sometimes called adrenaline. After norepinephrine has been produced, epinephrine can be synthesized in a single step. This is done with the aid of an enzyme called *phenylethanolamine N-methyltransferase*, which acts to add a methyl group to norepinephrine. The result is epinephrine which is illustrated in Fig. 12-9.

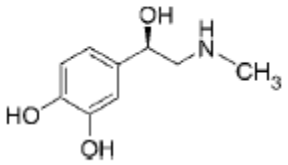


Figure 12-9 Epinephrine, an important neurotransmitter and hormone involved in stimulation of the sympathetic nervous system.

The catecholamines, including epinephrine and norepinephrine, act on cells through receptors on the cell membrane called *adrenergic receptors.* There are two major classes of adrenergic receptors, which can be denoted as *α*- and *β*-receptors. When epinephrine binds to β-adrenoreceptors, the following effects take place in the body via the activation of *adenylate cyclase:*

• Glycolysis is stimulated in skeletal muscle cells.

• Gluconeogenesis is stimulated in the liver.

• Lipolysis is stimulated in adipose tissue.

• Smooth muscles in blood vessels supplying skeletal muscles relax.

• Heart rate is increased.

• Smooth muscles in the bronchial tubes relax.

We see that the *same* receptors together with the *same* hormone (epinephrine) serves to achieve one goal—preparing the body for the flight or fight response. When epinephrine binds to *a*-adrenergic receptors, the following effects are noted:

• Smooth muscle contraction in blood vessels supplying the peripheral organs is stimulated.

• Smooth muscle *relaxation* in the lungs and gastrointestinal tract.

• Blood platelet aggregation is stimulated, preparing the body for clotting.

The α-adrenergic receptors can be further classifi ed into α1 and *α* 2 receptors. The *a*1 receptors act via a process called the *phosphoinositide cascade,* while the α2 receptors act by inhibition of an enzyme called *adenylate cyclase.*

In medicine, the action of a receptor is often characterized by the use of *agonists* and *antagonists.* An agonist is a substance that binds to a hormone receptor and induces a hormone response. In contrast, an antagonist binds a hormone receptor, but *does not* induce a hormone response. As a result the antagonist prevents the hormone from binding to a cell and blocks the action of the hormone. Agonists and antagonists can often be used as therapeutic agents. For

example, a chemical called *propranolol* blocks *a*-adrenoreceptors and hence acts as an antagonist. By noting the action of epinephrine on α-adrenoreceptors described above, the reader should not be surprised to learn that this compound can be used to treat high blood pressure in some cases.

Now let's consider how epinephrine acts at the cell membrane level. A sudden shock which stimulates the flight or fight response (such as someone cutting you off in traffic) results in the rapid release of epinephrine into the blood stream. This stimulates the liver to degrade glycogen making more glucose available to the body, and causes the production of glucose 6-phosphate in the skeletal muscle cells, giving them the energy they need to take action. These processes are mediated by cAMP.

At the cell membrane, epinephrine (the *first messenger*) binds to a receptor in the phospholipid bilayer which causes in conformational change in membrane bound *adenylate cyclase.* When activated, ATP can be converted into cAMP which acts as a *second messenger* inside the cell.

DNA-structure, properties and function

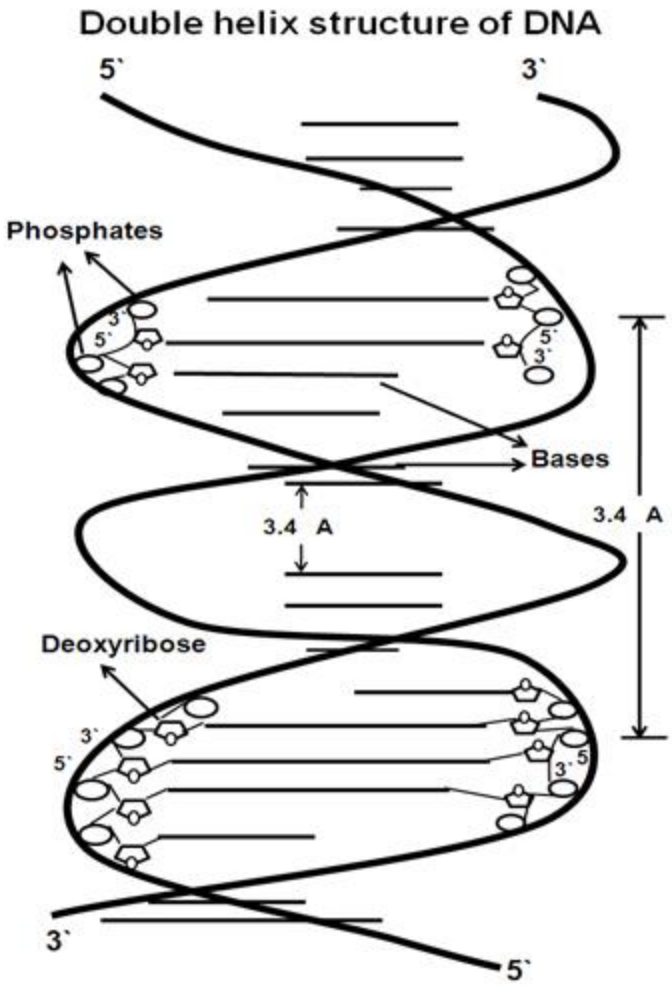
Structure of DNA :

• The discovery of DNA structure is one of the hall mark of the modern molecular biology.

• Based on the assumptions of Chargoff and utilizing X-ray diffraction data, obtained from crystals of DNA by Rosalind Franklin and Maurice Wilkins, James Watson and Francis Crick proposed a model for the structure of DNA in 1953.

• They established that DNA has a double helical structure comprising of two complementary antiparallel polynucleotide strands, wound around each other in a rightward direction.

• The backbone of the helix is sugar-phosphate and the bases are in the interior of the helix and extended at 90^0perpendicular to the axis of the helix. Bases from opposite helix pair with each other. Purines form base pairs with pyrimidene as a thumb rule- A will pair with T, and C with G. According to this pattern, known as Watson-Crick base-pairing.



Specific features of DNA structure :

• It is double helical structure. One polynucleotide chain forms one strand. Two such strands form double helix.

• Chain has sugar phosphate backbone and the bases are arranged perpendicular to the chain.

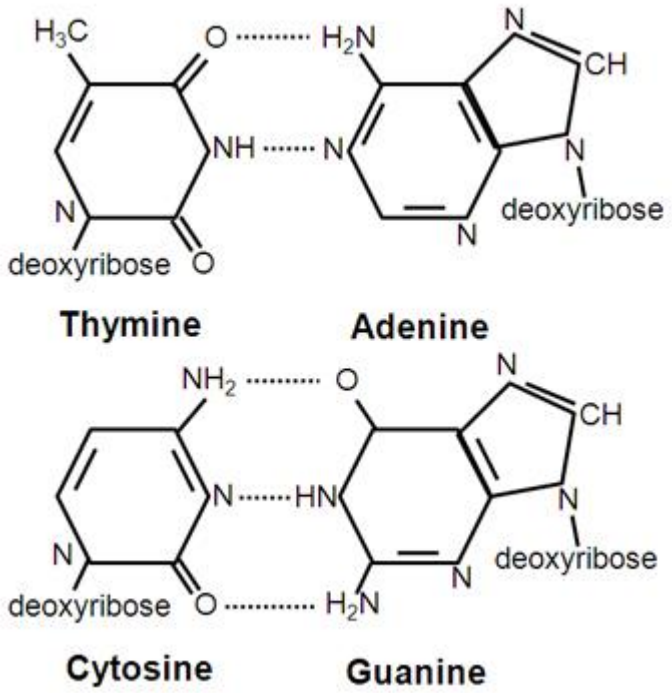
• Two strands are antiparallel to each other: one in 5' ---> 3' direction and the other in the 3' ---> 5' direction.

• A and T ; and G and C occur as complementary and form base pair with corresponding complementary base in opposite strand.

• One turn of the helix is 36 A and 10 base pairs are found per turn with rise of 3.6A.

• On the surface of double helix two deep grooves are found which are called major and **minor grooves**.

• Helix is right handed along the axis.
The bases pairs are hydrogen bonds with each other and impart stability to the structure. The base-pairs composed of G and C contain three H-bonds, whereas those of A and T contain two H-bonds. For this reason G-C base-pairs are stronger than A-T base-pairs. The outcome will be that DNA having more GC base pairs will be more stable than the one having more AT pairs.



• Bases are stacked over each other in the double helix.

• Hydrophobic interactions between stacked bases also stabilize the DNA.

• The sugar phosphate backbone of each strand is negatively charged (due to phosphate group (pKa being near to zero). These charges are stabilized by Mg2+.

Various conformations of DNA :

• One of the properties of the DNA is that it shows conformational flexibility, and could exist in alternative structural forms. The Watson-Crick structure is the B-form DNA or B-DNA.

• The B from is the most stable structure for a random sequence DNA molecule under physiological conditions and is therefore "the standard point of reference in any study of the properties of DNA".The B-DNA predominates in the cell.

• There are two other structural variants of DNA that have been well characterized in crystal

structures. They are the A-DNA and Z-DNA. These DNA variants differ in their helical sense, diameter, base pairs per helical turn, helix rise per base pair, base tilt normal to the helix axis, sugar pucker conformation, and glycosyl bond conformation.

Concept of GENE, GENOME and GENE EXPRESSION

DNA Replication.

• DNA replication, the basis for biological inheritance, is a fundamental process occurring in all living organisms to copy their DNA.

• In the process of "replication" each strand of the original double-stranded DNA molecule serves as template for the generation of the complementary strand.

• A number of enzymes are associated with the initiation and continuation of DNA synthesis.

• Proofreading activity by associated enzymes also ensures near perfect fidelity for DNA replication. $(dNMP)_n + dNTP \longrightarrow (dNMP)_{n+1} + PPi$ **Requirements :**

• Template : double stranded parent DNA.

• Substrate : Mg^{2+} and all four dNTP i.e. dATP, dTTP, dGTP, dCTP.

• Primer : short RNA fragment with a free 3´-OH end, wherein the initiation is started.

• Enzyme : DNA-dependent DNA polymerase (DDDP), other enzymes, protein factors.

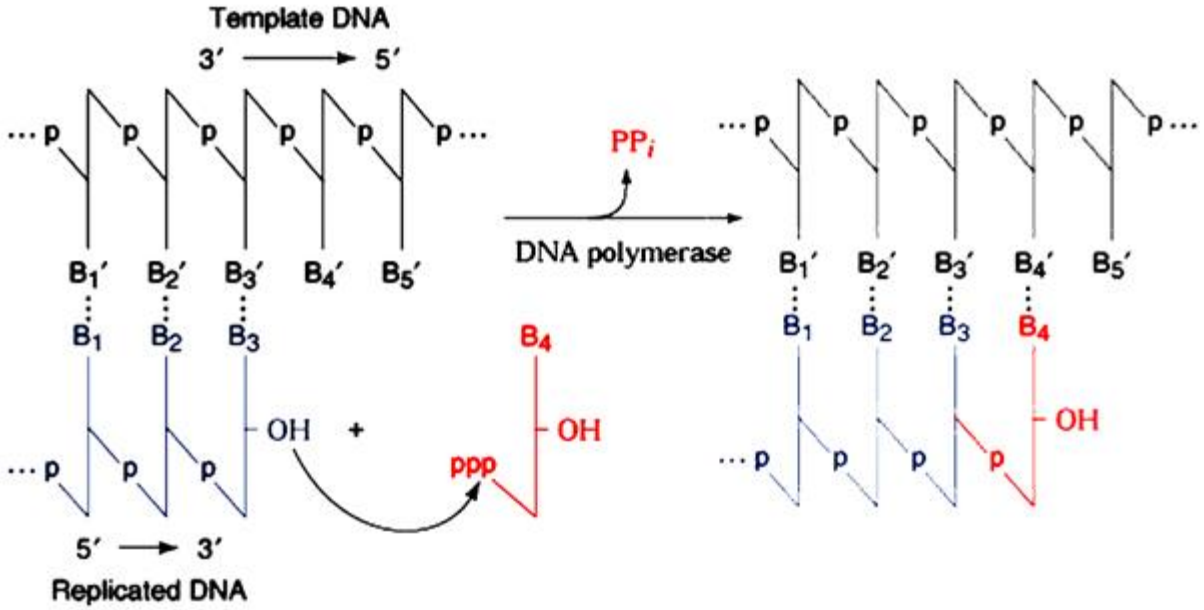
Features of replication :

• Semi-conservative.

• Semi-continuous.

• Bidirectional.

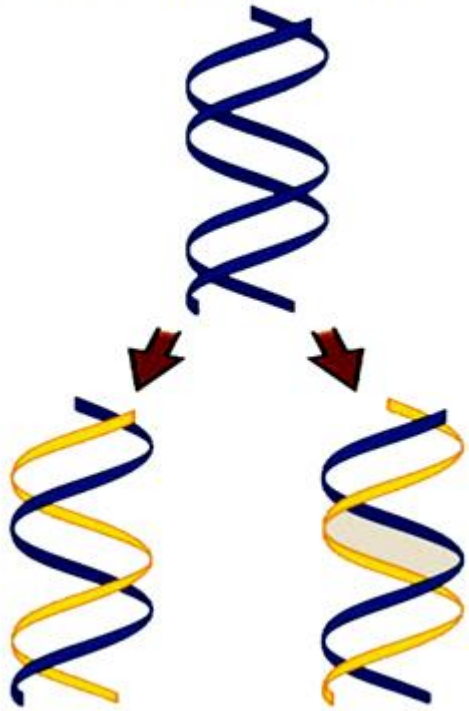
• High fidelity. Phosphodiester bond formation



: **B_1-$B_{1'}$, B_2-$B_{2'}$ and so on constitute complimentary base pairs like AT or GC**

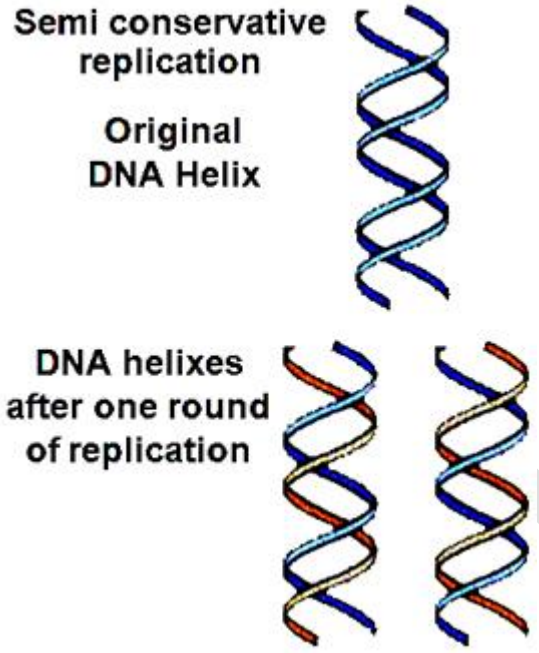
Mode of replication is semi-conservative : One of the strand of the parental DNA molecule is conserved in each new double helix, over which a new complementary daughter strand is synthesized. This ensures that genetic information is transferred from one generation to the next

One strand "old, One strand, new"



generation with a high fidelity. Semi conservative mode of replication was proved by Matthew Messelson and Franklin Stahl • Firstly, two strands of parent DNA unwind and then each of the strand acts as template to be copied. The

daughter strand get complementary base to the template and pair as per Watson and Crick base pairing. Thus the daughter DNA is essentially same in nucleotide sequence as



parent

Replication is Bi - Directional : Bi-directional nature of replication was proved by John Cairns.

In a cell, DNA replication begins at specific locations in the genome, called "origin". Unwinding of DNA at the origin, and synthesis of new strands, forms a Y-shape structure called the replication fork followed by the synthesis on each strand.

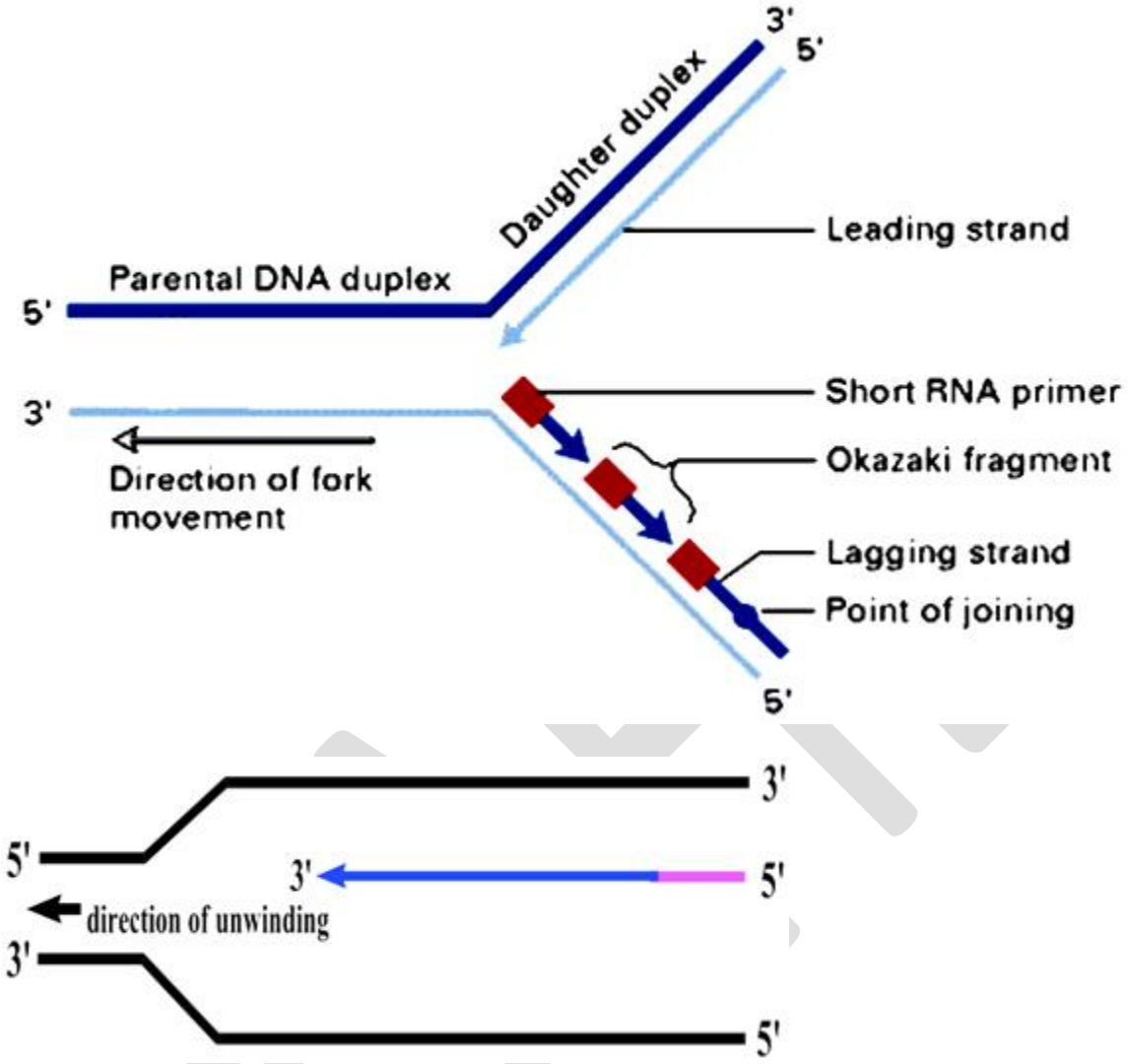
Once the double stranded DNA is opened at the origin, two replication forks are formed spontaneously. These two replication forks move in opposite directions as the synthesis proceeds. Therefore the replication is bidirectional but direction is 5'--> 3'. Replication Fidelity : Replication is crucial to the high accuracy of the genetic information transfer. Enzymes use two mechanisms to ensure the replication fidelity.

• Proofreading and real-time correction.

• Base selection. Semi-continuous Replication : • The daughter strands on two template strands are synthesized differently since the replication process obeys the principle that **DNA is synthesized from the 5´ end to the 3´end.**

• **Leading strand :** On the template having the 3´- end, the daughter strand is synthesized continuously in the 5'-3' direction. This strand is referred to as the **leading**

strand.



• **Lagging strand :** DNA is synthesized in discontinuous manner on template strand having the 5´- end. In this case small fragments are synthesized sequentially on the DNA. These DNA fragments are called **Okazaki fragments.** They are 1000 – 2000 nucleotides long for prokaryotes and 100-150 nucleotides long for eukaryotes. The daughter strand consisting of **Okazaki fragments** is called the **lagging strand.**

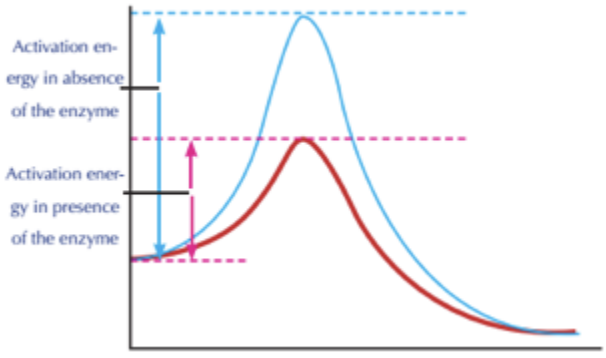
• Continuous synthesis of the leading strand and discontinuous synthesis of the lagging strand represent a unique feature of DNA replication. It is referred to as the semi-continuous replication.

Factors Affecting Enzyme Activity

- Biochemical reactions are necessary for growth, repairing damaged tissues, and obtaining energy and they take place in all living organisms' bodies. These reactions are called

'metabolism' and they happen all the time in living organisms. If they stop working, this leads to the death of the organism.

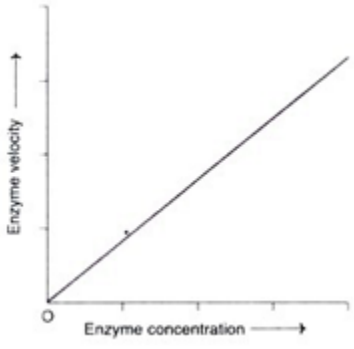
- All the reactions that occur in living organisms require high activation energy to take place. To reduce the cell's consumption of energy, there is a catalyst to ensure that the chemical reactions occur rapidly and reduce the activation of energy. This catalyst is the enzymes.



Enzyme activation energy

- Enzymes are biological catalysts made up of large protein molecules. They speed up the chemical reactions inside the cell. The enzyme is made up of a combination of amino acids which for a chain of polypeptides between each other.
- Enzymes are similar to other chemical catalysts. They participate in the reaction without getting affected. In other words, they speed up the chemical reactions inside the cells without getting consumed. Enzymes are affected by the hydrogen ion concentration (pH) and the temperature. Enzymes are highly specific compared to other catalysts, and each enzyme is specialized for one reactant substance. This reactant substance is called substrate, and it is specialized for one type of reaction or a few reactions. Enzymes lower the activation energy required to get the reaction started. Collectively, these are the most important properties of the enzyme.
- There are several factors that affect the speed of an enzyme's action, such as the concentration of the enzyme, the concentration of the substrate, temperature, hydrogen ion concentration (pH), and the presence of inhibitors.

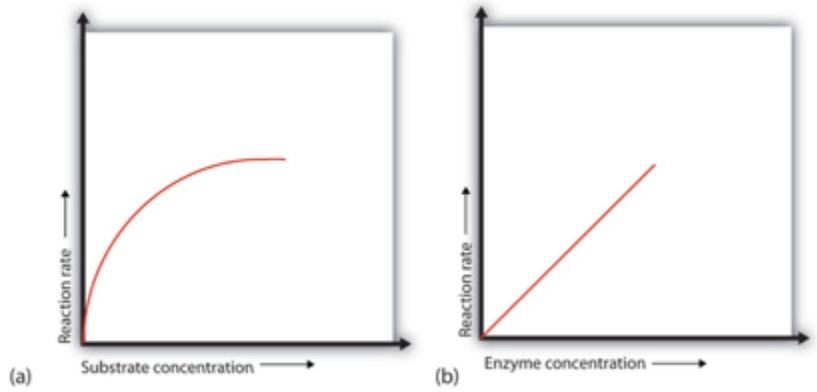
Factor 1: Concentration of Enzyme



Enzyme Concentration

- As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases. This property is used for determining the activities of serum enzymes during the diagnosis of diseases.

Factor 2: Concentration of Substrate

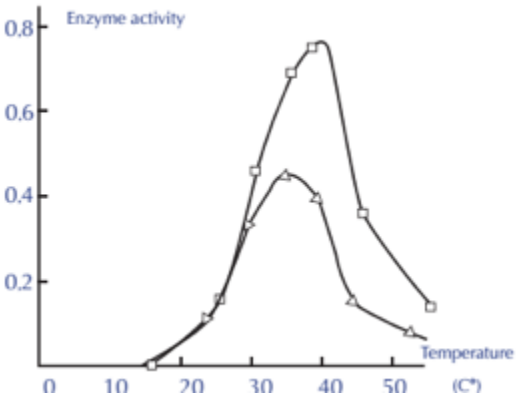


Substrate Enzyme Concentration

- In the presence of a given amount of enzyme, the rate of enzymatic reaction increases as the substrate concentration increases until a limiting rate is reached, after which further increase in the substrate concentration produces no significant change in the reaction rate. At this point, so much substrate is present that essentially all of the enzyme active sites have substrate bound to them.
- In other words, the enzyme molecules are saturated with substrate. The excess substrate molecules cannot react until the substrate already bound to the enzymes has reacted and been released (or been released without reacting).

Factor 3: Effect of Temperature

- The protein nature of the enzymes makes them extremely sensitive to thermal changes. Enzyme activity occurs within a narrow range of temperatures compared to ordinary chemical reactions. As you have seen, each enzyme has a certain temperature at which it is more active. This point is called the optimal temperature, which ranges between 37 to 40C°.
- The enzyme activity gradually lowers as the temperature rises more than the optimal temperature until it reaches a certain temperature at which the enzyme activity stops completely due to the change of its natural composition.
- On the other hand, if the temperature lowers below the optimal temperature, the enzyme activity lowers until the enzyme reaches a minimum temperature at which the enzyme activity is the least. The enzyme activity stops completely at 0C°, but if the temperature rises again, then the enzyme gets reactivated once more.



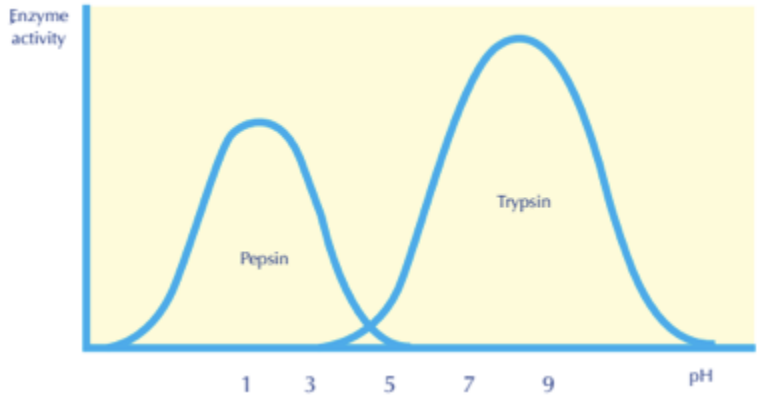
Enzyme activity and temperature

Factor 4: Effect of pH

- The potential of hydrogen (pH) is the best measurement for determining the concentration of hydrogen ion (H^+)in a solution. It also determines whether the liquid is acidic, basic or neutral. Generally, all liquids with a pH below 7 are called acids, whereas liquids with a pH above 7 are called bases or alkalines. Liquids with pH 7 are neutral and equal the acidity of pure water at 25 C°. You can determine pH of any solution using the pH indicators.

pH Indicators

- Enzymes are protein substances that contain acidic carboxylic groups ($COOH^-$) and basic amino groups (NH_2). So, the enzymes are affected by changing the pH value.
- Each enzyme has a pH value that it works at with maximum efficiency called the optimal pH. If the pH is lower or higher than the optimal pH, the enzyme activity decreases until it stops working. For example, pepsin works at a low pH, i.e, it is highly acidic, while trypsin works at a high pH, i.e, it is basic. Most enzymes work at neutral pH 7.4.



Enzyme PH activity

Factor 5: Effect of Activators

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

UNIT-IV

PART-A

MULTIPLE CHOICE QUESTIONS (EACH QUESTIONS CARRY ONE MARK)

ONLINE EXAMINATION (20 X1 =20 MARKS)

1. How do steroid hormones produce their effects in cells?

a) By activating key enzymes in metabolic pathway

b) By binding to intracellular receptors and promoting transcription of specific genes

c) By promoting degradation of specific m-RNAs

d) By activating translation of certain m-RNAs

Answer: b

2. An example of positive regulator

a) CAP

b) Lac 1 gene

c) Trp operon

d) Met operon

Answer: a

3. An example of negative regulator

a) CAP

b) Lac 1 gene

c) Nuclear receptors

d) Phosphorylated STAT proteins

Answer: b

4. Steroid regulatory proteins mediate the act by binding at

a) Zinc finger motif

b) Leucine zipper motif

c) Helix turn helix motif

d) Histone helix motif

Answer: a

5. Which out of the following statements is true about gene regulation in bacteria?

a) Repressor protein blocks transcription by binding to operator sequence

b) Activator proteins bind near promoters and increase the efficiency of transcription

c) Enhancers commonly regulate transcription

d) Genes with related functions are often grouped together and have a single start codon

Answer: a

6. Steroid hormones receptor binds to

a) Hormone response elements in m-RNA

b) Hormone response elements in DNA

c) Hormone response elements in proteins

d) Ribosomes to stimulate translation

Answer: b

7. Mode of action of steroid hormones involve

a) Stimulation of DNA replication

b) Stimulation of m-RNA transcription

c) Inhibition of protein synthesis

d) Secondary messenger

Answer: b

8. The drug antagonist of estrogen is

a) Tanoxifen

b) Metformin

c) Glucophage

d) Victoza

Answer: a

9. The drug used to terminate early pregnancies is

a) RU486

b) Metformin

c) Glucophage

d) Victoza

Answer: a

10. Plasma membrane protein predicted to have seen 7 transmembrane helices segment binds

a) Progesterone

b) Thyroid stimulating hormone

c) Insulin

d) Follicle stimulating hormone

Answer: a

11. DNA replication is

a) Conservative

b) Non-conservative

c) Semi-conservative

d) None

Answer: c

12. Semi-conservative DNA replication was first demonstrated in

a) Drosophila melanogaster

b) Escherichia coli

c) Streptococcus pneumonae

d) Drosophila melanogaster

Answer: a

13. Eukaryotes differ from prokaryote in mechanism of DNA replication due to

a) Use of DNA primer rather than RNA primer

b) Different enzyme for synthesis of lagging and leading strand

c) Discontinuous rather than semi-discontinuous replication

d) Unidirectional rather than semi-discontinuous replication

Answer: c

14. Which of the following is true about DNA polymerase?

a) It can synthesize DNA in the 5’ to 3’ direction

b) It can synthesize DNA in the 3’ to 5’ direction

c) It can synthesize mRNA in the 3’ to 5’ direction

d) It can synthesize mRNA in the 5’ to 3’ direction

Answer: a

15. The reaction in DNA replication catalyzed by DNA ligase is
a) Addition of new nucleotides to the leading strand
b) Addition of new nucleotide to the lagging strand
c) Formation of a phosphodiester bond between the 3'-OH of one Okazaki fragment and the 5'-phosphate of the next on the lagging strand
d) Base pairing of the template and the newly formed DNA strand

Answer: c

16. Which of the following reactions is required for proofreading during DNA replication by DNA polymerase III?
a) 5' to 3' exonuclease activity
b) 3' to 5' exonuclease activity
c) 3' to 5' endonuclease activity
d) 5' to 3' endonuclease activity

Answer: b

17. Which of the following enzymes remove supercoiling in replicating DNA ahead of the replication fork?
a) DNA polymerases
b) Helicases
c) Primases
d) Topoisomerases

Answer: d

18. DNA unwinding is done by
a) Ligase
b) Helicase
c) Topoisomerase
d) Hexonuclease

Answer: b

19. Which of the following enzymes is the principal replication enzyme in E. coli?
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b) DNA polymerase II

c) DNA polymerase III

d) None of these

Answer: c

20. The enzyme used to join bits of DNA is

a) DNA polymerase

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c) Endonuclease

d) Primase

Answer: b

Part-B (5 x2= 10 marks)

1. **Draw the structure of DNA.**
2. **What is Peptide Harmone?**
3. **What is steroid harmone?**
4. **What is Genetic code?**
5. **What is gemone?**
6. **What are the factors that affect the enzyme activity?**

Part-C (5x6=30 marks)

1. **Discuss the structure of DNA?**
2. **Write a note on DNA replication?**
3. **Write a note on Steroid harmone?**
4. **Explain the factors affecting enzyme activity?**
5. **Write a note on peptide harmone?**

KARPAGAM ACADEMY OF HIGHER EDUCATION

COIMBATORE-21

DEPARTMENT OF CHEMISTRY

SUBJECT: ANALYTICAL CLINICAL BIOCHEMISTRY

SUBJECT CODE: 17CHU404B

UNIT-IV

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a) DNA polymerase
b) DNA ligase
c) Endonuclease
d) Primase

Answer: b

221. Which of the following involves remarkable capacity of short segment of DNA to move from one place to another?
a) DNA transposition
b) DNA replication
c) Translation
d) Transcription

Answer: a

22. Which of the following process occurs between DNA molecules of very similar sequences?
a) Homologous genetic recombination
b) Site specific recombination
c) Non-homologous recombination
d) Replicative recombination

Answer: a

23. Which of the following process occurs in regions where no large –scale sequence similarity is apparent?
a) Homologous genetic recombination
b) Site specific recombination

c) Non-homologous recombination
d) Replicative recombination

Answer: c

24. Which of the following process generates a new copy of the transposable element at a new location of DNA?
a) Homologous genetic recombination
b) Site specific recombination
c) Non-homologous recombination
d) Replicative recombination

Answer: d

25. Which of the following occurs between particular short sequences present on otherwise dissimilar parental molecules?
a) Homologous genetic recombination
b) Site specific recombination
c) Non-homologous recombination
d) Replicative recombination

Answer: b

26. Which of the following promotes branch migration at higher rates than does Rec-A?
a) Rec-B
b) Rec-C
c) Rec-D
d) Ruv-A and Ruv-B

Answer: d

27. Which of the following is called a resolvase?
a) Ruv-C
b) Ruv-A
c) Ruv-B
d) Rec-A

Answer: a

28. Which of the following does not code for an enzyme having both helicase and nuclease activity?

a) Rec-A
b) Rec-B
c) Rec-C
d) Rec-D

Answer: a

29. The sequences of the recombination sites recognized by site-specific recombinases are
a) Partially asymmetric
b) Partially symmetric
c) Symmetric
d) Palindromic

Answer: a

30. Which of the following contain only the sequences required for transposition and the genes for proteins that promote the process?
a) Insertion sequences
b) Complex transposons
c) Transposons
d) Chromosomes
View Answer

Answer: a

31. Which of the following statements regarding splicing in eukaryotes is correct?
a) Several reactions in the splicing process involve hydrolysis of ATP
b) Exons are spliced out and introns are retained in the mature mRNA transcript
c) Splicing takes place in the cytosol
d) Small nuclear RNAs are retained in the mature mRNA transcript

Answer: a

32. Which of the following is not involved in the post transcriptional processing of t-RNA?
a) Base modulation
b) Attachment of CCA arm
c) Splicing
d) Attachment of poly-A tail

Answer: d

33. Incorrect statement about m-RNA

a) Cap is added to the 5’ end

b) Introns are removed and exons are spliced together

c) Histone mRNAs lack 5’ cap

d) Poly-A tail is added to the 3’ end

Answer: b

34. The first nucleic acid synthesizing enzyme discovered is

a) Polynucleotide phosphorylase

b) DNA polymerase

c) RNA polymerase

d) DNA ligase

Answer: a

35. 70S prokaryotic ribosome is the complex of

a) 30S + 50S

b) 30S + 40S

c) 20S + 60S

d) 20S + 30S

Answer: a

36. 80S eukaryotic ribosome is the complex of

a) 60S and 40S

b) 40S and 20S

c) 60S and 50S

d) 30S and 20S

Answer: a

37. The main function of t-RNA is

a) Proof reading

b) Inhibits protein synthesis

c) Identifies amino acids and transport them to ribosomes

d) None

Answer: c

38. One of the following best describes the cap modification of eukaryotic mRNA

a) Modified guanine nucleotide added to the 3’ end of the transcript

b) Modified guanine nucleotide added to the 5’ end of the transcript

c) String of adenine nucleotides added to the 3’ end of the transcript

d) String of adenine nucleotides added to the 5’ end of the transcript

Answer: b

39. The largest class of introns which are found in nuclear mRNA primary transcript is

a) Spliceosomal introns

b) Group I introns

c) Group II introns

d) Group IV introns

Answer: a

40. Which type of splicing reaction requires a guanine nucleoside or nucleotide cofactor that is not used as a source of energy?

a) Spliceosomal

b) Group I

c) Group II

d) Group IV

Answer: b

41. A codon contains how many nucleotides

a) 1

b) 2

c) 3

d) 4

Answer: c

42. The initiation codon is

a) AUG

b) UAA

c) UAG

d) UGA

Answer: a

43. The termination codon is not

a) AUG

b) UAA

c) UAG

d) UGA

View Answer

Answer: a

44. How many t-RNAs are required to translate all 61 codons?

a) 31

b) 32

c) 30

d) 29

Answer: b

45. Which position of a codon is said to wobble?

a) First

b) Second

c) Third

d) Fourth

Answer: c

46. In which of the following cases the first base of anticodon pairs with only one codon?

a) When the first base of anticodon is A or C

b) When the first base of anticodon is A or G

c) When the first base of anticodon is inosine

d) When the first base of anticodon is G or U

Answer: a

47. In which of the following cases the first base of anticodon pairs with two codons?

a) When the first base of anticodon is A or C

b) When the first base of anticodon is A or G

c) When the first base of anticodon is inosine

d) When the first base of anticodon is G or U

Answer: d

48. In which of the following cases the first base of anticodon pairs with three codons?
a) When the first base of anticodon is A or C
b) When the first base of anticodon is A or G
c) When the first base of anticodon is inosine
d) When the first base of anticodon is G or U

Answer: c

49. The genetic code translated the language of
a) Proteins into that of RNA
b) Amino acids into that of RNA
c) RNA into that of proteins
d) RNA into that of DNA

Answer: c

50. Wobble hypothesis was first proposed by
a) Nirenberg
b) Watson and Crick
c) Watson
d) Crick

Answer: d

51. By what factor chymotrypsin enhances the rate of peptide bond hydrolysis?
a) 10^7
b) 10^8
c) At least 10^9
d) 10^6

Answer: c

52. The active site of chymotrypsin consists of a catalytic triad of which of the following amino acid residues?
a) Serine, histidine and aspartate
b) Serine, histidine and glutamate
c) Threonine, histidine and aspartate
d) Methionine, histidine and aspartate

Answer: a

53. Which of the following statements are true about the reactions at the active center of chymotrypsin?
a) The aspartate residue gives an electron to histidine
b) The aspartate residue gives a proton to histidine
c) The aspartate residue keeps the histidine in the correct direction
d) A proton moves from the aspartate to serine to histidine in the catalytic triad of chymotrypsin

Answer: c

54. The polypeptide chains in chymotrypsin are linked by
a) Hydrogen bonds
b) Ionic bonds
c) Disulfide bond
d) SH-SH bond

Answer: c

55. Which of the following is false about chymotrypsin?
a) Hydrolytic cleavage of a peptide bond by chymotrypsin has two phases
b) It is activated in the presence of trypsin
c) It is synthesized in the thyroid gland
d) Polypeptide chains in chymotrypsin are linked by S-S bonds

Answer: c

56. Which of the following is true about the structure of hexokinase?
a) U-shaped
b) T-shaped
c) E-shaped
d) G-shaped

Answer: a

57. Which of the following is true?
a) Xylose is stereo chemically similar to glucose but one carbon shorter
b) Xylose binds to hexokinase in a position where it can be phosphorylated
c) Addition of xylose increases the rate of ATP hydrolysis
d) The binding of xylose is sufficient to induce a change in hexokinase to its active conformation

Answer: b

58. Which of the following catalyzes the reversible reaction of β-D-Glucose to glucose 6-phosphate?
a) Chymotrypsin
b) Hexokinase
c) Enolase
d) Trypsin

Answer: b

59. Which of the following is false about lysozyme?
a) It is an antibacterial agent found in tears and egg white
b) The substrate of lysozyme is peptidoglycan
c) Lysozyme cleaves (β1 → 4) glycosidic C-O bonds between two types of sugar residue in the molecule NAM and NAG
d) It is a bisubstrate enzyme

Answer: d

60. Which of the following is true about Michaelis-Menten kinetics?
a) K_m, the Michaelis constant, is defined as that concentration of substrate at which enzyme is working at maximum velocity
b) It describes single substrate enzymes
c) K_m, the Michaelis constant is defined as the dissociation constant of the enzyme-substrate complex
d) It assumes covalent binding occurs between enzyme and substrate

Answer: b

61. When the velocity of enzyme activity is plotted against substrate concentration, which of the following is obtained?
a) Hyperbolic curve
b) Parabola
c) Straight line with positive slope
d) Straight line with negative slope

Answer: a

62. Which of the following is the correct Line weaver-Burk equation?

a) $\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$

b) $\frac{1}{V_{max}} = \frac{K_m}{V_0[S]} + \frac{1}{V_0}$

c) $V_0 = \frac{V_{max}[S]}{K_m + [S]}$

d) $V_{max} = \frac{V_0[S]}{K_m + [S]}$

Answer: a

63. Which of the following statements is true about competitive inhibitors?

a) It is a common type of irreversible inhibition

b) In the presence of a competitive inhibitor, the Michaelis-Menten equation becomes

$$V_0 = \frac{V_{max}[S]}{\alpha K_m + [S]}$$

c) The apparent K_m decreases in the presence of inhibitor by a factor α

d) The maximum velocity for the reaction decreases in the presence of a competitive inhibitor

Answer: b

64. Which of the following statements is true about uncompetitive inhibitors?

a) They bind covalently at a site distinct from the substrate active site

b) In the presence of a uncompetitive inhibitor, the Michaelis-Menten equation becomes

$$V_0 = \frac{V_{max}[S]}{K_m + \alpha'[S]}$$

c) They increase the measured V_{max}

d) Apparent K_m also increases

Answer: b

65. The rate determining step of Michaelis-Menten kinetics is

a) The complex dissociation step to produce products

b) The complex formation step

c) The product formation step

d) None of the above

Answer: a

66. The molecule which acts directly on an enzyme to lower its catalytic rate is

a) Repressor

b) Inhibitor

c) Modulator

d) Regulator

Answer: b

67. Which of the following is an example for irreversible inhibitor?

a) Disulfiram

b) Oseltamivir

c) Protease inhibitors

d) DIPF

Answer: d

68. Which of the following is an example of reversible inhibitor?

a) DIPF

b) Penicillin

c) Iodoacetamide

d) Protease inhibitors

Answer: d

69. Where does inhibitor binds on enzyme in mixed inhibition?

a) At active site

b) Allosteric site

c) Does not bind on enzyme

d) Binds on substrate

Answer: b

70. The catalytic efficiency of two distinct enzymes can be compared based on which of the following factor?

a) K_m

b) Product formation

c) Size of the enzymes

d) pH of optimum value

Answer: a

12. What is the general mechanism of an enzyme?

a) It acts by reducing the activation energy

b) It acts by increasing the activation energy

c) It acts by decreasing the pH

d) It acts by increasing the pH

Answer: a

Unit V

Lecture Notes:

Syllabus

Unit V

Biochemistry of disease: A diagnostic approach by blood/ urine analysis.
Blood: Composition and functions of blood, blood coagulation. Blood collection and preservation of samples. Anaemia, Regulation, estimation and interpretation of data for blood sugar, urea, creatinine, cholesterol and bilirubin.
Urine: Collection and preservation of samples. Formation of urine. Composition and estimation of constituents of normal and pathological urine.

Introduction:

Blood makes up about 8% of the human body weight. It contains erythrocytes, leucocytes, thrombocytes (platelets) and plasma.

The volume percentage of all blood cells in the whole blood is about 45% of adults (hematocrit). The rest consists of liquid plasma (e.g. water, plasma proteins, electrolytes etc.).

The blood is composed of:

- Cells.
- Cell fragments.
- Aqueous solution (plasma).

Key Facts about Blood	
Functions	Transports gases (oxygen, carbon dioxide, nitrogen), nutrients, and hormones

	Helps with the maintenance of acid-base homeostasis Maintains a constant body temperature Thrombogenesis and thrombolysis
Erythrocytes	They are round and bioconcave cells without a nucleus, transporting oxygen bound to their heme groups
Leukocytes	Neutrophils, eosinophils, basophils, lymphocytes (B,T), and monocytes
Platelets	They derive from megakaryocytes and are responsible for homeostasis
Clinical	Anemia, leukemia

Function

Messenger & Waste Removal

Blood is the most important transport medium in the human body. It transports gases (oxygen, carbon dioxide, nitrogen etc.) as well as nutrients (metabolism) and end products of cell metabolism. Hence the blood has the task of assuring the exchange of substances. It provides the tissues with blood gases and nutrients and in exchange transports end products (e.g. carbon dioxide, urea, uric acid, creatinine etc.) to the eliminating organs (lung, liver, kidney). Furthermore, it carries chemical messengers (hormones) to their target organs.

Acid-Base Balance

The acid-base homeostasis is regulated in the blood through the diffusion of gases between alveoli and blood in the lung (alveolar diffusion) oxygen diffuses from the alveoli into the blood due to the concentration gradient. It is taken up by the carrying protein hemoglobin (hem = iron-containing, globin = protein). Contrariwise carbon dioxide diffuses from the blood into the alveoli due to its higher blood concentration where it is breathed out.

Oxygen Supply & Carbon Dioxide Removal

The blood transports the oxygen from the alveoli to the remotest cells of the body. Because of the higher gas pressure in the plasma (relative to the cells), it diffuses to the tissues.

Carbon dioxide diffuses from the cells into the blood due to the higher gas pressure in the tissue. Here it undergoes a chemical reaction and forms carbonic acid ($CO_2 + H_2O \rightarrow H_2CO_3$) which dissociates into hydrogen ion (H^+) and bicarbonate (HCO_3^-). Thus the metabolism end product carbon dioxide is transported in the form of carbonic acid (or rather hydrogen ion and bicarbonate). In the lung, the above mentioned chemical reaction reverses and carbon dioxide is exhaled.

To sum it up the blood regulates the acid-base homeostasis by the gas exchange. The blood is also responsible for the homeostasis, e.g. balancing the water between the blood capillaries on the one hand and intracellular and extracellular space on the other hand. It also maintains a constant body temperature.

Coagulation

Coagulation factors (proteins) are solved in the blood and stop bleeding after a complex (cascade-like) activation of coagulation factors through damage to blood vessels finally leading to the building of thrombus (thrombogenesis). Simultaneously, fibrinogen/fibrin prevents the pathological development of blood clots in the blood vessels. Blood coagulation and fibrinolysis influence each other and maintain a sensitive equilibrium.

Blood Cellular Components

Erythrocytes

The function of the erythrocytes is the transport of oxygen from the lung to the tissue by bonding oxygen to the iron-containing heme group of the hemoglobin. Erythrocytes are round and have a biconcave shape as they have no nucleus. An erythrocyte has a diameter of 8 to 10 µm. A healthy adult has about 5 million/µl erythrocytes. Also, the blood group antigens are expressed on the surface membrane of the erythrocytes.

Leukocytes

Unlike mature erythrocytes, leucocytes have a nucleus. Different types of leucocytes can be found in the blood:

- neutrophil granulocytes (banded and segmented)
- eosinophil granulocytes
- basophil granulocytes
- lymphocytes
- monocytes

The normal concentration of leucocytes ranges from 4,000 to 10,000 per µl, depending on age and health status. Both leucocytes and erythrocytes are descendants of pluripotent hematopoietic stem cells from the bone marrow.

The primary function of leucocytes is the immune defense. Especially lymphocytes (25 to 40% of leucocytes) are responsible for the adaptive immune response, the specific defense from pathogenic germs. The B lymphocytes produce antibodies, whereas T lymphocytes mediate the antibody production and the direct cellular immune response.

Monocytes (4 to 8% of leucocytes) have the task of phagocytosis (e.g. removing foreign materials, bacteria etc.) by producing extremely reactive free oxygen radicals which are capable of penetrating and destroying bacteria wall. Monocytes may differentiate into fixed macrophages (histiocytes) in connective tissue or into free macrophages.

Platelets

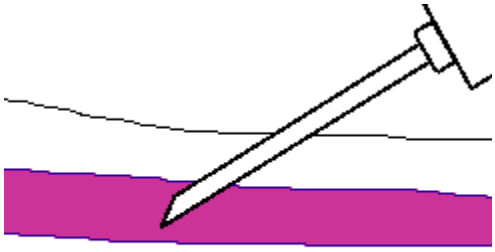
Platelets (thrombocytes) are another type of blood cells. They derive from megakaryocytes (bone barrow giant cells). Their task is the hemostasis when damage to blood vessels occurs (wound closure). The platelets adhere to the vascular wall of the damaged blood vessel and react with fibrin building a solid clot within 1 to 3 minutes (bleeding time). The physiological range for platelets is 150,000 – 400,000/µl.

Blood Specimen Collection and Processing

The first step in acquiring a quality lab test result for any patient is the specimen collection procedure. The venipuncture procedure is complex, requiring both knowledge and skill to perform. Several essential steps are required for every successful collection procedure:

Venipuncture Procedure:

1. A phlebotomist must have a professional, courteous, and understanding manner in all contact with all patients.
2. The first step to the collection is to positively identify the patient by two forms of identification; ask the patient to state and spell his/her name and give you his/her birth date. Check these against the requisition (paper or electronic).
3. Check the requisition form for requested tests, other patient information and any special draw requirements. Gather the tubes and supplies that you will need for the draw.
4. Position the patient in a chair, or sitting or lying on a bed.
5. Wash your hands.
6. Select a suitable site for venipuncture, by placing the tourniquet 3 to 4 inches above the selected puncture site on the patient. See below for venipuncture site selection "notes."
7. Do not put the tourniquet on too tightly or leave it on the patient longer than 1 minute.
8. Next, put on non-latex gloves, and palpate for a vein.
9. When a vein is selected, cleanse the area in a circular motion, beginning at the site and working outward. Allow the area to air dry. After the area is cleansed, it should not be touched or palpated again. If you find it necessary to reevaluate the site by palpation, the area needs to be re-cleansed before the venipuncture is performed.
10. Ask the patient to make a fist; avoid "pumping the fist." Grasp the patient's arm firmly using your thumb to draw the skin taut and anchor the vein. Swiftly insert the needle through the skin into the lumen of the vein. The needle should form a 15-30 degree angle with the arm surface. Avoid excess probing.

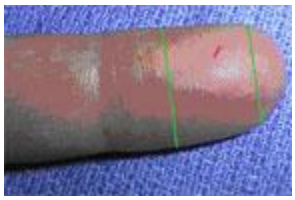


11. When the last tube is filling, remove the tourniquet.
12. Remove the needle from the patient's arm using a swift backward motion.
13. Place gauze immediately on the puncture site. Apply and hold adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes, tape a fresh piece of gauze or Band-Aid to the puncture site.
14. Dispose of contaminated materials/supplies in designated containers.

Note: The larger median cubital and cephalic veins are the usual choice, but the basilic vein on the dorsum of the arm or dorsal hand veins are also acceptable. Foot veins are a last resort because of the higher probability of complications.

Fingerstick Procedure:

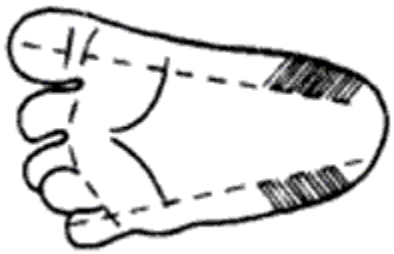
1. Follow steps #1 through #5 of the procedure for venipuncture as outlined above.
2. The best locations for fingersticks are the 3rd (middle) and 4th (ring) fingers of the non-dominant hand. Do not use the tip of the finger or the center of the finger. Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface. The 2nd (index) finger tends to have thicker, callused skin. The fifth finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold or cyanotic, swollen, scarred, or covered with a rash.
3. When a site is selected, put on gloves, and cleanse the selected puncture area.
4. Massage the finger toward the selected site prior to the puncture.
5. Using a sterile safety lancet, make a skin puncture just off the center of the finger pad. The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges.
6. Wipe away the first drop of blood, which tends to contain excess tissue fluid.



7. Collect drops of blood into the collection tube/device by gentle pressure on the finger. Avoid excessive pressure or “milking” that may squeeze tissue fluid into the drop of blood.
8. Cap, rotate and invert the collection device to mix the blood collected.
9. Have the patient hold a small gauze pad over the puncture site for a few minutes to stop the bleeding.
10. Dispose of contaminated materials/supplies in designated containers.
11. Label all appropriate tubes at the patient bedside.

Heelstick Procedure (infants):

The recommended location for blood collection on a newborn baby or infant is the heel. The diagram below indicates the proper area to use for heel punctures for blood collection.



1. Prewarming the infant's heel (42° C for 3 to 5 minutes) is important to increase the flow of blood for collection.
2. Wash your hands, and put gloves on. Clean the site to be punctured with an alcohol sponge. Dry the cleaned area with a dry gauze pad.
3. Hold the baby's foot firmly to avoid sudden movement.
4. Using a sterile blood safety lancet, puncture the side of the heel in the appropriate regions shown above. Make the cut across the heel print lines so that a drop of blood can well up and not run down along the lines.
5. Wipe away the first drop of blood with a piece of clean, dry cotton gauze. Since newborns do not often bleed immediately, use gentle pressure to produce a rounded drop of blood. Do not use excessive pressure because the blood may become diluted with tissue fluid.
6. Fill the required microtainer(s) as needed.
7. When finished, elevate the heel, place a piece of clean, dry cotton on the puncture site, and hold it in place until the bleeding has stopped. Apply tape or Band-Aid to area if needed.
8. Be sure to dispose of the lancet in the appropriate sharps container. Dispose of contaminated materials in appropriate waste receptacles.
9. Remove your gloves and wash your hands.

Order of Draw:

Blood collection tubes must be drawn in a specific order to avoid cross-contamination of additives between tubes. The recommended order of draw for plastic vacutainer tubes is:

1. First - blood culture bottle or tube (yellow or yellow-black top)
2. Second - coagulation tube (light blue top).
3. Third - non-additive tube (red top)

4. Last draw - additive tubes in this order:
    - SST (red-gray or gold top). Contains a gel separator and clot activator.
    - Sodium heparin (dark green top)
    - PST (light green top). Contains lithium heparin anticoagulant and a gel separator.
    - EDTA (lavender top)
    - Oxalate/fluoride (light gray top) or other additives

    NOTE: Tubes with additives must be thoroughly mixed. Clotting or erroneous test results may be obtained when the blood is not thoroughly mixed with the additive.

Labeling The Sample

All specimens must be received by the laboratory with a legible label containing at least two (2) unique identifiers.

The specimen must be labeled with the patient's full name (preferably last name first, then first name last) and one of the following:

- GHS medical record number (MRN) - for Geisinger locations, this is the required second identifier
- Patient's full date of birth (must include the month, day, and year)
- Unique requisition identifier/label

Areas to Avoid When Choosing a Site for Blood Draw:

Certain areas are to be avoided when choosing a site for blood draw:

- Extensive scars from burns and surgery - it is difficult to puncture the scar tissue and obtain a specimen.
- The upper extremity on the side of a previous mastectomy - test results may be affected because of lymphedema.
- Hematoma - may cause erroneous test results. If another site is not available, collect the specimen distal to the hematoma.
- Intravenous therapy (IV) / blood transfusions - fluid may dilute the specimen, so collect from the opposite arm if possible.
- Cannula/fistula/heparin lock - hospitals have special policies regarding these devices. In general, blood should not be drawn from an arm with a fistula or cannula without consulting the attending physician.
- Edematous extremities - tissue fluid accumulation alters test results.

Techniques to Prevent Hemolysis (which can interfere with many tests):

- Mix all tubes with anticoagulant additives gently (vigorous shaking can cause hemolysis) 5-10 times.
- Avoid drawing blood from a hematoma; select another draw site.
- If using a needle and syringe, avoid drawing the plunger back too forcefully.
- Make sure the venipuncture site is dry before proceeding with draw.
- Avoid a probing, traumatic venipuncture.
- Avoid prolonged tourniquet application (no more than 2 minutes; less than 1 minute is optimal).
- Avoid massaging, squeezing, or probing a site.
- Avoid excessive fist clenching.
- If blood flow into tube slows, adjust needle position to remain in the center of the lumen.

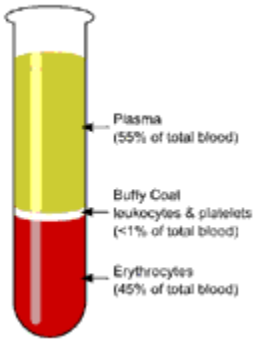
Blood Sample Handling and Processing:

Pre-centrifugation Handling - The first critical step in the lab testing process, after obtaining the sample, is the preparation of the blood samples. Specimen integrity can be maintained by following some basic handling processes:

- Fill tubes to the stated draw volume to ensure the proper blood-to-additive ratio. Allow the tubes to fill until the vacuum is exhausted and blood flow ceases.
- Vacutainer tubes should be stored at 4-25°C (39-77°F).
- Tubes should not be used beyond the designated expiration date.
- Mix all gel barrier and additive tubes by gentle inversion 5 to10 times immediately after the draw. This assists in the clotting process. This also assures homogenous mixing of the additives with the blood in all types of additive tubes.
- Serum separator tubes should clot for a full 30 minutes in a vertical position prior to centrifugation. Short clotting times can result in fibrin formation, which may interfere with complete gel barrier formation.

Blood Sample Centrifugation – It is recommended that serum be physically separated from contact with cells as soon as possible, with a maximum time limit of 2 hours from the time of collection.

- Complete gel barrier formation (gel barrier tubes) is time, temperature and G-force dependent. The uniformity of the barrier is time dependent; an incomplete barrier could result from shortened centrifugation times.



- In general, for a horizontal, swing-bucket centrifuge, the recommended spin time is 10 minutes. For a fixed-angle centrifuge, the recommended spin time is 15 minutes.
- NOTE: Gel flow may be impeded if chilled before or after centrifugation.
- Tubes should remain closed at all times during the centrifugation process.
- Place the closed tubes in the centrifuge as a "balanced load" noting the following:
    - Opposing tube holders must be identical and contain the same cushion or none at all.
    - Opposing tube holders must be empty or loaded with equally weighted samples (tubes of the same size and equal in fill).
    - If an odd number of samples is to be spun, fill a tube with water to match the weight of the unpaired sample and place it across from this sample.

Centrifuge Safety

- Interference with an activated centrifuge by an impatient employee can result in bodily injury in the form of direct trauma or aerosolization of hazardous droplets.
- Centrifuges must never be operated without a cover in place.
- Uncovered specimen tubes must not be centrifuged.
- Centrifuges must never be slowed down or stopped by grasping part(s) of the device with your hand or by applying another object against the rotating equipment.
- Be sure the centrifuge is appropriately balanced before activating. If an abnormal noise, vibration, or sound is noted while the centrifuge is in operation, immediately stop the unit (turn off the switch) and check for a possible load imbalance.
- Clean the centrifuge daily with a disinfectant and paper towel. Broken tubes or liquid spills must be cleaned immediately.

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What Is Anemia?

Anemia is a condition that develops when your blood lacks enough healthy red blood cells or hemoglobin. Hemoglobin is a main part of red blood cells and binds oxygen. If you have too few or abnormal red blood cells, or your hemoglobin is abnormal or low, the cells in your body will not get enough oxygen. Symptoms of anemia -- like fatigue -- occur because organs aren't getting what they need to function properly.

Anemia is the most common blood condition in the U.S. It affects about 5.6% of the people in the U.S. Women, young children, and people with chronic diseases are at increased risk of anemia. Important factors to remember are:

- Certain forms of anemia are hereditary and infants may be affected from the time of birth.
- Women in the childbearing years are particularly susceptible to iron-deficiency anemia because of the blood loss from menstruation and the increased blood supply demands during pregnancy.
- Older adults also may have a greater risk of developing anemia because of poor diet and other medical conditions.

There are many types of anemia. All are very different in their causes and treatments. Iron-deficiency anemia, the most common type, is very treatable with diet changes and iron supplements. Some forms of anemia -- like the mild anemia that develops during pregnancy -- are even considered normal. However, some types of anemia may present lifelong health problems.

What Causes Anemia?

There are more than 400 types of anemia, which are divided into three groups:

- Anemia caused by blood loss
- Anemia caused by decreased or faulty red blood cell production
- Anemia caused by destruction of red blood cells

Anemia Caused by Blood Loss

Red blood cells can be lost through bleeding, which often can occur slowly over a long period of time, and can go undetected. This kind of chronic bleeding commonly results from the following:

- Gastrointestinal conditions such as ulcers, hemorrhoids, gastritis (inflammation of the stomach), and cancer
- Use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin or ibuprofen, which can cause ulcers and gastritis
- Menstruation, especially if menstrual bleeding is excessive

ANEMIA

- **What Is Anemia?**
- Symptoms
- Diagnosis & Treatment

- Types

Anemia Caused by Decreased or Faulty Red Blood Cell Production

With this type of anemia, the body may produce too few blood cells or the blood cells may not function correctly. In either case, anemia can result. Red blood cells may be faulty or decreased due to abnormal red blood cells or a lack of minerals and vitamins needed for red blood cells to work properly. Conditions associated with these causes of anemia include the following:

- Sickle cell anemia
- Iron-deficiency anemia
- Vitamin deficiency
- Bone marrow and stem cell problems
- Other health conditions

Sickle cell anemia is an inherited disorder that, in the U.S. affects mainly African-Americans and Hispanic Americans. Red blood cells become crescent-shaped because of a genetic defect. They break down rapidly, so oxygen does not get to the body's organs, causing anemia. The crescent-shaped red blood cells can also get stuck in tiny blood vessels, causing pain.

Iron-deficiency anemia occurs because of a lack of the mineral iron in the body. Bone marrow in the center of the bone needs iron to make hemoglobin, the part of the red blood cell that transports oxygen to the body's organs. Without adequate iron, the body cannot produce enough hemoglobin for red blood cells. The result is iron-deficiency anemia. This type of anemia can be caused by:

- An iron-poor diet, especially in infants, children, teens, vegans, and vegetarians
- The metabolic demands of pregnancy and breastfeeding that deplete a woman's iron stores
- Menstruation
- Frequent blood donation
- Endurance training
- Digestive conditions such as Crohn's disease or surgical removal of part of the stomach or small intestine
- Certain drugs, foods, and caffeinated drinks

Vitamin-deficiency anemia may occur when vitamin B_{12} and folate are deficient. These two vitamins are needed to make red blood cells. Conditions leading to anemia caused by vitamin deficiency include:

- Megaloblastic anemia: Vitamin B12 or folate or both are deficient

- Pernicious anemia: Poor vitamin B12 absorption
- Dietary deficiency: Eating little or no meat may cause a lack of vitamin B12, while overcooking or eating too few vegetables may cause a folate deficiency.
- Other causes of vitamin deficiency: pregnancy, certain medications, alcohol abuse, intestinal diseases such as tropical sprue and celiac disease

During early pregnancy, sufficient folic acid can help prevent the fetus from developing neural tube defects such as spina bifida.

Bone marrow and stem cell problems may prevent the body from producing enough red blood cells. Some of the stem cells found in bone marrow develop into red blood cells. If stem cells are too few, defective, or replaced by other cells such as metastatic cancer cells, anemia may result. Anemia resulting from bone marrow or stem cell problems include:

- Aplastic anemia occurs when there's a marked reduction in the number of stem cells or absence of these cells. Aplastic anemia can be inherited, can occur without apparent cause, or can occur when the bone marrow is injured by medications, radiation, chemotherapy, or infection.
- Thalassemia occurs when the red cells can't mature and grow properly. Thalassemia is an inherited condition that typically affects people of Mediterranean, African, Middle Eastern, and Southeast Asian descent. This condition can range in severity from mild to life-threatening; the most severe form is called Cooley's anemia.
- Lead exposure is toxic to the bone marrow, leading to fewer red blood cells. Lead poisoning occurs in adults from work-related exposure and in children who eat paint chips, for example. Improperly glazed pottery can also taint food and liquids with lead.

Anemia associated with other conditions usually occurs when there are too few hormones necessary for red blood cell production. Conditions causing this type of anemia include the following:

- Advanced kidney disease
- Hypothyroidism
- Other chronic diseases, such as cancer, infection, lupus, diabetes, and rheumatoid arthritis
- Old age

Anemia Caused by Destruction of Red Blood Cells

When red blood cells are fragile and cannot withstand the routine stress of the circulatory system, they may rupture prematurely, causing hemolytic anemia. Hemolytic anemia can be present at birth or develop later. Sometimes there is no known cause. Known causes of hemolytic anemia may include:

- Inherited conditions, such as sickle cell anemia and thalassemia
- Stressors such as infections, drugs, snake or spider venom, or certain foods
- Toxins from advanced liver or kidney disease

- Inappropriate attack by the immune system (called hemolytic disease of the newborn when it occurs in the fetus of a pregnant woman)
- Vascular grafts, prosthetic heart valves, tumors, severe burns, exposure to certain chemicals, severe hypertension, and clotting disorders
- In rare cases, an enlarged spleen can trap red blood cells and destroy them before their circulating time is up.

Urine Specimens – an overview of collection methods, collection devices, specimen handling and transportation

This Focus Topic is the first of a two part series on urine specimen collection. Part 2 will cover sources of preanalytical artifact arising during urine collection, handling and transportation.

Urine has a long history as a specimen for analysis in clinical laboratories. After blood, urine is the most commonly used specimen for diagnostic testing, monitoring of disease status and detection of drugs. Urine testing, using both automated and traditional manual methods, is growing rapidly.[1] As with all clinical laboratory specimens, preanalytical error in urine specimens is often difficult to detect. Because of this, it is important for laboratories to have processes in place to ensure compliance with best practice in specimen collection, handling and transport – including the use of preservatives where appropriate.

Types of Urine Collection Methods

Urine specimens may be collected in a variety of ways according to the type of specimen required, the collection site and patient type.

Randomly Collected Specimens are not regarded as specimens of choice because of the potential for dilution of the specimen when collection occurs soon after the patient has consumed fluids.

First Morning Specimen is the specimen of choice for urinalysis and microscopic analysis, since the urine is generally more concentrated.

Midstream Clean Catch Specimens are strongly recommended for microbiological culture and antibiotic susceptibility testing because of the reduced incidence of cellular and microbial contamination.

Timed Collection Specimens may be required for quantitative measurement of certain analytes, including those subject to diurnal variation. Analytes commonly tested using timed collection include creatinine, urea, potassium, sodium, uric acid, cortisol, calcium, citrate, amino acids, catecholamines, metanephrines, vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid, protein, oxalate, copper,17-ketosteroids, and 17-

hydroxysteroids.

Collection from Catheters (e.g. Foley catheter)using a syringe, followed by transfer to a specimen tube or cup. Alternatively, urine can be drawn directly from the catheter to an evacuated tube using an appropriate adaptor.

Supra-pubic Aspiration may be necessary when a non-ambulatory patient cannot be catheterized or where there are concerns about obtaining a sterile specimen by conventional means.

Pediatric Specimens present many challenges. For infants and small children, a special urine collection bag can be adhered to the skin surrounding the urethral area.

Urine Collection Devices

An extensive array of urine collection products is available on the market. Information on features, intended use and instructions for use should be obtained from the device manufacturer and reviewed before being incorporated into a specimen collection protocol.

Urine Collection Containers (cups for collection and transport)

Urine collection container cups are available in a variety of shapes and sizes with lids that are either 'snap-on' or 'screw-on'. Leakage is a common problem with low quality products. To protect healthcare workers from exposure to the specimen and protect the specimen from exposure to contaminants, leak-proof cups should be utilized. Some urine specimen containers have closures with special access ports that allow closed-system transfer of urine directly from the collection device to the tube (further information)

Urine Collection Containers for 24-hour Collection

Urine collection containers for 24-hour specimens commonly have a 3 liter capacity. As for the urine collection cups above, closure types vary with some containers featuring anintegrated port for transfer of an aliquot of the specimen to an evacuated urine collection tube (further information). This provides the option for the laboratory to receive only the aliquot tube and specimen weight (with the large 24-hour container and contents discarded at the point of collection). Additional precautions need to be taken when a preservative is required (further information).

Urine Specimen Tubes

Urine specimens may be poured directly into tubes with 'screw-on' or 'snap-on' caps. Additionally,evacuated tubes, similar to those used in blood collection, are available. (further information)

Urine Specimen Collection and Transportation Guidelines

As for any type of clinical laboratory specimen, certain criteria for collection and transportation (further information) of urine specimens must be met to ensure high quality specimens free of preanalytical artifact are obtained consistently. Without this, accurate test results cannot be guaranteed.

Urine Specimen Preservation

For urinalysis and culture and sensitivity testing, CLSI Guidelines[2] recommend testing within two hours of collection. Different time limits may apply to specimens required for molecular testing of infectious agents (e.g. testing for*Neisseria gonorrhoeae*, *Chlamydia trachomatis*). For this type of testing, laboratories should ensure they are able to comply with specimen transportation conditions prescribed by the assay manufacturers. Where compliance with these and/or CLSI recommendations is not possible,consideration should be given to the use of a preservative (further information). Specimen collection tubes withp reservatives for chemical urinalysis (further information) and culture and antibiotic susceptibility are available (further information).

Urine Specimen Reception in the Laboratory

In addition to routine checks and precautions taken for all specimens received in the clinical laboratory, the following additional 'check items' apply to urine specimens.

Labels

Volume

Collection Date and Time

Collection Method

Specimen Preservation

Light Protection

Randomly Collected Specimens

Randomly collected specimens are suitable for urinalysis in the clinical chemistry laboratory and for microscopic analysis. However, they are not regarded as specimens of choice because of the potential for dilution of the specimen when collection occurs soon after the patient has consumed fluids. In this situation, analyte values may be artificially low. Of necessity, pediatric urine specimens for urinalysis and microscopy are often of this type.

First Morning Specimen is the Specimen

First morning specimens are the specimen of choice for urinalysis and microscopic analysis since the urine is generally more concentrated (due to the length of time the urine is allowed to remain in the bladder) and, therefore, contains relatively higher levels of cellular elements and analytes. Abnormal constituents are also likely to be present in higher concentration and, thus, more likely to be detected.

Midstream Clean Catch Specimens

Midstream specimens are strongly recommended for microbiological culture and antibiotic susceptibility testing because of the reduced incidence of cellular and microbial contamination. Following instruction from a healthcare professional, patients are required to follow a prescribed procedure commencing with cleansing the urethral area. The patient should then void the first portion of the urine stream into the toilet. These first steps significantly reduce the opportunities for contaminants to enter the urine stream during collection of the clinical specimen. The urine midstream is then collected into a clean container after which the remaining urine is voided into the toilet. This method of collection can be conducted at any time of day or night.

Timed Collection Specimens

Timed specimens may be required for quantitative measurement of certain analytes, including those subject to diurnal variation. Analytes commonly tested using timed

collection include creatinine, urea, potassium, sodium, uric acid, cortisol, calcium, citrate, amino acids, catecholamines, metanephrines, vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid, protein, oxalate, copper, 17-ketosteroids, and 17-hydroxysteroids. A timed collection allows measurement of the excretion of these substances in urine over a specified length of time, usually, but not always, 8 or 24 hours. In this collection method, the bladder is emptied prior to beginning the timed collection. Then, for the duration of the designated time period, all urine is collected and pooled into a collection container, with the final collection taking place at the very end of that period. Half an hour before the end of the collection period, it is helpful to ask the patient to drink a glass of water, so that the last urine specimen can be obtained. If no specimen is produced, then the total volume and time of collection cannot be determined. It is also important to caution the patient not to lose urine specimens to the toilet during defecation. When a 24-hour urine specimen is required for the assay of catecholamines, metanephrines and/or VMA, for the diagnosis of pheochromocytoma, which causes persistent or episodic hypertension, it is advisable to monitor the blood pressure of the patient and collect the urine specimen when the blood pressure is high, in order to improve the chance of a positive finding.

Timed specimens should be refrigerated during the collection period, unless otherwise directed by the laboratory. Accurate timing is very important as this information forms a critical part of the calculations performed to determine urine clearance values (e.g. creatinine clearance). Interpretations based on faulty calculations can result in improper diagnoses or medical treatment.

Collection from Catheters

Urine specimens can be collected from catheters (e.g. Foley catheter) using a syringe, followed by transfer to a specimen tube or cup. Alternatively, urine can be drawn directly from the catheter to an evacuated tube using an appropriate adaptor.

Direct draw adaptor for urine specimen collection from Foley catheter

Supra-pubic Aspiration

Supra-pubic aspiration may be necessary when a non-ambulatory patient cannot be catheterized or where there are concerns about obtaining a sterile specimen by conventional means. This procedure involves collection of the specimen by needle aspiration through the abdominal wall into the bladder.

Pediatric Specimens

Urine collection from pediatric patients presents many challenges. For infants and small children, a special urine collection bag can be adhered to the skin surrounding the urethral area. Once the collection is completed, the urine is poured into a collection cup or transferred directly into an evacuated tube with a transfer straw. Urine collected from a diaper is not recommended for laboratory testing since contamination from the diaper material may affect test results.

Urine Collection Containers

Urine collection containers with integrated port for transfer of specimen to evacuated urine collection tube

24 Hour Urine Collection Container

24 hour urine collection container with integrated port for transfer of specimen to evacuated urine collection tube. This provides the option for the laboratory to receive only the aliquot tube and specimen weight (with the large 24-hour container and contents discarded at the point of collection).

Preservatives for 24 Hour Specimens

When a preservative is required, it should be added to the collection container before the urine collection begins. Commonly used preservatives for 24 hour specimens are hydrochloric acid, boric acid, acetic acid, thymol and toluene. If more than one acceptable preservative is available for the analyte(s) being tested, the least hazardous one should, of course, be selected. Appropriate warning labels should be placed on the container to alert patients to possible harm arising from contact with the preservatives. This should be reinforced by appropriate instruction from the attending healthcare worker. A corresponding Material Safety Data Sheet (MSDS) should also be provided for the patient.

Urine Specimen Tubes

Evacuated tubes, similar to those used in blood collection, are available for urine collection. These can be filled using a straw device, from urine specimen containers with integrated transfer devices, or from direct sampling devices that are used to access catheter sampling ports.

Urine transfer 'straw' with adaptor for transfer of specimen to evacuated urine collection tube

Urine collection containers with integrated port for transfer of specimen to evacuated urine collection tube

Urinalysis tubes ae available in a variety of shapes: conical bottom, round bottom, or flat bottom. Conical bottom tubes offer advantages for microscopic examination of urine sediment. The laboratory's tube selection process must include consideration of centrifugation conditions and compatibility with automated instrument systems. Tube fill volumes are typically within the range of 4 to 10mL with dimensions of 13 x 75mm and 16 x 100mm.

Evacuated urine specimen collection tubes

Collection and Transport Guidelines

- All urine collection and/or transport containers should be clean and free of particles or interfering substances.
- The collection and/or transport container should have a secure lid and be leak-proof. Leak-proof containers reduce specimen loss and risk of healthcare worker exposure to the specimen while also protecting the specimen from contaminants.
- The use of containers that are made from break-resistant plastic is strongly recommended.
- The container material should not leach interfering substances into the specimen.
- Specimen containers must not be re-used.
- Specimen tubes should be compatible with automated systems and instruments used by the laboratory.
- Collection containers and/or specimen tubes should be compatible with pneumatic tube systems where these are used for urine specimen transport. Use of leak-proof containers is essential in this situation.

The CLSI Guidelines[2] makes the following recommendations for urine collection:

- Primary (routine) specimen containers to have a wide base and a capacity of at least 50 mL.
- 24 hour specimen containers to have a capacity of at least 3 litres.
- Sterile collection containers for all microbiology specimens
- Specimen containers to have secure closures to prevent specimen loss and to protect the specimen from contaminants.
- Amber colored containers for specimens required for assay of light sensitive analytes such as urobilinogen and porphyrins.

Urine Tubes Preservatives

For chemical urinalysis and conventional (culture based) microbiological testing, unpreserved specimens exceeding the two hour limit that have not been refrigerated should not be accepted for analysis due to potential bacterial overgrowth leading to disintegration of cells and casts*, invalidation of bacterial colony counts and errors in chemical urinalysis. When specimens for such testing are directly transferred from a collection cup to a tube containing a suitable preservative, a stable environment is provided for the specimen until testing can be conducted. Preservatives are also available for some molecular tests (e.g. BD UPT urine specimen tube for use with BD ProbeTec™ ET assay system) . When a decision to use a preservative is made – for any type of testing, potential interference with assay methods should be considered. Laboratories should validate all test procedures intended to be used for preserved specimens. Specimens may need to be split if various tests requiring different preservatives are requested.

***** Bacterial growth increases the pH of the urine leading to lysis of red blood cells and

white blood cells. Increased pH (alkalinity) can also cause casts to dissolve.

Chemical Urinalysis Preservatives

A variety of urine preservatives is available that allow urine to be maintained at room temperature while still providing urinalysis test results comparable to those achieved with fresh specimens or those stored under refrigerated conditions. Commonly used preservatives for chemical urinalysis specimens include tartaric acid, boric acid, chlorhexidine, ethyl paraben, thymol and sodium propionate (and 'cocktails' of these). Preservation times are typically within the range of 24 to 72 hours. Claims for the duration of stability for specific analytes should be obtained from the manufacturer.

Culture and Antibiotic Susceptibility Preservatives

Preservatives for culture and antibiotic susceptibility testing are designed to maintain the specimen in a state equivalent to that which would be achieved with refrigeration by deterring the proliferation of organisms that could result in a false positive culture or bacterial overgrowth. Careful attention must be given to the formulation of these preservatives to achieve this objective. There is evidence to suggest that non-pH buffered boric acid may be harmful to certain organisms and that buffered boric acid preservatives can reduce the harmful effects of the preservative on the organisms[3]. Preserved urine specimens can be stored at room temperature until the time of testing. Product claims regarding duration of preservative potency should be obtained from the manufacturer.

Labels

If the collection container is used for transport, the label should be placed on the container and not on the lid, since the lid can be mistakenly placed on a different container. Note that some labels are unsuitable for specimens stored under refrigerated conditions because of a lack of adhesion at low temperatures.

Volume

It is important for specimen collection personnel to ensure there is sufficient volume to perform the required tests. For specimens in preservative tubes, the fill volume must be correct. As above, under-filling or over-filling these tubes may adversely affect test result accuracy.

Collection Time and Date

Collection time and date must be shown on the specimen label. For timed specimens, both the start and stop times of the collection must be shown. The time at which the

specimen was received in the laboratory must also be documented for verification of proper handling and transport after collection.

Return to article

Collection Method

The method of collection should be confirmed when the specimen is received in the laboratory to ensure the type of specimen submitted meets the needs of the required test(s). An example of an optimum specimen/test match would be a first morning specimen for urinalysis and microscopic examination.

Specimen Preservation

If the specimen is not received within two hours of collection, specimen reception personnel must confirm that a tube containing an appropriate preservative has been used. Confirmation that the specimen is received within the allowable time for the particular preservative tube used is required.

Light Protection

Specimens submitted for testing of light-sensitive analytes must be collected in containers that protect the specimen from light.

Urine is a liquid byproduct of the body secreted by the kidneys through a process called urination and excreted through the urethra. The normal chemical composition of urine is mainly water content, but it also includes nitrogenous molecules, such as urea, as well as creatinine and other metabolic waste components. Other substances may be excreted in urine due to injury or infection of the glomeruli of the kidneys, which can alter the ability of the nephron to reabsorb or filter the different components of blood plasma.

Normal Chemical Composition of Urine

Urine is an aqueous solution of greater than 95% water, with a minimum of these remaining constituents, in order of decreasing concentration:

- Urea 9.3 g/L.
- Chloride 1.87 g/L.
- Sodium 1.17 g/L.

- Potassium 0.750 g/L.
- Creatinine 0.670 g/L .
- Other dissolved ions, inorganic and organic compounds (proteins, hormones, metabolites).

Urine is sterile until it reaches the urethra, where epithelial cells lining the urethra are colonized by facultatively anaerobic gram-negative rods and cocci. Urea is essentially a processed form of ammonia that is non-toxic to mammals, unlike ammonia, which can be highly toxic. It is processed from ammonia and carbon dioxide in the liver.

ABNORMAL TYPES OF URINE

There are several conditions that can cause abnormal components to be excreted in urine or present as abnormal characteristics of urine. They are mostly referred to by the suffix -uria. Some of the more common types of abnormal urine include:

- Proteinuria—Protein content in urine, often due to leaky or damaged glomeruli.
- Oliguria—An abnormally small amount of urine, often due to shock or kidney damage.
- Polyuria—An abnormally large amount of urine, often caused by diabetes.
- Dysuria—Painful or uncomfortable urination, often from urinary tract infections.
- Hematuria—Red blood cells in urine, from infection or injury.
- Glycosuria—Glucose in urine, due to excess plasma glucose in diabetes, beyond the amount able to be reabsorbed in the proximal convoluted tubule.

Summary

Urine is a liquid by-product of the body secreted by the kidneys through a process called urination and excreted through the urethra. It is an aqueous solution of greater than 95% water. Other constituents include urea, chloride, sodium, potassium, creatinine and other dissolved ions, and inorganic and organic compounds. Urea is a non-toxic molecule made of toxic ammonia and carbon dioxide. Any abnormal constituents found in urine are an indication of disease.

References:

Text Book:

Vladimir Bartos by Clinical *Biochemistry* First Edition by Charles University Prague.

Possible Questions

Part-A (20 x 1 = 20 marks)

1. Which of the following white blood cells is capable of phagocytosis?

a) Basophil **b)** Eosinophil **c)** Lymphocyte **d)** Neutrophil

Answer:d)

2. What would happen to red blood cells if the haem group were removed from haemoglobin?

a) Red blood cells would not be able to bind oxygen. **b)** Red blood cells would not be able to reproduce. **c)** White blood cells would not be able to reproduce. **d)** Blood clot formation would be inhibited.

Answer:a)

3. The hormone erythropoietin stimulates red blood cell production in the red bone marrow. Where in the body is erythropoietin produced?

a) Spleen **b)** Kidney **c)** Liver **d)** Thyroid

Answer:b) Kidney

4. Which of the following statements about erythrocytes is correct?

a) They fight infection. **b)** They clot blood. **c)** They lack a nucleus. **d)** They are produced in the spleen.

Answer:c)

5. Where does haematopoiesis take place?

a) Lungs **b)** Pancreas **c)** Liver **d)** Bone marrow.

answer: d)

6. The formation of a blood clot is known as which of the following?

a) Coagulation **b)** Chemotaxis **c)** Leucopoiesis **d)** Erythropoiesis

answer:a)

7. Platelets are formed from what type of cell?

a) Melanocytes **b)** Macrophages **c)** Astrocytes **d)** Megakaryocytes

answer:d)

8. Which of the following is the function of white blood cells?

a) Transport oxygen. **b)** Maintain homeostasis. **c)** Defend against infection. **d)** Produce haemoglobin.

answer:c)

9. An increased white blood cell count is indicative of which disease?

a) Lupus **b)** Leukaemia **c)** Anaemia **d)** Melanoma

answer:b)

10. The process of coagulation is classically divided into how many pathways?

a) 3 **b)** 5 **c)** 2 **d)** 4

Answer: a)

11.Which of the following are the possible blood groups of the offspring of the parents with blood group O and AB

a) O,A,B and AB b)A and B c) A,B and AB d) O and AB

Answer: (b)

12. White blood cells act

a) as a defence against of energy blood b) as a source of energy c) for clotting blood

d) as a medium for oxygen transport from lung to tissues

Answer: a)

13. In which one of the following is extra blood stored and is released when shortage occurs?

a) adrenal gland b) Pancreas c) Spleen d) thyroid gland

Answer: c)

14. The average life span of red blood corpuscles is about

a) 100-200 days b) 100-120 days c)160-180 days d)150-200days

Answer:b)

15. From which one of the following chambers of human heart is the oxygenated blood pumped in to aorta?

a) Right atrium b) Right ventricle c) Left atrium d) Left ventricle

Answer: d)

16. Which of the following white blood cells is capable of phagocytosis?

a) Basophil b) Eosinophil c) Lymphocyte d) Neutrophil

Answer: d)

17. In which instrument is used for measuring blood pressure?

a) Electrocardiogram b) Anemometer c) Stethoscope d) Sphygmanometer

Answer: d)

18. What is the percentage of water in blood plasma?

a) 90 b) 80 c) 98 d) 60

Answer: a)

19. Universal receivers can receive blood from

a) Group AB only b) Group O only c)Group A,AB d) Group o,A,B,AB

Answer: d)

20. Pulmonary artery carries

a) Pure blood from lungs b) Pure blood to lungs c) Impure blood to lungs d) Impure blood from lungs

Answer: c)

Part-B (5x 2= 10 marks)

1. What is blood coagulation?
2. Explain blood composition?
3. What are the main functions of blood?
4. What is Anemia?
5. Explain Erythrocytes

Part-C (5x 6= 30 marks)

1. Write a note on Blood collection and preservation of samples?
2. What are the Types of Urine Collection Methods? Explain

3. Explain the process of Blood Specimen Collection and Processing.
4. Explain Fingerstick Procedure
5. Write a note on Blood Sample Handling and Processing:
6. Explain the Normal Chemical Composition of Urine.

KARPAGAM ACADEMY OF HIGHER EDUCATION

COIMBATORE-21

DEPARTMENT OF CHEMISTRY

SUBJECT: ANALYTICAL CLINICAL BIOCHEMISTRY

SUBJECT CODE: 17CHU404B

UNIT-V

PART-A

MULTIPLE CHOICE QUESTIONS (EACH QUESTIONS CARRY ONE MARK)

ONLINE EXAMINATION (20 X1 =20 MARKS)

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Answer: d)

20. Pulmonary artery carries

a) Pure blood from lungs b) Pure blood to lungs c) Impure blood to lungs d) Impure blood from lungs

Answer: c)

21. The system responsible for transporting blood around the body is :

a)Urinary system b)Circulatory system [True] c) Lymphatic system d) Digestive system
Answer :b)

22. A protein in the plasma which contributes to the osmotic pressure of blood is _____.

a) elastin b) prothrombin c) albumin d) thrombin

Answer :c)

23. A woman with blood type O has a baby with blood type O. The father _____.

a) must be type O only. b) could be A, B or O, but in no way AB. c) could possibly be AB. d) is Rh-..

Answer :b)

24. Active macrophages that work in long-term cleanup of tissues are called _____.

a) neutrophils b) eosinophils c) basophils d) lymphocytes e) monocytes

Answer :e)

25. Although it carries much oxygen, _____ is more attracted to pesticides and carbon monoxide.

a) hemoglobin b) plasma c) thrombin d) white blood cells

Answer :a)

26. The normal pH of blood is in the range of ____; therefore, a pH of 7.10 would be a condition called _____.

a) 7.00 – 7.15, alkalosis b) 7.35 – 7.45, acidosis c) 7.25 – 7.55, acidosis d) 7.35 – 7.55, alkalosis e) 7.25 – 7.55, alkalosis

Answer :b)

27. Which of the following is NOT a function of blood?

a) to regulate body temperature. b) to increase fluid loss. c) to transport nutrients. d) to regulate ph.

Answer :b)

28. Each of the following is a characteristic of red blood cells EXCEPT that they _____.

a) live about 30 days. b) are formed in the red bone marrow. c) are anucleate. d) contain hemoglobin.

Answer :a)

29. Each of the following occurs when blood clots after a cut EXCEPT:

a) platelets release clotting factor.

b) thromboplastin converts prothrombin into thrombin.

c) thrombin converts fibrin into fibrinogen.

d) fibrin filaments trap cells to produce a clot.

Answer :c)

30. Fragments of megakaryocytes that rupture into pieces are responsible for clotting are called _____.

a) wbcs b) rbcs c) antibodies d) platelets

Answer :d)

31. Hematopoiesis primarily occurs in:

a) most flat bones and the epiphyses of certain long bones.

b) the kidneys.

c) the liver.

d) irregular bones.

Answer :a)

32. In a normal sample of centrifuged blood, the buffy coat accounts for:

a) approximately 10 percent of whole blood.

b) all of the white blood cells and plasma.

c) the top portion of the centrifuged blood.

d) approximately 1 percent of blood volume.

Answer :d)

33. Neutrophils, eosinophils, and basophils are alike in that they _____.

a) lack a defined nucleus. b) are granulocytes. c) release histamine.
d) are the only phagocytic leukocytes.

Answer :b)

34. The fluid that leaks into the tissues from the blood is _____.

a) useless. b) harmful. c) lymph. d) plasma.

Answer :c)

35. The largest of the WBCs is the

a) eosinophil. b) monocyte. c) basophil. d) neutrophil. e) lymphocyte.

Answer :d)

36. Which WBC has a very thin rim of cytoplasm and a large, spherical nucleus?

a) neutrophil b) eosinophil c) basophil d) lymphocyte e) monocyte

Answer :a)

37. Which WBC increases during allergic reactions and parasitic worm infections?

a) eosinophils b) basophils c) neutrophils d) lymphocytes e) monocytes

Answer : a)

38. Which WBC releases histamine at sites of inflammation?

a) neutrophil b) eosinophil c) basophil d) lymphocyte e) monocyte

Answer :c)

39. Unlike red blood cells, white blood cells _____.

a) contain hemoglobin b) are biconcave c) have a nucleus d) live for one week

Answer :c)

40. When oxygen levels are low, the _____ is stimulated to release _____.

a) liver, calcitonin b) bone, oxygen c) kidney, erythropoietin d) bone, erythropoietin

Answer :c)

41. Approximately 55 percent of blood is _____.

a) plasma b) red blood cells c) white blood cells d) lymph

Answer :a)

42. Considering normal urine composition, urea content is

a)7.7g/l b)8.7g/l c)9.3g/l d)10.2g/l

Answer :c)

43. In normal urine composition, potassium ions content is

a)0.863g/l b)0.658g/l c)0.925g/l d)0.750g/l

Answer :d)

44. Considering normal urine composition, chloride ions content is

a)4.2g/l b)1.87g/l c)2.5g/l d)3.6g/l

Answer :b)

45. In normal urine composition, sodium ions content is

a)2.27g/l b)1.17g/l c)3.2g/l d)3.6g/l

Answer :b)

46. Considering normal urine composition, water content in percentage is

a) 95% b)98% c)97% d)93%

Answer :a)

47. The main excretory organs in man are

a) Kidney b) Nephridia c) Trachea d) Lungs

Answer :a)

48. The number of uriniferous tubules in each kidney of man is

a) about 10,000 b)about 5,000 c) numerous d) about1.0×10^{-6}

Answer :d)

49. Conversion of excess of amino acids into urea is done in

a) Lungs b) Large intestine c)Liver d) Cloaca

Answer :c)

50. The waste matter (urea) are transported by

a) Blood b) Lymph c) RBC d)Liver

Answer: a)

51. Columns of Bertin are found in

a) Testes b) Overies c) Kidney d) Liver

Answer: c)

52. Man is

a) Ammonotelic b) Ureotelic c) Uricotelic d) Urochrome

Answer: b)

53. The yellow color of urine is due to

a. Uric acid b) Urea c) Urochrome d) Melalin

Answer: c)

54. Malpighian body is present in

a) Skin b) Kidney c) Testes d) Overies

Answer: b)

55. Certain carbonates and Phosphates are removed by

a)) Skin b) Kidney c) Liver d) None of the above

Answer:d)

56. Mineral impurities in blood are removed by

a) Lungs b) Kidney c) Spleen d) Liver

Answer: b)

57. Insufficient blood supply in human body is referred as

a) Ischemia b) Hyperemia c) Hemostasis d) Hemorrhage

Answer:a)

58. The pH of human blood is

a) 7.4 b) 7.2 c) 7.8 d) 6.6

Answer:a)

59. In the human body, red blood cells are produced in

a)Liverb) Voluntary muscles c) Pancreas d) bone marrow

Answer:d)

60. Which is the anti-coagulant substance in blood?

a)Fibrinogen b) Thrombin c) Globin d) Heparin

Answer:d)

Subject Code: 17CHU404B Reg.No…….

KARPAGAM ACADEMY OF HIGHER EDUCATION

COIMBATORE-21

(For the candidates admitted from 2017& onwards)

DEPARTMENT OF CHEMISTRY

B.Sc Degree Examination

Semester-IV

INTERNAL TEST-I(December 2018)

SUBJECT TITLE: ANALYTICAL CLINICAL BIOCHEMISTRY

Elective Paper

Date: 18.12.2018 AN Subject Code : 17CHU404B

Time : 2 Hours Maximum : 50 marks

Part-A (20 x1= 20 Marks)

Answer all the questions

1. Lactate fermentation

(a) is used by yeast to replenish NAD+.

(b) is used by animal cells to replenish NAD+.

(c) is used by animal cells to replenish NADH.

(d) is used by animal cells to replenish NAD+, under aerobic conditions

2. The NET production of ATP in glycolysis is

(a) 2 ATP molecules. (b) 4 ATP molecules.

(c) 6 ATP molecules. (d) 38 ATP molecules.

3. If a cell needs to continue using glycolysis for energy, it must replenish its supply of

(a) NAD+ molecules. (b) NADH molecules. (c) Protons. (d) H+ molecules

4. In the pay-off phase of glycolysis, the total number of ATP molecules produced is

(a) 1. (b) 2. (c) 4. (d) 6.

5. In the first step of the citric acid cycle

(a) acetyl-CoA is bound to the enzyme citrate synthase, and then it undergoes a condensation reaction with oxaloacetate.

(b) acetyl-CoA undergoes a free condensation reaction with oxaloacetate.

(c) oxaloacetate is bound to the enzyme citrate synthase and then it undergoes a condensation reaction with acetyl-CoA.

(d) oxaloacetate is bound to the enzyme acetate dehydrogenase, and then it undergoes a condensation reaction with acetyl-CoA.

6. Isocitrate dehydrogenase

(a) is activated by high concentrations of ATP and NADH.

(b) is activated by high concentrations of ATP and NADPH.

(c) is unaffected by high concentrations of NADPH.

(d) is inhibited by high concentrations of high-energy compounds.

7. The electron transport chain

(a) generates 5 ATP molecules.

(b) prepares a proton gradient which makes ATP production possible.

(c) utilizes large amounts of oxygen, so is damaging to the cells.

(d) establishes an electron gradient, making ATP production possible.

8. The rate of breakdown of metabolites is termed as

a) Metabolic state b) Metabolism c) Steady state d) Homeostasis

9. Which of the following type of metabolite is used for generating glucose under severe starvation conditions?

a) Amino acids b) Fats c) Glycogen d) Starch

10. One of the following statements about the control of enzyme activity by phosphorylation is correct

a) Phosphorylation of an enzyme results in conformational change

b) Phosphorylation of an enzyme occurs only at specific tyrosine residues

c) Phosphorylation of an enzyme is carried out by phosphoprotein phosphatases

d) Enzyme control by phosphorylation is irreversible

11. Which of the following enzyme catalyzes the first step of glycolysis?

a) Hexokinase b) Pyruvate kinase c) Glucokinase d) Phosphofructokinase-1

12. The general term used for the anaerobic degradation of glucose to obtain energy is

a) Anabolism b) Oxidation c) Fermentation d) Metabolism

13. Cleavage of Fructose 1, 6-biophosphate yields

a) two aldoses b) two ketoses c) an aldose and a ketose d) only a ketose

14. The substrate used in the last step of glycolysis is

a) Glyceraldehyde 3-phosphate b) Pyruvate c) Phosphoenolpyruvate d)1, 3-bisphosphoglycerate

15. Glycolysis converts

a) Glucose into pyruvate b) Glucose into phosphoenolpyruvate

c) Fructose into pyruvate d) Fructose into phosphoenolpyruvate

16. Peptide bond is a

a) Covalent bond b) Ionic bond c) Metallic bond d) Hydrogen bond

17. The factor which does not affect pK_a value of an amino acid is

a) the loss of charge in the α-carboxyl and α-amino groups

b) the interactions with other peptide R groups

c) Other environmental factors

d) Molecular weight

18. The average molecular weight of an amino acid residue in a protein is about

a) 128 b) 118 c) 110 d) 120

19. Unfolding of a protein can be termed as

a) Renaturation b) Denaturation c) Oxidation d) Reduction

20. The salt which produces salting out effect during extraction of proteins is

a) $NH_4 SO_4$ b) $(NH_4)_2 SO_4$ c) $(NH_4)_3 SO_4$ d) NaCl

Part-B (3 x2= 6 Marks)

Answer all the questions

21. What is meant by metabolism?

22. What is Cori cycle?

23. Define Isoelectric pH.

Part-B (3 x 8= 24 Marks)

Answer all the questions

24. a) Write a note on Glycolysis pathway?

OR

b) Explain alcoholic fermentation?

25. a) Write a note on Citric acid cycle?

OR

b) Write the biological importance of carbohydrate metabolism?

26. a) Discuss the structure of protein?

OR

b) Explain the classification of Protein.

Subject Code: 17CHU404B **Reg.No……..**

KARPAGAM ACADEMY OF HIGHER EDUCATION

COIMBATORE-21

(For the candidates admitted from 2017& onwards)

DEPARTMENT OF CHEMISTRY

B.Sc Degree Examination

Semester-IV

INTERNAL TEST-I(December 2018)

SUBJECT TITLE: ANALYTICAL CLINICAL BIOCHEMISTRY

Elective Paper

Date: 18.12.2018 AN **Subject Code : 17CHU404B**

Time : 2 Hours **Maximum : 50 marks**

Answer Key

Part-A (20 x1= 20 Marks)

Answer all the questions

1. Lactate fermentation

(a) is used by yeast to replenish NAD+.

(b) is used by animal cells to replenish NAD+.

(c) is used by animal cells to replenish NADH.

(d) is used by animal cells to replenish NAD+, under aerobic conditions

Answer: b

2. The NET production of ATP in glycolysis is

(a) 2 ATP molecules. (b) 4 ATP molecules.

(c) 6 ATP molecules. (d) 38 ATP molecules.

Answer:a

3. If a cell needs to continue using glycolysis for energy, it must replenish its supply of

(a) NAD+ molecules. (b) NADH molecules. (c) Protons. (d) H+ molecules

Answer: a

4. In the pay-off phase of glycolysis, the total number of ATP molecules produced is

(a) 1. (b) 2. (c) 4. (d) 6.

Answer:c

5. In the first step of the citric acid cycle

(a) acetyl-CoA is bound to the enzyme citrate synthase, and then it undergoes a condensation reaction with oxaloacetate.

(b) acetyl-CoA undergoes a free condensation reaction with oxaloacetate.

(c) oxaloacetate is bound to the enzyme citrate synthase and then it undergoes a condensation reaction with acetyl-CoA.

(d) oxaloacetate is bound to the enzyme acetate dehydrogenase, and then it undergoes a condensation reaction with acetyl-CoA.

Answer:c

6. Isocitrate dehydrogenase

(a) is activated by high concentrations of ATP and NADH.

(b) is activated by high concentrations of ATP and NADPH.

(c) is unaffected by high concentrations of NADPH.

(d) is inhibited by high concentrations of high-energy compounds.

Answer: d

7. The electron transport chain

(a) generates 5 ATP molecules.

(b) prepares a proton gradient which makes ATP production possible.

(c) utilizes large amounts of oxygen, so is damaging to the cells.

(d) establishes an electron gradient, making ATP production possible.

Answer:b

8. The rate of breakdown of metabolites is termed as

a) Metabolic state b) Metabolism c) Steady state d) Homeostasis

Answer: c

9. Which of the following type of metabolite is used for generating glucose under severe starvation conditions?

a) Amino acids b) Fats c) Glycogen d) Starch

Answer:a

10. One of the following statements about the control of enzyme activity by phosphorylation is correct

a) Phosphorylation of an enzyme results in conformational change

b) Phosphorylation of an enzyme occurs only at specific tyrosine residues

c) Phosphorylation of an enzyme is carried out by phosphoprotein phosphatases

d) Enzyme control by phosphorylation is irreversible

Answer: a

11. Which of the following enzyme catalyzes the first step of glycolysis?

a) Hexokinase b) Pyruvate kinase c) Glucokinase d) Phosphofructokinase-1

Answer:a

12. The general term used for the anaerobic degradation of glucose to obtain energy is

a) Anabolism b) Oxidation c) Fermentation d) Metabolism

Answer:c

13. Cleavage of Fructose 1, 6-biophosphate yields

a) two aldoses b) two ketoses c) an aldose and a ketose d) only a ketose

Answer:c

14. The substrate used in the last step of glycolysis is

a) Glyceraldehyde 3-phosphate b) Pyruvate c) Phosphoenolpyruvate d)1, 3-bisphosphoglycerate

Answer:c

15. Glycolysis converts

a) Glucose into pyruvate b) Glucose into phosphoenolpyruvate

c) Fructose into pyruvate d) Fructose into phosphoenolpyruvate

Answer:a

16. Peptide bond is a

a) Covalent bond b) Ionic bond c) Metallic bond d) Hydrogen bond

Answer:a

17. The factor which does not affect pK_a value of an amino acid is
a) the loss of charge in the α-carboxyl and α-amino groups
b) the interactions with other peptide R groups
c) Other environmental factors
d) Molecular weight

Answer:d

18. The average molecular weight of an amino acid residue in a protein is about
a) 128 b) 118 c) 110 d) 120

Answer:c

19. Unfolding of a protein can be termed as
a) Renaturation b) Denaturation c) Oxidation d) Reduction

Answer:b

20. The salt which produces salting out effect during extraction of proteins is
a) $NH_4\ SO_4$ b) $(NH_4)_2\ SO_4$ c) $(NH_4)_3\ SO_4$ d) NaCl

Answer:b

Part-B (3 x2= 6 Marks)

Answer all the questions

21. What is meant by metabolism?

Metabolism is the sum of all chemical reactions in the body. **Metabolism** is the term used to describe the body's capture and use of energy and nutrients to sustain life. Diabetes is a **metabolic** disease because it affects the body's ability to capture glucose from food for use by the cells.

22. What is Cori cycle?

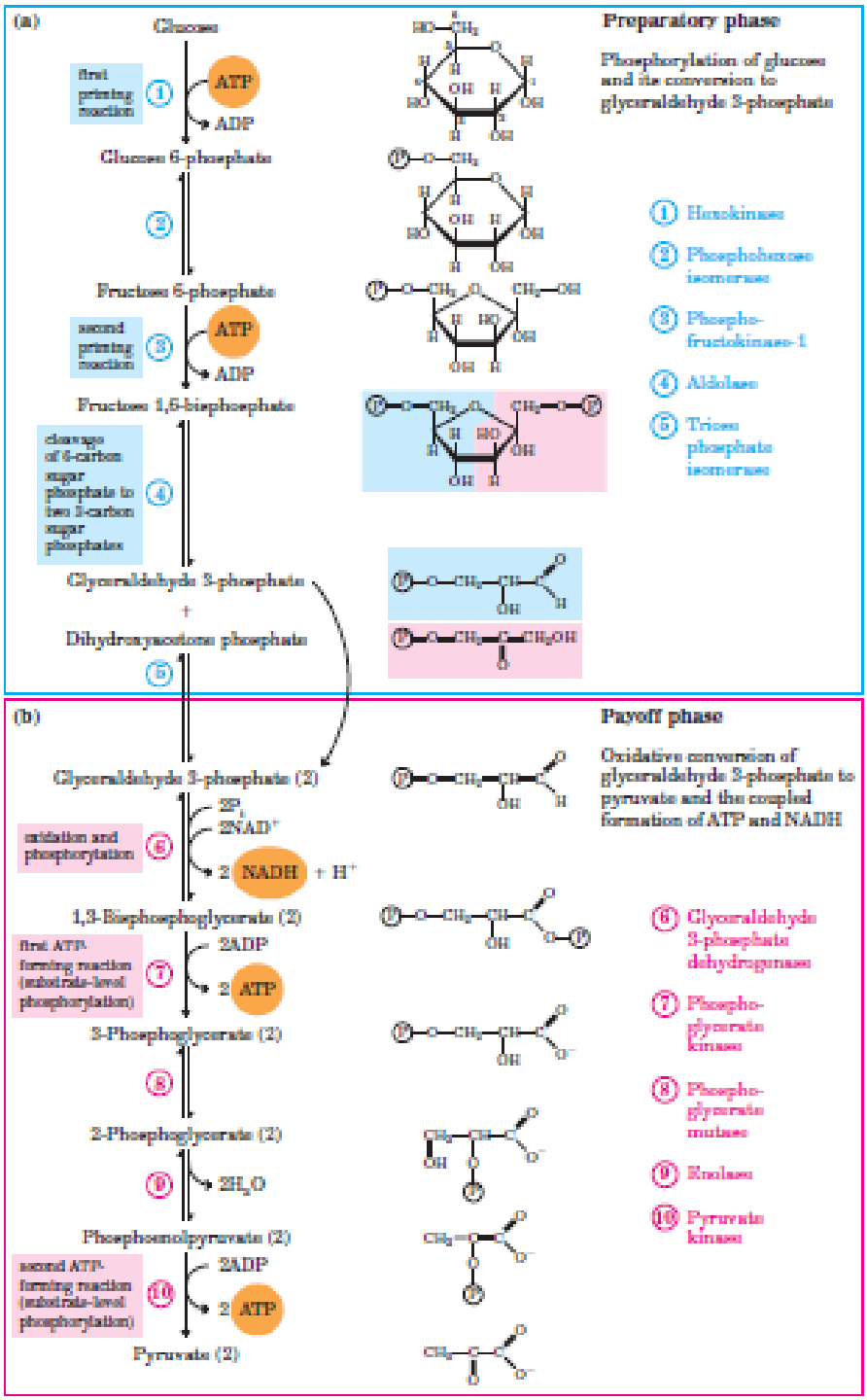
The Cori cycle (also known as the Lactic acid cycle), named after its discoverers, Carl Ferdinand Coriand Gerty Cori,refers to the metabolic pathway in which lactate produced by anaerobic glycolysis in the muscles moves to the liver and is converted to glucose, which then returns to the muscles and is metabolized back to lactate.

23. Define Isoelectric pH.

The isoelectric point (pI, pH(I), IEP), is the pH at which a particular molecule carries no net electrical charge or is electrically neutral in the statistical mean.

Part-B (3 x 8= 24 Marks)
Answer all the questions

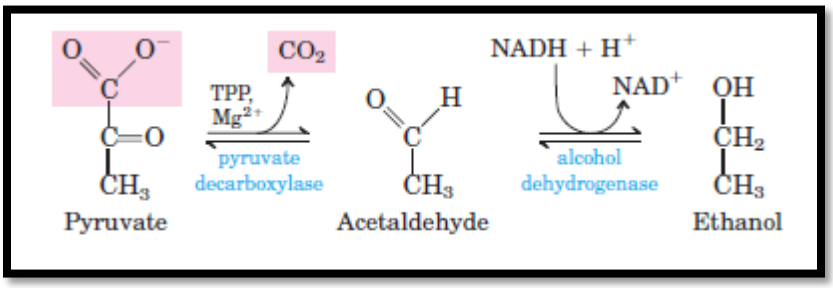
24. a) Write a note on Glycolysis pathway?



OR

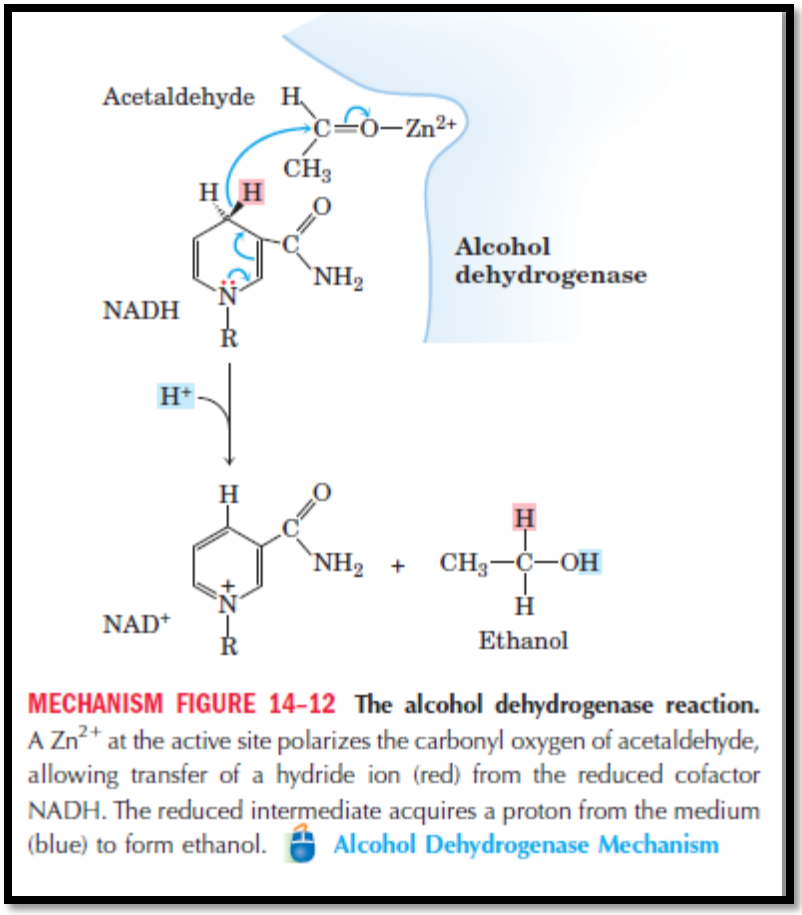
b) Explain alcoholic fermentation?

Yeast and other microorganisms ferment glucose to ethanol and CO_2, rather than to lactate. Glucose is converted to pyruvate by glycolysis, and the pyruvate is converted to ethanol and CO_2 in a two-step process:



In the first step, pyruvate is decarboxylated in an irreversible reaction catalyzed by **pyruvate decarboxylase.** This reaction is a simple decarboxylation and does not involve the net oxidation of pyruvate. Pyruvate decarboxylase requires Mg2+ and has a tightly bound coenzyme, thiamine pyrophosphate, discussed below.

In the second step, acetaldehyde is reduced to ethanol through the action of **alcohol dehydrogenase,** with the reducing power furnished by NADH derived from the dehydrogenation of glyceraldehyde 3-phosphate. This reaction is a well-studied case of hydride transfer from NADH (Fig. 14–12).



MECHANISM FIGURE 14–12 **The alcohol dehydrogenase reaction.** A Zn^{2+} at the active site polarizes the carbonyl oxygen of acetaldehyde, allowing transfer of a hydride ion (red) from the reduced cofactor NADH. The reduced intermediate acquires a proton from the medium (blue) to form ethanol. Alcohol Dehydrogenase Mechanism

Ethanol and CO2 are thus the end products of ethanol fermentation, and the overall equation is

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i \longrightarrow 2\ \text{ethanol} + 2\text{CO}_2 + 2\text{ATP} + 2\text{H}_2\text{O}$$

25. a) Write a note on Citric acid cycle?

The citric acid cycle with the production of *acetyl-CoA*, which is the fuel used to drive the cycle. For example, acetyl-CoA is produced from pyruvate using the enzyme pyruvate dehydrogenase, coenzyme A (CoASH), and one NAD+. The product of this reaction is acetyl-CoA with the liberation of 1 CO_2 molecule + NADH. The acetyl-CoA molecule is the input molecule for the citric acid cycle, but *oxaloacetate* is also required. The latter molecule is not considered "fuel" for the cycle because it is a recycled product used in the reaction.

We can summarize the essential facts of the citric acid cycle in the following way:

• The citric acid cycle is an eight-step reaction.

• It requires 8 enzymes.

• The fi nal product is oxaloacetate.

• Two NADH molecules are produced.

• One GTP molecule is produced.

• One FADH2 molecule is produced.

• The cycle is accompanied by the liberation of 2 CO2 molecules.

The eight enzymes utilized in the citric acid cycle are:

1. Citrate synthase
2. Aconitase
3. Isocitrate dehydrogenase
4. *a*-Ketoglutarate dehydrogenase
5. Succinyl-CoA synthetase
6. Succinate dehydrogenase
7. Fumarase

8. Malate dehydrogenase

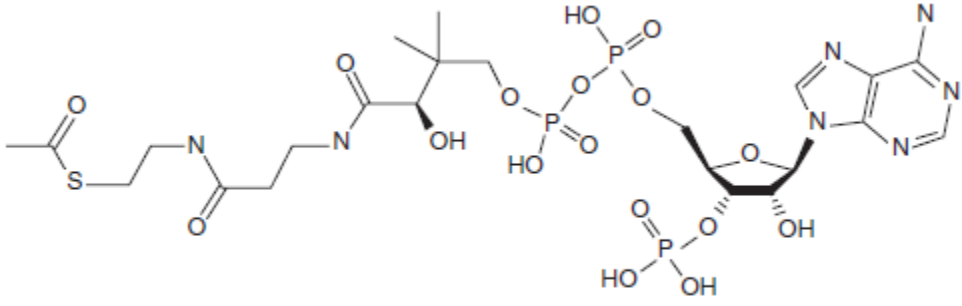


Figure 11-1 Acetyl-CoA, the input or "fuel" for the citric acid cycle.

The production of acetyl-CoA for the citric acid cycle must occur inside the mitochondria because it is unable to cross the mitochondrial membrane from the cytosol. As a result, other molecules must be utilized. It turns out that pyruvate, fatty acids, and some amino acids are able to cross the mitochondrial membrane where they can be used by the mitochondria to produce acetyl-CoA.

STEPS IN THE CITRIC ACID CYCLE

Now let's consider the eight steps of the citric acid cycle.

Step 1: Acetyl-CoA → Citrate

In the fi rst step of the cycle, the enzyme citrate synthase catalyzes the condensation of acetyl-CoA (Fig. 11-1) with oxaloacetate (Fig. 11-2). This produces a molecule called *citrate*, and liberates coenzyme A. One water molecule is also required. Note that citrate is a tertiary alcohol that cannot be oxidized very easily (Fig. 11-3), so it must be further processed. This is done in the second step of the cycle

Step 2: Citrate → Isocitrate

In step 2 of the cycle, there are two substeps used to generate isocitrate, which is easier to oxidize. First, the enzyme aconitase catalyzes the dehydration of the citrate molecule producing an intermediate molecule called *cis-aconitate*.

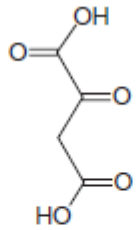


Figure 11-2 Oxaloacetate, a molecule required in the first step of the citric acid cycle and produced in the final or eighth step.

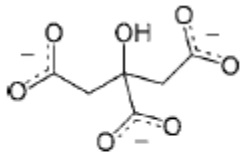


Figure 11-3 The citrate molecule.

26.

The cis-aconitate molecule, remaining bound to the enzyme, is then hydrated to produce the product of the second step, isocitrate. The net effect of this reaction is that the hydroxyl group on citrate is moved from the third carbon atom to the fourth carbon atom, producing an isomer of citrate that is easier to oxidize.

Step 3: Isocitrate → α-Ketoglutamate

The next step in the citric acid cycle also has two substeps or phases. First, isocitrate is oxidized by the enzyme isocitrate dehydrogenase producing *oxalosuccinate*. Like cis-aconitate in step 2, this molecule is an intermediate that never dissociates from the enzyme. Instead it undergoes further processing. In the second phase of step two, the enzyme decarboxylates oxalosuccinate to produce *a*-ketoglutamate. In step 3, one NADH is produced in the fi rst phase and a molecule of CO2 is release in the second phase.

Step 4: _-Ketoglutamate → Succinyl-CoA

The next step in the reaction, which is catalyzed by *a*-ketoglutamate dehydrogenase, decarboxylates *a*-ketoglutamate producing succinyl-CoA (Fig. 11-4). One NAD+ and 1 molecule of coenzyme A are required for this step of the reaction, which produces 1 NADH and 1 molecule of CO2.

Step 5: Succinyl-CoA → Succinate

In this step, a high energy GTP molecule is produced. This is substrate level phosphorylation. The reaction is catalyzed by the molecule succinyl-CoA synthetase, and the coenzyme A molecule consumed in the production of succinyl-CoA is released (see Fig. 11-5).

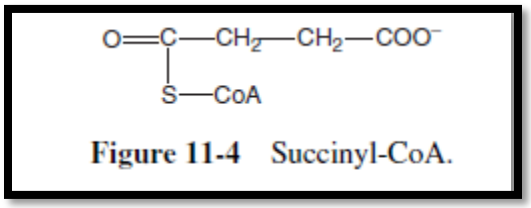


Figure 11-4 Succinyl-CoA.

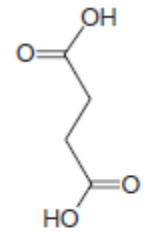


Figure 11-5 Succinate.

Steps 1 to 5 can be considered to be the fi rst part of the citric acid cycle. Two NADH, 1 GTP and 2 CO_2 molecules have been produced. In the second part of the cycle, succinate will be oxidized back to the starting product in the reaction, oxaloacetate, which can then be used in another round of the cycle. This oxidation requires three steps and produces 1 FADH2 and 1 NADH molecule.

Step 6: Succinate → Fumarate

In this step, succcinate dehydrogenase oxidizes the succinate molecule producing fumarate. One FAD is utilized in this reaction, and succinate dehydrogenase eliminates 2

hydrogens in the oxidation of the central single bond of the succinate molecule. The coenzyme FAD is therefore reduced to FADH2 in the process (see Fig. 11-6).

Step 7: Fumarate → L-Malate

In this step, fumarate hydratase or *fumarase* catalyzes a reversible reaction in which fumarate is transformed into malate. One molecule of water is required, in a process which transforms the central double bond of fumarate into a single bond. The top carbon of the double bond is changed from CH ‡ CHOH and the bottom carbon of the double bond is transformed from CH ‡ CH2 (see Fig. 11-7).

Step 8: Malate → Oxaloacetate

We have now reached the fi nal step in the cycle. In this last step, the molecule oxaloacetate is regenerated from malate, allowing the cycle to start anew (provided there is a supply of acetyl-CoA). The enzyme which catalyzes this step is malate dehydrogenase, and 1 NADH molecule is produced.

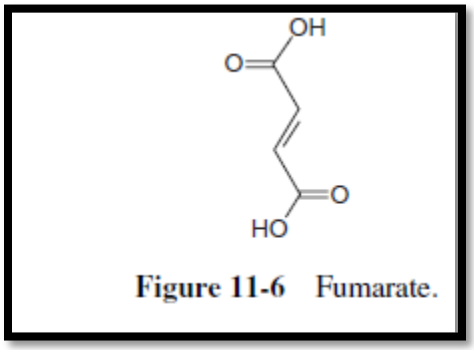


Figure 11-6 Fumarate.

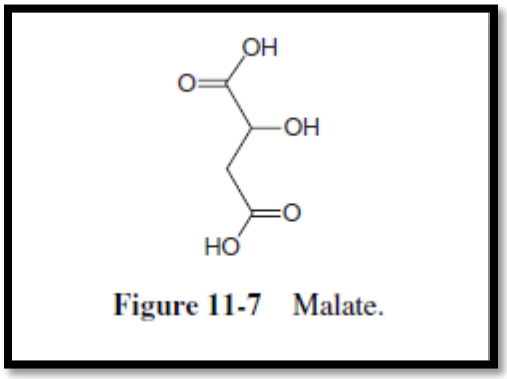


Figure 11-7 Malate.

OR

b) Write the biological importance of carbohydrate metabolism?

Glucose occupies a central position in the metabolism of plants, animals, and many microorganisms. It is the complete oxidation of glucose to carbon dioxide and water proceeds with a standard free-energy change of _2,840 kJ/mol. By storing glucose as a high molecular weight polymer such as starch or glycogen, a cell can stockpile large quantities of hexose units while maintaining a relatively low cytosolic osmolarity. When energy demands increase, glucose can be released from these intracellular storage polymers and used to produce ATP either aerobically or anaerobically.

Glucose is not only an excellent fuel, it is also a remarkably versatile precursor, capable of supplying a huge array of metabolic intermediates for biosynthetic reactions. A bacterium such as *Escherichia coli* can obtain from glucose the carbon skeletons for every amino acid, nucleotide, coenzyme, fatty acid, or other metabolic intermediate it needs for growth. A comprehensive study of the metabolic fates of glucose would encompasshundreds or thousands of transformations. In animals and vascular plants, glucose has three major fates: it may be stored (as a polysaccharide or as sucrose); oxidized to a three-carbon compound (pyruvate) via glycolysis to provide ATP and metabolic intermediates; or oxidized via the pentose phosphate (phosphogluconate) pathway to yield ribose 5-phosphate for nucleic acid synthesis and NADPH for reductive biosynthetic processes.

27. a) Discuss the structure of protein?

Protein structures generally are described at four levels: primary, secondary, tertiary, and quaternary. A *primary structure* is simply the two-dimentional linear sequence of amino acids in the peptide chain. See Fig. 6-6.

The secondary structure is formed as the protein begins to twist in accordance with chemical forces within the primary chain. The hydrogen in the amine groups and the oxygen in the carboxyl groups of the backbone form hydrogen bonds that stabilize the secondary structures. Secondary structure commonly takes one of two forms. One form is a left-handed helix that is developed as the molecule twines around itself. This form is called an *a-helix* (Fig. 6-7).

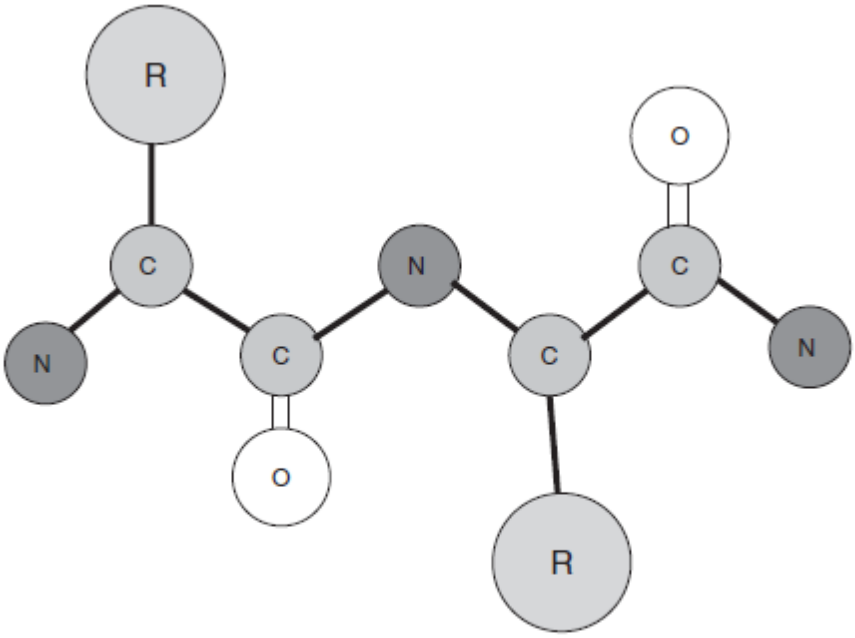


Figure 6-6 Protein primary structure.

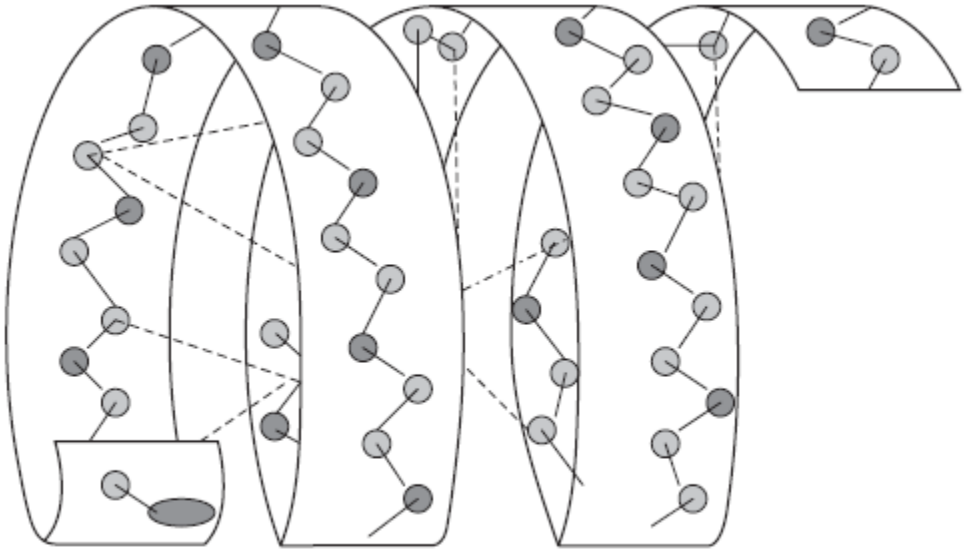


Figure 6-7 α-Helix.

As is usual with biological terminology the alpha designation has nothing whatsoever to do with this confi guration, but is part of the name because this was the first configuration discovered. In the *a*-helix, stabilizing hydrogen bonds formed between amine and carboxyl groups on the carbon backbone are tight. The side chains protrude from the exterior of the helix.

Globular proteins typically contain the *a*-helix form. As the name implies, globular proteins are globelike in form and do not have structural strength. Hormones are globular proteins. Also, some structural proteins also consist of *a*-helices. For example, *keratin* is a family of structural *fibrous proteins* that include *a*-keratin. Hair and other *a*-keratins consist of coiled single protein strands, which are then further coiled into superhelical structures. These structures can stretch out and return to the original confi guration. The supercoils contain a high percentage of cysteine residues that form stabilizing cross-links. The sulfur from the cysteine accounts for the distinct odor of burning hair. Extensive cysteine cross-links between *a*-coils form the hard structures of hooves and nails.

OR

b) Explain the classification of Protein.

Classification of Proteins:

I. Simple proteins

(i) Albumins:

Soluble in water, coagulable by heat and 1 precipitated at high salt concentrations.

Examples – Serum albumin, egg albumin, lactalbumin (Milk), leucosin (wheat), legumelin (soyabeans).

(ii) Globulins:

Insoluble in water, soluble in dilute salt 1 solutions and precipitated by half 1 saturated salt solutions.

Examples – Serum globulin, vitellin (egg yolk), tuberin (potato), myosinogen (muscle), legumin (peas).

(iii) Glutelins:

Insoluble in water but soluble in dilute 1 acids and alkalis. Mostly found in plants.

Examples – Glutenin (wheat), oryzenin (rice).

(iv) Prolamines: Insoluble in water and absolute alcohol 1 but soluble in 70 to 80 per cent alcohol.

Examples – Gliadin (wheat), zein (maize).

(v) Protamines:

Basic proteins of low molecular weight. 1 Soluble in water, dilute acids and alkalis, j Not coagulable by heat.

Examples – Salmine (salmon sperm).

(vi) Histones:

Soluble in water and insoluble in very I dilute ammonium hydroxide.

Examples – Globin of hemoglobin and thymus histones.

(vii) Scleroproteins:

Insoluble in water, dilute acids and alkalis.

Examples – Keratin (hair, horn, nail, hoof and feathers), collagen (bone, skin), elastin (ligament).

II. Conjugated Proteins

(i) Nucleoproteins:

Composed of simple basic proteins (protamines or histones) with nucleic acids, I found in nuclei. Soluble in water.

Examples – Nucleoprotamines and nucleohistones.

(ii) Lipoproteins:

Combination of proteins with lipids, such ‘ as fatty acids, cholesterol and 1 phospholipids etc.

Examples – Lipoproteins of egg-yolk, milk and cell membranes, lipoproteins of blood.

(iii) Glycoproteins:

Combination of proteins with carbohydrate (mucopolysaccharides).

Examples – Mucin (saliva), ovomucoid (egg white), osseomucoid (bone), tendomucoid (tendon).

(iv) Phosphoproteins:

Contain phosphorus radical as a | prosthetic group.

Examples – Caseinogen (milk), ovovitellin (egg yolk).

(v) Metalloproteins:

Contain metal ions as their prosthetic | groups. The metal ions generally are Fe, I Co. Mg, Mn, Zn, Cu etc.

Examples – Siderophilin (Fe), ceruloplasmin (Cu).

(vi) Chromoproteins:

Contain porphyrin (with a metal ion) as | their prosthetic groups.

Examples – Haemoglobin , myoglobin, catalase, peroxidase, cytochromes.

(vii) Flavoproteins:

Contain riboflavin as their prosthetic 1 groups.

Examples – Flavoproteins of liver and kidney.

III. Derived Protein

A. Primary derivatives

(i) Proteans:

Derived in the early stage of protein hydrolysis by dilute acids, enzymes or alkalis.

Examples – Fibrin from fibrinogen.

(ii) Metaproteins:

Derived in the later stage of protein hydrolysis by slightly stronger acids and alkalis.

Examples – Acid and alkali metaproteins.

(iii) Coagulated:

They are denatured proteins formed by the action of heat. X-rays, ultraviolet rays etc.

Cooked proteins, coagulated albumins.

B. Secondary derivatives

(i) Proteoses:

Formed by the action of pepsin or trypsin. Precipitated by saturated solution of ammonium sulphate, incoagulable by heat.

Examples – Albumose from albumin, globulose from globulin.

(ii) Peptones: .

Further stage of cleavage than the proteoses. Soluble in water, incoagulable by heat and not precipitated by saturated ammonium sulphate solutions.

(iii) Peptides:

Compounds containing two or more amino acids. They may be di-, tri-, and porypeptides.

Examples – Glycyl-alanine, leucyl-glutamic acid.

4. Protein Hydrolyzing Enzymes:

i. Pepsin:

In Gastric Juice.

ii. Trypsin, Chymotrypsin and Carboxypeptidases:

In Pancreatic Juice.

iii. Amino-peptidases, Dipeptidases and Poly-peptidases:

In intestinal juice.

Subject Code: 17CHU404B Reg.No…….

KARPAGAM ACADEMY OF HIGHER EDUCATION

COIMBATORE-21

(For the candidates admitted from 2017& onwards)

DEPARTMENT OF CHEMISTRY

B.Sc Degree Examination

Semester-IV

INTERNAL TEST-II(FEBRUARY-2019)

SUBJECT TITLE: ANALYTICAL CLINICAL BIOCHEMISTRY

Elective Paper

Date: 5.2.2019 AN Subject Code : 17CHU404B

Time : 2 Hours Maximum : 50 marks

Part-A (20 x1= 20 Marks)

Answer all the questions

1. β-pleated sheets are the examples of
a) Primary structure b) Secondary structure c) Tertiary structure d) Quaternary structure

2. A coiled peptide chain held in place by hydrogen bonding between peptide bonds in the same chain is
a) Primary structure b) α-helix c) β-pleated sheets d) Tertiary structure

3. A structure that has hydrogen bonds between polypeptide chains arranged side by side is
a) Primary structure b) α-helix c) β-pleated sheets d) Tertiary structure

4. Which of the following are known as helix breakers?
a) Proline and glycine b) Isoleucine and leucine c) Valine d) Threonine

5. A process by which a protein structure assumes its functional shape or conformation is
a) Denaturing b) Folding c) Synthesis d) Hydrolysis

6. Process of folding does not depend on
a) Concentration of salts b) PH c) Solute d) Solvent

7. As folding progresses which of the following does not take place?
a) Entropy decreases b) Amount of protein in native state increases
c) Free energy increases d) Amount of protein in native state decreases

8. Which technique is preferred in the separation of fatty acyl methyl esters from a mixture?

a) Gas-liquid chromatography b) Absorption chromatography

c) TLC d) Centrifugation

9. The dye used in TLC for detecting separated lipids by spraying the plate is

a) Mordant b) Alizarin c) Rhodamine d) Fuchsin

10. Which of the following is not a phospholipase?

a) A b) C c) D d) K

11. In which type of chromatography lipids are carried up a silica gel coated pate by a rising solvent front, less polar travels farther than the more polar ones?

a) Absorption chromatography b) Thin layer chromatography

c) Gas-liquid chromatography d) HPLC

12. Conversion of acetyl co-A to malonyl co-A requires which of the following?

a) NADPH b) H_2O c) Folic acid d) Biotin

13. The prosthetic group of acyl carrier protein is

a) 4'-phosphopantetheine b) 3'-phosphopantetheine c) 2'-phosphopantetheine

d) 1'-phosphopantetheine

14. Which of the following is an essential fatty acid?

a) Linolenic b) Palmitic c) Oleic d) Stearic

15. Which of the following is a polar derivative of cholesterol?

a) Bile salt b) Oestrogen c) Vitamin D d) Progesterone

16. The number of milligrams of KOH required to neutralize the free and combined fatty acid in one gram of a given fat is called

a) Saponification number b) Iodine number c) Acid number d) Polenske number

17. Which of the following is a storage form of lipid?

a) Glycolipid b) Phospholipid c) Sufolipid d) Triacyl glycerol

18. Out of the following, cholesterol does not serve as a precursor for which compounds?

a) Vitamin D b) Sex hormones c) Bile salts d) Bile pigments

19. Which of the following is a sphingophospholipid?

a) Lecithin b) Sphingomyelin c) Plasmolegen d) Cardiolipin

20. Which of the following group of membrane lipids predominate in plant cells?

a) Galactolipids b) Sphingolipids c) Glycerophospholipids d) Archaebacterial ether lipids

PART-B (3 X 2= 6 MARKS)

ANSWER ALL THE QUESTIONS

21. Explain the role of metals in enzyme action.

22. What is meant by coenzyme?

23. What is meant by lipoprotein?

PART-C (3 X 8 = 28 MARKS)

ANSWER ALL THE QUESTIONS

24. (a). Describe the classification and nomenclature of enzymes?

OR

(b). Write an account of various factors affecting enzyme activity?

25.(a). Describe the mechanism of enzyme action?

OR

(b). Explain the synthesis of Triacylglecerol.

26.(a). Write a note on disorders of plasma lipoprotein?

OR

(b). Discuss the metabolism of Phospholipids?

Subject Code: 17CHU404B
Reg.No…….

KARPAGAM ACADEMY OF HIGHER EDUCATION
COIMBATORE-21
(For the candidates admitted from 2017& onwards)
DEPARTMENT OF CHEMISTRY
B.Sc Degree Examination
Semester-IV
INTERNAL TEST-II(FEBRUARY-2019)
SUBJECT TITLE: ANALYTICAL CLINICAL BIOCHEMISTRY
Elective Paper

Date: 5.2.2019 AN **Subject Code : 17CHU404B**
Time : 2 Hours **Maximum : 50 marks**
Part-A (20 x1= 20 Marks)
Answer all the questions

1. β-pleated sheets are the examples of
a) Primary structure b) **Secondary structure** c) Tertiary structure d) Quaternary structure

2. A coiled peptide chain held in place by hydrogen bonding between peptide bonds in the same chain is
a) Primary structure b) **α-helix** c) β-pleated sheets d) Tertiary structure

3. A structure that has hydrogen bonds between polypeptide chains arranged side by side is
a) Primary structure b) α-helix c) **β-pleated sheets** d) Tertiary structure

4. Which of the following are known as helix breakers?
a) **Proline and glycine** b) Isoleucine and leucine c) Valine d) Threonine

5. A process by which a protein structure assumes its functional shape or conformation is
a) Denaturing b) **Folding** c) Synthesis d) Hydrolysis

6. Process of folding does not depend on
a) Concentration of salts b) PH c) **Solute** d) Solvent

7. As folding progresses which of the following does not take place?
a) Entropy decreases b) Amount of protein in native state increases
c) Free energy increases d) **Amount of protein in native state decreases**

8. Which technique is preferred in the separation of fatty acyl methyl esters from a mixture?
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PART-B (3 X 2= 6 MARKS)

ANSWER ALL THE QUESTIONS

21. Explain the role of metals in enzyme action.

Metal ions play important roles in the biological function of many enzymes. The various modes of metal-protein interaction include metal-, ligand-, and enzyme-bridge complexes. Metals can serve as electron donors or acceptors, Lewis acids or structural regulators. Those that participate directly in the catalytic mechanism usually exhibit anomalous physicochemical characteristics reflecting their entatic state. Carboxypeptidase A, liver alcohol dehydrogenase, aspartate transcarbamoylase and alkaline phosphatase exemplify the different roles of metals in metalloenzymes while the nucleotide polymerases point to the essential role of zinc in maintaining normal growth and development.

22. What is meant by coenzyme?

Coenzymes are small molecules. They cannot by themselves catalyze a reaction but they can help enzymes to do so. In technical terms, **coenzymes** are organic nonprotein molecules that bind with the protein molecule (apoenzyme) to form the active enzyme (holoenzyme).

23. What is meant by lipoprotein?

Lipoproteins are basically a core full of fat and cholesterol, along with a lipid membrane that contains proteins called apolipoproteins. There are many types of lipoproteins, but the two most important ones are called LDL (Low DensityLipoprotein) and HDL (High Density Lipoprotein).

PART-C (3 X 8 = 28 MARKS)

ANSWER ALL THE QUESTIONS

24. (a). Describe the classification and nomenclature of enzymes?

Naming and Classification

Except for some of the originally studied enzymes such as pepsin, rennin, and trypsin, most enzyme names end in "ase". The International Union of Biochemistry (I.U.B.) initiated standards of enzyme nomenclature which recommend that enzyme names indicate both the substrate acted upon and the type of reaction catalyzed. Under this system, the enzyme uricase is called urate: O_2oxidoreductase, while the enzyme glutamic oxaloacetic transaminase (GOT) is called L-aspartate: 2-oxoglutarate aminotransferase.

Enzymes can be classified by the kind of chemical reaction catalyzed.

1. Addition or removal of water
    A. Hydrolases - these include esterases, carbohydrases, nucleases, deaminases, amidases, and proteases
    B. Hydrases such as fumarase, enolase, aconitase and carbonic anhydrase
2. Transfer of electrons
    A. Oxidases

    B. Dehydrogenases
3. Transfer of a radical
    A. Transglycosidases - of monosaccharides
    B. Transphosphorylases and phosphomutases - of a phosphate group
    C. Transaminases - of amino group
    D. Transmethylases - of a methyl group
    E. Transacetylases - of an acetyl group
4. Splitting or forming a C-C bond
    A. Desmolases
5. Changing geometry or structure of a molecule
    A. Isomerases
6. Joining two molecules through hydrolysis of pyrophosphate bond in ATP or other tri-phosphate
    A. Ligases

OR

(b). Write an account of various factors affecting enzyme activity?

Factors Affecting Enzyme Activity

Knowledge of basic enzyme kinetic theory is important in enzyme analysis in order both to understand the basic enzymatic mechanism and to select a method for enzyme analysis. The conditions selected to measure the activity of an enzyme would not be the same as those selected to measure the concentration of its substrate. Several factors affect the rate at which enzymatic reactions proceed - temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators.

Enzyme Concentration

In order to study the effect of increasing the enzyme concentration upon the reaction rate, the substrate must be present in an excess amount; i.e., the reaction must be independent of the substrate concentration. Any change in the amount of product formed over a specified period of time will be dependent upon the level of enzyme present. Graphically this can be represented as:

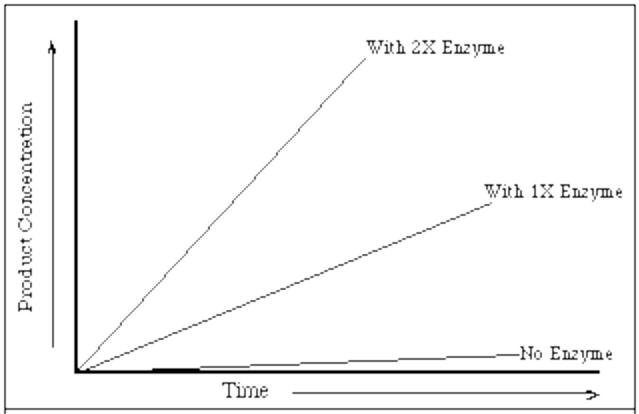


FIGURE 4
"Zero order" reaction rate is independent of substrate concentration.

These reactions are said to be "zero order" because the rates are independent of substrate concentration, and are equal to some constant k. The formation of product proceeds at a rate which is linear with time. The addition of more substrate does not serve to increase the rate. In zero order kinetics, allowing the assay to run for double time results in double the amount of product.

Table I: Reaction Orders with Respect to Substrate Concentration

Order	**Rate Equation**	**Comments**
zero	rate = k	rate is independent of substrate concentration
first	rate = k[S]	rate is proportional to the first power of substrate concentration
second	rate = $k[S][S]=k[S]^2$	rate is proportional to the square of the substrate concentration
second	rate = $k[S_1][S_2]$	rate is proportional to the first power of each of two reactants

The amount of enzyme present in a reaction is measured by the activity it catalyzes. The relationship between activity and concentration is affected by many factors such as temperature, pH, etc. An enzyme assay must be designed so that the observed activity is proportional to the amount of enzyme present in order that the enzyme concentration is the only limiting factor. It is satisfied only when the reaction is zero order.

In Figure 5, activity is directly proportional to concentration in the area AB, but not in BC. Enzyme activity is generally greatest when substrate concentration is unlimiting.

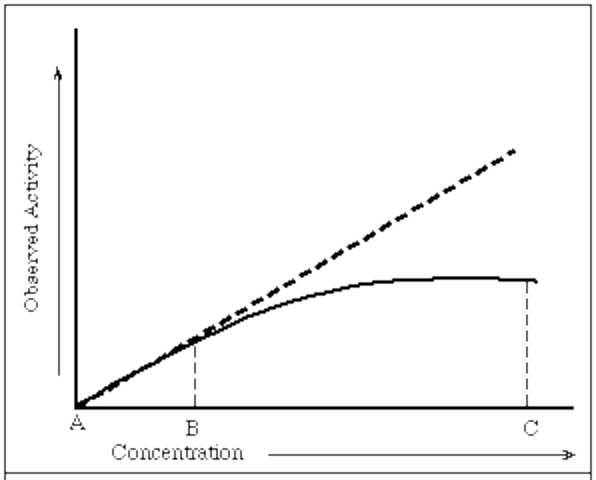


FIGURE 5
Activity vs. Concentration

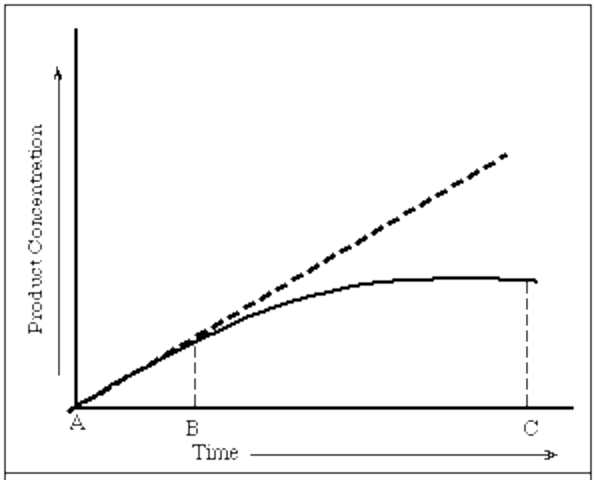


FIGURE 6
Reaction rate limited by substrate concentration

When the concentration of the product of an enzymatic reaction is plotted against time, a similar curve results, Figure 6.

Between A and B, the curve represents a zero order reaction; that is, one in which the rate is constant with time. As substrate is used up, the enzyme's active sites are no longer saturated, substrate concentration becomes rate limiting, and the reaction becomes first order between B and C.

To measure enzyme activity ideally, the measurements must be made in that portion of the curve where the reaction is zero order. A reaction is most likely to be zero order initially since substrate concentration is then highest. To be certain that a reaction is zero order, multiple measurements of product (or substrate) concentration must be made.

Figure 7 illustrates three types of reactions which might be encountered in enzyme assays and shows the problems which might be enountered if only single measurements are made.

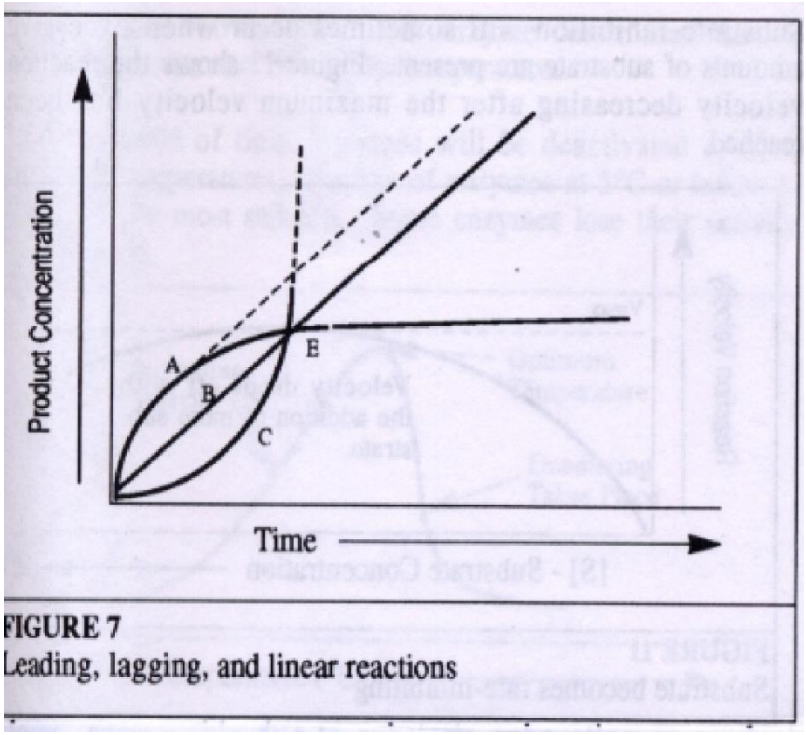


FIGURE 7
Leading, lagging, and linear reactions

B is a straight line representing a zero order reaction which permits accurate determination of enzyme activity for part or all of the reaction time. A represents the type of reaction that was shown in Figure 6. This reaction is zero order initially and then slows, presumably due to substrate exhaustion or product inhibition. This type of reaction is sometimes referred to as a "leading" reaction. True "potential" activity is represented by the dotted line. Curve C represents a reaction with an initial "lag" phase. Again the dotted line represents the potentially measurable activity. Multiple determinations of product concentration enable each curve to be plotted and true activity determined. A single end point determination at E would lead to the false conclusion that all three samples had identical enzyme concentration.

Substrate Concentration

It has been shown experimentally that if the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity (delta A/delta T). This is represented graphically in Figure 8.

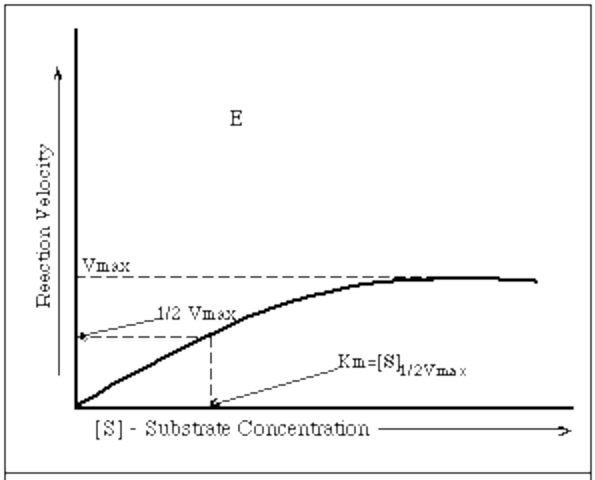


FIGURE 8
Effect of substrate concentration

It is theorized that when this maximum velocity had been reached, all of the available enzyme has been converted to ES, the enzyme substrate complex. This point on the graph is designated Vmax. Using this maximum velocity and equation (7), Michaelis developed a set of mathematical expressions to calculate enzyme activity in terms of reaction speed from measurable laboratory data.

$$E + S \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} ES \underset{K_{2}}{\overset{K_{+2}}{\rightleftharpoons}} P + E \qquad [7]$$

The Michaelis constant Km is defined as the substrate concentration at 1/2 the maximum velocity. This is shown in Figure 8. Using this constant and the fact that Km can also be defined as:

$K_m = K_{-1} + K_2 / K_{+1}$

K^{+1}, K^{-1} and K^{+2} being the rate constants from equation (7). Michaelis developed the following

$$V_1 = \frac{V_{max}[S]}{K_m + [S]}$$

where

V_t = the velocity at any time

[S] = the substrate concentration at this time

V_{max} = the highest under this set of experimental conditions (pH, temperature, etc.)

K_m = the Michaelis constant for the particular enzyme being investigated

Michaelis constants have been determined for many of the commonly used enzymes. The size of Km tells us several things about a particular enzyme.

- A small Km indicates that the enzyme requires only a small amount of substrate to become saturated. Hence, the maximum velocity is reached at relatively low substrate concentrations.
- A large Km indicates the need for high substrate concentrations to achieve maximum reaction velocity.
- The substrate with the lowest Km upon which the enzyme acts as a catalyst is frequently assumed to be enzyme's natural substrate, though this is not true for all enzymes.

- **Effects of Inhibitors on Enzyme Activity**
- Enzyme inhibitors are substances which alter the catalytic action of the enzyme and consequently slow down, or in some cases, stop catalysis. There are three common types of enzyme inhibition - competitive, non-competitive and substrate inhibition.
- Most theories concerning inhibition mechanisms are based on the existence of the enzyme-substrate complex ES. As mentioned earlier, the existence of temporary ES structures has been verified in the laboratory.
- Competitive inhibition occurs when the substrate and a substance resembling the substrate are both added to the enzyme. A theory called the "lock-key theory" of enzyme catalysts can be used to explain why inhibition occurs.

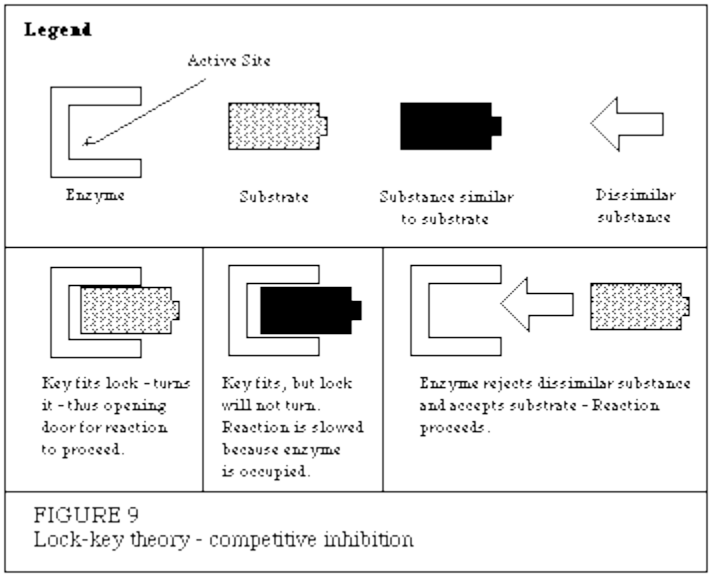


FIGURE 9
Lock-key theory - competitive inhibition

- The lock and key theory utilizes the concept of an "active site." The concept holds that one particular portion of the enzyme surface has a strong affinity for the substrate. The substrate is held in such a way that its conversion to the reaction products is more favorable. If we consider the enzyme as the lock and the substrate the key (Figure 9) - the key is inserted in the lock, is turned, and the door is opened and the reaction proceeds. However, when an inhibitor which resembles the substrate is present, it will compete with the substrate for the position in the enzyme lock. When the inhibitor wins, it gains the lock position but is unable to open the lock. Hence, the observed reaction is slowed down because some of the available enzyme sites are occupied by the inhibitor. If a dissimilar substance which does not fit the site is present, the enzyme rejects it, accepts the substrate, and the reaction proceeds normally.

- Non-competitive inhibitors are considered to be substances which when added to the enzyme alter the enzyme in a way that it cannot accept the substrate. Figure 10.

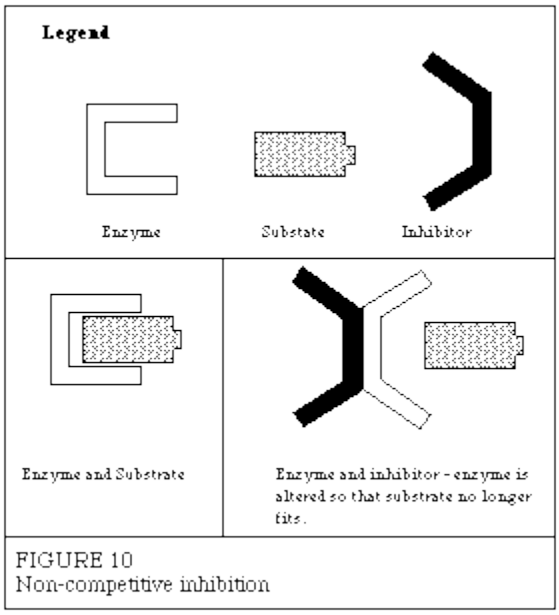


FIGURE 10
Non-competitive inhibition

- Substrate inhibition will sometimes occur when excessive amounts of substrate are present. Figure 11 shows the reaction velocity decreasing after the maximum velocity has been reached.

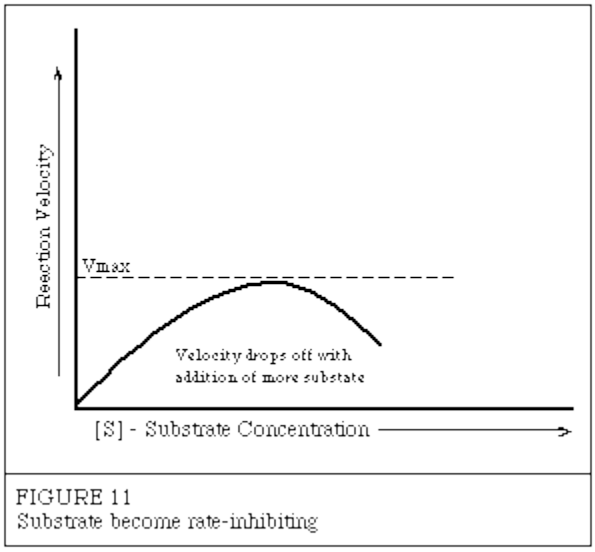


FIGURE 11
Substrate become rate-inhibiting

- Additional amounts of substrate added to the reaction mixture after this point actually decrease the reaction rate. This is thought to be due to the fact that there are so many substrate molecules competing for the active sites on the enzyme surfaces that they block the sites (Figure 12) and prevent any other substrate molecules from occupying them.

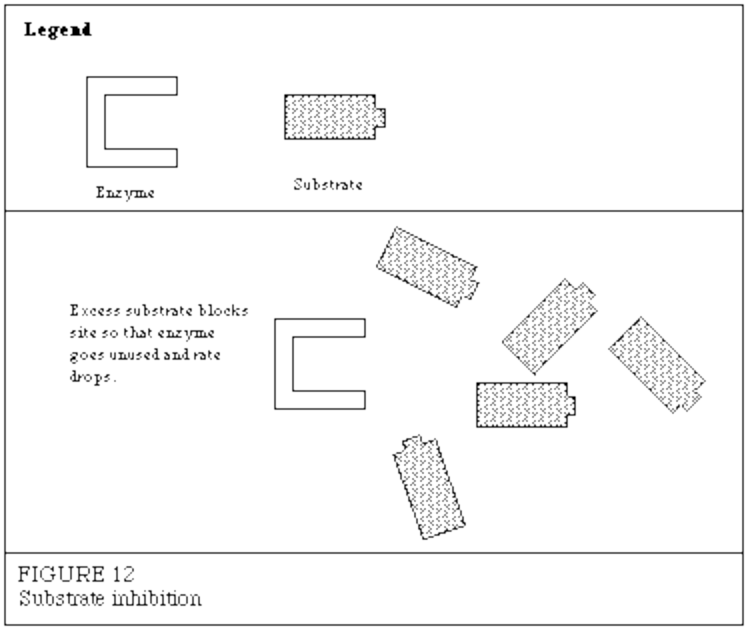


FIGURE 12
Substrate inhibition

- This causes the reaction rate to drop since all of the enzyme present is not being used.
- **Temperature Effects**

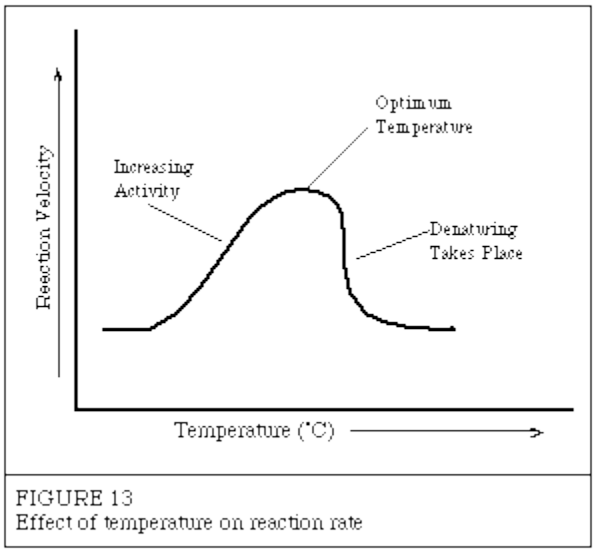


FIGURE 13
Effect of temperature on reaction rate

- Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised. A ten degree Centigrade rise in temperature will increase the activity of most enzymes by 50 to 100%. Variations in reaction temperature as small as 1 or 2 degrees may introduce changes of 10 to 20% in the results. In the case of enzymatic reactions, this is complicated by the fact that many enzymes are adversely affected by high temperatures. As shown in Figure 13, the reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature. Because most animal enzymes rapidly become denatured at temperatures above 40°C, most enzyme determinations are carried out somewhat below that temperature.

- Over a period of time, enzymes will be deactivated at even moderate temperatures. Storage of enzymes at 5°C or below is generally the most suitable. Some enzymes lose their activity when frozen.
- **Effects of pH**
- Enzymes are affected by changes in pH. The most favorable pH value - the point where the enzyme is most active - is known as the optimum pH. This is graphically illustrated in Figure 14.

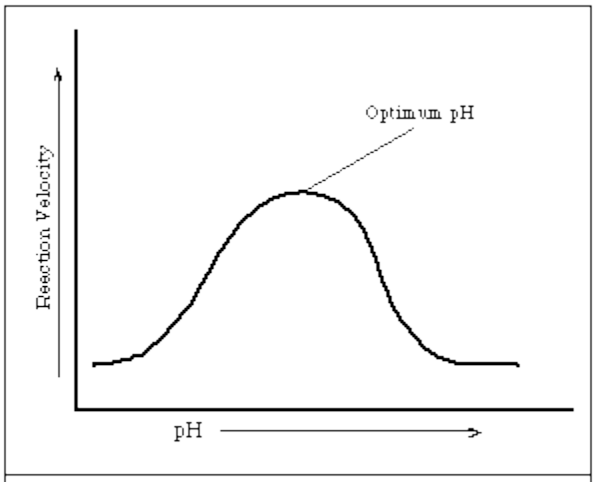


FIGURE 14
Effect of pH on reaction rate

- Extremely high or low pH values generally result in complete loss of activity for most enzymes. pH is also a factor in the stability of enzymes. As with activity, for each enzyme there is also a region of pH optimal stability.
- The optimum pH value will vary greatly from one enzyme to another, as Table II shows:

Table II: pH for Optimum Activity

Enzyme	pH Optimum
Lipase (pancreas)	8.0
Lipase (stomach)	4.0 - 5.0
Lipase (castor oil)	4.7
Pepsin	1.5 - 1.6
Trypsin	7.8 - 8.7
Urease	7.0

Table II: pH for Optimum Activity

Enzyme	pH Optimum
Invertase	4.5
Maltase	6.1 - 6.8
Amylase (pancreas)	6.7 - 7.0
Amylase (malt)	4.6 - 5.2
Catalase	7.0

- In addition to temperature and pH there are other factors, such as ionic strength, which can affect the enzymatic reaction. Each of these physical and chemical parameters must be considered and optimized in order for an enzymatic reaction to be accurate and reproducible.

25.(a). Describe the mechanism of enzyme action?

Mechanism of Enzyme Action

Introduction - Enzyme Characteristics:

The basic mechanism by which enzymes catalyze chemical reactions begins with the binding of the **substrate** (or substrates) to the active site on the enzyme. The **active site** is the specific region of the enzyme which combines with the substrate. The binding of the substrate to the enzyme causes changes in the distribution of electrons in the chemical bonds of the substrate and ultimately causes the reactions that lead to the formation of products. The products are released from the enzyme surface to regenerate the enzyme for another reaction cycle.

The **active site** has a unique geometric shape that is complementary to the geometric shape of a substrate molecule, similar to the fit of puzzle pieces. This means that enzymes specifically react with only one or a very few similar compounds.

Lock and Key Theory:

The specific action of an enzyme with a single substrate can be explained using a **Lock and Key**analogy first postulated in 1894 by Emil Fischer. In this analogy, the lock is the enzyme and the key is the substrate. Only the correctly sized **key (substrate) fits** into the **key hole (active site)** of the **lock (enzyme)**.

Smaller keys, larger keys, or incorrectly positioned teeth on keys (incorrectly shaped or sized

substrate molecules) do not fit into the lock (enzyme). Only the correctly shaped key opens a particular lock. This is illustrated in graphic on the left.

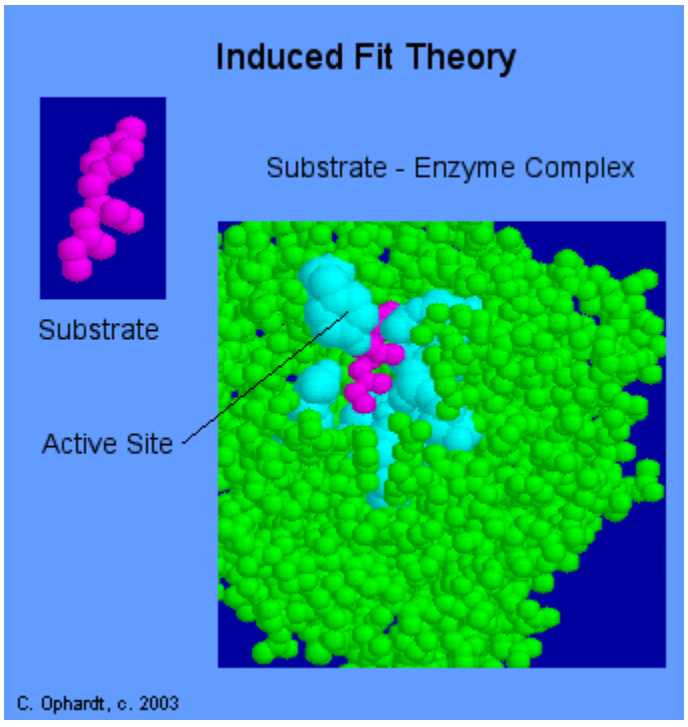
QUES: Using a diagram and in your own words, describe the various lock and key theory of enzyme action in relation to a correct and incorrect substrate.

Induced Fit Theory:

Not all experimental evidence can be adequately explained by using the so-called rigid enzyme model assumed by the lock and key theory. For this reason, a modification called the induced-fit theory has been proposed.

The induced-fit theory assumes that the substrate plays a role in determining the final shape of the enzyme and that the enzyme is partially flexible. This explains why certain compounds can bind to the enzyme but do not react because the enzyme has been distorted too much. Other molecules may be too small to induce the proper alignment and therefore cannot react. Only the proper substrate is capable of inducing the proper alignment of the active site.

In the graphic on the left, the substrate is represented by the magenta molecule, the enzyme protein is represented by the green and cyan colors. The cyan colored protein is used to more sharply define the active site. The protein chains are flexible and fit around the substrate.



Carboxypeptidase

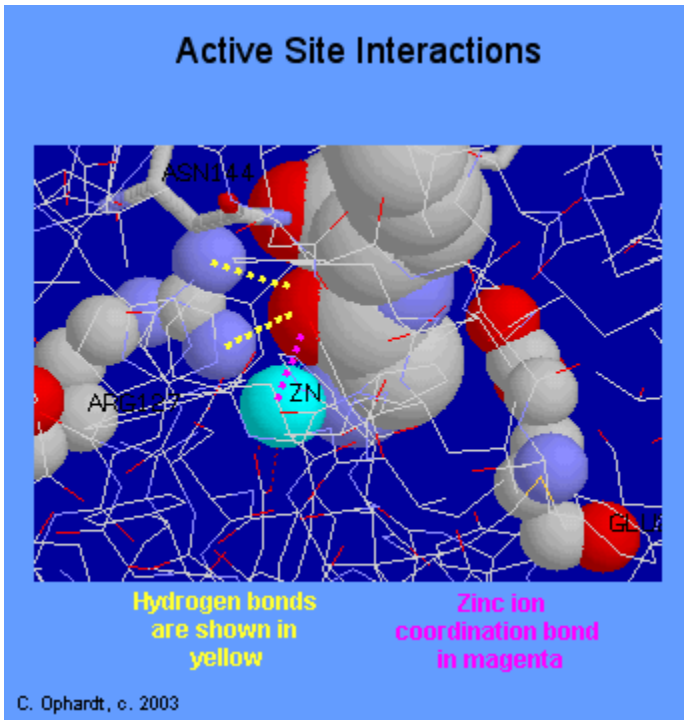
Nature of Active Site and Substrate Interaction:

Enzymes have varying degrees of specificity. Some enzymes have absolute specificity for one substrate and no others, while other enzymes react with substrates with similar functional groups, side chains, or positions on a chain. The least specific enzymes catalyze a reaction at a particular chemical bond regardless of other structural features.

Much experimental work is devoted to gaining an understanding of the nature of the active site in an enzyme. Since enzymes are proteins, the nature of amino acid side chains in the vicinity of the active site is important. The specific amino acid side chains have been determined for many enzymes. The active site for carboxypeptidase A will be used to illustrate the principles involved as shown in the graphic on the left.

The substrate (space filling gray,blue red) can interact with the active site through opposite charges, hydrogen bonding (shown in yellow), hydrophobic non-polar interaction, and coordinate covalent bonding to the metal ion activator as shown in magenta. The numbers behind the amino acids indicate the sequence position of the amino acid in the protein. The white lines represent the wire frames of the other amino acids in the enzyme.

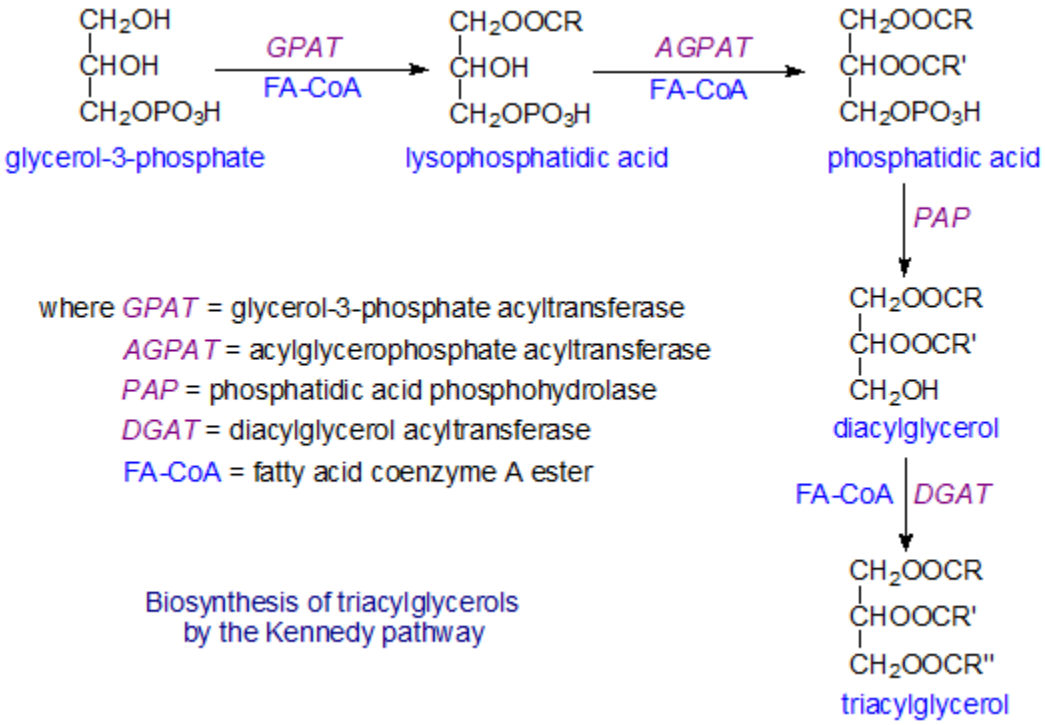
The carbonyl bond is activated by interaction with the Zn ions. This leads to the addition of -OH from water to the carbonyl to produce an acid and the ultimate rupture of the C-N bond.



OR

(b). Explain the synthesis of Triacylglecerol.

Three main pathways for triacylglycerol biosynthesis are known, the *sn*-glycerol-3-phosphate and dihydroxyacetone phosphate pathways, which predominates in liver and adipose tissue, and a monoacylglycerol pathway in the intestines. In maturing plant seeds and some animal tissues, a fourth pathway has been recognized in which a diacylglycerol transferase is involved. The most important route to triacylglycerols is the *sn*-glycerol-3-phosphate or **Kennedy pathway**, first described by Professor Eugene Kennedy and colleagues in the 1950s, by means of which more than 90% of liver triacylglycerols are produced.



Biosynthesis of triacylglycerols by the Kennedy pathway

In this pathway, the main source of the glycerol backbone has long been believed to be *sn*-glycerol-3-phosphate produced by the catabolism of glucose (glycolysis) or to a lesser extent by the action of the enzyme glycerol kinase on free glycerol. However, there is increasing evidence that a significant proportion of the glycerol is produced *de novo* by a process known as glyceroneogenesis via pyruvate. Indeed, this may be the main source in adipose tissue.

Subsequent reactions occur primarily in the endoplasmic reticulum. First, the precursor *sn*-glycerol-3-phosphate is esterified by a fatty acid coenzyme A ester in a reaction catalysed by a glycerol-3-phosphate acyltransferase (GPAT) at position *sn*-1 to form lysophosphatidic acid, and this is in turn acylated by an acylglycerophosphate acyltransferase (AGPAT) in position *sn*-2 to form a key intermediate in the biosynthesis of all glycerolipids - **phosphatidic acid**. Numerous isoforms of these enzymes are known; they are expressed with specific tissue and membrane distributions and they are regulated in different ways.

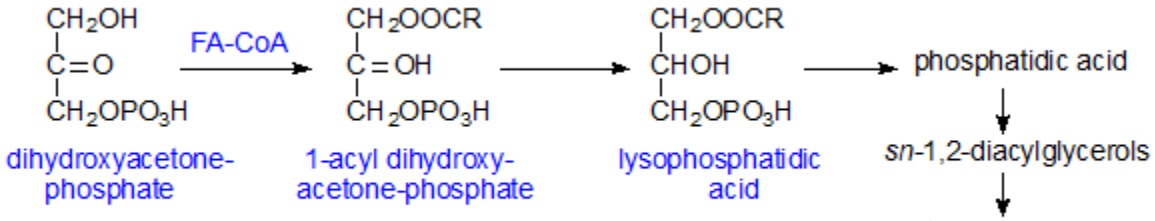
The phosphate group is removed by an enzyme (or family of enzymes) phosphatidic acid phosphohydrolase (PAP or 'phosphatidate phosphatase' or 'lipid phosphate phosphatase'). PAP is also important as it produces ***sn*-1,2-diacylglycerols** as essential intermediates in the biosynthesis not only of triacylglycerols but also of phosphatidylcholine and phosphatidylethanolamine (and of monogalactosyldiacylglycerols in plants). In contrast to the activity responsible for phospholipid biosynthesis in mammals, much of the phosphatase activity leading to triacylglycerol biosynthesis resides in three related cytoplasmic proteins, termed lipin-1, lipin-2 and lipin-3, which were characterized before the nature of their enzymatic activities were determined. The lipins are tissue specific, and each appears to have distinctive expression and functions, but lipin-1 (PAP1) in three isoforms (designated 1α, 1β and 1γ) accounts for all the PAP activity in adipose tissue and skeletal muscle. While it occurs mainly in the cytosolic compartment of cells, it is translocated to the endoplasmic reticulum in response to elevated levels of fatty acids within cells although it does not have trans-membrane domains. Lipin-1 activity requires Mg^{2+} ions and is inhibited by *N*-ethylmaleimide, whereas the membrane-bound activity responsible for synthesising diacylglycerols as a phospholipid intermediate is independent of Mg^{2+} concentration and is not sensitive to the inhibitor.

Perhaps surprisingly, lipin-1 has a dual role in that it operates in collaboration with known nuclear receptors as a transcriptional coactivator to modulate lipid metabolism (lipin 1α) while lipin 1β is associated with induction of lipogenic genes such as fatty acid synthase, stearoyl CoA desaturase and DGAT. Abnormalities in lipin-1 expression are known to be involved in some human disease states that may lead to the metabolic syndrome. Lipin 2 is a similar phosphatidate phosphohydrolase, which is present in liver and brain and is regulated dynamically by fasting and obesity (in mice), while lipin 3 is found in the gastrointestinal tract and liver.

In the final step in this pathway, the resultant 1,2-diacyl-*sn*-glycerol is acylated by diacylglycerol acyltransferases (DGAT), which can utilize a wide range of fatty acyl-CoA esters to form the triacyl-*sn*-glycerol. In fact there are two DGAT enzymes, which are structurally and functionally distinct. In animals, DGAT1 is located mainly in the endoplasmic reticulum and is expressed in skeletal muscle, skin and intestine, with lower levels of expression in liver and adipose tissue. It is believed to have dual topology contributing to triacylglycerol synthesis on both sides of the membrane of the endoplasmic reticulum, but esterifying only pre-formed fatty acids of exogenous origin. Perhaps surprisingly, DGAT1 is the only one present in the epithelial cells that

synthesise milk fat in the mammary gland. Orthologues of this enzyme are present in most eukaryotes, other than yeasts, and it is especially important in plants. Also DGAT1 can utilize a wider range of substrates, including monoacylglycerols, long-chain alcohols (for wax synthesis) and retinol. DGAT2 is the main form of the enzyme in hepatocytes and adipocytes (lipid droplets), although it is expressed much more widely in tissues. It is associated with distinct regions of the endoplasmic reticulum, at the surface of lipid droplets and in mitochondria, and it esterifies fatty acids of both endogenous and exogenous origin. DGAT2 is believed to have a targeting domain that enables it to tether between the endoplasmic reticulum and lipid droplet thereby channelling triacylglycerols from the synthesis site in the endoplasmic reticulum to the nascent lipid droplet, where they accumulate and lead to the expansion of the latter (see **below**). Both enzymes are important modulators of energy metabolism, although DGAT2 appears to be especially important in controlling the homeostasis of triacylglycerols *in vivo*. As the glycerol-3-phosphate acyltransferase (GPAT) has the lowest specific activity of these enzymes, this step may be the rate-limiting one. However, DGATs are the dedicated triacylglycerol-forming enzymes, and they are seen as the best target for pharmaceutical intervention in obesity and attendant ailments; clinical studies of DGAT1 inhibitors are at an early stage.

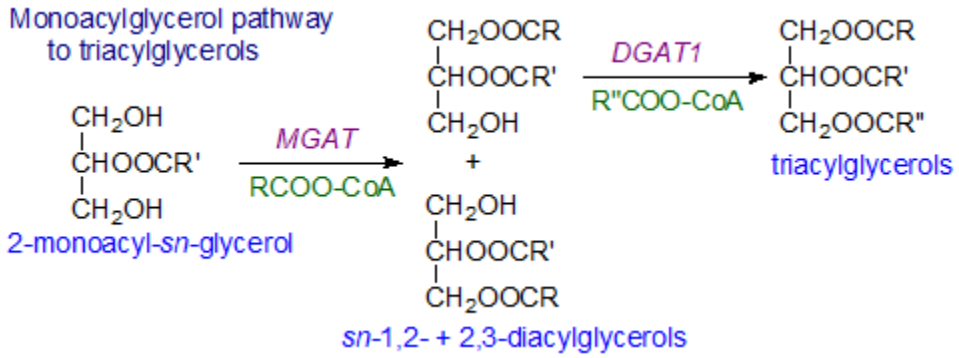
In a second pathway for triacylglycerol biosynthesis, dihydroxyacetone-phosphate in peroxisomes or endoplasmic reticulum can be acylated by a specific acyltransferase to form 1-acyl dihydroxyacetone-phosphate, which is reduced by dihydroxyacetone-phosphate oxido-reductase to lysophosphatidic acid, which can then enter the pathway above to triacylglycerols. The precursor dihydroxyacetone-phosphate is important also as part of the biosynthetic route to **plasmalogens**, and neutral plasmalogens can be significant components of cytoplasmic droplets in many mammalian cells types but not adipose tissue.



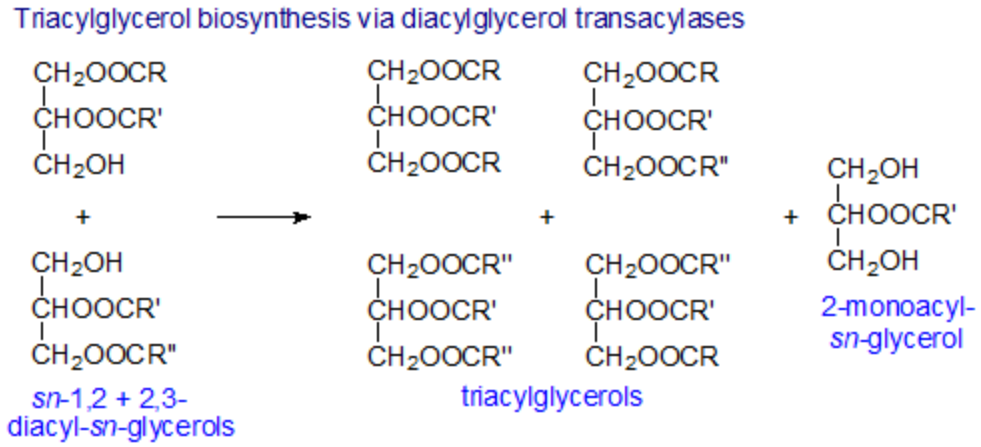
Biosynthesis of triacylglycerols via dihydroxyacetone-phosphate

In the enterocytes of intestines after a meal, up to 75% of the triacylglycerols are formed via a **monoacylglycerol pathway**. 2-Monoacyl-*sn*-glycerols and free fatty acids released from dietary triacylglycerols by the action of pancreatic lipase within the intestines (see below) are taken up by the enterocytes. There, the monoacylglycerols are first acylated by an acyl coenzyme A:monoacylglycerol acyltransferase with formation of *sn*-1,2-diacylglycerols mainly as the first intermediate in the process, though some *sn*-2,3-diacylglycerols (~10%) are also produced (DGAT1 can also acylate monoacylglycerols). 1-Monoacylglycerols can also be synthesised by

the acylation of glycerol and these can also be acylated. There are three isoforms of the monoacylglycerol acyltransferase in humans of which MGAT2 is most active in the intestines and liver and MGAT1 in adipose tissue. Finally, the acyl coenzyme A:diacylglycerol acyltransferase (DGAT1) reacts with the *sn*-1,2-diacylglycerols only to form triacylglycerols.



In a fourth biosynthetic pathway, which is less well known, triacylglycerols are synthesised by a transacylation reaction between two racemic diacylglycerols that is independent of acyl-CoA. The reaction was first detected in the endoplasmic reticulum of intestinal micro villus cells and is catalysed by a diacylglycerol transacylase. Both diacylglycerol enantiomers participate in the reaction with equal facility to transfer a fatty acyl group with formation of triacylglycerols and a 2-monoacyl-*sn*-glycerol. A similar reaction has been observed in seed oils.



It has been suggested that this enzyme may function in remodelling triacylglycerols post synthesis, especially in oil seeds, and it is possible that it may be involved in similar processes in the liver and adipose tissue, where extensive hydrolysis/re-esterification is known to occur. There is evidence for selectivity in the biosynthesis of different molecular species in a variety of tissues and organisms, which may be a consequence of the varying biosynthetic pathways. Also in adipose tissue, fatty acids synthesised *de novo* are utilized in different ways from those from external sources in that they enter positions *sn*-1 and 2 predominantly, while a high proportion of

the oleic acid synthesised in the tissue by desaturation of exogenous stearic acid is esterified to position *sn*-3.

In prokaryotes, the glycerol-3-phosphate pathway of triacylglycerol biosynthesis only occurs, but in yeast both glycerol-3-phosphate and dihydroxyacetone-phosphate can be the primary precursors and synthesis takes place in cytoplasmic lipid droplets and the endoplasmic reticulum. In plants, the glycerol-3-phosphate pathway is most important, but these process are discussed below in greater detail.

Among other potential routes to the various intermediates, lysophosphatidic acid and phosphatidic acid can be synthesised in mitochondria, but must then be transported to the endoplasmic reticulum before they enter the pathway for triacylglycerol production. 1,2-Diacyl-*sn*-glycerols are also produced by the action of phospholipase C on phospholipids.

In the glycerol-3-phosphate and other pathways, the starting material is of defined stereochemistry and each of the enzymes catalysing the various steps in the process is distinctive and can have preferences for particular fatty acids (as their coenzyme A esters) and for particular fatty acid combinations in the partially acylated intermediates. It should not be surprising, therefore, that natural triacylglycerols exist in enantiomeric forms with each position of the *sn*-glycerol moiety esterified by different fatty acids, as discussed in **Triacylglycerols - Part 1**.

While triacylglycerols are essential for normal physiology, an excessive accumulation in human adipose tissue and other organs results in obesity and other health problems, including insulin resistance, steatohepatitis and cardiomyopathy. Accordingly, there is considerable pharmaceutical interest in drugs that affect triacylglycerol biosynthesis and metabolism.

26.(a). Write a note on disorders of plasma lipoprotein?

The clinical importance of lipoprotein disorders derives chiefly from the role of lipoproteins in atherogenesis and its associated risk of coronary and peripheral vascular disease. The greatly increased risk of acute pancreatitis associated with severe hypertriglyceridemia is an additional indication for intervention. Disordered lipid metabolism is also a critical element in nonalcoholic fatty liver disease. Characterization of dyslipidemia is important for selection of appropriate treatment and may provide clues to underlying primary clinical disorders.

Atherosclerosis

Atherosclerosis is the leading cause of death in the United States. Abundant epidemiologic evidence establishes its multifactorial character and indicates that the effects of the multiple risk factors are at least additive. Risk factors include hyperlipidemia, hypertension, smoking, diabetes, physical inactivity, decreased levels of high-density lipoproteins (HDL), hyperhomocysteinemia, and hypercoagulable states. Atheromas are complex lesions containing cellular elements, collagen, and lipids. The progression of the lesion is chiefly attributable to its

content of unesterified cholesterol and cholesteryl esters. Cholesterol in the atheroma originates in circulating lipoproteins. Atherogenic lipoproteins include low-density (LDL), intermediate density (IDL), very low density lipoproteins (VLDL), and Lp(a) species, all of which contain the B-100 apolipoprotein (Apo B-100). Chylomicron remnants containing apoB-48 are also atherogenic. All of these are subject to oxidation by reactive oxygen species in the tissues and also by lipoxygenases secreted by macrophages in atheromas. Oxidized lipoproteins cause impairment of endothelial cell-mediated vasodilation and stimulate endothelium to secrete monocyte chemoattractant protein-1 (MCP-1) and adhesion molecules that recruit monocytes to the lesion. Tocopherols (vitamin E) are natural antioxidants that localize in the surface monolayers of lipoproteins, exerting resistance to oxidation. Increased oxidative stress such as that induced by smoking depletes the tocopherol content. Oxidation of lipoproteins stimulates their endocytosis via scavenger receptors on macrophages and smooth muscle cells, leading to the formation of foam cells. Recent studies strongly support a role of vitamin D in prevention of atherosclerosis, probably by influencing inflammatory activity of macrophages.

Hypertension increases access of lipoproteins to the subintima. Smoking accelerates atherogenesis by reducing HDL and increasing thrombogenesis by platelets—in addition to its pro-oxidant effect. Activated platelets release platelet-derived growth factor (PDGF), stimulating proliferation and migration of cells of smooth muscle origin into the lesion.

OR

(b). Discuss the metabolism of Phospholipids?

Subject Code: 17CHU404B Reg.No…….

KARPAGAM ACADEMY OF HIGHER EDUCATION

COIMBATORE-21

(For the candidates admitted from 2017& onwards)

DEPARTMENT OF CHEMISTRY

B.Sc Degree Examination

Semester-IV

SUBJECT TITLE: ANALYTICAL CLINICAL BIOCHEMISTRY

Elective Paper

Date: March-2019 Subject Code : 17CHU404B

Time: 2 Hours Maximum: 50 marks

Internal Test -III

PART-A (20 X1= 20 MARKS)

ANSWER ALL THE QUESTIONS

1. Steroid hormones receptor binds to

a) Hormone response elements in m-RNA b) Hormone response elements in DNA

c) Hormone response elements in proteins d) Ribosomes to stimulate translation

2. Mode of action of steroid hormones involve

a) Stimulation of DNA replication b) Stimulation of m-RNA transcription

c) Inhibition of protein synthesis d) Secondary messenger

3. The normal pH of blood is in the range of ____; therefore, a pH of 7.10 would be a condition called _____.

a) 7.00 – 7.15, alkalosis b) 7.35 – 7.45, acidosis c) 7.25 – 7.55, acidosis d) 7.35 – 7.55, alkalosis

4. Which of the following is NOT a function of blood?

a) to regulate body temperature. b) to increase fluid loss. c) to transport nutrients. d) to regulate ph.

5. Each of the following is a characteristic of red blood cells EXCEPT that they _____.

a) live about 30 days. b) are formed in the red bone marrow. c) areanucleate. d) contain hemoglobin.

6. Each of the following occurs when blood clots after a cut EXCEPT:

a) Platelets release clotting factor. b) thromboplastin converts prothrombin into thrombin.

c) Thrombin converts fibrin into fibrinogen. d) Fibrin filaments trap cells to produce a clot.

7. The drug antagonist of estrogen is

a) Tanoxifen b) Metformin c) Glucophage d) Victoza

8. The drug used to terminate early pregnancies is

a) RU486 b) Metformin c) Glucophage d) Victoza

9. A protein in the plasma which contributes to the osmotic pressure of blood is _____.

a) elastin b) prothrombin c) albumin d) thrombin

10. A woman with blood type O has a baby with blood type O. The father _____.
a) must be type O only. b) could be A, B or O, but in no way AB.
c) could possibly be AB. d) is Rh-..

11. Active macrophages that work in long-term cleanup of tissues are called _____.
a) neutrophils b) eosinophils c)lymphocytes d) monocytes

12. Although it carries much oxygen, _____ is more attracted to pesticides and carbon monoxide.
a) hemoglobin b) plasma c) thrombin d) white blood cells

13. Eukaryotes differ from prokaryote in mechanism of DNA replication due to
a) Use of DNA primer rather than RNA primer
b) Different enzyme for synthesis of lagging and leading strand
c) Discontinuous rather than semi-discontinuous replication
d) Unidirectional rather than semi-discontinuous replication

14. Which of the following is true about DNA polymerase?
a) It can synthesize DNA in the 5' to 3' direction
b) It can synthesize DNA in the 3' to 5' direction
c) It can synthesize mRNA in the 3' to 5' direction
d) It can synthesize mRNA in the 5' to 3' direction

15. In which instrument is used for measuring blood pressure?
a) Electrocardiogram b) Anemometer c) Stethoscope d) Sphygmanometer

16. What is the percentage of water in blood plasma?
a) 90 b) 80 c) 98 d) 60

17. Universal receivers can receive blood from
a) Group AB only b) Group O only c)Group A,AB d) Group o,A,B,AB

18. Pulmonary artery carries
a) Pure blood from lungs b) Pure blood to lungs c) Impure blood to lungs
d) Impure blood from lungs

19. The average life span of red blood corpuscles is about
a) 100-200 days b) 100-120 days c)160-180 days
d)150-200days

20. From which one of the following chambers of human heart is the oxygenated blood pumped in to aorta?
a) Right atrium b) Right ventricle c) Left atrium d) Left ventricle

PART-B (3 X 2= 6 MARKS)
ANSWER ALL THE QUESTIONS

21. What is Telomerase?
22.Different types of leucocytes can be found in the blood. What are they?
23. What is RNA primer?

PART-C (3 X 8 = 24 MARKS)
ANSWER ALL THE QUESTIONS

24.(a). Give an account of recombination of DNA.

OR

(b). Give an account of telomeres and their role in senescence and cancer.

25.(a). Discuss different types of DNA damages and their repair mechanism.

OR

(b). Discuss the types of Anemia?

26.(a). Write a note on Blood collection and preservation of samples?

OR

(b).What is the abnormal types of urine? Explain.

Subject Code: 17CHU404B
Reg.No…….

KARPAGAM ACADEMY OF HIGHER EDUCATION
COIMBATORE-21
(For the candidates admitted from 2017& onwards)
DEPARTMENT OF CHEMISTRY
B.Sc Degree Examination
Semester-IV
INTERNAL TEST-III(FEBRUARY-2019)
SUBJECT TITLE: ANALYTICAL CLINICAL BIOCHEMISTRY
Elective Paper

Date:12 .3.2019 AN **Subject Code : 17CHU404B**
Time : 2 Hours **Maximum : 50 marks**
Part-A (20 x1= 20 Marks)
Answer all the questions

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a) Right atrium b) Right ventricle c) Left atrium **d) Left ventricle**

PART-B (3 x 2= 6 marks)

21. What is Telomerase?

Telomerase, also called terminal transferase, is a ribonucleoprotein that adds a species-dependent telomere repeat sequence to the 3' end of telomeres. A telomere is a region of repetitive sequences at each end of eukaryotic chromosomes in most eukaryotes.

22. Different types of leucocytes can be found in the blood? What are they?

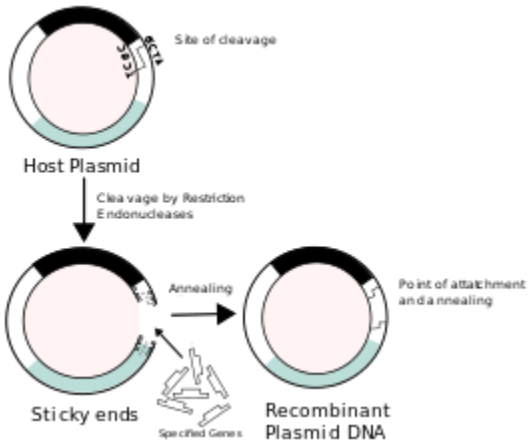
There are five different leukocytes that accomplish specific tasks based on their abilities and the type of invaders they are fighting. They are called neutrophils, basophils, eosinophils, monocytes, and lymphocytes.

23. What is RNA primer?

A primer is a short single strand of RNA or DNA (generally about 18-22 bases) that serves as a starting point for DNA synthesis. It is required for DNA replication because the enzymes that catalyze this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA. The polymerase starts replication at the 3′-end of the primer, and copies the opposite strand.

PART-C (3 x 8 = 24 marks)

24. a) Give an account of recombination of DNA?



Recombinant DNA (**rDNA**) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome. Recombinant DNA in a living organism was first achieved in 1972 by Herbert Boyer, of the University of California at San Francisco, and Stanley Cohen, at Stanford University, who used E. coli restriction enzymes to insert foreign DNA into plasmids.

Recombinant DNA is the general name for a piece of DNA that has been created by the combination of at least two strands. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure, and differ only in the nucleotide sequence within that identical overall structure. Recombinant DNA molecules are sometimes called **chimeric DNA**, because they can be made of material from two different species, like the mythical chimera. R-DNA technology uses palindromic sequences and leads to the production of sticky and blunt ends.

The DNA sequences used in the construction of recombinant DNA molecules can originate from any species. For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA, and incorporated into recombinant molecules. Using recombinant DNA technology and synthetic DNA, literally any DNA sequence may be created and introduced into any of a very wide range of living organisms.

Proteins that can result from the expression of recombinant DNA within living cells are termed *recombinant proteins*. When recombinant DNA encoding a protein is introduced into a host organism, the recombinant protein is not necessarily produced. Expression of foreign proteins requires the use of specialized expression vectors and often necessitates significant restructuring by foreign coding sequences. Recombinant DNA differs from genetic recombination in that the former results from artificial methods in the test tube, while the latter is a normal biological process that results in the remixing of existing DNA sequences in essentially all organisms.

b). Give an account of telomeres and their role in senescence and cancer.

The telomerase RNP and telomere complex present multiple potential targets for the design of new anticancer strategies .Telomerase may be a challenging target since its inhibition should exhibit a lag phase: the lack of telomerase should not affect cell growth rates until progressive telomere shortening with each cell division eventually causes cells to die or undergo growth arrest. Although it has been correctly suggested that this approach would not be sufficient by itself in patients with a large tumor burden, it may be a unique approach to patients with minimal residual disease. Importantly, normal somatic cells that lack telomerase expression should be largely unaffected by anti-telomerase therapy. Although telomerase inhibitors should possess great specificity, it is hoped they will also display low toxicity and few side effects. The most likely use of telomerase inhibitors would be as an adjuvant treatment in combination with surgery, radiation treatment and typical chemotherapy, when tumor burden is minimal. It is also possible that telomerase inhibitors could be used following standard therapies in which there is no clinical evidence of residual disease in order to treat possible micrometastases, and thus prevent cancer relapse. These situations will require prolonged treatment, so it will be important that the drugs have a low toxicity profile and are easily administered.

The primary unwanted effect of telomerase inhibition therapy may be on telomerase-positive reproductive cells and other proliferative cells of renewal tissues. Cells from such tissues generally have much longer telomeres than most tumor cell populations. Furthermore, stem cells of renewal tissues should be much less affected than dividing tumor cells; they proliferate only occasionally, and telomere shortening should not occur in the absence of cell division. Because the most primitive stem cell populations only rarely divide, their telomeres should shorten at a much slower rate than telomerase-inhibited, proliferating cancer cells. After the cancer cells have shortened their telomeres and died, anti-telomerase therapy could be discontinued and telomerase activity in reproductive and stem cells would be restored. Thus, anti-telomerase therapy is likely to eliminate the proliferative potential of cancer cells before the telomere lengths in normal reproductive and stem cells shorten sufficiently to disrupt their function.

Another avenue is to kill telomerase-expressing cells. Immunotherapy directed against telomerase positive cells is currently under investigation. This approach has the advantage of abolishing the lag phase that is required with the classic mode of telomerase inhibition. However, this treatment might also prove to be toxic to normal stem cells expressing telomerase.

It is still too early to know with certainty whether telomerase inhibitors will become a treatment option against cancer. There is concern about the emergence of alternative mechanisms of telomere maintenance and whether there will be side effects on normal hematopoietic and germline cells. These and other questions will only be answered when anti-telomerase drugs are moved into animal and human clinical trials.

25.(a). Discuss different types of DNA damages and their repair mechanism.

DNA damage

DNA damage, due to environmental factors and normal metabolic processes inside the cell, occurs at a rate of 10,000 to 1,000,000 molecular lesions per cell per day. While this constitutes only 0.000165% of the human genome's approximately 6 billion bases (3 billion base pairs), unrepaired lesions in critical genes (such as tumor suppressor genes) can impede a cell's ability to carry out its function and appreciably increase the likelihood of tumor formation and contribute to tumour heterogeneity.

The vast majority of DNA damage affects the primary structure of the double helix; that is, the bases themselves are chemically modified. These modifications can in turn disrupt the molecules' regular helical structure by introducing non-native chemical bonds or bulky adducts that do not fit in the standard double helix. Unlike proteins and RNA, DNA usually lacks tertiary structure and therefore damage or disturbance does not occur at that level. DNA is, however, supercoiled and wound around "packaging" proteins called histones (in eukaryotes), and both superstructures are vulnerable to the effects of DNA damage.

DNA damage can be subdivided into two main types:

1. endogenous damage such as attack by reactive oxygen species produced from normal metabolic byproducts (spontaneous mutation), especially the process of oxidative deamination
    1. also includes replication errors
2. exogenous damage caused by external agents such as
    1. ultraviolet [UV 200–400 nm] radiation from the sun
    2. other radiation frequencies, including x-rays and gamma rays
    3. hydrolysis or thermal disruption
    4. certain plant toxins
    5. human-made mutagenic chemicals, especially aromatic compounds that act as DNA intercalating agents
    6. viruses

The replication of damaged DNA before cell division can lead to the incorporation of wrong bases opposite damaged ones. Daughter cells that inherit these wrong bases carry mutations from which the original DNA sequence is unrecoverable (except in the rare case of a back mutation, for example, through gene conversion).

Types

There are several types of damage to DNA due to endogenous cellular processes:

1. *oxidation* of bases [e.g. 8-oxo-7,8-dihydroguanine (8-oxoG)] and generation of DNA strand interruptions from reactive oxygen species,
2. *alkylation* of bases (usually methylation), such as formation of 7-methylguanosine, 1-methyladenine, 6-O-Methylguanine
3. *hydrolysis* of bases, such as deamination, depurination, and depyrimidination.
4. "bulky adduct formation" (e.g., benzo[a]pyrene diol epoxide-dG adduct, aristolactam I-dA adduct)
5. *mismatch* of bases, due to errors in DNA replication, in which the wrong DNA base is stitched into place in a newly forming DNA strand, or a DNA base is skipped over or mistakenly inserted.
6. Monoadduct damage cause by change in single nitrogenous base of DNA
7. Diadduct damage

Damage caused by exogenous agents comes in many forms. Some examples are:

1. *UV-B light* causes crosslinking between adjacent cytosine and thymine bases creating *pyrimidine dimers*. This is called direct DNA damage.
2. *UV-A light* creates mostly free radicals. The damage caused by free radicals is called indirect DNA damage.
3. *Ionizing radiation* such as that created by radioactive decay or in *cosmic rays* causes breaks in DNA strands. Intermediate-level ionizing radiation may induce irreparable DNA damage (leading to replicational and transcriptional errors needed for neoplasia or may trigger viral interactions) leading to pre-mature aging and cancer.
4. *Thermal disruption* at elevated temperature increases the rate of depurination (loss of purine bases from the DNA backbone) and single-strand breaks. For example, hydrolytic depurination is seen in the thermophilic bacteria, which grow in hot springs at 40–80 °C. The rate of depurination (300 purine residues per genome per generation) is too high in these species to be repaired by normal repair machinery, hence a possibility of an adaptive response cannot be ruled out.
5. *Industrial chemicals* such as vinyl chloride and hydrogen peroxide, and environmental chemicals such as polycyclic aromatic hydrocarbons found in smoke, soot and tar create a huge diversity of DNA adducts- ethenobases, oxidized bases, alkylated phosphotriesters and crosslinking of DNA, just to name a few.

UV damage, alkylation/methylation, X-ray damage and oxidative damage are examples of induced damage. Spontaneous damage can include the loss of a base, deamination, sugar ring puckering and tautomeric shift.

DNA repair is a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encode its genome. In human cells, both normal metabolic activities and environmental factors such as radiation can cause DNA damage, resulting in as many as 1 million individual molecular lesions per cell per day. Many of these lesions cause structural damage to the DNA molecule and can alter or eliminate the cell's ability to transcribe the gene that the affected DNA encodes. Other lesions induce potentially

harmful mutations in the cell's genome, which affect the survival of its daughter cells after it undergoes mitosis. As a consequence, the DNA repair process is constantly active as it responds to damage in the DNA structure. When normal repair processes fail, and when cellular apoptosis does not occur, irreparable DNA damage may occur, including double-strand breaks and DNA crosslinkages (interstrand crosslinks or ICLs). This can eventually lead to malignant tumors, or cancer as per the two hit hypothesis.

The rate of DNA repair is dependent on many factors, including the cell type, the age of the cell, and the extracellular environment. A cell that has accumulated a large amount of DNA damage, or one that no longer effectively repairs damage incurred to its DNA, can enter one of three possible states:

1. an irreversible state of dormancy, known as senescence
2. cell suicide, also known as apoptosis or programmed cell death
3. unregulated cell division, which can lead to the formation of a tumor that is cancerous

The DNA repair ability of a cell is vital to the integrity of its genome and thus to the normal functionality of that organism. Many genes that were initially shown to influence span have turned out to be involved in DNA damage repair and protection.

OR

(b). Discuss the types of Anemia?

The most common types of anaemia are

- Iron deficiency anaemia
- Thalassaemia
- Aplastic anaemia
- Haemolytic anaemia
- Sickle cell anaemia
- Pernicious anaemia
- Fanconi anaemia

Iron Deficiency Anaemia

Overview

The most common form of anaemia is iron deficiency anaemia which is usually due to chronic blood loss caused by excessive menstruation. Increased demands for iron, such as foetal growth in pregnancy, and children undergoing rapid growth spurts in infancy and adolescence, can also cause iron deficiency anaemia.

This condition is treated with iron supplementation as well as the treatment of the underlying cause of the iron deficiency.

Causes

Iron deficiency occurs when the rate of loss or use of iron is more than its rate of absorption and use. The reasons for this are

- Chronic blood loss: Most commonly due to excessive menstruation or bleeding into or from the gut as a result of a peptic ulcer, gastritis, haemorrhoids or in children, worm infestation.
- Increased use of iron: In pregnancy, due to the growth of the foetus or children undergoing rapid growth spurts in infancy and adolescence.
- Decreased absorption of iron
    - after a partial or total removal of the stomach;
    - lack of stomach acid;
    - chronic diarrhoea; or
    - malabsorption.

Signs and symptoms

The most common symptoms of chronic anaemia include tiredness, weakness, shortness of breath and sometimes, a fast heartbeat. The tongue may also become smooth, shiny and inflamed - this is called glossitis. Angular stomatitis (erosion, tenderness and swelling at the corners of the mouth) may also occur. In some instances, the patient also suffers from pica, a craving for strange foods such as starch, ice and clay.

The symptoms of the underlying cause of the iron deficiency may be present such as heavy menstrual bleeding or abdominal pain due to peptic ulceration.

Treatment

Treatment for iron-deficiency anaemia will depend on the cause and severity of the condition. Treatments may include dietary changes and supplements, medicines, and surgery. Severe iron-deficiency anaemia may require treatment in hospital, blood transfusions, iron rejections, or intravenous iron therapy.

Risk

Infants and young children, women, and adults who have internal bleeding are at highest risk for iron-deficiency anaemia.

Aplastic Anaemia

Overview

Aplastic anaemia is a blood disorder in which the body's bone marrow doesn't make enough new blood cells. This may result in a number of health problems including arrhythmias, an enlarged heart, heart failure, infections and bleeding.

Aplastic anaemia is a rare but serious condition. It can develop suddenly or slowly and tends to worsen with time, unless the cause is found and treated.

Causes

Damage to the bone marrow's stem cells causes aplastic anaemia. In more than half of people who have aplastic anaemia, the cause of the disorder is unknown.

A number of acquired diseases, conditions, and factors can cause aplastic anaemia including

- Toxins, such as pesticides, arsenic, and benzene
- Radiation and chemotherapy
- Medicines such as chloramphenicol
- Infectious diseases such as hepatitis, Epstein-Barr virus, cytomegalovirus, parvovirus B19, and HIV
- Autoimmune disorders such as lupus and rheumatoid arthritis

Inherited conditions, such as Fanconi anaemia, Shwachman-Diamond syndrome, dyskeratosis congenital and Diamond-Blackfan anaemia may also cause aplastic anaemia.

Signs and symptoms

The most common symptoms of aplastic anaemia are

- Fatigue
- Shortness of breath
- Dizziness
- Headache
- Coldness in your hands or feet
- Pale skin, gums and nail beds
- Chest pains

Treatment

Treatment for aplastic anaemia includes blood transfusions, blood and marrow stem cell transplants, and medication. These treatments can prevent or limit complications, relieve symptoms, and improve quality of life.

In some cases, a cure may be possible. Blood and marrow stem cell transplants may cure the disorder. Removing a known cause of aplastic anaemia, such as exposure to a toxin, may also cure the condition.

Risk

People of all ages can get aplastic anaemia. However, it is most common in adolescents, young adults and the elderly. Men and women are equally likely to have it.

A person's risk for aplastic anaemia is higher if you have

- Been exposed to toxins
- Taken certain medicines or had radiation or chemotherapy treatment
- Certain infectious diseases, autoimmune disorders, or inherited conditions

Haemolytic Anaemia

Overview

Haemolytic anaemia is a condition in which red blood cells are destroyed and removed from the bloodstream before their normal lifespan is up. A number of diseases, conditions and factors can cause the body to destroy its red blood cells. Haemolytic anaemia can lead to various health problems such as fatigue, pain, arrhythmias, an enlarged heart and heart failure.

There are many types of haemolytic anaemias – some of which are inherited and others that are acquired.

Inherited haemolytic anaemias include

- Sickle cell anaemia
- Thalassaemias
- Hereditary spherocytosis
- Hereditary elliptocytosis
- Glucose-6-phosphate dehydrogenase (G6PD) deficiency
- Pyruvate kinase deficiency

Acquired haemolytic anaemias include

- Immune haemolytic anaemia
  - Autoimmune haemolytic anaemia
  - Alloimmune haemolytic anaemia
  - Drug-induced haemolytic anaemia
- Mechanical haemolytic anaemias
- Paroxysmal nocturnal haemoglobinuria
- Certain infections and substances can also damage red blood cells and lead to haemolytic anaemia

Causes

The immediate cause of haemolytic anaemia is the early destruction of red blood cells. A number of diseases, conditions, and factors can cause the body to destroy its red blood cells. These causes can be inherited or acquired. Sometimes, the cause of haemolytic anaemia isn't known.

- In **inherited haemolytic anaemias**, the genes that control how red blood cells are made are faulty. Different types of faulty genes account for the different types of inherited haemolytic anaemias. In each type of inherited haemolytic anaemia, the body makes abnormal red blood cells. The problem with the red blood cells may involve the haemoglobin, cell membrane, or enzymes that maintain healthy red blood cells.
- In **acquired haemolytic anaemias**, the body makes normal red blood cells, however, some disease, condition, or factor destroys the cells too early. Examples include immune disorders, infections and reactions to medicines or blood transfusions.

Signs and Symptoms

The most common symptom of all types of anaemia is fatigue. A low red blood cell count can also cause shortness of breath, dizziness, headache, coldness in your hands or feet, pale skin, gums and nail beds, as well as chest pain.

Symptoms of haemolytic anaemia include

- Jaundice
- Pain in the upper abdomen
- Leg ulcers and pain
- A severe reaction to a blood transfusion

Treatment

Treatments for haemolytic anaemia include blood transfusions, medicines, plasmapheresis, surgery, blood and marrow stem cell transplants and lifestyle changes.

People who have mild haemolytic anaemia may not need treatment, as long as the condition doesn't worsen. People with severe haemolytic anaemia usually need ongoing treatment.

Risk

Haemolytic anaemia can affect people of all ages, races and sexes.

Thalassaemia

Overview

Thalassaemias are inherited blood disorders which cause the body to make fewer healthy red blood cells and less haemoglobin (an iron-rich protein in red blood cells).

The two major types of thalassaemia are alpha- and beta thalassaemia. The most severe form of alpha thalassaemia is known as alpha thalassaemia major or hydrops fetalis, while the severe form of beta thalassaemia is known as thalassaemia major or Cooley's anaemia.

Thalassaemias affect both males and females and occur most often in people of Italian, Greek, Middle Eastern, Asian, and African descent. Severe forms are usually diagnosed in early childhood and are lifelong conditions.

Causes

Haemoglobin in red blood cells has two kinds of protein chains: alpha globin and beta globin. If your body doesn't make enough of these protein chains, red blood cells don't form properly and can't carry enough oxygen.

Genes control how the body makes haemoglobin protein chains. When these genes are missing or altered, thalassaemias occur.

Thalassaemias are inherited disorders – they are passed on from parents to their children through genes. People who get abnormal haemoglobin genes from one parent but normal genes from the other are carriers. Carriers often have no signs of illness other than mild anaemia. However, they can pass the abnormal genes on to their children.

Signs and symptoms

Symptoms of thalassaemias are caused by a lack of oxygen in the blood stream. This occurs because the body doesn't make enough healthy red blood cells and haemoglobin. The severity of symptoms depends on the severity of the disorder:

- People who have alpha or beta thalassaemia can have mild anaemia, which can make you feel tired.
- People with beta thalassaemia intermedia have mild to moderate anaemia. They may also have other health problems including: slowed growth and delayed puberty; bone problems; and an enlarged spleen.
- People with haemoglobin H disease or beta thalassaemia major have severe thalassaemia. Symptoms occur within the first two years of life and include severe anaemia and other serious health problems
    - Pale and listless appearance
    - Poor appetite
    - Dark urine
    - Slowed growth and delayed puberty
    - Jaundice

- Enlarged spleen, liver and heart
- Bone problems

Treatment

Treatment for thalassaemias depends on the type and severity of the disorder. People who are carriers or who have alpha or beta thalassaemia need little or no treatment.

Three standard treatments are used to treat moderate and severe forms of thalassaemia, these include blood transfusions, iron chelation therapy, and folic acid supplements.

Risk

Family history and ancestry are the two risk factors for thalassaemias.

Sickle Cell Anaemia

Overview

Sickle cell anaemia is a serious disease in which the body makes sickle-shaped ("C"-shaped) red blood cells. Normal red blood cells are disk-shaped and move easily through your blood vessels. Red blood cells contain the protein haemoglobin (an iron-rich protein that gives blood its red colour and carries oxygen from the lungs to the rest of the body).

Sickle cells contain abnormal haemoglobin that causes the cells to have a sickle shape, which don’t move easily through the blood vessels – they are stiff and sticky and tend to form clumps and get stuck in the blood vessels.

The clumps of sickle cells block blood flow in the blood vessels that lead to the limbs and organs. Blocked blood vessels can cause pain, serious infections, and organ damage.

In sickle cell anaemia, a lower-than-normal number of red blood cells occurs because sickle cells don't last very long. Sickle cells usually die after about 10 to 20 days and the body can't reproduce red blood cells fast enough to replace the dying ones, which causes anaemia.

Causes

Sickle cell anaemia is an inherited, lifelong disease. People who have the disease inherit two copies of the sickle cell gene – one from each parent.

Signs and Symptoms

The most common symptoms of sickle cell anaemia are linked to anaemia and pain.

Common symptoms for anaemia include

- Fatigue
- Shortness of breath
- Dizziness
- Headache
- Coldness in the hands and feet
- Pale skin
- Chest pain

Sudden pain throughout the body is a common symptom of sickle cell anaemia. This pain is called a "sickle cell crisis", and often affects the bones, lungs, abdomen, and joints.

Treatment

Sickle cell anaemia has no widely-available cure. However, treatments can help relieve symptoms and treat complications. The goals of treating sickle cell anaemia are to relieve pain, prevent infections, eye damage and strokes, and control complications.

Bone marrow transplants may offer a cure in a small number of sickle cell anaemia cases.

Risk

Sickle cell anaemia is most common in people whose families descended from Africa, South or Central American, Caribbean islands, Mediterranean countries, India and Saudi Arabia.

Pernicious Anaemia

Overview

Pernicious anaemia is a condition in which the body can't make enough healthy red blood cells because it doesn't have enough vitamin B12 (a nutrient found in certain foods). People who have pernicious anaemia can't absorb enough vitamin B12 due to a lack of intrinsic factor (a protein made in the stomach). However, other conditions and factors can also cause vitamin B12 deficiency.

Causes

- A lack of intrinsic factor is a common cause of pernicious anaemia as the body can't absorb enough vitamin B12.
- Some pernicious anaemia occurs because the body's small intestine can't properly absorb vitamin B12 which may be due to the wrong bacteria in the small intestines; certain diseases that interfere with vitamin B12 absorption; certain medicines; surgical removal of part of the small intestine; and tapeworm infection.
- Sometimes people develop pernicious anaemia because they don't get enough vitamin B12 in their diets.

Signs and symptoms

Apart from the symptoms of anaemia (fatigue, dizziness, etc.), the vitamin B12 deficiency may also have some serious symptoms such as

- Nerve damage
- Neurological problems such as confusion, dementia, depression, and memory loss.
- Symptoms in the digestive tract include nausea and vomiting, heartburn, abdominal bloating and gas, constipation or diarrhoea, loss of appetite, and weight loss.
- An enlarged liver
- A smooth, beefy red tongue
- Infants who have vitamin B12 deficiency may have poor reflexes or unusual movements, such as face tremors.

Treatment

Pernicious anaemia is treated by replacing the missing vitamin B12 in the body. People who have this disease may need lifelong treatment.

Risk

You are at higher risk for pernicious anaemia if you

- Have a family history of the condition.
- Have had part or all of your stomach removed.
- Have certain autoimmune disorders that involve the endocrine glands, such as Addison's disease, type 1 diabetes, Graves' disease, and vitiligo.
- Have had part or all of your small intestine removed.
- Have certain intestinal diseases or disorders that prevent your body from properly absorbing vitamin B12.
- Take medicines that prevent your body from properly absorbing vitamin B12.
- Are a strict vegetarian who doesn't eat any animal or diary products and doesn’t take a vitamin B12 supplement, or if you eat poorly overall.

Fanconi Anaemia

Overview

Fanconi anaemia, or FA, is a rare, inherited blood disorder that leads to bone marrow failure. FA is a type of aplastic anaemia that prevents your bone marrow from making enough new blood cells for your body to work normally. FA can also cause your bone marrow to make many abnormal blood cells. This can lead to serious health problems, such as leukemia.

FA is a blood disorder, but it can also affect many of the body's organs, tissues, and systems. Children who inherit FA are at higher risk of being born with birth defects, and people who have FA are at higher risk of some cancers and other serious health problems.

FA is an unpredictable disease. The average lifespan for people with FA is between 20 and 30 years. The most common causes of death related to FA are bone marrow failure, leukemia, and solid tumours.

Causes

FA is an inherited disease – it is passed on from parents to children through the genes. At least 13 faulty genes are associated with FA. FA develops when both parents pass the same faulty FA gene to their child. People who have only one faulty gene are FA carriers which means they don't have FA, but they can pass the faulty gene to their children.

Signs and symptoms

The symptoms of FA include

- Anaemia
- Bone marrow failure
- Birth defects
- Developmental or eating problems

Treatment

Treatment for FA is based on a person's age and how well or poorly the person's bone marrow makes new blood cells.

The four main types of treatment for FA are

- Blood and marrow stem cell transplant
- Androgen therapy
- Synthetic growth factors
- Gene therapy

Risk

FA occurs in all racial and ethnic groups and affects men and women equally. You are at an increased risk of developing the disease if you have a family history of FA.

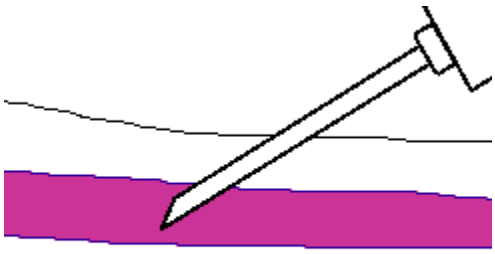
26.(a). Write a note on Blood collection and preservation of samples?

Blood Specimen Collection and Processing

The first step in acquiring a quality lab test result for any patient is the specimen collection procedure. The venipuncture procedure is complex, requiring both knowledge and skill to perform. Several essential steps are required for every successful collection procedure:

Venipuncture Procedure:

1. A phlebotomist must have a professional, courteous, and understanding manner in all contact with all patients.
2. The first step to the collection is to positively identify the patient by two forms of identification; ask the patient to state and spell his/her name and give you his/her birth date. Check these against the requisition (paper or electronic).
3. Check the requisition form for requested tests, other patient information and any special draw requirements. Gather the tubes and supplies that you will need for the draw.
4. Position the patient in a chair, or sitting or lying on a bed.
5. Wash your hands.
6. Select a suitable site for venipuncture, by placing the tourniquet 3 to 4 inches above the selected puncture site on the patient. See below for venipuncture site selection "notes."
7. Do not put the tourniquet on too tightly or leave it on the patient longer than 1 minute.
8. Next, put on non-latex gloves, and palpate for a vein.
9. When a vein is selected, cleanse the area in a circular motion, beginning at the site and working outward. Allow the area to air dry. After the area is cleansed, it should not be touched or palpated again. If you find it necessary to reevaluate the site by palpation, the area needs to be re-cleansed before the venipuncture is performed.
10. Ask the patient to make a fist; avoid "pumping the fist." Grasp the patient's arm firmly using your thumb to draw the skin taut and anchor the vein. Swiftly insert the needle through the skin into the lumen of the vein. The needle should form a 15-30 degree angle with the arm surface. Avoid excess probing.



11. When the last tube is filling, remove the tourniquet.
12. Remove the needle from the patient's arm using a swift backward motion.

13. Place gauze immediately on the puncture site. Apply and hold adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes, tape a fresh piece of gauze or Band-Aid to the puncture site.
14. Dispose of contaminated materials/supplies in designated containers.

    Note: The larger median cubital and cephalic veins are the usual choice, but the basilic vein on the dorsum of the arm or dorsal hand veins are also acceptable. Foot veins are a last resort because of the higher probability of complications.

OR

(b).What is the abnormal types of urine? Explain.

There are several conditions that can cause abnormal components to be excreted in urine or present as abnormal characteristics of urine. They are mostly referred to by the suffix -uria. Some of the more common types of abnormal urine include:

- Proteinuria—Protein content in urine, often due to leaky or damaged glomeruli.
- Oliguria—An abnormally small amount of urine, often due to shock or kidney damage.
- Polyuria—An abnormally large amount of urine, often caused by diabetes.
- Dysuria—Painful or uncomfortable urination, often from urinary tract infections.
- Hematuria—Red blood cells in urine, from infection or injury.
- Glycosuria—Glucose in urine, due to excess plasma glucose in diabetes, beyond the amount able to be reabsorbed in the proximal convoluted tubule.